cobas® TaqScreen MPX Test
for use on the cobas s 201 system

FOR *IN VITRO* DIAGNOSTIC USE.

cobas® TaqScreen MPX Test	**MPX**	96 Tests	P/N: 04584252 190
cobas® TaqScreen MPX Control Kit	**MPX CTL**	6 Sets	P/N: 04626303 190
cobas® TaqScreen Wash Reagent	**TS WR**	5.1 L	P/N: 04404220 190

For testing cadaveric specimens with the **cobas**® TaqScreen MPX Test, the following kit is required by the user, in addition to the kits above:

cobas® TaqScreen Cadaveric Specimen Diluent Kit	**CADV SPEC**	96 Tests	P/N: 05002125 190

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INTENDED USE

The **cobas®** TaqScreen MPX Test, for use with the **cobas s** 201 system, is a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA and Hepatitis B Virus (HBV) DNA in human plasma.

This test is intended for use to screen donor samples for HIV-1 Group M RNA, HCV RNA and HBV DNA in plasma specimens from individual human donors, including donors of Whole Blood, blood components, source plasma and other living donors.

This test is also intended for use in testing plasma specimens to screen individual organ donors when specimens are obtained while the donor's heart is still beating and in testing blood 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 specimens. For donations of Whole Blood and blood components, plasma specimens may be tested individually or in pools comprised of not more than six equal aliquots of individual specimens. For donors of hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood or cord blood, and for donors of donor lymphocytes for infusion (DLI), plasma may be tested in pools comprised of equal aliquots of not more than 6 individual donor specimens. For donations from cadaveric (non-heart-beating) organ and tissue donors, specimens may only be screened as individual specimens. For donations of source plasma, plasma may be tested in pools comprised of up to 96 individual donations. This test is intended to be used in conjunction with licensed serology tests for HIV, HCV and HBV.

Whereas this test can detect HIV-1 Group O RNA and HIV-2 RNA, detection of HIV-1 Group O RNA or HIV-2 RNA in donor specimens negative for anti-HIV-1 Group O antibodies or anti-HIV-2 antibodies, respectively, has not been demonstrated in clinical studies.

This test is not intended for use as an aid in diagnosis of infection with HIV, HCV or HBV.

SUMMARY AND EXPLANATION OF THE TEST

A major concern regarding the transfusion of blood and blood components is the potential for transmission of viral infections, particularly with Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV). These agents are primarily transmitted by exposure to contaminated blood or blood and plasma products, exposure to certain body tissues or fluids, by sexual contact or by an infected mother to the newborn child.

HIV-1 is prevalent globally, with an estimated overall prevalence of 1.1% (0.56% in North America and 0.25% in Western Europe). Persons infected with HIV-1 can experience a brief, initially acute, flu-like illness associated with high levels of viremia in peripheral blood within 3-6 weeks of initial infection.[1] There are currently three principal genetic groups for HIV-1: Group M (main), Group N (non-M-non-O), and Group O (outlier). Group M is highly prevalent and is divided into 9 subtypes as well as several circulating recombinant forms (CRFs).[2]

HIV-2 was first isolated in 1986 from patients in West Africa. Both HIV-1 and HIV-2 have the same modes of transmission and are associated with similar opportunistic infections and Acquired Immunodeficiency Syndrome (AIDS).[3] The prevalence of HIV-2 in some African nations reaches more than 1%, and HIV-2 is a growing concern in certain parts of Europe and India.[4] The first case of HIV-2 infection in the United States was diagnosed in 1987. The Centers for Disease Control and Prevention (CDC) advise that continued surveillance is needed to monitor HIV-2 in the US population.[3]

HCV is considered to be the principal etiologic agent responsible for 90%–95% of the post transfusion non-A and non-B hepatitis cases.[5,6] HCV occurs globally but the incidence is not well known because the infection is generally asymptomatic. However, the reported prevalence varies from 0.5 – 2.0% in Western Europe to as high as 20% in Egypt. More than 2 billion people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus.[7,8,9,10,11] Three quarters of the world's population live in areas where there are high levels of infection. Every year there are over 4 million acute clinical cases of HBV.

Serological screening assays have greatly reduced, but not eliminated, the risk of transmission of viral infections by transfusion of blood and blood products. Testing of whole blood and source plasma donations for HBV was initiated with HBsAg assays in the early 1970s and anti-HBc in the 1980s. In addition to HBV screening, blood and plasma donations are routinely tested for HIV-1 and HIV-2 by screening with enzyme immunoassays (EIAs) and for anti-HCV by EIAs. Public demand for higher standards of screening for infectious agents in transfusion products has fueled the advancement of nucleic acid test (NAT) technology. Studies have shown that testing for viral nucleic acids (HIV-1 RNA,[12,13,14] HCV RNA[5,13-16] and HBV DNA[17-20]) can further reduce the transmission risk of these agents in blood donations made during the seroconversion window period. This window period has been estimated as 22 days on average, but may be as long as 6 months, for HIV-1.[21] With the implementation of HIV-1 mini-pool NAT testing, the infectious window period has been significantly shortened and the current risk of HIV-1 transmission is estimated to be approximately 1 in 2 million donations.[13,14,15] Similarly, the introduction of HCV RNA NAT reduced the antibody negative window period by approximately 60 days,[13-16] with a current estimated risk of approximately 1 – 2 in 1 million donations. HBV DNA NAT screening is being increasingly adopted. Studies from countries with low, moderate and high HBV prevalence have demonstrated NAT yield from window period and late stage HBV-infected donors, thus demonstrating reduced incidence of transfusion transmitted HBV.[15,19,20,22,23]

To improve the efficiency of testing for multiple targets, a multiplex (MPX) polymerase chain reaction (PCR) for simultaneous detection of multiple viruses has been developed. In MPX PCR, more than one target sequence is amplified and detected by using multiple pairs of primers and probes in one reaction tube.

The **cobas**® TaqScreen MPX Test is a qualitative multiplex test that enables the simultaneous detection of HIV-1 Group M and Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in infected pooled and individual plasma donations. The **cobas**® TaqScreen MPX Test uses a generic nucleic acid preparation technique on the COBAS® AmpliPrep Instrument. HIV-1 Groups M and O RNA, HIV-2 RNA, HCV RNA and HBV DNA are amplified and detected using automated, real time PCR on the COBAS® TaqMan® Analyzer. The test incorporates an Internal Control for monitoring test performance in each individual test as well as the AmpErase enzyme to reduce potential contamination by previously amplified material (amplicon). The **cobas**® TaqScreen MPX Test does not discriminate which virus was detected in a specimen. The COBAS® AmpliScreen HIV-1 Test, v1.5, COBAS® AmpliScreen HCV Test, v2.0 and COBAS® AmpliScreen HBV Test are used for individual viral target identification (Discriminatory Testing) of HIV-1 Group M, HCV and HBV. Individual viral target identification test procedures for HIV-1 Group O and HIV-2 are not available from Roche.

PRINCIPLES OF THE PROCEDURE

The **cobas**® TaqScreen MPX Test used on the **cobas s** 201 system is based on 4 major processes:

1. Automated Specimen Pooling and Control Pipetting using the HAMILTON Microlab® STAR/STARlet IVD Pipettor
2. Automated Specimen Preparation using the COBAS® AmpliPrep Instrument
3. Automated Amplification of Nucleic Acid and Real Time Automated Detection of PCR products using the COBAS® TaqMan® Analyzer
4. Automated Data Management using the Pooling and Data Management (PDM) Software

Automated Specimen Pooling and Pipetting using the HAMILTON Microlab STAR/STARlet IVD Pipettor

The HAMILTON Microlab STAR/STARlet IVD Pipettor automates pipetting of pools and individual donor specimens, transfer of aliquots to Deep Well Plates (optional) and pipetting of Test Controls. The **cobas s** 201

system is used for resolution testing of reactive pools and to identify the reactive individual specimens. The **cobas s** 201 system is designed to process specimens in batches. A batch is defined as a collection of specimens and controls that are pipetted, extracted, amplified and detected together. When the pipetting of a batch is completed on the HAMILTON Microlab STAR/STARlet IVD Pipettor, the entire sample rack is transferred into the COBAS® AmpliPrep Instrument for the next phase of the process.

Note: For testing of cadaveric specimens, the specimen should first be manually diluted 1:5 in cobas TaqScreen Cadaveric Specimen Diluent (CADV SPEC DIL) prior to pipetting using the HAMILTON Microlab STAR/STARlet IVD Pipettor.

Automated Specimen Preparation using the COBAS® AmpliPrep Instrument

Nucleic acids from the targets and added Armored RNA Internal Control (IC) molecules (which serves as a specimen preparation and amplification/detection process control) are simultaneously processed. The **cobas®** TaqScreen MPX Test contains reagents that accomplish five sequential steps on the COBAS® AmpliPrep Instrument. The Protease solution digests proteins to promote lysis, inactivate nucleases and facilitate the release of RNA and DNA from viral particles. Addition of Lysis Reagent to the specimen results in viral lysis and nuclease inactivation by denaturation of proteins. RNA and DNA are released and simultaneously protected from nucleases. The released nucleic acids bind to the silica surface of the added Magnetic Glass Particles. This is mainly due to the net positive charge on the glass particle surface and net negative charge of the nucleic acids under the chaotropic salt concentration and ionic strength of the Lysis reaction. Wash Reagent removes unbound substances and impurities such as denatured proteins, cellular debris and potential PCR inhibitors (such as hemoglobin, etc.), and reduces the salt concentration. Purified nucleic acids are released from the Magnetic Glass Particles at an elevated temperature with Elution Buffer.

Automated Amplification of Nucleic Acid using the COBAS® TaqMan® Analyzer

After isolation of the purified nucleic acids from human plasma during automated specimen preparation, **cobas®** TaqScreen MPX Master Mix (MPX MMX) is used for the amplification and detection of HIV-1 (Groups M and O), HIV-2 and HCV RNA, HBV DNA and IC RNA. Once activated by the addition of manganese acetate, the **cobas®** TaqScreen MPX Master Mix permits reverse transcription (for RNA targets), followed by PCR amplification of highly conserved regions of HIV-1 (Groups M and O), HIV-2 and HCV RNA, HBV DNA and IC RNA using specific primers. Concurrent detection of the amplified nucleic acid is accomplished by the generation of fluorescent signals from 5'-nucleolytic degradation of HIV-1 (Groups M and O), HIV-2, HCV, HBV and IC-specific probes, also present in the Master Mix. Two fluorescent dyes are used: one dye labels the IC probe and a second dye labels all target specific probes permitting multiplex combined identification of the viral targets and independent identification of the IC.

Reverse Transcription and PCR Amplification

Reverse transcription and amplification reactions are performed with a thermostable recombinant enzyme, Z05 DNA Polymerase. In the presence of manganese (Mn^{2+}), Z05 DNA Polymerase has reverse transcriptase and DNA polymerase activities. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture.

PCR amplification is accomplished using the Z05 DNA Polymerase which extends the annealed primers along the target templates to produce a double-stranded DNA (amplicon). This process is repeated for multiple cycles, with each cycle doubling the amount of amplicon DNA. Amplification occurs only in the region of the target genomes between the primers; the entire genomes are not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas®** TaqScreen MPX Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[24], but not DNA containing deoxythymidine or RNA containing ribouridine.[25,26] Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate with thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA

non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55ºC and therefore does not destroy target amplicon formed after PCR.

Real time Automated Detection of PCR Products using the COBAS® TaqMan® Analyzer

During PCR Amplification, the intermittent high temperature during the cycling denatures the Target and IC amplicon to form single stranded DNA. The specific detection oligonucleotide probes hybridize to the single stranded form of the amplified DNA. Amplification, Hybridization and Detection occur simultaneously.

Detection of PCR Products[27,28]

The **cobas**® TaqScreen MPX MMX contains detection probes which are specific for HIV-1 (Groups M and O), HIV-2, HCV, HBV or IC nucleic acid. Each detection probe is labeled with 1) one of two fluorescent dyes which act as a reporter and 2) another dye which acts as a quencher. One specific reporter dye is associated with the viral specific probes and is measured at a defined wavelength. A second different reporter dye is associated with the IC specific probe and is measured at a different wavelength. A single type of quencher dye is used in all probes. This system permits detection of all the amplified virus targets at one wavelength and simultaneous detection of amplified IC nucleic acid at another wavelength.

Before PCR amplification begins, the probes are intact and the reporter dye fluorescence is suppressed by the quencher dye due to Förster-type energy transfer. During PCR amplification, the probes hybridize to specific single stranded DNA sequences and are cleaved by the 5' to 3' nuclease activity of the Z05 DNA Polymerase at the same time that amplification is occurring. Once the reporter and quencher dyes are separated by this cleavage, the fluorescent activity of the reporter dye is unmasked. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased.

Real time detection of PCR products is accomplished by measuring the fluorescence of the released reporter dyes representing the viral targets and IC independently.

Automated Data Management using the PDM Software

Roche PDM Data Manager allows the user to review and report results. The Roche PDM Data Manager assigns test results for all tests as non-reactive, reactive or invalid. In addition to retrieving and examining PCR results, the Roche PDM software allows the operator to print reports, search for results, accept donor results and optionally transmit results to an LIS.

MATERIALS PROVIDED BY ROCHE

Three kits are required for the detection of HIV-1 (Groups M and O), HIV-2 and HCV RNA and HBV DNA in plasma specimens: 1) **cobas**® TaqScreen MPX Test, 2) **cobas**® TaqScreen MPX Control Kit, and 3) **cobas**® TaqScreen Wash Reagent. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

cobas® TaqScreen MPX Test (P/N: 04584252 190)	**MPX**	**96 Tests**

MPX CS1
(MPX Magnetic Glass Particles Reagent Cassette)

MPX CS2
(MPX Lysis Reagent Cassette)

MPX CS3
(MPX Multi-Reagent Cassette)

MPX CS4
(MPX Test-Specific Reagent Cassette)

cobas® TaqScreen MPX Control Kit **MPX CTL** **6 Sets**
(P/N: 04626303 190)

HIV-1 M (+) C
(HIV-1 M Positive Control)

HIV-1 O (+) C
(HIV-1 O Positive Control)

HIV-2 (+) C
(HIV-2 Positive Control)

HCV (+) C
(HCV Positive Control)

HBV (+) C
(HBV Positive Control)

TS (–) C
[**cobas®** TaqScreen Negative Control (Human Plasma)]

cobas® TaqScreen Wash Reagent **TS WR** **5.1L**
(P/N: 04404220 190)

TS WR
(**cobas®** TaqScreen Wash Reagent)

NOTE: For detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in cadaveric specimens, the following kit is required and provided, in addition to the kits mentioned above: cobas TaqScreen Cadaveric Specimen Diluent.

cobas® TaqScreen Cadaveric Specimen Diluent Kit **CADV SPEC** 96 Tests
(P/N: 05002125 190)

CADV SPEC DIL
(**cobas®** TaqScreen Cadaveric Specimen Diluent)

OTHER MATERIALS REQUIRED BUT SOLD SEPARATELY (May Be Purchased From Roche)

This test must be run on the **cobas s** 201 system. The **cobas s** 201 system must be installed by a Roche Diagnostics Field Service Representative, and used as a complete system configuration. Individual **cobas s** 201 system components cannot be used as stand-alone devices, nor may other components be substituted. The **cobas s** 201 system utilizes the components listed below. Please refer to the Product Information Card for additional details.

Instrumentation and Software for cobas s 201 system — Configuration C or Configuration C Maintenance Release 1 (MR1)

- HAMILTON Microlab STAR/STARlet IVD Pipettor, Pooling Manager Workstation and software
- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer (with EX48 upgrade)
- AMPLILINK software datastation and software v3.1.2 or v3.2.1
- Roche PDM Data Manager Server, Data Manager workstation and software v2.0.10 or v2.0.14
- **cobas s** 201 system — Configuration C or Configuration C (MR1) Operator's Manual
- **cobas s** 201 system — Configuration C or Configuration C (MR1) Known Issues List

Racks and Disposables

- COBAS® AmpliPrep Sample Racks (SK24) (P/N: 28122172001)
- COBAS® AmpliPrep SPU-racks (P/N: 28122806001)
- COBAS® AmpliPrep Reagent Racks (P/N: 28122199001)

- Sample Processing Units (SPU): (P/N: 03755525001)
- Sample Input Tubes (S-tubes) with Barcode Clips (P/N: 03137040001)
- Racks of K-tips (P/N: 03287343001)
- K-tube Box of 12 x 96 (P/N: 03137082001)
- COBAS® TaqMan® K-carrier (P/N: 28150397001)
- High Volume CO-RE Tips (1000 µL), filter (P/N: 04639642001)
- Deep Well Plates with Barcode Labels (P/N: 04639634001)
- Deep Well Plate Sealing Mats (P/N: 04789288001)
- Sample Carrier for 24 Test Tubes (P/N: 04639502001)
- Sample Carrier for 32 Test Tubes (P/N: 04639529001)
- Tip Carrier (P/N: 04639545001)
- Deep Well Plate Carrier (P/N: 04639553001)
- SK24 Rack Carrier (P/N: 04639600001)
- Microcide SQ™ or HAMILTON Disinfectant Spray (P/N: 04592557001)
- Disposable gloves, powderless

REAGENTS

cobas® TaqScreen MPX Test **MPX** **96 Tests**
(P/N: 04584252 190)

MPX CS1 **2 x 48 Tests**

MGP 2 x 7.0 mL
(Magnetic Glass Particles)
Magnetic glass particles
93% Isopropanol

Xi  93% (w/w) Isopropanol
Irritant

F  93% (w/w) Isopropanol
Highly
Flammable

MPX CS2 **2 x 48 Tests**

LYS 2 x 78 mL
(Lysis Reagent)
Sodium citrate dihydrate
42.5% Guanidine thiocyanate
< 14% Polydocanol
0.9% Dithiothreitol

Xn  42.5% (w/w) Guanidine thiocyanate
Harmful

MPX CS3 **2 x 48 Tests**

Pase 2 x 3.8 mL
(Proteinase Solution)
TRIS buffer
< 0.05% EDTA
Calcium chloride
Calcium acetate
≤ 7.8% Proteinase
Glycerol

Xn  ≤ 7.8% (w/w) Proteinase
Harmful

EB 2 x 7.0 mL
(Elution Buffer)
TRIS buffer
0.2% Methylparaben

MPX CS4 **2 x 48 Tests**

MPX MMX-R1 2 x 3.0 mL
(MPX Master Mix Reagent 1)
Tris buffer
Potassium acetate
Glycerol
Manganese acetate
Betaine
0.08% Sodium azide

MPX MMX-R2 2 x 2.5 mL
(MPX Master Mix Reagent 2)
Tricine buffer
Potassium chloride
Potassium hydroxide
< 21% Dimethyl sulfoxide
Glycerol
EDTA
Tween 20
Igepal CA630
< 0.09% dATP, dGTP, dCTP, dUTP, dTTP
< 0.01% Upstream and downstream HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, HBV primers
< 0.01% Fluorescent-labeled HIV-1, HIV-2, HCV, HBV probes
< 0.01% Fluorescent-labeled Internal Control probe
< 0.01% Oligonucleotide aptamer
< 0.07% Z05 DNA Polymerase (microbial)
< 0.4% AmpErase [uracil-N-glycosylase] enzyme (microbial)
0.08% Sodium azide

MPX IC 2 x 15 mL
(MPX Internal Control)
TRIS buffer
≤ 0.002% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide
< 0.001% Non-infectious, synthetic internal control RNA encapsulated in MS2 bacteriophage coat protein

cobas® TaqScreen MPX Control Kit **6 Sets**
(P/N: 04626303 190)

MPX CTL

HIV-1 M (+) C 6 x 1.6 mL
(HIV-1 M Positive Control)
< 0.001% Non-infectious, synthetic HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

HIV-1 O (+) C 6 x 1.6 mL
(HIV-1 O Positive Control)
< 0.001% Non-infectious, synthetic HIV-1 Group O RNA encapsulated in MS2 bacteriophage coat protein.
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

HIV-2 (+) C 6 x 1.6 mL
(HIV-2 Positive Control)
< 0.001% Non-infectious, synthetic HIV-2 RNA encapsulated in MS2 bacteriophage coat protein
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

HCV (+) C 6 x 1.6 mL
(HCV Positive Control)
< 0.001% Non-infectious, synthetic HCV RNA encapsulated in MS2 bacteriophage coat protein
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

HBV (+) C 6 x 1.6 mL
(HBV Positive Control)
0.001% Non-infectious, synthetic HBV DNA encapsulated in Lambda bacteriophage coat protein
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

TS (–) C 6 x 1.6 mL
[**cobas**® TaqScreen Negative Control (Human Plasma)]
Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2 and HBsAg; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-Methyl-2H-isothiazol-3-one

Irritant
R43: May cause sensitization by skin contact

cobas® TaqScreen Wash Reagent **TS WR** **5.1 L**
(P/N: 04404220 190)

TS WR
(**cobas**® TaqScreen Wash Reagent)
Sodium citrate dihydrate
0.1% Methylparaben

cobas® TaqScreen Cadaveric Specimen Diluent Kit **96 Tests**
(P/N: 05002125 190) **CADV SPEC**

CADV SPEC DIL 4 x 100 mL
(**cobas**® TaqScreen Cadaveric Specimen Diluent)
EDTA

STORAGE AND HANDLING REQUIREMENTS

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

B. Do not freeze reagents or controls.

C. Store **MPX CS1, MPX CS2, MPX CS3** and **MPX CS4** at 2 to 8°C. Unused, these reagents are stable until the expiration date indicated.

D. After initial use, reagents are stable for 30 days at 2 to 8°C or until the expiration date, whichever comes first.

E. Reagents can be used for up to 6 instrument runs and up to a maximum of 40 cumulative hours on the COBAS® AmpliPrep Instrument. Reagents must be stored at 2 to 8°C between uses. The AMPLILINK software monitors the cumulative hours of the reagent cassettes on the COBAS® AmpliPrep Instrument, and blocks the cassettes from being used once the 40 cumulative hours are reached.

F. Reagents are stable for a total of 24 continuous hours on the COBAS® AmpliPrep Instrument. The AMPLILINK software does not monitor the continuous hours of the reagent cassettes on the COBAS® AmpliPrep Instrument, nor the number of instrument runs the cassettes have been used for. It is the user's responsibility to discard the reagent cassettes once the 24 continuous hours or 6 instrument runs are reached.

G. Store **HIV-1 M (+) C, HIV-1 O (+) C, HIV-2 (+) C, HCV (+) C, HBV (+)** C and **TS (–) C** at 2 to 8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

H. Store **TS WR** at 15 to 30°C. Unopened **TS WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days at 15 to 30°C or until the expiration date, whichever comes first.

I. Store **CADV SPEC DIL** at 15 to 30°C. The diluent is stable until the expiration date indicated. Once opened, any unused diluent remaining in the container must be discarded.

PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the test.[29,30] Only personnel proficient in the use of the **cobas®** TaqScreen MPX Test and trained in handling infectious materials should perform this procedure. Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute bleach). Follow by wiping the surface with 70% Ethanol.

B. **CAUTION: HIV-1 M (+) C, HIV-1 O (+) C, HIV-2 (+) C, HCV (+) C, HBV (+) C and TS (–) C contain plasma derived from human blood. The source material has been tested and found non-reactive for the presence of antibody to HIV-1/2, antibody to HCV, and HBsAg. Source material has also been tested using the cobas® TaqScreen MPX Test. Testing of negative human plasma by PCR methods showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 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 with a freshly prepared solution of dilute 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. **MPX MMX-R1, MPX MMX-R2** and **MPX IC** contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide 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. The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. False positive results may occur if cross contamination of specimens is not prevented during specimen handling and processing.

G. Use only supplied or specified required disposables to ensure optimal test performance.

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

I. Before use, visually inspect each reagent cassette, control tube and Wash Reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.

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

K. Do not use a **cobas**® TaqScreen MPX Test, **cobas**® TaqScreen MPX Control Kit, **cobas**® TaqScreen Wash Reagent or **cobas**® TaqScreen Cadaveric Specimen Diluent Kit after its expiration date. Do not interchange, mix, or combine reagents from different kits or different lots. Do not load mixed reagent lots on the COBAS® AmpliPrep Instrument.

L. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

M. Avoid contact of reagents 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 LYS, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.**

N. Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may a ffect optimal test performance.

O. The use of excessively hemolyzed living donor specimens should be avoided.

P. Red blood cell contamination of plasma specimens (>2.5%) may inhibit the **cobas**® TaqScreen MPX Test.

Q. Do not use any component with damaged barcode labels at any phase of testing.

CONTROL AND REAGENT PREPARATION

A. Equilibrate **cobas**® TaqScreen MPX Control Kit to room temperature for 30 minutes prior to loading controls onto the HAMILTON Microlab STAR/STARlet IVD Pipettor. Equilibrate **cobas**® TaqScreen MPX Test reagents in the COBAS® AmpliPrep Instrument for 30 minutes prior to use.

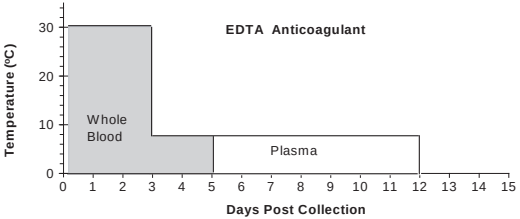
SPECIMEN COLLECTION, STORAGE AND POOLING

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

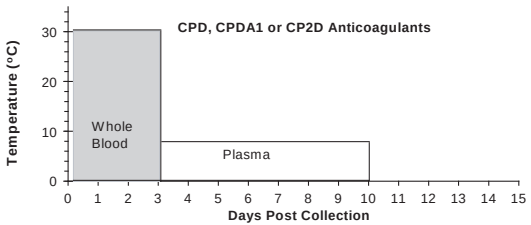
Living Donor Specimens

A. Plasma specimens collected using EDTA, CPD, CPDA1, CP2D, ACDA and 4% Sodium Citrate may be used with the **cobas**® TaqScreen MPX Test. Follow the specimen collection tube manufacturer instructions. Specimen stability is affected by elevated temperatures.

B. Blood collected in EDTA may be stored for up to 72 hours at 2 – 30°C followed by up to 48 hours at 2 – 8°C prior to plasma separation. For storage longer than five days, separate the plasma from the red blood cells by centrifugation at 800 – 1,600 x g for 20 minutes. Following removal, plasma may be stored for up to seven days at 2 – 8°C followed by up to 30 days at ≤ -18ºC. EDTA plasma may be frozen and thawed a maximum of three (3) times.



C. Blood collected in CPD, CPDA1 or CP2D may be stored for up to 72 hours at 2 – 30°C prior to plasma separation. For storage longer than 72 hours, separate the plasma from the red blood cells by centrifugation at 800 – 1,600 x g for 20 minutes. Following removal, plasma may be stored for up to seven days at 2 – 8°C followed by up to 30 days at ≤ -18°C. CPD, CPDA1, and CP2D plasma may be frozen and thawed a maximum of three (3) times.



D. Apheresis plasma in ACDA or 4% Sodium Citrate anticoagulants may be stored for up to 72 hours from time of draw at 2 – 30°C. Apheresis plasma may be stored for up to 30 days at ≤ -18°C.

E. The following plasma volume guidelines are based on pipetting from 13 x 100 mm glass or plastic donor tubes on the HAMILTON Microlab STAR/STARlet IVD Pipettor. The listed volumes are for plasma on top of packed red blood cells, and are for use when running the **cobas**® TaqScreen MPX Test.

Pool Type	Minimum Plasma Volume
Primary Pool of 6 *	3 mL
Primary Pool of 1 *	3 mL
Repeat Pool From Tube	1.5 mL
Resolution Pool from Tube	2 mL

**Includes creation of Deep Well Plate*

F. **Do not freeze whole blood.**

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

H. Covered Deep Well Plates may be stored at 2 to 8 °C for up to seven days from the date the plasma was removed from the red blood cells. Alternatively, covered Deep Well Plates may be stored at ≤ -18ºC for up to 7 days.

I. No adverse effect on assay performance was observed when plasma specimens were subjected to three (3) freeze/thaw cycles.

J. Equilibrate pooled or individual donor specimens to room temperature before using.

K. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of specimens and etiologic agents.[31]

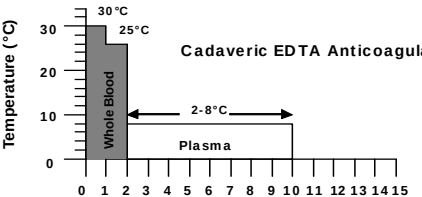
L. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**

Cadaveric Specimens

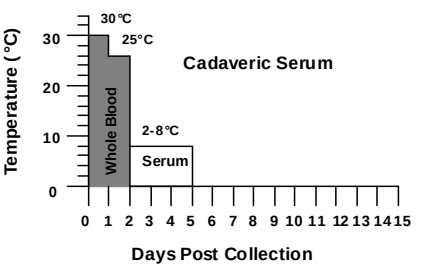
A. When testing specimens from cadaveric donors, specimens displaying a straw to pink color are classified as Moderately Hemolyzed and specimens displaying a red to dark-red or brown color are classified as Highly Hemolyzed specimens.

B. Cadaveric blood specimens collected in EDTA anticoagulant tubes or in serum tubes may be used with the **cobas**® TaqScreen MPX Test. Follow the sample tube manufacturer instructions. Specimen stability is affected by elevated temperatures.

C. Cadaveric blood collected in EDTA anticoagulant may be stored for up to 24 hours at 2-30ºC followed by up to 24 hours at 2-25ºC prior to plasma separation. For storage longer than 48 hours, separate the plasma from the red blood cells by centrifugation at 800 - 1,600 x g for 20 minutes. Following removal, plasma may be stored for up to eight days at 2-8ºC followed by up to 30 days at ≤ -18ºC. Cadaveric EDTA plasma may be frozen and thawed a maximum of three (3) times.



D. Cadaveric blood collected as serum may be stored for up to 24 hours at 2-30ºC followed by up to 24 hours at 2-25ºC prior to separation. For storage longer than 48 hours, separate the serum from the clot by centrifugation at 800 – 1,600 x g for 20 minutes. Following removal, serum may be stored for up to three days at 2-8ºC followed by up to 15 days at ≤-18ºC. Cadaveric serum may be frozen and thawed a maximum of three (3) times.



E. The stability of cadaveric specimens stored at ≤ -18ºC was determined using approximately 52 moderately or highly hemolyzed specimens each for cadaveric EDTA plasma and serum. These

specimens were spiked with HBV, HCV or HIV and were evaluated in duplicate at each time point. The non-spiked aliquot of each specimen was tested on Day 0 to verify that the specimen was non-reactive with the cobas TaqScreen MPX Test. The remaining aliquots of cadaveric EDTA plasma and serum specimens were spiked with HBV, HCV or HIV-1 Group M using one of five singly infected clinical specimens of known titers for each target, or Roche Primary Standards for HIV-1 Group O or HIV-2. Results for EDTA Plasma and serum are shown as follows.

Summary of Stability at ≤ -18ºC for Cadaveric EDTA Plasma and Serum Specimens with the cobas TaqScreen MPX Test

Target	Matrix	Day 0	After Storage for 15 Days at ≤ -18°C	After Storage for 31 Days at ≤ - 18°C
HBV	Plasma	104/104	104/104	104/104
	Serum	104/104	103/104	102/104
HCV	Plasma	104/105*	103/104	104/104
	Serum	102/102	98/102	99/102
HIV-1 Group M	Plasma	66/67**	64/66	64/66
	Serum	68/68	67/68	65/68
HIV-1 Group O	Plasma	22/23**	22/22	21/22
	Serum	22/23***	22/22	20/22
HIV-2	Plasma	20/20	20/20	20/20
	Serum	20/20	19/20	18/20

* Two additional replicates were tested due to one invalid and one non-reactive result from initial Day 0 testing for one HCV spiked EDTA plasma specimen. The additional replicates were reactive.
** Additional replicates were tested due to a non-reactive result from initial Day 0 testing for one HIV-1 Group M and one HIV-1 Group O spiked EDTA plasma specimen. For both targets, the additional replicate was reactive.
*** An additional replicate was tested, due to one non-reactive result from initial Day 0 testing for one HIV-1 Group O spiked serum specimen, and the additional replicate was reactive

F. Cadaveric specimens diluted 1:5 in the **cobas**® TaqScreen Cadaveric Specimen Diluent may be stored for up to 1 day at 2-8ºC and after remixing (by pipetting up and down four (4) times in each tube), may be tested with the **cobas**® TaqScreen MPX Test.

G. The storage of cadaveric specimens diluted 1:5 in the cobas TaqScreen Cadaveric Specimen Diluent at ≤ -18ºC has not been assessed.

H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of specimens and etiologic agents.[31]

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

SPECIMEN POOLING AND PIPETTING

1. The **cobas s** 201 system utilizes the HAMILTON Microlab STAR/STARlet IVD Pipettor for all pipetting and pooling activities. The HAMILTON Microlab STAR/STARlet IVD Pipettor performs barcode scanning and pooling operations from equal aliquots of specimen volume to form pools.

2. If reactive pools are detected by the **cobas**® TaqScreen MPX Test, the HAMILTON Microlab STAR/STARlet IVD Pipettor is used to pipette the individual specimens from either Deep Well Plates or original specimen tubes for Resolution Testing.

3. For source plasma, the **cobas s** 201 system Configuration C for large pools is used for testing pools of up to 96 individual donations. Pools of 96 are created by the HAMILTON Microlab STAR/STARlet IVD Pipettor by means of two pooling runs. The first pooling run creates intermediate pools of 12 donors and the second pooling run creates the pool of 96 from the intermediate pools of 12. If reactive pools of 96 are detected by the **cobas** TaqScreen MPX Test, the individual reactive specimens are identified using either 2D Pooling or Confirmation Pooling procedures, see the *cobas s 201 System Operator's Manual*.

PROCEDURAL NOTES

A. Equipment

1. Prepare the **cobas s** 201 system for use according to instructions in the ***cobas s*** *201 system Operator's Manual*.

2. Perform recommended maintenance on instruments to ensure proper functioning.

B. Reagents

1. The **cobas**® TaqScreen MPX Test reagents must be equilibrated for 30 minutes in the COBAS® AmpliPrep Instrument prior to use. The **cobas**® TaqScreen MPX Control Kit and **cobas**® TaqScreen Wash Reagent must be at room temperature before use. See Storage and Handling Requirements Section for reagent storage conditions.

2. Each **cobas**® TaqScreen MPX Test kit contains sufficient material for processing a total of 96 tests which are recommended to be run in batches consisting of up to 24 tests per SK24 rack. One replicate of the negative control [**TS (-) C**] and one replicate of each positive control [**HIV-1 M (+) C, HIV-1 O (+) C, HIV-2 (+) C, HCV (+) C** and **HBV (+) C**] must be processed with each batch or SK24 rack.

3. Each cobas TaqScreen Cadaveric Specimen Diluent kit contains sufficient material for processing a total of 96 tests which are recommended to be run in batches consisting of up to 24 tests per SK24 rack. One replicate of the Negative Control and one replicate of each Positive Control must be processed with each batch or SK24 rack. Controls are processed in the same way when testing living donor and cadaveric specimens with the cobas TaqScreen MPX Test.

4. All controls are for single use only.

5. The system will prevent the use of reagents which have exceeded the allowed runs or hours on the COBAS® AmpliPrep Instrument (more than 40 cumulative hours and 30 days after initial use), reagents which have expired or mixed cassettes from a set of four cassettes previously used on the system.

C. Specimen Processing

1. Avoid contaminating gloves when handling specimens and controls.

2. Care should be used to avoid contamination of specimens and **TS (–) C** with Positive Controls.

D. Individual Viral Target Identification (Discriminatory Testing)

1. Any individual specimen identified as reactive using the **cobas**® TaqScreen MPX Test should be further tested to determine which virus was initially detected using the COBAS® AmpliScreen Tests (the COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test) as Discriminatory Testing. Additional tests may be performed to identify HIV-1 Group O and HIV-2. Roche does not provide individual viral target identification test procedures for HIV-1 Group O and HIV-2.

2. Living Donor: For the COBAS® AmpliScreen individual viral target identification test procedures, 200 µL of the individual reactive specimen is extracted using the Standard Specimen Processing Procedure for neat specimens, resulting in 200 µL of processed specimen of which 50 µL is used for amplification with each of the COBAS® AmpliScreen Tests. Refer to Step B, Specimen and Control Preparation, in the INSTRUCTIONS FOR USE section of the Package Inserts for the COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test for the Standard Specimen Processing Procedure.

3. Cadaveric Donor: For the COBAS® AmpliScreen viral target identification test procedures, 200 µL of the reactive cadaveric specimen is extracted using the Multiprep Specimen Processing Procedure for cadaveric specimens, resulting in 200 µL of processed specimen of which 50 µL is used for amplification with each of the COBAS® AmpliScreen Tests. Refer to Step B, Specimen and Control Preparation, in the INSTRUCTIONS FOR USE section of the Package Inserts for the COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test for the Multiprep Specimen Processing Procedure.

INSTRUCTIONS FOR USE

The **cobas s** 201 system includes four major processes: Specimen and Control Pipetting on the HAMILTON Microlab STAR/STARlet IVD Pipettor, Specimen preparation on the COBAS® AmpliPrep Instrument using the **cobas**® TaqScreen MPX Test, Amplification/Detection on the COBAS® TaqMan® Analyzer and Data Management.

The **cobas s** 201 system configuration allows up to six HAMILTON Microlab STAR/STARlet IVD Pipettors, five Data Manager Workstations and ten AMPLILINK datastations. Each AMPLILINK datastation allows up to three COBAS® AmpliPrep Instruments and two COBAS® TaqMan® Analyzers. Refer to the ***cobas s*** *201 system Operator's Manual* for detailed information regarding the **cobas s** 201 system configuration.

Each **cobas**® TaqScreen MPX Test kit contains eight cassettes: two **MPX CS1** cassettes with Magnetic Glass Particles, two **MPX CS2** cassettes with Lysis Reagent, two **MPX CS3** cassettes with Protease and Elution Buffer, and two **MPX CS4** cassettes with IC, MMX Reagent 1 and MMX Reagent 2. This test kit is to be used in conjunction with the **cobas**® TaqScreen MPX Control Kit and the **cobas**® TaqScreen Wash Reagent and, for processing cadaveric specimens, with the cobas TaqScreen Cadaveric Specimen Diluent Kit.

Note: Do not open the cassettes.

Note: Do not pool reagents from different lots or from different bottles of the same lot.

Note: Do not mix reagents (including cassettes) from different kits. Do not load mixed reagent lots on the COBAS® AmpliPrep Instrument.

Note: Do not separate control tubes from adapters (the plastic control tube holder).

Note: The PDM Software tracks and enforces that a batch is run on a single COBAS® AmpliPrep Instrument and COBAS® TaqMan® Analyzer linked to the same AMPLILINK datastation.

Note: Do not separate batches across more than one COBAS® AmpliPrep Instrument or COBAS® TaqMan® Analyzer.

Perform all required maintenance as described in the ***cobas s*** *201 system Operator's Manual*.

Refer to the ***cobas s*** *201 system Operator's Manual* for detailed instructions for use. It is important that the user follow the instructions in the ***cobas s*** *201 system Operator's Manual* for proper performance of the assay.

A. Pipetting Controls and Specimens on the HAMILTON Microlab STAR/STARlet IVD Pipettor

Note: Avoid contaminating gloves when preparing the specimens and controls.

Note: Mix controls by gentle inversion three times, avoiding the creation of bubbles.

Note: For testing of cadaveric specimens, Deep Well Plate usage is disabled at the time of installation of the cobas s 201 system.

A1. Perform startup procedures on the HAMILTON Microlab STAR/STARlet IVD Pipettor, then start the Roche PDM Pooling Wizard following the on-screen instructions.

A2. Use caution not to damage the identifier barcode on specimen tubes and control tube adapters. If damaged, the system will not be able to recognize the specimens or controls.

A3. Uncap the control tubes and load the specimens, consumables and controls onto the HAMILTON Microlab STAR/STARlet IVD Pipettor. When the specimens, consumables, and controls have been loaded, the instrument transfers controls and specimens into S-tubes. Specimens and controls are stable for up to 6 hours at 30°C in the open tubes and an additional 6 hours at 30°C in the S-tubes.

A4. For individual cadaveric specimens, pipette 2000 µL of **CADV SPEC DIL** into appropriately labeled 13 x 100 mm tubes, add 500 µL of cadaveric specimen to each individual tube and mix each specimen by pipetting up and down four (4) times. Then load the diluted cadaveric specimens, consumables and controls onto the HAMILTON Microlab STAR/STARlet IVD Pipettor. When the diluted cadaveric specimens, consumables and controls have been loaded, the instrument transfers controls and diluted specimens into S-tubes. Diluted cadaveric specimens and MPX controls are stable for up to 6 hours at 2-30ºC in the open tubes.

A5. When the pipetting run is completed, review alarms and print the pooling report(s). Inspect pools and Deep Well Plate wells. Invalidate pools and/or wells if red blood cell contamination is observed or if volumes are inconsistent.

A6. Cap the S-tubes and transfer the SK24 rack(s) to the COBAS® AmpliPrep Instrument for nucleic acid extraction. Once transferred to the COBAS® AmpliPrep Instrument, all viral targets and controls are stable for 6 hours at 40°C in the S-tubes.

A7. Seal and store the Deep Well Plates (if plates were created during the pipetting run). All viral targets are stable in the Deep Well Plates for 7 days at 2-8°C or for 7 days at ≤ -18°C.

A8. Remove and store the donor tubes. Refer to "Specimen Collection, Storage and Pooling" Section for conditions.

A9. Remove and discard the control tubes. (Control tubes are single use only.)

B. Preparation and Loading of cobas® TaqScreen MPX Test Reagents

Note: Use caution so as to not damage the cassette labels. The barcode reader on the COBAS® AmpliPrep Instrument automatically reads the barcode label of each cassette when the reagent racks are loaded onto the instrument.

B1. Equilibrate reagents for 30 minutes in the COBAS® AmpliPrep Instrument before the first specimen is to be processed. No other Reagent preparation is required.

B2. Prior to start, a sufficient number of all cassettes must be loaded to accommodate the total number of specimens that will be processed during continuous operation of the COBAS® AmpliPrep Instrument. Each cassette contains enough reagents for 48 tests. Refer to the ***cobas s** 201 system Operator's Manual* for information regarding loading of reagents for continuous operation.

B3. Place the **MPX CS1** cassette into a reagent rack, ensuring the cassette barcode is in line with the rack barcode located to the right side of the rack. **MPX CS1** cassettes must be loaded together on a separate reagent rack from the other cassettes.

B4. Load the reagent rack containing **MPX CS1** into rack position A. Do not load mixed reagent lots on the instrument.

B5. Place one set of **MPX CS2, MPX CS3** and **MPX CS4** cassettes for each **MPX CS1** cassette into a reagent rack(s), ensuring the cassette barcodes are in line with the rack barcode located to the right side of the rack.

B6. Load the reagent rack(s) into rack position B, C, D or E.

B7. LED lights on the COBAS® AmpliPrep Instrument Status bar will turn green when all required kit components are loaded and recognized by the system.

C. Extraction of Nucleic Acids from the Pipetted Specimens and Controls

Note: Perform the following steps on a clean bench surface.

C1. Remove the wrap from Sample Processing Unit (SPU) bundle, leaving tape and plastic cover intact.

C2. With the large tab of the SPU Rack facing toward the operator, insert SPU bundle flush with the right side of the SPU rack.

C3. Remove tape and plastic cover from SPUs seated in the rack. Ensure all SPUs are pressed down, level and fully seated in rack. Elevated SPUs may cause an instrument failure. Do not apply pressure to the S-tip in the SPU.

C4. Slide loaded SPU racks into COBAS® AmpliPrep Instrument SPU positions J, K or L until the rack is inserted completely and recognized. The instrument will hold up to 72 SPUs at a time. Load at least the number of SPUs needed for run or insert more as needed.

C5. Remove cellophane wrapping from manufacturer loaded K-tube and K-tip racks being careful not to tip the racks. Ensure that all are properly seated.

C6. Slide at least the required number of K-tube and K-tip racks into COBAS® AmpliPrep Instrument positions M, N, O or P.

C7. Create orders using AMPLILINK software at Workstation.

C8. Load SK24 racks containing HAMILTON Microlab STAR/STARlet IVD Pipettor pipetted specimens into COBAS® AmpliPrep Instrument Positions F, G or H. Slide in until rack is locked. Check system status Sample window to ensure all specimens on each rack were recognized.

C9. Check AMPLILINK software to ensure adequate reagents and consumables are loaded for desired specimen preparation.

C10. Press Start on AMPLILINK software workstation to begin the COBAS® AmpliPrep Instrument Specimen Preparation Procedure.

C11. Any unused K-tips and K-tubes will remain locked within the COBAS® AmpliPrep Instrument for use in the next run.

D. Amplification and Detection

Note: The Working Master Mix plus processed specimens contained in the SK24 rack has a limited stability. The COBAS® TaqMan® Analyzer must be ready to accept samples as soon as the COBAS® AmpliPrep Instrument is finished with the Specimen Preparation Procedure.

D1. Transfer the SK24 rack containing processed specimens to the COBAS® TaqMan® Analyzer. The COBAS® TaqMan® Analyzer will automatically start the amplification and detection. The run on the COBAS® TaqMan® Analyzer must begin within 1 hour of completion of specimen preparation for that SK24 rack. Results for samples from SK24 racks not transferred within 1 hour will be invalid.

D2. When the amplification and detection is completed on the COBAS® TaqMan® Analyzer, the analyzed K-tubes are automatically disposed of in the waste bin.

D3. Accept the results on the AMPLILINK software workstation.

D4. The results are transferred automatically to the PDM software.

E. Reviewing and Releasing Results

E1. Start the Roche PDM Workstation.

E2. Retrieve Unevaluated Batches on the "Review Batches" tab at the Data Manager workstation.

E3. Review Alarms by highlighting a batch and then clicking "Next".

E4. Review Control Results on the "Controls Review" tab. Refer to the Quality Control Section for control validity criteria.

E5. Review Pool Result on the "Alarms Review" tab for the selected batch. Non-Reactive pools can be invalidated manually by the user if required. Donor specimens in an invalid pool must be retested.

E6. Review and Release Donors on the "Donor Review" tab for the selected batch.

E7. Print reports and send to Laboratory Information System (LIS), if applicable.

QUALITY CONTROL

1. One replicate of the Negative Control [**TS (–) C**] and one replicate of each of the five Positive Controls [**HIV-1 M (+) C, HIV-1 O (+) C, HIV-2 (+) C, HCV (+) C** and **HBV (+) C**] must be processed with each batch.

2. Batch Status: A Batch Status is assigned "Complete, Valid" when the batch controls are valid. If any control within a batch is invalid, the entire batch is invalid. The invalidation of results based on control failures is performed automatically by the PDM software.

   a. Negative Control

      For a batch to be valid, the Negative Control [**TS (–) C**] must be valid. If the interpreted result for the Negative Control is invalid, the entire batch is invalid, and must be repeated.

   b. Positive Controls

For a batch to be valid, the 5 Positive Controls (**HIV-1 M (+) C, HIV-1 O (+) C, HIV-2 (+) C, HCV (+) C** and **HBV (+) C**) must be valid. If the interpreted result for any Positive Control is invalid, the entire batch is invalid and must be repeated.

3. Internal Control for Donor Specimens

   a. For a donor specimen to have a valid non-reactive (-) test result, the associated Internal Control must be valid, otherwise, the non-reactive result is invalid and the donor specimen must be retested

   b. For a donor specimen to have a valid reactive test result, the associated Internal Control may be either valid or invalid.

RESULTS

1. Specimen Results are valid only if the batch containing them is valid. See Quality Control Section for acceptance criteria. Two parameters are measured for each specimen, one for the viral target and another for the Internal Control

2. Final donor results for the **cobas**® TaqScreen MPX Test are reported by the PDM Software as follows:

Status	Meaning
Complete Non-Reactive	The donor is non-reactive for the tested analyte(s).
Complete Reactive	The donor is reactive for the tested analyte(s).
Complete Unresolved	The viability time limit expired before the donor was assigned a status of reactive or non-reactive. No additional testing can be performed on the system for this donor. (See the ***cobas s*** *201 system Operator's Manual* for a description of the viability time limit).

3. Donors that require additional testing: donor tubes whose pool status or individual donation status is invalid have a status of "Repeat Needed" and donor tubes included in a reactive pool have a status of "Resolution Needed".

Repeat Needed

Donor tubes with a pool status or individual donation status of "invalid" require repeat testing as part of a repeat pool or in single determination.

Pool Testing — Resolution Needed

When testing as part of a pool and a pool is reported as reactive by the **cobas s** 201 system, the associated donors within that pool are given the status "Resolution needed" and the HAMILTON Microlab STAR/STARlet IVD Pipettor is used to pipette the individual specimens from either the Deep Well Plates or the original specimen tubes for Resolution Testing. The **cobas**® TaqScreen MPX Test is used to identify the individual reactive specimen using the same methods (tested in singlicate) as for the pooled specimens. Any individual specimens found to be reactive should be further tested using the COBAS® AmpliScreen Tests for viral target identification (Discriminatory Testing).

If one or more of the individual specimens from a pool is reactive, the reactive specimen(s) is reported as "Complete Reactive" and the remaining negative specimens associated with the positive pool are reported as "Complete, Non-reactive". If all of the individual donor specimens in that pool test negative, the specimens in that pool are all reported as "Complete, Non-reactive."

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

For source plasma pools of up to 96 individual donations, reactive pools are resolved to the individual reactive specimens using either 2D Pooling or Confirmation Pooling procedures, see the *cobas s 201 System Operator's Manual*.

LIMITATIONS

Procedural Limitations

1. The **cobas**® TaqScreen MPX Test has been evaluated only for use in combination with the **cobas**® TaqScreen MPX Control Kit, the **cobas**® TaqScreen Wash Reagent and the **cobas s** 201 system.

2. For testing of cadaveric specimens, the **cobas**® TaqScreen MPX Test has been evaluated only for use in combination with the **cobas**® TaqScreen Cadaveric Specimen Diluent Kit, the **cobas**® TaqScreen MPX Control Kit, the **cobas**® TaqScreen Wash Reagent and the **cobas s** 201 system.

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

4. Reliable results are dependent on adequate specimen collection and proper transport procedures.

5. Only the HAMILTON Microlab STAR/STARlet IVD Pipettor has been validated for use with the **cobas**® TaqScreen MPX Test, for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the ***cobas s*** *201 system Operator's Manual* and the User Manual for the HAMILTON Microlab STAR/STARlet IVD Pipettor.

Limitations of the Test

1. Detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and 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.

2. Though rare, mutations within the highly conserved regions of a viral genome covered by the **cobas**® TaqScreen MPX Test primers and/or probe may result in failure to detect a virus.

3. These studies were conducted in a population where HIV-1 Group O and HIV-2 yield cases would not be expected to be detected. The ability of the **cobas**® TaqScreen MPX Test to detect window period cases for HIV-1 Group O and HIV-2 has not been evaluated.

4. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.

PERFORMANCE CHARACTERISTICS

Living Donor Specimens

Reproducibility

The Reproducibility of the **cobas**® TaqScreen MPX Test using the **cobas s** 201 system was established by testing 12-member randomized, blinded panels composed of 2 negative plasma samples, and 2 positive plasma samples each for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV at concentrations of approximately 0.54X and 3X the Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test for each virus.

Testing was performed at 3 sites with 1 operator at each site using 3 lots of **cobas**® TaqScreen MPX Test kit and 1 **cobas s** 201 system. At each site 4 panels and 4 control sets were tested each day for 5 days with each of the 3 reagent kit lots, for a total of 180 tests of each sample in the panel (2,160 total tests).

All valid reproducibility data was evaluated by calculating the percentage of reactive test results for each panel member. The data were analyzed by kit lot and site/operator.

This study demonstrated consistent performance of the **cobas**® TaqScreen MPX Test across kit lot and testing site/operator on different days (Table 1). The invalid batch rate in this study was 5.8% and the invalid rate for the individual sample was 0.14%.

Table 1
cobas® TaqScreen MPX Test — Reproducibility Results

Virus	Concentration	Kit Lot	Reactive Results by Reagent Lot	Test Site/Operator	Reactive Results by Testing Site/Operator
Negative	0	A	0.0% (0/120)	1	0.0% (0/120)
		B	0.0% (0/120)	2	0.0% (0/120)
		C	0.0% (0/120)	3	0.0% (0/120)
HIV-1 Group M	0.54 X LOD*	A	75.0% (45/60)	1	75.0% (45/60)
		B	80.0% (48/60)	2	71.7% (43/60)
		C	75.0% (45/60)	3	83.3% (50/60)
HIV-1 Group O	0.54 X LOD	A	96.7% (58/60)	1	100.0% (60/60)
		B	98.3% (59/60)	2	95.0% (57/60)
		C	98.3% (59/60)	3	98.3% (59/60)
HIV-2	0.54 X LOD	A	83.3% (50/60)	1	73.3% (44/60)
		B	81.7% (49/60)	2	80.0% (48/60)
		C	73.3% (44/60)	3	85.0% (51/60)
HCV	0.54 X LOD	A	81.4% (48/59)	1	71.7% (43/60)
		B	78.3% (47/60)	2	71.2% (42/59)
		C	61.7% (37/60)	3	78.3% (47/60)
HBV	0.54 X LOD	A	98.3% (58/59)	1	96.7% (58/60)
		B	98.3% (59/60)	2	98.3% (58/59)
		C	95.0% (57/60)	3	96.7% (58/60)
HIV-1 Group M	3 X LOD	A	100.0% (60/60)	1	98.3% (59/60)
		B	98.3% (59/60)	2	100.0% (60/60)
		C	100.0% (60/60)	3	100.0% (60/60)
HIV-1 Group O	3 X LOD	A	100.0% (60/60)	1	100.0% (60/60)
		B	100.0% (60/60)	2	100.0% (60/60)
		C	100.0% (60/60)	3	100.0% (60/60)
HIV-2	3 X LOD	A	100.0% (59/59)	1	100.0% (60/60)
		B	100.0% (60/60)	2	100.0% (60/60)
		C	100.0% (60/60)	3	100.0% (59/59)
HCV	3 X LOD	A	100.0% (60/60)	1	100.0% (60/60)
		B	100.0% (60/60)	2	100.0% (60/60)
		C	100.0% (60/60)	3	100.0% (60/60)
HBV	3 X LOD	A	100.0% (60/60)	1	100.0% (60/60)
		B	100.0% (60/60)	2	100.0% (60/60)
		C	100.0% (60/60)	3	100.0% (60/60)

** Limit of Detection*

The Reproducibility of the **cobas**® TaqScreen MPX Test was also evaluated using a 6-member panel composed of 1 negative panel member and 1 positive panel member each for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV at a concentration of approximately 1X the Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test for each virus.

Testing was performed at 1 site with 3 operators, using 3 lots of the **cobas**® TaqScreen MPX Test kit. Each operator utilized a unique partial **cobas s** 201 system (i.e., a COBAS® AmpliPrep Instrument and COBAS® TaqMan® Analyzer with AMPLILINK datastation). Four replicates of each of the 6 panel members were tested over 5 days with each reagent kit lot, by each of the 3 instrument-operator pairs. A minimum of 60 valid replicates of each panel member was tested with each reagent kit lot.

All valid reproducibility data was evaluated by calculating the percentage of reactive test results for each panel member. The data was analyzed by kit lot and instrument-operator pair.

This study demonstrated consistent performance of the **cobas**® TaqScreen MPX Test across kit lot and instrument-operators on different days at 1X LOD (Table 2).

Table 2
cobas® TaqScreen MPX Test – Reproducibility Results at 1X LOD

Virus	Concentration	Kit Lot	Reactive Results by Reagent Lot	Instrument-Operator Pair	Reactive results by Instrument-Operator Pair
Negative	0	1	0.0% (0/60)	1	0.0% (0/60)
		2	1.7% (1/60)	2	0.0% (0/60)
		3	0.0% (0/60)	3	1.7% (1/60)
HIV-1 Group M	1 X LOD*	1	100.0% (60/60)	1	100.0% (60/60)
		2	98.3% (59/60)	2	98.3% (59/60)
		3	98.3% (59/60)	3	98.3% (59/60)
HIV-1 Group O	1 X LOD	1	100.0% (60/60)	1	98.3% (59/60)
		2	98.3% (59/60)	2	100.0% (60/60)
		3	100.0% (60/60)	3	100.0% (60/60)
HIV-2	1 X LOD	1	100.0% (60/60)	1	100.0% (60/60)
		2	100.0% (60/60)	2	100.0% (60/60)
		3	98.3% (59/60)	3	98.3% (59/60)
HCV	1 X LOD	1	100.0% (60/60)	1	100.0% (60/60)
		2	100.0% (60/60)	2	100.0% (60/60)
		3	98.3% (59/60)	3	98.3% (59/60)
HBV	1 X LOD	1	100.0% (60/60)	1	98.3% (59/60)
		2	98.3% (59/60)	2	100.0% (60/60)
		3	100.0% (60/60)	3	100.0% (60/60)

** Limit of Detection*

Analytical Sensitivity — WHO International Standards/Roche Standards/CBER Standard

The Limits of Detection (LOD) of the **cobas**® TaqScreen MPX Test for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA were determined using the following standards: the WHO INTERNATIONAL STANDARD FOR HEPATITIS B VIRUS DNA FOR NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) ASSAYS (NIBSC code 97/746)[32], the WHO SECOND INTERNATIONAL STANDARD FOR HEPATITIS C VIRUS RNA FOR GENOMIC AMPLIFICATION TECHNOLOGY ASSAYS (NIBSC code 96/798)[33] and Roche Standards for HIV-1 Group M RNA, HIV-1 Group O RNA and HIV-2 RNA. The Roche Secondary Standard for HIV-1 Group M RNA is a commercially available, cultured virus stock (HIV-1 LAV 8E5, PN 227, Boston Biomedica, Inc.) traceable to the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC code 97/656) calibrated by the COBAS® TaqMan® HIV-1 Test For Use With The High Pure System. No international standards are currently available for HIV-1 Group O RNA and HIV-2 RNA. The Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA are commercially available cultured virus stocks, PN 242O (Boston Biomedica, Inc.) and Cat. No. 10-127-000 (Advanced Biotechnologies, Inc.). The Roche HIV-1 Group O RNA

and HIV-2 RNA Standards are traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot O1 and to the CBER HIV-2 RNA Lot Release Panel ISD, respectively.

For the WHO and Roche standards, 3 independent dilution series of each viral standard were prepared with normal, virus-negative human plasma. Each dilution series was tested using 3 different lots of the **cobas®** TaqScreen MPX Test kit with 22 replicates per lot, for a total of 198 replicates per concentration. PROBIT analysis on the combined data from all replicates tested for each virus was used to estimate the 95% LOD and two-sided 95% fiducial confidence intervals (Table 8).

Table 3 to Table 7 summarize the overall results of the Analytical Sensitivity Study. The commonly used conversion factors for International Units (IU) to copies for HIV-1 Group M RNA, HCV RNA and HBV DNA are 0.6 copies/IU[34], 2.7 copies/IU[35] and 5.0 copies/IU[36], respectively.

Table 3
Analytical Sensitivity Summary: Roche Secondary Standard for HIV-1 Group M

HIV-1 Group M RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
190	196	196	100%	98.5%
60	193	197	98.0%	95.4%
50	187	195	95.9%	92.7%
40	180	195	92.3%	88.4%
20	143	196	73.0%	67.2%
6	72	196	36.7%	31.0%

Table 4
Analytical Sensitivity Summary: Roche Primary Standard for HIV-1 Group O

HIV-1 Group O RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
293	194	194	100%	98.5%
147	198	198	100%	98.5%
117	196	198	99.0%	96.9%
93.8	191	197	97.0%	94.1%
29.3	98	198	49.5%	43.4%
9.4	46	189	24.3%	19.3%

Table 5
Analytical Sensitivity Summary: Roche Primary Standard for HIV-2

HIV-2 RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
320	194	194	100.0%	98.5%
107	194	195	99.5%	97.6%
82.8	186	189	98.4%	95.9%
61.4	188	195	96.4%	93.4%
34.7	154	192	80.2%	74.9%
10.7	79	194	40.7%	34.8%

Table 6
Analytical Sensitivity Summary: WHO Second International Standard for HCV (96/798)

HCV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
30.0	188	192	97.9%	95.3%
15.4	183	184	99.5%	97.4%
11.3	183	186	98.4%	95.9%
10.0	180	193	93.3%	89.5%
3.0	128	192	66.7%	60.6%
1.0	67	192	34.9%	29.2%

Table 7
Analytical Sensitivity Summary: WHO International Standard for HBV (97/746)

HBV DNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
12.0	193	193	100.0%	98.5%
5.0	194	197	98.5%	96.1%
4.0	186	193	96.4%	93.3%
3.6	186	197	94.4%	90.9%
1.3	121	192	63.0%	56.9%
0.4	47	191	24.6%	19.5%

Table 8
PROBIT Analysis for Viral Standards

Analyte	Standard	Units	Average 95% LOD	95% Lower Limit	95% Upper Limit
HIV-1 Group M	Roche Secondary Standard	IU/mL	49	42.4	58.1
HIV-1 Group O	Roche Primary Standard	Copies/mL	89	56	217
HIV-2	Roche Primary Standard	Copies/mL	59.3	51.9	69.7
HCV	WHO Second International Standard	IU/mL	11	7.0	21.7
HBV	WHO International Standard	IU/mL	3.8	3.3	4.4

Analytical Sensitivity — CBER Panels for HIV-1 Group M, HCV, HBV, HIV-1 Group O and CBER HIV-2 Panel Stock

The FDA CBER Panels for HIV-1 Group M, HCV, HBV and HIV-1 Group O were tested using the **cobas**® TaqScreen MPX Test. Three replicates of the HIV-1 Group M, HCV and HBV panel members and 4 replicates of the HIV-1 Group O panel members were tested. For the HIV-1 Group M panel, the **cobas**® TaqScreen MPX Test detected 33% (1/3) of the replicates at 10 copies/mL and 100% (3/3) of the replicates at 50, 100 and 500 copies/mL (Table 9). For the HCV panel, the **cobas**® TaqScreen MPX Test detected 66% (2/3) of the replicates

at 5 copies/mL, 33% (1/3) of the replicates at 10 copies/mL and 100% (3/3) of the replicates at 50 and 100 copies/mL (Table 10). For the HBV panel, the **cobas®** TaqScreen MPX Test detected 100% (3/3) of the replicates at 10, 50, 100 and 500 copies/mL (Table 11). For the HIV-1 Group O panel, the **cobas®** TaqScreen MPX Test detected 100% (4/4) of the replicates at 25, 100 and 1000 copies/mL (Table 12). All negative panel members were non-reactive.

The FDA CBER HIV-2 panel stock used in the preparation of the CBER HIV-2 RNA Lot Release Panel ISD, was diluted to 5 concentrations and tested with the **cobas®** TaqScreen MPX Test (Table 13). This stock was estimated by CBER to be at 100,000 copies/mL of HIV-2 RNA. The **cobas®** TaqScreen MPX Test detected 100% (73/73) of the replicates at 142 copies/mL, 99.2% (119/120) of the replicates at 100 copies/mL, 99.2% (119/120) of the replicates at 71 copies/mL, 93.3% (112/120) of the replicates at 50.2 copies/mL and 90.8% (108/119) of the replicates at 35.5 copies/mL (Table 13). PROBIT analysis on the combined data from all replicates tested was used to estimate the 95% Limit of Detection (LOD) and two-sided 95% fiducial confidence intervals (Table 14). The rates of reactivity determined for the replicates of these FDA CBER panels are consistent with each of the average 95% LOD values shown in Table 8.

Table 9
CBER HIV-1 Group M Panel Results

	CBER HIV-1 Group M Panel Member Test Results				
CBER HIV-1 Group M Panel	B4 0 c/mL	B2 10 c/mL	B6 50 c/mL	B5 100 c/mL	B9 500 c/mL
cobas® TaqScreen MPX Test Results	0% (0/3)	33% (1/3)	100% (3/3)	100% (3/3)	100% (3/3)

Table 10
CBER HCV Panel Results

	CBER HCV Panel Member Test Results				
CBER HCV Panel	2 0 c/mL	10 5 c/mL	9 10 c/mL	8 50 c/mL	7 100 c/mL
cobas® TaqScreen MPX Test Results	0% (0/3)	66% (2/3)	33% (1/3)	100% (3/3)	100% (3/3)

Table 11
CBER HBV Panel Results

	CBER HBV Panel Member Test Results				
CBER HBV Panel	1 0 c/mL	2 10 c/mL	4 50 c/mL	3 100 c/mL	5 500 c/mL
cobas® TaqScreen MPX Test Results	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

Table 12
CBER HIV-1 Group O Panel Results

	CBER HIV-1 Group O Panel Member Test Results		
CBER HIV-1 Group O Panel	O1 1000 c/mL	O2 100 c/mL	O3 25 c/mL
cobas® TaqScreen MPX Test Results	100% (4/4)	100% (4/4)	100% (4/4)

Table 13
Analytical Sensitivity Summary for the CBER HIV-2 Panel Stock with the cobas® TaqScreen MPX Test

HIV-2 RNA Concentration* (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
142	73	73	100.0%	96.0%
100	119	120	99.2%	96.1%
71	119	120	99.2%	96.1%
50.2	112	120	93.3%	88.3%
35.5	108	119	90.8%	85.2%

**The CBER HIV-2 panel stock, estimated at 100,000 copies/mL, was diluted to these concentrations*

Table 14
Probit Analysis Results with the CBER HIV-2 Panel Stock

Analyte	Units	Average 95% LOD	95% Lower Limit	95% Upper Limit
CBER HIV-2	Copies/mL	48.9	40.3	60.3

Genotype/Subtype Sensitivity and Inclusivity

The performance of the **cobas®** TaqScreen MPX Test was determined on the subtypes of HIV-1 Group M, HIV-1 Group O, HIV-1 Group N and HIV-2, and the genotypes of HCV and HBV.

HIV-1 Group M

Fifty HIV-1 Group M clinical specimens and 25 cultured isolates with known subtype (7 subtype A, 15 subtype AE recombinants, 10 subtype AG recombinants, 12 subtype B, 9 subtype C, 7 subtype D, 4 subtype F, 8 subtype G, 2 subtype H and 1 subtype J) were quantified with the COBAS® AMPLICOR® HIV-1 MONITOR Test, v1.5, diluted with normal, virus-negative human plasma to 3X and 1X the Limit of Detection (LOD) of the **cobas®** TaqScreen MPX Test for HIV-1 Group M, and tested with the **cobas®** TaqScreen MPX Test. All 50 clinical specimens and 25 cultured isolates were reactive at both 3X and 1X LOD.

Table 15
HIV-1 Group M Specimens and Cultured Isolates

Subtype	Clinical Specimens	Culture Supernatants	Total Isolates	Reactive at 3X LOD	Reactive at 1X LOD
A	3	4	7	100% (7/7)	100% (7/7)
AE	10	5	15	100% (15/15)	100% (15/15)
AG	10	0	10	100% (10/10)	100% (10/10)
B	10	2	12	100% (12/12)	100% (12/12)
C	7	2	9	100% (9/9)	100% (9/9)
D	2	5	7	100% (7/7)	100% (7/7)
F	1	3	4	100% (4/4)	100% (4/4)
G	4	4	8	100% (8/8)	100% (8/8)
H	2	0	2	100% (2/2)	100% (2/2)
J	1	0	1	100% (1/1)	100% (1/1)

HIV-1 Group O and HIV-1 Group N

Eight HIV-1 Group O cultured isolates and 1 HIV-1 Group N cultured isolate were tested. For the HIV-1 Group O isolates, 7 out of 8 isolates were detected at dilutions below 100 copies/mL. Since no molecular method was available to quantify the HIV-1 Group N isolate, half-log dilutions of the culture stock were prepared in normal, virus-negative human plasma and each dilution was tested with the **cobas®** TaqScreen MPX Test. The HIV-1 Group N isolate was detected in all dilutions up to 3.3×10^{-10}.

HIV-2

Nine HIV-2 cultured isolates (5 subtype A, 1 subtype A/B, 1 subtype B and 2 with unknown subtype) and 11 HIV-2 clinical specimens (unknown subtype) were tested. Half-log dilutions of the cultured stocks and clinical specimens were prepared in normal, virus-negative human plasma, and each dilution was tested with the **cobas**® TaqScreen MPX Test.

Five HIV-2 subtype A cultured isolates were detected with the **cobas**® TaqScreen MPX Test in all dilutions up to 1 x 10^{-8} to 3.3 x 10^{-9}. The HIV-2 subtype A/B isolate was detected in dilutions up to 1 x 10^{-9}. The HIV-2 subtype B isolate was detected in dilutions up to 3.3 x 10^{-9}. The 2 HIV-2 cultured isolates of unknown subtype were detected in dilutions up to 3.3 x 10^{-9}. All 11 HIV-2 clinical specimens (unknown subtype) were detected with the **cobas**® TaqScreen MPX Test at dilutions up to 1:100 (2 specimens), 1:30 (3 specimens), 1:10 (2 specimens), 1:3 (3 specimens) and undiluted (1 specimen).

HCV

Seventy-four HCV clinical specimens with known genotype (8 genotype 1a, 13 genotype 1b, 1 genotype 2, 7 genotype 2a, 1 genotype 2a/c, 9 genotype 2b, 8 genotype 3a, 1 genotype 3a/b, 6 genotype 4, 1 genotype 4a, 1 genotype 4b/4c, 1 genotype 4h, 2 genotype 5, 9 genotype 5a, 2 genotype 6 and 4 genotype 6a) were quantified with the COBAS® AMPLICOR® HCV MONITOR Test, v2.0, diluted with normal, virus-negative human plasma to 3X and 1X the Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test for HCV, and tested with the **cobas**® TaqScreen MPX Test. All 74 of the isolates were reactive at 3X LOD. At 1X LOD 73 of the 74 isolates were reactive.

Table 16
HCV Specimens

Genotype	Clinical Specimens	Reactive at 3X LOD	Reactive at 1X LOD
1a	8	100% (8/8)	100% (8/8)
1b	13	100% (13/13)*	92% (12/13)*
2	1	100% (1/1)	100% (1/1)
2a	7	100% (7/7)	100% (7/7)
2a/c	1	100% (1/1)	100% (1/1)
2b	9	100% (9/9)	100% (9/9)
3a	8	100% (8/8)	100% (8/8)
3a/b	1	100% (1/1)	100% (1/1)
4	6	100% (6/6)	100% (6/6)
4a	1	100% (1/1)	100% (1/1)
4b/4c	1	100% (1/1)	100% (1/1)
4h	1	100% (1/1)	100% (1/1)
5	2	100% (2/2)	100% (2/2)
5a	9	100% (9/9)	100% (9/9)
6	2	100% (2/2)	100% (2/2)
6a	4	100% (4/4)	100% (4/4)

** One genotype 1b isolate was reactive at 3X LOD, but not reactive at 1X LOD*

HBV

Sixty-four HBV clinical specimens and 1 molecular clone with known genotype (13 genotype A, 9 genotype B, 10 genotype C, 9 genotype D, 14 genotype E, 6 genotype F, 2 genotype G and 2 genotype H) were quantified with the COBAS® AMPLICOR® HBV MONITOR Test, diluted with normal, virus-negative human plasma to 3X and 1X the Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test for HBV, and tested with the **cobas**® TaqScreen MPX Test. All 65 specimens were reactive at both 3X and 1X LOD.

Table 17
HBV Specimens

Genotype	Clinical Specimens	Reactive at 3X LOD	Reactive at 1X LOD
A	13	100% (13/13)	100% (13/13)
B	9	100% (9/9)	100% (9/9)
C	10	100% (10/10)	100% (10/10)
D	9	100% (9/9)	100% (9/9)
E	14	100% (14/14)	100% (14/14)
F	6	100% (6/6)	100% (6/6)
G	2*	100% (2/2)	100% (2/2)
H	2**	100% (1/1)**	100% (2/2)

** One genotype G sample was a plasmid DNA rather than a clinical specimen*

*** One genotype H isolate was not tested at 3X LOD due to insufficient volume*

Seroconversion Panels

The performance of the **cobas**® TaqScreen MPX Test during seroconversion was determined for HIV-1 Group M, HCV and HBV using 60 commercially available seroconversion panels. No seroconversion panels are available for HIV-1 Group O and HIV-2.

HIV-1 Seroconversion Panels

Twenty commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HIV antibody were tested with the **cobas**® TaqScreen MPX Test. Each sample was tested neat and diluted 1:6 to simulate testing in pools of 6 donors. The **cobas**® TaqScreen MPX Test results were compared to the results obtained with a research use only HIV-1/2 and Group O ChLIA and the Abbott HIVAB HIV-1/HIV-2 EIA.

The **cobas**® TaqScreen MPX Test on neat samples detected HIV RNA prior to detection of HIV antibody with either the research use only HIV-1/2 and Group O ChLIA or the Abbott HIVAB HIV-1/HIV-2 EIA in 20 of 20 panels. The **cobas**® TaqScreen MPX Test on neat samples detected HIV RNA 5 to 25 days prior to detection of HIV antibody with the research use only HIV-1/2 and Group O ChLIA and 5 to 89 days prior to detection of HIV antibody with the Abbott HIVAB HIV-1/HIV-2 EIA.

The **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HIV RNA prior to detection of HIV antibody with either the research use only HIV-1/2 and Group O ChLIA or the Abbott HIVAB HIV-1/HIV-2 EIA in 20 of 20 panels. The **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HIV RNA 5 to 25 days prior to detection of HIV antibody with the research use only HIV-1/2 and Group O ChLIA and 5 to 89 days prior to detection of HIV antibody with the Abbott HIVAB HIV-1/HIV-2 EIA.

Table 18
Performance of cobas® TaqScreen MPX Test on HIV Seroconversion Panels

HIV Seroconversion Panel	Days Earlier Detection Than HIV-1/2 Antibody with			
	Abbott HIVAB HIV-1/HIV-2 EIA		Research use only HIV-1/2 and Group O ChLIA	
	cobas® TaqScreen MPX Test			
	Neat	1:6	Neat	1:6
1	15	15	15	15
2	14	14	14	14
3	18	18	18	18
4	5	5	5	5
5	9	9	14	14
6	10	10	10	10
7	17	11	17	11
8	7	7	10	10
9	10	10	10	10
10	13	13	13	13
11	19	19	19	19
12	25	25	25	25
13	19	14	19	14
14	7	7	7	7
15	19	19	19	19
16	89	89	9	9
17	7	7	7	7
18	14	11	14	11
19	11	7	11	7
20	15	15	15	15

HCV Seroconversion Panels

Twenty commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HCV antibody were tested with the **cobas**® TaqScreen MPX Test. Each sample was tested neat and diluted 1:6 to simulate testing in pools of 6 donors. The **cobas**® TaqScreen MPX Test results were compared to the results obtained with the Abbott PRISM® HCV Assay and ORTHO® HCV Version 3.0 ELISA Test System.

The **cobas**® TaqScreen MPX Test on neat samples detected HCV RNA prior to detection of HCV antibody with either the Abbott PRISM HCV Assay or the ORTHO HCV Version 3.0 ELISA Test System in 19 of 20 panels and on the same day as detection of HCV antibody in 1 of 20 panels. The **cobas**® TaqScreen MPX Test on neat samples detected HCV RNA 0 to 97 days prior to detection of HCV antibody with the Abbott PRISM HCV Assay or 0 to 97 days prior to detection of HCV antibody with the ORTHO HCV Version 3.0 ELISA Test System. The **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HCV RNA prior to detection of HCV antibody with either the Abbott PRISM HCV Assay or the ORTHO HCV Version 3.0 ELISA Test System in 19 of 20 panels and on the same day as detection of HCV antibody in 1 of 20 panels. The **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HCV RNA 0 to 97 days prior to detection of HCV antibody with the Abbott PRISM HCV Assay or 0 to 97 days prior to detection of HCV antibody with the ORTHO HCV Version 3.0 ELISA Test System.

Table 19
Performance of cobas® TaqScreen MPX Test on HCV Seroconversion Panels

HCV Seroconversion Panel	Days Earlier Detection than HCV Antibody			
	ORTHO HCV Version 3.0 ELISA		Abbott PRISM HCV	
	cobas® TaqScreen MPX Test			
	Neat	1:6	Neat	1:6
1	97	97	97	97
2	85	85	85	85
3	16	16	13	13
4	7	7	7	7
5	7	7	3	3
6	27	27	24	24
7	19	19	13	13
8	8	8	8	8
9	14	14	14	14
10	9	9	7	7
11	23	23	16	16
12	21	21	18	18
13	39	39	37	37
14	32	32	32	32
15	31	31	28	28
16	23	23	23	23
17	39	33	39	33
18	32	32	32	32
19	38	38	38	38
20	0	0	0	0

HBV Seroconversion Panels

Twenty commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HBsAg were tested with the **cobas®** TaqScreen MPX Test. Each sample was tested neat and diluted 1:6 to simulate testing in pools of 6 donors. The **cobas®** TaqScreen MPX Test results were compared to the results obtained with the Abbott PRISM® HBsAg Assay and the ORTHO® HBsAg ELISA Test System 3.

The **cobas®** TaqScreen MPX Test on neat samples detected HBV DNA prior to detection of HBsAg with the Abbott PRISM HBsAg Assay in 17 of 20 panels, on the same day as detection of HBsAg in 2 of 20 panels and after detection of HBsAg in 1 of 20 panels.

Compared to ORTHO HBsAg ELISA Test System 3, the **cobas®** TaqScreen MPX Test on neat samples detected HBV DNA prior to detection of HBsAg in 19 of 20 panels and after detection of HBsAg in 1 of 20 panels. The **cobas®** TaqScreen MPX Test on neat samples detected HBV DNA -36 to 35 days prior to detection of HBsAg with Abbott PRISM HBsAg Assay and -5 to 35 days prior to detection of HBsAg with the ORTHO HBsAg ELISA Test System 3.

The **cobas®** TaqScreen MPX Test on 6-fold diluted samples detected HBV DNA prior to detection of HBsAg with the Abbott PRISM HBsAg Assay in 15 of 20 panels, on the same day as detection of HBsAg in 3 of 20 panels and after detection of HBsAg in 2 of 20 panels.

Compared to ORTHO HBsAg ELISA Test System 3, the **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HBV DNA prior to detection of HBsAg in 17 of 20 panels, on the same day as detection of HBsAg in 2 of 20 panels and after detection of HBsAg in 1 of 20 panels. The **cobas**® TaqScreen MPX Test on 6-fold diluted samples detected HBV DNA -23 to 35 days prior to detection of HBsAg with Abbott PRISM HBsAg Assay and -7 to 43 days prior to detection of HBsAg with the ORTHO HBsAg ELISA Test System 3.

Table 20
Performance of cobas® TaqScreen MPX Test on HBV Seroconversion Panels

HBV Seroconversion Panel	Days Earlier Detection Than HBsAg			
	ORTHO HBsAg ELISA Test System 3		Abbott PRISM HBsAg	
	cobas® TaqScreen MPX Test			
	Neat	1:6	Neat	1:6
1	-5	-7	22	20
2	18	16	16	14
3	35	35	35	35
4	31	24	21	14
5	7	43	-36*	0
6	21	0	21	0
7	8	8	4	4
8	13	13	9	9
9	14	14	7	7
10	18	18	7	7
11	28	16	26	14
12	7	7	2	2
13	19	19	12	12
14	21	12	23	14
15	21	9	19	7
16	26	0	8	-18
17	12	12	0	0
18	18	18	7	7
19	35	12	0	-23*
20	21	17	18	14

** The Abbott PRISM HBsAg Assay was reactive in the first bleed of the series and the interval between the first reactive bleed with the **cobas**® TaqScreen MPX Test and prior bleed was 36 days for Panel 5 and 23 days for Panel 19.*

Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the **cobas**® TaqScreen MPX Test was evaluated by testing a panel of 17 microorganisms, including 12 viral isolates, 4 bacterial strains and 1 yeast isolate. The microorganisms were added to normal, virus-negative, human plasma and tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV added to a concentration of 3X the limit of detection of the **cobas**® TaqScreen MPX Test for each virus.

Non-reactive results were obtained with the **cobas**® TaqScreen MPX Test on all of the microorganism samples without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV. The tested microorganisms do not cross-react with the **cobas**® TaqScreen MPX Test. Reactive results were obtained on all of the microorganism samples with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV. The tested microorganisms do not interfere with the **cobas**® TaqScreen MPX Test.

Table 21
Microorganisms Tested

Analytical Specificity — Microorganisms Tested		
Adenovirus 2	Human Herpes Virus 6	*Staphylococcus aureus*
Cytomegalovirus	Human T-Lymphotrophic Virus; Type I	*Candida albicans*
Epstein Barr Virus	Human T-Lymphotrophic Virus; Type II	*Propionibacterium acnes*
Varicella-Zoster Virus	Hepatitis A Virus	*Staphylococcus epidermis*
Herpes Simplex Virus Type 1	Influenza Virus	*Staphylococcus haemolyticus*
Herpes Simplex Virus Type 2	Hepatitis G Virus (GBV-C)	

Analytical Specificity — Other Disease States

Plasma specimens from each of the following disease categories (Cytomegalovirus infection (20), Hepatitis A Virus infection (15), Epstein Barr Virus infection (15) and autoimmune diseases, i.e., Anti-Nuclear Antibodies (5), Rheumatoid Arthritis (10)) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV added to a concentration of 3X the limit of detection of the **cobas**® TaqScreen MPX Test for each virus. The **cobas**® TaqScreen MPX Test yielded non-reactive results for all of the disease state specimens without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV, except for one CMV specimen which was initially reactive and upon retest yielded non-reactive. The **cobas**® TaqScreen MPX Test yielded reactive results for all of the disease state specimens with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV. These disease states did not interfere with the sensitivity or specificity of the **cobas**® TaqScreen MPX Test.

Potentially Interfering Substances

Endogenous Interfering Substances

Plasma specimens with abnormally high levels of triglycerides (up to 3186 mg/dL), hemoglobin (up to 472 mg/dL), unconjugated bilirubin (up to 62 mg/dL), albumin (up to 9.6 g/dL) or human DNA (up to 0.4 mg/dL) were tested with the **cobas**® TaqScreen MPX Test. For each condition, 10 plasma specimens were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV added to a concentration of 3X the limit of detection for each virus. These endogenous substances did not interfere with the sensitivity or specificity of the **cobas**® TaqScreen MPX Test.

Ten plasma specimens with red blood cells added to abnormally high levels, (up to 10% v/v) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV added to a concentration of 3X the limit of detection of the **cobas**® TaqScreen MPX Test for each virus. Plasma with red blood cells added to 2.5% (v/v) did not interfere with the sensitivity or specificity of the **cobas**® TaqScreen MPX Test. Plasma with red blood cells added to 5.0% (v/v) reduced the sensitivity of the **cobas**® TaqScreen MPX Test for detection of HCV (80% detection rate). Plasma with red blood cells added to 10.0% (v/v) reduced the sensitivity of the **cobas**® TaqScreen MPX Test for detection of HBV (30% detection rate), HCV (40% detection rate), HIV-1 Group O (50% detection rate) and HIV-2 (90% detection rate).

Exogenous Interfering Substances

Normal, human plasma specimens containing abnormally high concentrations of acetaminophen (1324 µmol/L), acetylsalicylic acid (3.62 mmol/L), atorvastatin (600 Eq/L), fluoxetine (11.2 µmol/L), loratadine (0.78 µmol/L), nadolol (3.88 µmol/L), naproxen (2170 µmol/L), paroxetine (3.04 µmol/L), sertraline (1.96 µmol/L), ascorbic acid (342 µmol/L), ibuprofen (2425 µmol/L) and phenylephrine HCl (491 µmol/L) were tested with the **cobas**® TaqScreen MPX Test. For each condition, two plasma specimens were tested with HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV added to a concentration of 3X the limit of detection for each virus. In addition, for each condition, at least two viral negative plasma specimens were tested with the abnormally high concentrations of the potentially interfering substances. These exogenous substances did not interfere with the sensitivity or specificity of the **cobas**® TaqScreen MPX Test.

Cadaveric Donor Specimens

Reproducibility

Twenty individual cadaveric EDTA plasma specimens were spiked with either HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV using the Roche Secondary Standard for HIV-1 Group M, Roche Primary Standards for HIV-1 Group O and HIV-2, Roche Secondary Standard for HCV and the WHO International Standard for HBV NIBSC (97/746) to a final concentration of approximately 3X LOD as appropriate to the level of hemolysis for each specimen. Also, twenty individual living donor EDTA plasma specimens were spiked with the same standards to a level that was approximately 3X LOD in pools of six. The cadaveric specimens were manually diluted 1:5 with **cobas**® TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure and the living donor specimens were diluted 1:6 into negative EDTA plasma by the Hamilton Star as part of the living donor procedure for evaluation of pools of 6. Each of the 20 living donor and cadaveric specimens were tested by 2 operators using three different **cobas**® TaqScreen MPX Test kit lots. Each operator was paired with a **cobas s** 201 system to create two operator-instrument pairs.

All valid reproducibility data was analyzed by comparing the reactive rates of living donor and cadaveric specimens spiked with HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV and tested across three kit lots (Table 22) and two operator-instrument pairs (Table 23). The results were evaluated using 1-Tailed Fisher's Exact Test and no significant differences were observed in the reactive rates for living donor and cadaveric specimens. Fisher's Exact Test p-values greater than 0.05 are not considered statistically significant.

Table 22
Summary of the cobas® TaqScxreen MPX Test Reproducibilty of Cadaveric Specimens and Living Donor Specimens - Results by Kit Lot

Viral Target	Donor Type	Lot # 1	Lot # 2	Lot # 3	1-tailed p-value living donor vs. cadaveric specimens by target
HIV-1 Group M	Cadaveric	40 / 40 100%	40 / 40 100%	40 / 40 100%	1
	Living	40 / 40 100%	39 / 39* 100%	39 / 39* 100%	
HIV-1 Group O	Cadaveric	39 / 40 97.5%	40 / 40 100%	40 / 40 100%	1
	Living	39 / 39* 100%	40 / 40 100%	40 / 40 100%	
HIV-2	Cadaveric	40 / 40 100%	40 / 40 100%	40 / 40 100%	1
	Living	39 / 39* 100%	40 / 40 100%	40 / 40 100%	
HCV	Cadaveric	40 / 40 100%	40 / 40 100%	39 / 39* 100%	0.5
	Living	38 / 39* 97.4%	40 / 40 100%	40 / 40 100%	
HBV	Cadaveric	40 / 40 100%	40 / 40 100%	40 / 40 100%	1
	Living	39 / 39* 100%	40 / 40 100%	39 / 39* 100%	
1-Tailed p-value living vs. cadaveric specimens by reagent lot		0.75	1	1	

*One replicate was invalid

Table 23
Reproducibility Summary of Cadaveric Specimens and Living Donor Specimens - Results by Operator-Instrument Pair

Viral Target	Donor Type	Operator-Instrument # 1	Operator-Instrument # 2	1-tailed p-value living vs. cadaveric specimems by target
HIV-1 Group M	Cadaveric	60 / 60 100%	60 / 60 100%	1
	Living	60 / 60 100%	58 / 58** 100%	
HIV-1 Group O	Cadaveric	59 / 60 98.3%	60 / 60 100%	1
	Living	59 / 59* 100%	60 / 60 100%	
HIV-2	Cadaveric	60 / 60 100%	60 / 60 100%	1
	Living	59 / 59* 100%	60 / 60 100%	
HCV	Cadaveric	60 / 60 100%	59 / 59* 100%	0.5
	Living	59 / 59* 100%	59 / 60 98.3%	
HBV	Cadaveric	60 / 60 100%	60 / 60 100%	1
	Living	58 / 58** 100%	60 / 60 100%	
1-Tailed p-value living vs. cadaveric specimens by instrument-operator pair		1	0.5	

*One replicate was invalid
**Two replicates were invalid

Specificity

Seventy individual sero-negative living donor (pre-mortem) and seventy-one individual sero-negative cadaveric (post-mortem) EDTA plasma specimens were divided into three groups and each group of specimens was tested with one of three lots of the **cobas**® TaqScreen MPX Test. The cadaveric specimens were divided into three groups of 25, 21 and 25 specimens and manually diluted 1:5 with **cobas**® TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure. The living donor specimens were divided into three groups of 25, 20 and 25 specimens and manually diluted 1:6 with a pool of EDTA plasma (negative for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA) prior to testing, in order to simulate the pools of six testing procedure for living donors with the **cobas**® TaqScreen MPX Test.

For the cadaveric specimens, seventy valid results were obtained using three kit lots. Three specimens were initially reactive when tested. Upon repeat testing in duplicate, two of the three specimens remained reactive and one was non-reactive. Confirmatory testing with the COBAS® AmpliScreen Tests for HIV-1, HCV and HBV confirmed that all three specimens were reactive for HCV RNA. These three reactive specimens were therefore excluded from the specificity analysis as they were true viral positive specimens. For the living donor specimens, seventy valid results were obtained using the same three kit lots. All seventy results were non-reactive. The summary of the specificity test results are presented in Table 24.

Table 24
Summary of Testing with the cobas® TaqScreen MPX Test for Specificity of Cadaveric and Living Donor Specimens

	Cadaveric Donor Specimens (Diluted 1:5 in cobas® TaqScreen Cadaveric Specimen Diluent)			Living Donor Specimens (Diluted 1:6 in negative EDTA Plasma)		
Reagent Lot	**Number of Valid Results**	**Number of Non-reactive Results**	**Number of Reactive Results**	**Number of Valid Results**	**Number of Non-Reactive Results**	**Number of Reactive Results**
Lot # 1	24	24	0	25	25	0
Lot # 2	21	21	2*	20	20	0
Lot # 3	25	25	1**	25	25	0
Total	**70**	**70**	**3**	**70**	**70**	**0**
Specificity	**100%*** **			**100%**		

*Upon retesting in duplicate, one specimen was non-reactive and one specimen was reactive; both specimens were reactive with the COBAS® AmpliScreen HCV Test, v2.0.
**Upon retesting in duplicate, this specimen was reactive; this specimen was reactive with the COBAS® AmpliScreen HCV Test, v2.0.
***Specificity was calculated after removal of the 3 specimens that were confirmed reactive with the COBAS® AmpliScreen HCV Test, v2.0.

Analytical Sensitivity in Cadaveric Specimens

A limiting dilution study of the **cobas®** TaqScreen MPX Test for the HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV viral targets in cadaveric specimens were assessed using the following standards: WHO INTERNATIONAL STANDARD FOR HEPATITIS B VIRUS DNA FOR NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) ASSAYS (NIBSC code 97/746)[32], and Roche Standards for HCV, HIV-1 Group M, HIV-1 Group O and HIV-2. The Roche Secondary Standard for HCV was prepared at Roche from a commercially available clinical specimen (Millenium Biotech Inc., Ft. Lauderdale, FL) and calibrated to the WHO 1st International Standard for HCV (NIBSC code 96/790) using the COBAS AMPLICOR HCV MONITOR Test, v2.0. The Roche Secondary standard for HIV-1 Group M RNA is a commercially available, cultured virus stock (HIV-1 LAV 8E5, PN 227, Boston Biomedica, Inc.) traceable to the WHO 1st International Standard for HIV-1 RNA for NAT (NIBSC code 97/656) calibrated by the COBAS TaqMan HIV-1 Test For Use With The High Pure System. No international standards are currently available for HIV-1 Group O and HIV-2. The Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA are commercially available cultured virus stocks, PN242O (Boston Biomedica, Inc.) and Cat, No. 10-127-000 (Advanced Biotechnologies, Inc.) The Roche Primary Standards for HIV-1 Group O and HIV-2 were both calibrated to FDA/CBER panels (CBER HIV-1 Subtype RNA Reference Panel #1 Lot O1 and CBER HIV-2 RNA Lot Release Panel ISD) using the **cobas®** TaqScreen MPX Test.

For each target, six panel members were prepared by dilution of the HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV virus standards into a Moderately Hemolyzed cadaveric EDTA plasma pool and a Highly Hemolyzed sample matrix. For the Moderately Hemolyzed pool, panels were prepared for each virus standard by dilution into a pool of virus-negative cadaveric EDTA plasma that consisted of individual cadaveric specimens determined to fall into the category of Moderately Hemolyzed specimens (having a straw to pink colored appearance). The Highly Hemolyzed Sample Matrix was an artificial matrix consisting of aged-lysed whole blood mixed with EDTA plasma to represent a Highly Hemolyzed clinical specimen (red to dark-red or brown in appearance). The Highly Hemolyzed Sample Matrix was verified to be negative for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, HBV prior to preparing the dilution panels. Two lots of the **cobas®** TaqScreen MPX Test were used for the testing of the Moderately Hemolyzed panels and one lot of the **cobas®** TaqScreen MPX Test was used for testing the Highly Hemolyzed panels. Table 25 to Table 34 summarize the results.

Table 25
Analytical Sensitivity Summary: Roche Secondary Standard for HIV-1 Group M in Moderately Hemolyzed Cadaveric Sample Plasma

HIV-1-M RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
525	66	66	100%	95.6%
400	65	66	98.5%	93.0%
350	64	66	97.0%	90.8%
300	64	66	97.0%	90.8%
240	43	44	97.7%	89.7%
90	34	44	77.3%	64.5%

Table 26
Analytical Sensitivity Summary: Roche Secondary Standard for HIV-1 Group M in Highly Hemolyzed Cadaveric Sample Matrix

HIV-1-M RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
500	44	44	100%	93.4%
400	44	44	100%	93.4%
350	43	44	97.7%	89.7%
300	40	44	90.9%	80.4%
240	43	44	97.7%	89.7%
90	31	44	70.5%	57.2%

Table 27
Analytical Sensitivity Summary: Roche Primary Standard for HIV-1 Group O in Moderately Hemolyzed Cadaveric Sample Plasma

HIV-1-O RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
733	65	66	98.5%	93.0%
615	63	66	95.5%	88.7%
563	63	66	95.5%	88.7%
422	60	66	90.9%	82.8%
316	38	43	88.4%	77.1%
129	19	44	43.2%	30.4%

Table 28
Analytical Sensitivity Summary: Roche Primary Standard for HIV-1 Group O in Highly Hemolyzed Cadaveric Sample Matrix

HIV-1-O RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
1348	44	44	100%	93.4%
1055	40	44	90.9%	80.4%
967	44	44	100%	93.4%
879	39	44	88.6%	77.6%
703	42	44	95.5%	86.4%
264	23	44	52.3%	38.9%

Table 29
Analytical Sensitivity Summary: Roche Primary Standard for HIV-2 in Moderately Hemolyzed Cadaveric Sample Plasma

HIV-2 RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
801	65	65	100%	95.5%
534	65	66	98.5%	93.0%
481	65	66	98.5%	93.0%
401	65	66	98.5%	93.0%
267	40	43	93.0%	82.9%
134	35	44	79.6%	67.0%

Table 30
Analytical Sensitivity Summary: Roche Primary Standard for HIV-2 in Highly Hemolyzed Cadaveric Sample Matrix

HIV-2 RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
801	44	44	100%	93.4%
534	43	43	100%	93.3%
481	44	44	100%	93.4%
401	44	44	100%	93.4%
267	41	44	93.2%	83.3%
134	35	44	79.5%	67.0%

Table 31
Analytical Sensitivity Summary: Roche Secondary Standard for HCV in Moderately Hemolyzed Cadaveric Sample Plasma

HCV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
180	66	66	100%	95.6%
120	64	66	97.0%	90.8%
100	62	66	93.9%	86.7%
90	64	66	97.0%	90.8%
70	38	44	86.4%	74.8%
25	26	44	59.1%	45.6%

Table 32
Analytical Sensitivity Summary: Roche Secondary Standard for HCV in Highly Hemolyzed Cadaveric Sample Matrix

HCV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
200	44	44	100%	93.4%
170	44	44	100%	93.4%
150	43	43	100%	93.3%
130	41	44	93.2%	83.3%
100	44	44	100%	93.4%
40	38	44	86.4%	74.9%

Table 33
Analytical Sensitivity Summary: WHO International Standard for HBV in Moderately Hemolyzed Cadaveric Sample Plasma

HBV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
55	66	66	100%	95.6%
45	66	66	100%	95.6%
35	66	66	100%	95.6%
30	64	66	97.0%	90.8%
20	41	44	93.2%	83.3%
10	36	44	81.8%	69.6%

Table 34
Analytical Sensitivity Summary: WHO International Standard for HBV in Highly Hemolyzed Cadaveric Sample Matrix

HBV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one sided)
130	44	44	100%	93.4%
85	44	44	100%	93.4%
75	43	44	97.7%	89.7%
65	43	44	97.7%	89.7%
50	42	43	97.7%	89.4%
20	30	44	68.2%	54.8%

Sensitivity

HBV, HCV, HIV-1 Group M

Sixty randomly selected cadaveric EDTA plasma specimens non-reactive for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV RNA and HBV DNA and classified by hemolysis level [Moderately Hemolyzed specimen (having a straw-color to pink color) or a Highly Hemolyzed specimen (having a red to dark-red or brown appearance)], were divided evenly into 5 clinical specimen spiking groups with12 specimens per group. Each cadaveric specimen within a group was spiked with one of five unique HIV-1 Group M, HCV, or HBV singly infected clinical specimens of known titer, to a final concentration of approximately three times the Limit of Detection (3X LOD) for the appropriate level of hemolysis. Spiked cadaveric specimens were stored up to 7 days at 2-8°C prior to testing. Each specimen was manually diluted 1:5 with the cobas TaqScreen Cadaveric Specimen Diluent prior to testing. Three reagent lots were used for this study and each group was divided between each lot for a total of 20 specimens per reagent kit lot per target. For the sixty cadaveric specimens tested for each target in this study, the reactive rate was 100% [95% CI: 94 – 100%] for HICV-1 Group M, HCV and HBV with the **cobas**® TaqScreen MPX Test. A summary of the sensitivity test results are presented in Table 35.

HIV-1 Group O, HIV-2

For HIV-1 Group O and HIV-2 viral targets, the Roche Primary Standards were used to spike cadaveric EDTA plasma specimens at approximately 3X LOD for the appropriate level of hemolysis [Moderately Hemolyzed specimen (having a straw-color to pink color) or a Highly Hemolyzed specimen (having a red to dark-red or brown appearance)] . Consequently, these targets only had one spiking group. Spiked cadaveric specimens were stored up to 7 days at 2-8°C prior to testing. Each specimen was manually diluted 1:5 with the **cobas**® TaqScreen Cadaveric Specimen Diluent prior to testing. Three reagent lots were used for this study. Each group was divided between the three lots to yield a total of 12 specimens tested for HIV-1 Group O and HIV-2. For the twelve cadaveric specimens tested for HIV-1 Group O and HIV-2, the reactive rate was 100% [95% CI: 73.5-100%] with the **cobas**® TaqScreen MPX Test. A summary of the sensitivity results are presented in Table 35.

Table 35
Summary of Sensitivity for the cobas® TaqScreen MPX Test with Moderately Hemolyzed (MH) and Highly Hemolyzed (HH) Cadaveric Specimens

Reagent Lot	Hemolysis Level	HIV-1 Group M	HIV-1 Group O	HIV-2	HCV	HBV
Lot 1	MH	14/14	3/3	3/3	14/14	14/14
	HH	6/6	1/1	1/1	6/6	6/6
	Total	**20/20**	**4/4**	**4/4**	**20/20**	**20/20**
Lot 2	MH	13/13	3/3	3/3	13/13	13/13
	HH	7/7	1/1	1/1	7/7	7/7
	Total	**20/20**	**4/4**	**4/4**	**20/20**	**20/20**
Lot 3	MH	16/16	3/3	3/3	16/16	16/16
	HH	4/4	1/1	1/1	4/4	4/4
	Total	**20/20**	**4/4**	**4/4**	**20/20**	**20/20**
Sensitivity		**100%**	**100%**	**100%**	**100%**	**100%**
		94.0 – 100% CI	73..5 – 100% CI	73.5 – 100% CI	94.0 – 100% CI	94.0 – 100% CI

CLINICAL PERFORMANCE

Living Donor Specimens

Clinical Specificity

Reactivity in Whole Blood Donor Population

Clinical specificity of the **cobas®** TaqScreen MPX Test was evaluated by testing plasma samples from randomly selected whole blood donations at three external laboratory sites. Testing was performed on both plasma from individual blood donations and with pooled specimens prepared from equal aliquots of plasma from individual blood donations. A total of 72,281 blood donations were tested with the **cobas®** TaqScreen MPX Test resulting in a clinical specificity of 99.98% (72,266/72,281; 95% CI = 99.97 to 99.99). The invalid batch rate for pooled and individual specimen testing was 11.3% and 10.1%, respectively [Note that for individual specimen batches, 12% (8/66) of the invalid batches were due to termination of testing prior to completion of the batch due to a need to resume routine testing at the site]. The invalid rate for pooled and individual specimen results was 5.6% and 4.4%, respectively. For donor specimens tested in a pool format, the reactive pools were resolved by further testing of the individual blood donations comprising the pools. During the clinical specificity testing, there was one donor identified as a "yield" case, a donor determined to be HBV DNA positive which was not detected by the laboratory's current test methods.

Table 36
Pool Reactivity in Volunteer Whole Blood Donors

Category	Pools	Percentage
Pools tested	10,090	100%
Non-Reactive pools	10,054	99.64%
Initially reactive pools	36	0.36%
Initial reactive pools with donor status positive*	23	0.23%
Positive pools due to yield case	1	0.01%
Initial reactive pools with donor status negative (false positive)*	12	0.12%

** Donor status was assigned to each donor based upon results of serology and COBAS® AmpliScreen Test results and, in rare cases, results of alternate NAT*

Yield Case

One donor (a male, first-time donor who was not immunized against HBV) had negative results with the COBAS® AmpliScreen HIV-1, HCV and HBV Tests when tested in pools of 24, was negative by serology for HIV, HCV and HBc and negative for HBsAg, but was reactive with the **cobas®** TaqScreen MPX Test. Discriminatory testing on the index donation was positive with the COBAS® AmpliScreen HBV Test when tested individually and negative with individual COBAS® AmpliScreen HIV-1, v1.5 and COBAS® AmpliScreen HCV, v2.0 Tests, but the donation was positive for anti-HBs. This donation was negative by HBV Super Quant™ (National Genetics Institute), but positive by the more sensitive HBV UltraQual™ 2000 assay (National Genetics Institute). The donor was enrolled into the follow-up study and follow-up testing was positive for the **cobas®** TaqScreen MPX Test or a modified COBAS® AmpliScreen HBV Test, and positive for anti-HBs (Table 37). These results indicate an occult carrier.

The modified COBAS® AmpliScreen HBV Test procedure used a 1.0 mL sample volume and processed the sample using the Multiprep Specimen Processing Procedure. Following this single extraction, 3 amplification/detections were performed, each requiring 50 µL of the 200 µL of processed specimen. A positive result for any of the 3 replicates indicates that the specimen contains at least 2 IU/mL (10 copies/mL) of HBV DNA. This compares to a sensitivity of 16 IU/mL (80 copies/mL) when using the Standard Specimen Processing Procedure for the COBAS® AmpliScreen HBV Test.

Table 37
Results of Follow-up Testing for the Yield Case Donor

Day of Test	**Day 0**	**Day 19**	**Day 33**	**Day 43**	**Day 59**
cobas® TaqScreen MPX Test (Neat)	Reactive	Reactive	Reactive	Non-Reactive	Reactive
COBAS® AmpliScreen HBV Test (Neat)	Positive	Negative	Negative	Negative	Negative
Modified COBAS® AmpliScreen HBV Test (Multiprep Procedure x 3)	Not Tested	3/3	1/3	1/3	1/3
HBsAg	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive
Anti-HBc	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive	Non-Reactive
Anti-HBs	Reactive	Reactive	Reactive	Reactive	Reactive

Studies in High Risk Populations

Plasma specimens from a high risk population, which included individuals at risk for infection with HIV, HCV, and/or HBV due to injection drug use, multiple sexual partners, diagnosis of a STD (other than HIV or HBV), recipient of blood, blood component or transplant and dialysis were tested neat with the **cobas®** TaqScreen MPX Test. Testing was performed at four external clinical sites with two lots of reagents.

These plasma specimens were also tested neat using the Standard Specimen Processing Procedure with the licensed COBAS® AmpliScreen Tests (COBAS® AmpliScreen HIV-1 Test, v1.5, COBAS® AmpliScreen HCV Test, v2.0 and COBAS® AmpliScreen HBV Test). Testing was performed at two sites with three lots of reagents.

HIV High Risk Population

Out of 789 specimens tested in the HIV high risk population, 327 specimens were reactive with the **cobas®** TaqScreen MPX Test and 317 specimens were positive with at least one of the COBAS® AmpliScreen Tests. Of these 789 specimens, 103 specimens (13%) were positive for HIV, 243 specimens (31%) were positive for HCV and 46 specimens (6%) were positive for HBV.

The **cobas®** TaqScreen MPX Test showed greater detection rate than the three licensed COBAS® AmpliScreen Tests (Table 38 and Table 39).

Table 38
Summary of Performance of the cobas® TaqScreen MPX Test for HIV High Risk Specimens — Comparison to the Combined Licensed COBAS® AmpliScreen Test Results, Neat

	COBAS® AmpliScreen Positive	**COBAS® AmpliScreen Negative**	**Total**
MPX Reactive	305	22	327
MPX Non-Reactive	12	450	462
Total	317	472	789

Table 39
Performance of the cobas® TaqScreen MPX Test for HIV High Risk Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Neat

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	11
	P	P	N	31
	P	N	N	52
	P	N	P	6
	N	P	P	15
	N	N	P	7
	N	P	N	182
	N	N	N	22
	N/A	P	N	1
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	3
	P	N	P	0
	N	P	P	1
	N	N	P	6
	N	P	N	2
	N	N	N	450
	N/A	N	N	2*

Note: P=Positive and N=Negative

N/A = Not Available

** Specimens for which 1 or more COBAS® AmpliScreen Test results were not available, and the available results were negative, were excluded from Table 38.*

HCV High Risk Population

Out of 468 specimens tested in the HCV high risk population, 224 specimens were reactive with the **cobas®** TaqScreen MPX Test and 223 specimens were positive with at least one of the COBAS® AmpliScreen Tests. Of these 468 specimens, 52 specimens (11%) were positive for HIV, 196 specimens (42%) were positive for HCV and 26 specimens (6%) were positive for HBV.

Table 40
Summary of Performance of the cobas® TaqScreen MPX Test for HCV High Risk Specimens — Comparison to the Combined Licensed COBAS® AmpliScreen Test Results, Neat

	COBAS® AmpliScreen Positive	**COBAS® AmpliScreen Negative**	**Total**
MPX Reactive	218	6	224
MPX Non-Reactive	5	239	244
Total	223	245	468

Table 41
Performance of the cobas® TaqScreen MPX Test for HCV High Risk Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Neat

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	6
	P	P	N	24
	P	N	N	18
	P	N	P	2
	N	P	P	13
	N	N	P	3
	N	P	N	151
	N	N	N	6
	N/A	P	N	1
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	2
	P	N	P	0
	N	P	P	0
	N	N	P	2
	N	P	N	1
	N	N	N	239

Note: P=Positive and N=Negative

N/A = Not Available

HBV High Risk Population

Out of 1147 specimens tested in the HBV high risk population, 384 specimens were reactive with the **cobas®** TaqScreen MPX Test and 383 specimens were positive with at least one of the COBAS® AmpliScreen Tests. Of these 1147 specimens, 113 specimens (10%) were positive for HIV, 283 specimens (25%) were positive for HCV and 68 specimens (6%) were positive for HBV.

Table 42
Summary of Performance of the cobas® TaqScreen MPX Test for HBV High Risk Specimens — Comparison to the Combined Licensed COBAS® AmpliScreen Test Results, Neat

	COBAS® AmpliScreen Positive	**COBAS® AmpliScreen Negative**	**Total**
MPX Reactive	356	28	384
MPX Non-Reactive	27	736	763
Total	383	764	1147

Table 43
Performance of the cobas® TaqScreen MPX Test for HBV High Risk Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Neat

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	10
	P	P	N	35
	P	N	N	57
	P	N	P	7
	N	P	P	19
	N	N	P	14
	N	P	N	213
	N	N	N	28
	N/A	P	N	1
	N/A	N	N/A	1*
	N/A	N/A	N/A	1**
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	4
	P	N	P	0
	N	P	P	0
	N	N	P	17
	N	P	N	5
	N	N	N	736
	N/A	N	N	2*
	N	N/A	P	1
	N/A	N/A	N/A	1**

Note: P=Positive and N=Negative
N/A = Not Available

**Specimens for which 1 or more COBAS® AmpliScreen Test results were not available, and the available results were negative, were excluded from Table 42.*

***Specimens for which all 3 COBAS® AmpliScreen Test results were not available were excluded from Table 42.*

Studies in Seropositive Populations

HIV-1, HCV and HBV confirmed seropositive plasma specimens were tested diluted 1:6 (to simulate testing of pools) with the **cobas**® TaqScreen MPX Test. Testing was performed at six external clinical sites with two lots of reagents. Patients on therapy were not excluded from the study.

These plasma specimens were also tested diluted 1:24 using the Multiprep Specimen Processing Procedure with the licensed COBAS® AmpliScreen Tests (COBAS® AmpliScreen HIV-1 Test, v1.5, COBAS® AmpliScreen HCV Test, v2.0 and COBAS® AmpliScreen HBV Test). Testing was performed at two sites with three lots of reagents.

HIV-1 Seropositive Population

Out of 733 specimens tested in the HIV-1 seropositive population, 576 (79%) specimens were reactive with the **cobas**® TaqScreen MPX Test and 461 (63%) specimens were positive with the COBAS® AmpliScreen HIV-1 Test, v1.5.

The **cobas**® TaqScreen MPX Test showed greater detection rate than the licensed COBAS® AmpliScreen HIV-1 Test, v1.5 (Table 44 and Table 45).

Table 44
Summary of Performance of the cobas® TaqScreen MPX Test for HIV-1 Confirmed Seropositive Specimens — Comparison to the Licensed COBAS® AmpliScreen HIV-1 Test, v1.5 Results, Diluted*

	COBAS® AmpliScreen HIV-1 v1.5 Positive	**COBAS® AmpliScreen HIV-1 v1.5 Negative**	**Total**
MPX Reactive	441	135	576
MPX Non-Reactive	20	137	157
Total	461	272	733

Tested diluted 1:6 with **cobas® TaqScreen MPX Test and 1:24 with COBAS® AmpliScreen HIV-1 Test, v1.5.*

Table 45
Performance of the cobas® TaqScreen MPX Test for HIV-1 Confirmed Seropositive Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Diluted*

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	3
	P	P	N	5
	P	N	N	379
	P	N	P	54
	N	P	P	0
	N	N	P	15
	N	P	N	3
	N	N	N	117
	N	N	N/A	1**
	N	N/A	N	1**
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	16
	P	N	P	4
	N	P	P	0
	N	N	P	19
	N	P	N	0
	N	N	N	118
	N	N/A	N	2**

** Tested diluted 1:6 with **cobas**® TaqScreen MPX Test and diluted 1:24 with COBAS® AmpliScreen Tests.*
***Specimens for which 1 or more COBAS® AmpliScreen Test results were not available, and the available results were negative, were excluded from Table 44.*
Note: P=Positive and N=Negative
N/A = Not Available

HCV Seropositive Population

Out of 961 specimens tested in the HCV seropositive population, 831 (86%) specimens were reactive with the **cobas**® TaqScreen MPX Test and 797 (83%) specimens were positive with the COBAS® AmpliScreen HCV Test, v2.0 (Table 46 and Table 47).

The **cobas**® TaqScreen MPX Test showed greater detection rate than the licensed COBAS® AmpliScreen HCV Test, v2.0 (Table 46 and Table 47).

Table 46
Summary of Performance of the cobas® TaqScreen MPX Test for HCV Confirmed Seropositive Specimens — Comparison to the Licensed COBAS® AmpliScreen HCV Test, v2.0 Results, Diluted*

	COBAS® AmpliScreen HCV v2.0 Positive	**COBAS® AmpliScreen HCV v2.0 Negative**	**Total**
MPX Reactive	789	42	831
MPX Non-Reactive	8	122	130
Total	797	164	961

Tested diluted 1:6 with **cobas® TaqScreen MPX Test and 1:24 with COBAS® AmpliScreen HCV Test, v2.0.*

Table 47
Performance of the cobas® TaqScreen MPX Test for HCV Confirmed Seropositive Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Diluted*

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	3
	P	P	N	4
	P	N	N	0
	P	N	P	0
	N	P	P	96
	N	N	P	4
	N	P	N	686
	N	N	N	38
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	2
	P	N	P	0
	N	P	P	2
	N	N	P	24
	N	P	N	6
	N	N	N	96

** Tested diluted 1:6 with **cobas**® TaqScreen MPX Test and diluted 1:24 with COBAS® AmpliScreen Tests.*

Note: P=Positive and N=Negative

HBV Seropositive Population

Out of 391 specimens tested in the HBV seropositive population, 377 (96%) specimens were reactive with the **cobas**® TaqScreen MPX Test and 360 (92%) specimens were positive with the COBAS® AmpliScreen HBV Test.

The **cobas**® TaqScreen MPX Test showed greater detection rate than the licensed COBAS® AmpliScreen HBV Test (Table 48 and Table 49).

Table 48
Summary of Performance of the cobas® TaqScreen MPX Test for HBV Confirmed Seropositive Specimens — Comparison to the Licensed COBAS® AmpliScreen HBV Test Results, Diluted*

	COBAS® AmpliScreen HBV Positive	**COBAS® AmpliScreen HBV Negative**	**Total**
MPX Reactive	354	23	377
MPX Non-Reactive	6	8	14
Total	360	31	391

Tested diluted 1:6 with **cobas® TaqScreen MPX Test and 1:24 with COBAS® AmpliScreen HBV Test.*

Table 49
Performance of the cobas® TaqScreen MPX Test for HBV Confirmed Seropositive Specimens — Comparison to the Individual Licensed COBAS® AmpliScreen Test Results, Diluted*

MPX	**COBAS® AmpliScreen HIV-1**	**COBAS® AmpliScreen HCV**	**COBAS® AmpliScreen HBV**	**Total**
Reactive	P	P	P	0
	P	P	N	0
	P	N	N	1
	P	N	P	8
	N	P	P	3
	N	N	P	343
	N	P	N	2
	N	N	N	20
	N/A	N	N/A	1**
Non-Reactive	P	P	P	0
	P	P	N	0
	P	N	N	1
	P	N	P	0
	N	P	P	0
	N	N	P	6
	N	P	N	0
	N	N	N	7

** Tested diluted 1:6 with **cobas**® TaqScreen MPX Test and diluted 1:24 with COBAS® AmpliScreen Tests.*

***Specimens for which 1 or more COBAS® AmpliScreen Test results were not available, and the available results were negative, were excluded from Table 48.*

Note: P=Positive and N=Negative
N/A = Not Available

Seropositive Population — HIV-1 Group O

A total of 22 HIV-1 Group O specimens, characterized by serology and/or DNA sequencing, were tested using the **cobas**® TaqScreen MPX Test. Of the 22 specimens tested, 5 specimens were tested both neat and diluted 1:6 and the remaining 17 specimens were tested diluted 1:6 with the **cobas**® TaqScreen MPX Test. As shown in Table 50, 19 of the 22 specimens were reactive and 3 specimens were non-reactive when tested diluted 1:6 with the **cobas**® TaqScreen MPX Test. The 3 non-reactive specimens were subsequently confirmed to have undetectable viral load (<60 copies/mL), using the Abbott Real Time HIV-1 Test (Research Use Only) and were excluded from the sensitivity analysis. The sensitivity of the **cobas**® TaqScreen MPX Test for both the neat and 1:6 diluted HIV-1 Group O specimens was 100%, 4 out of 4 and 19 out of 19, respectively, as shown in Table 50.

Table 50
Sensitivity of the cobas® TaqScreen MPX Test with a Seropositive Population of HIV-1 Group O Specimens

	Total	MPX Reactive	MPX Non-reactive	Sensitivity	95% C.I.	
HIV-1 Group O (Neat)	5	4*	0	100.0%	82.4%	100.0%
HIV-1 Group O (1:6 Diluted)	22	19**	3***	100.0%	82.4%	100.0%

**One of the 5 specimens was invalid on initial testing with insufficient volume for a repeat test*

***There was 1 HIV-1 Group O/HIV-1 Group M co-infected specimen (as shown by positive HIV-1 Group M serology and sequence analysis), 1 HIV-1 Group O/HCV co-infected specimen (as shown by positive results with the COBAS® AmpliScreen HCV Test, v2.0), and 1 HIV-1 Group O/HBV co-infected specimen (as shown by positive results with the COBAS® AmpliScreen HBV Test)*

**** All 3 non-reactive specimens had undetectable HIV-1 viral load (<60 copies/mL) using the Abbott Real Time HIV-1 Test (Research Use Only) and were excluded from the sensitivity analysis*

Seropositive Population — HIV-2

A total of 200 HIV-2 seropositive specimens (HIV-2 positive using the BioRad Multispot HIV-1/HIV-2 Rapid Test) were purchased from a commercial vendor. These specimens were tested at Roche Molecular Systems, both neat and diluted 1:6 in EDTA plasma, using the **cobas**® TaqScreen MPX Test. Of these 200 specimens, 93 (46.5%) were reactive neat and 69 (34.5%) were reactive diluted 1:6 using the **cobas**® TaqScreen MPX Test.

The 200 neat HIV-2 seropositive specimens were also tested using a quantitative nucleic acid testing (NAT) assay (Research Use Only test developed by Dr. Florence Damond, Hopital Bichat Claude Bernard, Paris, France) with a limit of detection of 100 HIV-2 RNA copies/mL[37], and a total of 43 out of the 200 specimens had detectable HIV-2 RNA. The sensitivity of the **cobas**® TaqScreen MPX Test with HIV-2 seropositive/NAT positive specimens, tested neat was 100% (Table 37). For HIV-2 seropositive/NAT positive specimens diluted 1:6, the **cobas**® TaqScreen MPX Test detected 40 of 43 specimens tested (Table 51).

Table 51
Sensitivity of the cobas® TaqScreen MPX Test with a NAT-Positive* — Seropositive Population of HIV-2 Specimens

	Total	Alternate NAT Positive	MPX Reactive	MPX Non-reactive	Sensitivity	95% C.I.	
HIV-2 (Neat)	200	43**	43	0	100.0%	91.8%	100.0%
HIV-2 (1:6 Diluted)	200	43*	40	3	93.0%*	80.9%	98.5%

** HIV-2 NAT RNA quantification test (Research Use Only) developed by Dr. Florence Damond (Hopital Bichat Claude Bernard, Paris, France) and was run on neat specimens only.*

*** One HIV-2 specimen had a viral load of 117 copies/mL and was reactive for 5 out of 8 replicates when tested neat with the **cobas**® TaqScreen MPX Test.*

Validation of Discriminatory Testing using the COBAS® AmpliScreen Tests with the Standard Specimen Processing Procedure

Any individual reactive specimen identified as reactive using the **cobas**® TaqScreen MPX Test should be further tested to determine which virus was initially detected using the COBAS® AmpliScreen Tests (the COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test) as Discriminatory Testing. For the COBAS® AmpliScreen individual viral target identification test procedures, 200 µL of the individual reactive specimen is extracted using the Standard Specimen Processing Procedure for neat specimens, resulting in 200 µL of processed specimen of which 50 µL is used for amplification with each of the COBAS® AmpliScreen Tests. Refer to Step B, Specimen and Control Preparation, in the INSTRUCTIONS FOR USE section of the Package Inserts for the COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test for the Standard Specimen Processing Procedure.

HIV, HCV and HBV seropositive specimens were tested neat with the **cobas**® TaqScreen MPX Test and with the three COBAS® AmpliScreen Tests used as Discriminatory Tests (COBAS® AmpliScreen HIV-1 Test, v1.5, the COBAS® AmpliScreen HCV Test, v2.0 and the COBAS® AmpliScreen HBV Test). The data in Table 52 show that 91% (568/627) of HIV seropositive specimens that were **cobas**® TaqScreen MPX Test reactive were also positive with the COBAS® AmpliScreen HIV-1 Test, v1.5, 98% (809/825) of HCV seropositive specimens that were **cobas**® TaqScreen MPX Test reactive were also positive with the COBAS® AmpliScreen HCV Test, v2.0 and 97% (333/342) of HBV seropositive specimens that were **cobas**® TaqScreen MPX Test reactive were also positive with the COBAS® AmpliScreen HBV Test. Note that some **cobas**® TaqScreen MPX Test reactive specimens were reactive on two or three of the discriminatory HIV-1, HCV and HBV tests, indicating possible dual or triple infections with these viruses.

Table 52
Validation of Discriminatory Testing using the
COBAS® AmpliScreen Tests with the Standard Specimen Processing Procedure

	Sample Number	**COBAS® AmpliScreen HIV-1 Positive**	**COBAS® AmpliScreen HCV Positive**	**COBAS® AmpliScreen HBV Positive**
HIV Seropositive / MPX Reactive	627	568 (91%*)	33	86
HCV Seropositive / MPX Reactive	825	11	809 (98%*)	97
HBV Seropositive / MPX Reactive	342	30	21	333 (97%*)

Note: Numbers in ( ) indicate the percentage of seropositive specimens that were **cobas® TaqScreen MPX Test reactive and also positive with the individual COBAS® AmpliScreen Test.*

If an individual specimen identified as reactive using the **cobas**® TaqScreen MPX Test was found to be non-reactive with all three COBAS® AmpliScreen Tests, the specimen was considered "reactive and non-discriminated".

PERFORMANCE CHARACTERISTICS of Source Plasma

Clinical Performance

A total of 1,130 pools of 96 representing 108,216 donations from 24,238 source plasma donors were included in the analysis of pool reactivity (note that 178 pools had between 90 to 95 members). Of these 1,130 pools, 1094 (96.8%) were non-reactive with the **cobas** TaqScreen MPX Test and 36 (3.2%) were reactive. Of the 1094 non-reactive pools, 1046 pools contained all status negative donations and 13 pools contained at least one status positive donation. Of these 13 pools, 12 pools each contained a donation-status positive donation due to a positive anti-HCV serology result or an anti-HIV-1/2 serology result (in 4 of these cases, the serology result was confirmed to be false positive), and 1 pool contained a donation that was status positive due to a positive COBAS AmpliScreen HBV Test result in a pool (this donation was **cobas** TaqScreen MPX Test reactive when tested individually). An additional 35 non-reactive pools (3.1%) contained at least one donation with a status of unresolved.

Of the 36 **cobas** TaqScreen MPX Test reactive pools, 29 pools contained at least one status positive donation and 7 pools contained all status negative donations. Of these 29 pools, there were 5 pools each with a donation identified as a yield case. All 5 of these yield case donations were **cobas** TaqScreen MPX reactive in pools and negative by COBAS AmpliScreen Tests (in pools) and serology (individual donation). These donations were positive by COBAS AmpliScreen Tests (4 COBAS AmpliScreen HBV Test positive and 1 COBAS AmpliScreen HIV-1 Test v1.5 positive) either in pools on subsequent donations or when tested individually on follow-up. These donors were also COBAS AmpliScreen HBV or HIV-1 Test positive when tested individually on the initial donation. The test results for the 1130 pools are summarized in Table 53.

Table 53
cobas TaqScreen MPX Test Reactivity with Pools of up to 96 Source Plasma Donations

Category	No. of Pools	Percentage of Pools Tested
Total pools of 96* tested:	1130	100
Non-Reactive pools**	1,094	96.8
Non-reactive pools with all donations status negative	1,046	92.6
Non-reactive pools with at least one status-positive donation	13	1.2
Non-reactive pools with at least one donation with status of unresolved	35	3.1
Reactive pools**	36	3.2
Reactive pools with at least one status-positive donation	29	2.6
Reactive pools with all donations status-negative (false reactive pools)	7	0.6

** Note that 178 out of 1130 pools had between 90 to 95 members.*

***Donation status was assigned based on initial and additional (if performed) COBAS AmpliScreen Test results, serology results, and follow-up testing (if applicable).*

The clinical specificity of the **cobas** TaqScreen MPX Test for source plasma was determined by analysis of 107,170 evaluable donations from 24,194 donors tested in pools. Evaluable donations had valid **cobas** TaqScreen MPX and COBAS AmpliScreen HBV, HCV and HIV-1 Test results from testing in pools, and valid serology results (across analytes) from testing of individual donations. Of these 107,170 evaluable donations, 107,127 were assigned a donation status of negative, of which 107,127 were **cobas** TaqScreen MPX non-reactive, following resolution testing using the Pooled Testing Algorithm, for a clinical specificity of 100%. The 95% confidence interval is 99.984% to 100%, which is the most conservative calculation using the 24,900 donors, many of whom had multiple bleeds which are represented in the 107,170 donations used to calculate the overall specificity. Seven false **cobas** TaqScreen MPX reactive pools of 96 resolved to contain all **cobas** TaqScreen MPX non-reactive donations following resolution testing using the Pooled Testing Algorithm.

Non-Clinical Performance

Seroconversion Panels

Thirty seroconversion panels (10 HBsAg seroconversion panels, 10 anti-HCV seroconversion panels, and 10 anti-HIV-1 seroconversion panels) were tested neat (undiluted) and at a 1:96 dilution.

HIV-1 Seroconversion Panels

All HIV-1 panels (100%) tested neat had a reactive result by the **cobas** TaqScreen MPX Test before any of the serology tests. The median ranged from 21 to 42 days earlier detection by the **cobas** TaqScreen MPX Test. Also all panels (100%) tested at a 1:96 dilution had a reactive result by the **cobas** TaqScreen MPX Test before any of the serology tests. The median for the panels diluted 1:96 ranged from 9 to 28 days earlier detection by the **cobas** TaqScreen MPX Test.

Table 54
Performance of cobas TaqScreen MPX Test on HIV-1 Seroconversion Panels

	No. of Days Earlier Detection Than HIV Antibody									
	Abbott HIV-1 EIA		bioMerieux Vironostika HIV-1 Microelisa System		Genetic Systems rLAV EIA		Abbott HIV-1/2 EIA		Genetic Systems HIV-1/2	
	cobas TaqScreen MPX Test									
	Neat	1:96	Neat	1:96	Neat	1:96	Neat	1:96	Neat	1:96
Mean	22	12	40	27	27	16	21	10	28	19
Median	21	12	42	28	32	14	22	9	24	14
Min, Max	10, 33	5, 18	16, 67	16, 36	7, 40	7, 30	2, 38	2, 28	14, 49	14, 37

Note: min = minimum; max = maximum.

HCV Seroconversion Panels

All HCV panels (100%) tested neat by the **cobas** TaqScreen MPX Test had a reactive result before either the Abbott 2.0 Anti-HCV serology test (median = 37 days) or the Ortho ELISA Anti-HCV EIA 3.0 serology test (median = 32 days). Also all panels (100%) tested at a 1:96 dilution had a reactive result before either the Abbott 2.0 Anti-HCV serology test (median = 34 days) or the Ortho ELISA Anti-HCV EIA 3.0 serology test (median = 32 days).

Table 55
Performance of cobas TaqScreen MPX Test on HCV Seroconversion Panels

	No. of Days Earlier Detection Than HCV Antibody			
	Abbott 2.0 Anti-HCV		Ortho ELISA Anti-HCV EIA 3.0	
	cobas TaqScreen MPX Test			
	Neat	1:96	Neat	1:96
Mean	34	32	31	28
Median	37	34	32	32
Min, Max	3, 49	3, 49	7, 39	7, 39

Note: min = minimum; max = maximum.

HBV Seroconversion Panels

All HBV panels (100%) tested neat had a reactive result by the **cobas** TaqScreen MPX Test before either the Abbott Auszyme Monoclonal HBsAg serology test (median = 32 days) or the Genetic Systems HBsAg (2.0) Test (median = 37 days). Of the panels tested at a 1:96 dilution, 90% (9/10) had a reactive result by the **cobas** TaqScreen MPX Test before the Abbott Auszyme Monoclonal HBsAg serology test (median = 18 days) and 100% had a reactive result by the **cobas** TaqScreen MPX Test before the Genetic Systems HBsAg (2.0) Test (median = 15 days). For the one panel tested at a 1:96 dilution where Abbott Auszyme Monoclonal HbsAg test reactive results were detected before reactive results with the **cobas** TaqScreen MPX Test, the Abbott Auszyme Monoclonal HBsAg test was reactive on days 21 and 25, non-reactive on days 28, 35 and 39, and then reactive on day 39 whereas reactivity with the **cobas** TaqScreen MPX Test was detected at day 35 and later.

Table 56
Performance of cobas TaqScreen MPX Test on HBV Seroconversion Panels

	No. of Days Earlier Detection Than HBsAg			
	Abbott Auszyme Monoclonal HBsAg		**Genetic Systems HBsAg (2.0)**	
	cobas TaqScreen MPX Test			
	Neat	**1:96**	**Neat**	**1:96**
Mean	33	15	36	17
Median	32	18	37	15
Min, Max	11, 51	-14, 33	14, 47	3, 40

Note: min = minimum; max = maximum.

REFERENCES

1. Kahn JO, Walker BD. Current Concepts: acute human immunodeficiency virus type 1 infection. N Engl J Med. 1998; **339**:33-39.

2. McCutchan FE. Global Epidemiology of HIV. Journal of Medical Virology, **78**:S7-S12 (2006).

3. Centers for Disease Control and Prevention (www.cdc.gov). Fact Sheet – Human Immunodeficiency Virus Type 2. October 1998.

4. Reeves JD and Doms WR. Human Immunodeficiency Virus Type 2. Journal of General Virology (2002), **83**:1253-1265.

5. Choo Q-L, Weiner AJ, Overby LR, et al. Hepatitis C virus: the major causative agent of viral non-A, non-B hepatitis. Br Med Bull. 1990; **46(2)**:423-441.

6. Alter HJ. Descartes before the horse: I clone, therefore I am: the hepatitis C virus in current perspective. Ann Intern Med. 1991; **115(8)**:644-649.

7. Chisari FV, Ferrari C. Viral Hepatitis. In: Nathanson N et al., eds. Viral Pathogenesis, Philadelphia, Lippincott-Raven, 1997: 745-748.

8. Hollinger FB, Liang TJ. Hepatitis B Virus. In: Knipe DM et al., eds. Fields Virology, 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001: 2971-3036.

9. Mahoney FJ, Kane M. Hepatitis B Vaccine. In: Plotkin SA and Orenstein WA, eds. Vaccines, 3rd ed. Philadelphia, W.B. Saunders Company, 1999: 158-182.

10. Viral Hepatitis Prevention Board. Prevention and Control of Hepatitis B in the Community. Communicable Disease Series, 1996, 1.

11. World Health Organization. Introduction of Hepatitis B Vaccine into Childhood Immunization Services. Geneva, WHO, 2001 (unpublished document WHO/V&B/01.31 available on request from Department of Vaccines and Biologicals, World Health Organization, 1211 Geneva 27, Switzerland).

12. Murthy KK, Henrard DR, Eichberg JW, et al. Redefining the HIV-infectious window period in the chimpanzee model: evidence to suggest that viral nucleic acid testing can prevent blood-borne transmission. Transfusion. 1999; **39**:688-693.

13. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV infections among antibody-negative blood donors by nucleic acid-amplification testing. N Engl J Med. 2004; **351**:760-768.

14. Busch MP, Glynn SA, Stramer SL, et al. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion. 2005; **45**:254-264.

15. Offergeld R, Faensen D, Ritter S, Hamouda O. Human immunodeficiency virus, hepatitis C and hepatitis B infections among blood donors in Germany 2000-2002: risk of virus transmission and the impact of nucleic acid amplification testing. Euro Surveill. 2005; **10(2)**:8-11.

16. Hitzler WE, Runkel S. Routine HCV PCR screening of blood donations to identify early HCV infection in blood donors lacking antibodies to HCV. Transfusion. 2001; **41**:333-337.

17. Roth WK, Weber M, Petersen D, et al. NAT for HBV and anti-HBc testing increase blood safety. Transfusion. 2002; **42**:869-875.

18. Biswas R, Tabor E, Hsia CC, et al. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion. 2003; **43**:788-798.

19. Minegishi K, Yoshikawa A, Kishimoto S, et al. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang. 2003; **84**:287-291.

20. Kleinman SH, Strong DM, Tegtmeier GE, et al. Hepatitis B virus (HBV) DNA screening of blood donations in minipools with the COBAS® AmpliScreen HBV test. Transfusion. 2005; **45**:1247-1257.

21. Busch MP, Lee LL, Satten GA, et al. Time course of detection of viral and serologic markers preceding human immunodeficiency type 1 seroconversion: implications for screening blood and tissue donors. Transfusion. 1995; **35**:91-97.

22. Yoshikawa A, Gotanda Y, Itabashi M, et al. Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang. 2005; **88**:77-86.

23. Comanor L and Holland P. Hepatitis B virus blood screening: unfinished agendas. Vox Sanguinis (2006) **91**:1-12.

24. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990; **93**:125-128.

25. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature. 1995; **373**:487-493.

26. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. Cell. 1995; **80**:869-878.

27. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY). 1992;10:413-417.

28. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Res. 1996; **6**:986-994.

29. Richmond JY, McKinney RW, eds. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication Number (CDC) 99-8395, 1999.

30. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections. Approved Guideline-Third Edition. CLSI Document M29-A3. Wayne, PA:CLSI, 2005.

31. International Air Transport Association. Dangerous Goods Regulations. 41st ed. Quebec, Canada. 2000.

32. Saldanha J, Gerlich W, Lelie N, Dawson P, Heermann K, Heath A & The WHO Collaborative Study Group: An international collaborative study to establish a World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. Vox Sang. 2001; **80**:63-71.

33. Saldanha J, Heath A, Aberham C, Albrecht J, Gentili G, Gessner M and Pisani G: WHO Collaborative Study to Establish a Replacement WHO International Standard for HCV RNA NAT Assays. WHO/BS/03.1958 Expert Committee on Biological Standardization, Geneva, 17 to 21 February 2003.

34. Palla P, Vatteroni M L, Vacri L, Maggi F and Baicchi U. HIV-1 NAT minipool during the pre-conversion window period: detection of a repeat blood donor. Vox Sanguinis (2006) **90**:59-62.

35. Pawlotsky J M. Use and interpretation of virological tests for Hepatitis C. Hepatology (2002) **36**:S65-S73.

36. Garson J A, Grant P R, Ayliffe U et al., Real-time PCR quantitation of hepatitis B virus DNA using automated sample preparation and murine cytomegalovirus internal control. J. Virol. Methods (2005) **126**:207-213.

37. Damond F, Collin G, Descamps D, et al., Improved Sensitivity of Human Immunodeficiency Virus Type 2 Subtype B Plasma Viral Load Assay. Journal of Clinical Microbiology, 2005; Vol. **43**:4234-4236.

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