cobas®
Rx Only

cobas® MPX

Multiplex HIV, HCV & HBV nucleic acid test for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

IVD

cobas® MPX – 96	P/N: 06998909190
cobas® MPX – 480	P/N: 06998917190
cobas® MPX Control Kit	P/N: 06999069190
cobas® NHP Negative Control Kit	P/N: 07002220190
cobas® NHP Negative Control Kit	P/N: 09051554190
cobas omni MGP Reagent	P/N: 06997546190
cobas omni Specimen Diluent	P/N: 06997511190
cobas omni Lysis Reagent	P/N: 06997538190
cobas omni Wash Reagent	P/N: 06997503190

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Intended use

cobas® MPX, for use on **cobas**® 6800 and **cobas**® 8800 Systems, is a qualitative in vitro nucleic acid 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 and serum. The **cobas**® MPX test simultaneously detects and discriminates for HIV, HCV, and HBV. The assay does not discriminate between HIV-1 Group M, HIV-1 Group O, and HIV-2.

This test is intended for use to screen donor samples for HIV-1 Group M RNA, HCV RNA, and HBV DNA in plasma and serum samples from individual human donors, including donors of Whole Blood, blood components, Source Plasma and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating or from cadaveric (non-heart beating) donors. Plasma and serum from all donors may be screened as individual samples. For donations of whole blood and blood components, plasma and serum samples may be tested individually or plasma may be tested in pools comprised of not more than six individual samples. 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 not more than six individual samples. For donations of source plasma, samples may be tested in pools comprised of not more than 96 individual samples. For all other donors, samples may only be screened as individual samples.

This test is intended to be used in conjunction with licensed serology tests for HIV-1, HCV, and HBV.

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

This test is not intended for use on samples of cord blood.

cobas® MPX can be considered a supplemental test that confirms HIV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HIV and reactive for HIV on the **cobas**® MPX test.

cobas® MPX can be considered a supplemental test that confirms HCV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HCV and reactive for HCV on the **cobas**® MPX test.

cobas® MPX can be considered a supplemental test that confirms HBV infection for specimens that are repeatedly reactive on a licensed donor screening test for Hepatitis B surface antigen, and reactive for HBV on the **cobas**® MPX test.

Summary and explanation of the test

Background: Screening of blood for transfusion-transmitted viral infections

A major concern regarding the transfusion of blood and blood components is the potential for transmission of viral infections, particularly with HIV-1, HIV-2, HCV, and 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 her 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).[1] 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 to 6 weeks of initial infection. 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).[2-4]

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).[5,6] 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.[7-11] The first case of HIV-2 infection in the United States (US) 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.[12]

HCV is considered to be the principal etiologic agent responsible for 90% to 95% of post transfusion non-A and non-B hepatitis cases.[12-15] The reported prevalence of HCV varies from 0.5 to 2.0% in Western Europe[16] and between 6% and 40% in Egypt.[17]

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.[18-20] Both HCV and HBV can result in chronic liver disease, and these viruses are the most common cause of liver cirrhosis and cancer, accounting for 78% of cases globally.[21]

Rationale for NAT testing

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 antibodies to HIV and HCV using enzyme immunoassays (EIAs).[22, 23] A residual transmission risk exists from blood donations made during the seroconversion window period, which has been estimated to be approximately 19 days, 65 days and 36 days for HIV-1, HCV and HBV, respectively.[24] Testing for the viral nucleic acids (HIV-1 RNA, HCV RNA, and HBV DNA), using nucleic acid amplification technology (NAT) can substantially reduce this risk.[25,26] With the introduction of NAT, the current residual risk of transfusion in the US is 1:1.5 million for HIV-1, 1:1.2 million for HCV and 1:280,000-1:355,000 for HBV.[27,28] Similar estimates for Germany, where NAT testing was introduced in 1999, give an estimated residual risk of transfusion transmitted infections of 1:4.3 million, 1:10.9 million and 1:360,000, for HIV-1, HCV and HBV respectively.[25] In addition, in the case of HBV, NAT testing will also interdict donors with an occult HBV infection in which HBV DNA is detectable but HBsAg is absent,[29] and in vaccinated donors with a breakthrough, subclinical infection.[30-32]

Explanation of the test

cobas® MPX is a qualitative multiplex test that is run on the **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® MPX enables the simultaneous detection and discrimination of HIV RNA, HCV RNA, HBV DNA, and the internal control in a single test of an infected, individual donation or pooled plasma from individual donations. The test does not discriminate between HIV-1 Group M, HIV-1 Group O, and HIV-2.

Principles of the procedure

cobas® MPX is based on real time PCR technology on a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection system. The **cobas**® 6800/8800 Systems consists of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for all tests as non-reactive, reactive, or invalid. Results can be reviewed directly on the system screen, and printed as a report, or sent to a Laboratory Information Management System (LIMS) or other result management system.

Samples can either be tested individually or, optionally, can be tested in pools consisting of multiple samples. The **cobas p** 680 instrument, or **cobas**® **Synergy** software with the Hamilton MICROLAB® STAR IVD (**cobas**® **Synergy** Core), may optionally be used in a pre-analytical step if pooling is to be performed.

Nucleic acid from the sample and added armored RNA internal control (IC) (which serve as the sample preparation and

amplification/detection process control) is simultaneously extracted. In addition the test utilizes four kit controls: three positive and a negative control. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the donor sample is achieved by the use of virus-specific forward and reverse primers which are selected from highly conserved regions of the viral nucleic acid. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[33-35] Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR master mix, when heated in the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® MPX master mix contains detection probes which are specific for HIV-1 (Groups M and O), HIV-2, HCV, HBV, and IC nucleic acid. The specific HIV, HCV, HBV, and IC detection probes are each labeled with one of four unique fluorescent dyes which act as a reporter. Each probe also has a fifth dye which acts as a quencher. The four reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HIV, HCV, and HBV targets and the IC.[36, 37] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Since the four specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HIV, HCV and HBV targets and the IC are possible.

Reagents and materials

cobas® MPX reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® MPX

Store at 2-8°C
96 test cassette (P/N 06998909190)
480 test cassette (P/N 06998917190)

Kit components	Reagent ingredients	Quantity per kit 96 tests	Quantity per kit 480 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	13 mL	38 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL	14.5 mL
MPX Master Mix Reagent 2 (MPX MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, Tween 20, EDTA, < 0.06% dATP, dGTP, dCTP, < 0.14% dUTP, < 0.01% upstream and downstream HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, HBV, and internal control primers, < 0.01% fluorescent-labeled HIV, HCV, and HBV probes, < 0.01% fluorescent-labeled internal control probe, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL	17.5 mL

Table 2 cobas® MPX Control Kit

Store at 2-8°C
(P/N 06999069190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
MPX Multi-Positive Control (MPX M (+) C)	< 0.001% Synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% Synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (4 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)
MPX HIV-1 O Positive Control (MPX O (+) C)	< 0.001% Synthetic (armored) HIV-1 Group O RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (4 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
MPX HIV-2 Positive Control (MPX 2 (+) C)	< 0.001% Synthetic (armored) HIV-2 RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (4 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

** Hazardous substance

Table 3 cobas® NHP Negative Control Kit

Store at 2-8°C
(P/N 07002220190)

(P/N 09051554190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)

* Product safety labeling primarily follows EU GHS guidance
** Hazardous substance

cobas omni reagents for sample preparation

Table 4 **cobas omni** reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® MPX test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

*** Hazardous substance

Reagent storage and handling requirements

Opened reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® MPX – 96	2–8°C
cobas® MPX – 480	2–8°C
cobas® MPX Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **s**ystem allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® MPX – 96	Date not passed	30 days from first usage	Max 10 runs	Max 8 hours
cobas® MPX – 480	Date not passed	30 days from first usage	Max 20 runs	Max 20 hours
cobas® MPX Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

* Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

[a] Single use reagents

Additional materials required

Table 7 Material and consumables for use on **cobas**® 6800/8800 Systems

Material	**P/N**
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container	07094361001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® MPX analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system. The **cobas**® **Synergy** software shall be installed, if applicable.

Table 8 Instrumentation

Equipment	**P/N**
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Options for pipetting and pooling	**P/N**
cobas p 680 Instrument	06570577001
cobas® **Synergy** software Dongle	07788339001
Hamilton MICROLAB® STAR IVD	04640535001

Refer to the **cobas**® 6800/8800 Systems Operator's Manual and **cobas p** 680 instrument Operator's Manual, or to the **cobas**® **Synergy** software User Assistance, for additional information about primary and secondary sample tubes accepted on the instruments.
Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- All samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[38,39] Only personnel proficient in handling infectious materials and the use of **cobas**® MPX, **cobas**® 6800/8800 Systems and optionally **cobas p** 680 instrument or the Hamilton MICROLAB® STAR IVD with **cobas**® **Synergy** Core should perform this procedure.
- All human-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 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® MPX Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Do not freeze whole blood.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- The use of excessively hemolyzed living donor specimens should be avoided.
- Specimens with excessively high levels of human DNA may cause invalid test results.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, 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.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® MPX kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.

- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® MPX kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls. Change gloves if contaminated by sample, control, or reagents.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 System Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, storage, and pooling

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all donor samples at specified temperatures.
Sample stability is affected by elevated temperatures.

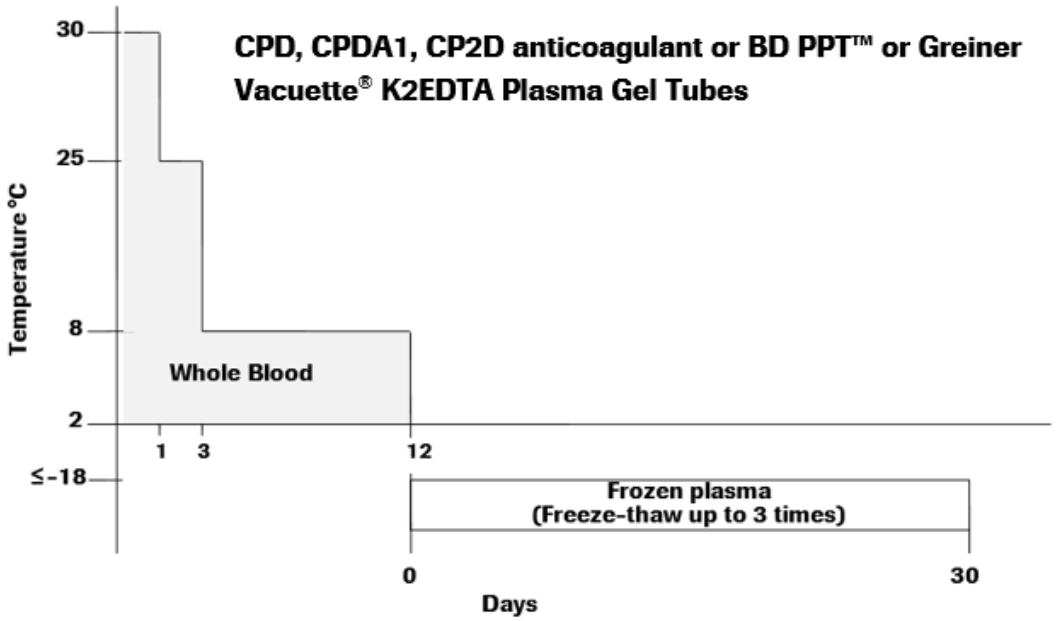
- It is recommended that serum samples are tested within 8 hours of centrifugation at 1600 x g for 20 minutes or are tested within 24 hours of high-speed centrifugation (e.g., 2600 x g for 20 minutes).

Living donor blood samples

- Plasma collected in EDTA, CPD, CPDA1, CP2D and 4% sodium citrate anticoagulant and serum collected in serum clot tubes may be used with **cobas**® MPX. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Prior to loading on the **cobas**® 6800/8800 Systems or an instrument for optional pooling, samples collected in Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) or Greiner Vacuette® K2EDTA Plasma Gel Tubes may undergo additional centrifugation at 600 x g for 5 minutes.
- Blood collected in CPD, CPDA1, CP2D anticoagulant, Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) or Greiner Vacuette® K2EDTA Plasma Gel Tubes may be stored for up to 12 days with the following conditions:
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

  Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 30 days at ≤-18°C with three freeze/thaw cycles. Refer to Figure 1.

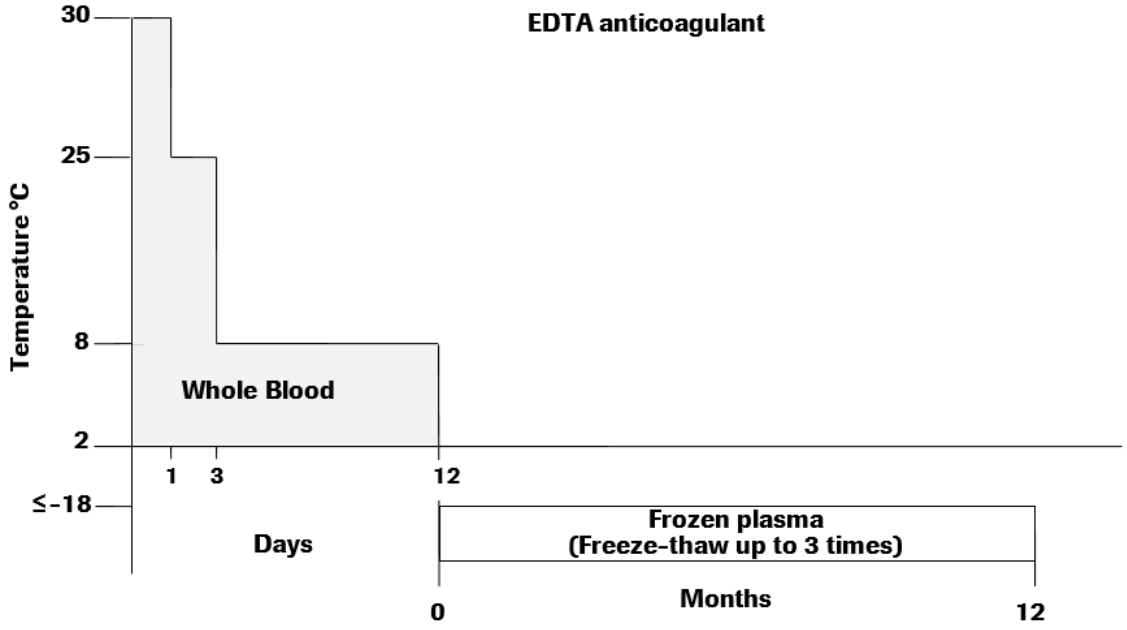
Figure 1 Sample storage conditions for living donor sample



- Blood collected in EDTA anticoagulant may be stored for up to 12 days with the following conditions:
  - Samples must be centrifuged within 72 hours of draw.
  - For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 12 months at ≤-18°C with three freeze/thaw cycles. Refer to Figure 2.

Figure 2 Sample conditions for living donor sample



- Blood collected in serum clot tubes may be stored for up to 7 days at 2-8°C with the following conditions,
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, samples are stored at 2-8°C. In addition, serum separated from the cells may be stored for up to 30 days at ≤-18°C with three freeze/thaw cycles.

- Plasma in 4% sodium citrate anticoagulant may be stored under the following two conditions:
    - Store for up to 30 days at 2-8°C. For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours. In addition, plasma may be stored at ≤ -18°C for up to 12 months with two freeze/thaw cycles. Refer to Figure 3.
    - Store for up to 18 days at 2-8°C. For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours. In addition, plasma may be stored at ≤ -18° C for up to 12 months with three freeze/thaw cycles. Refer to Figure 4.

Figure 3 Sample storage conditions for plasma

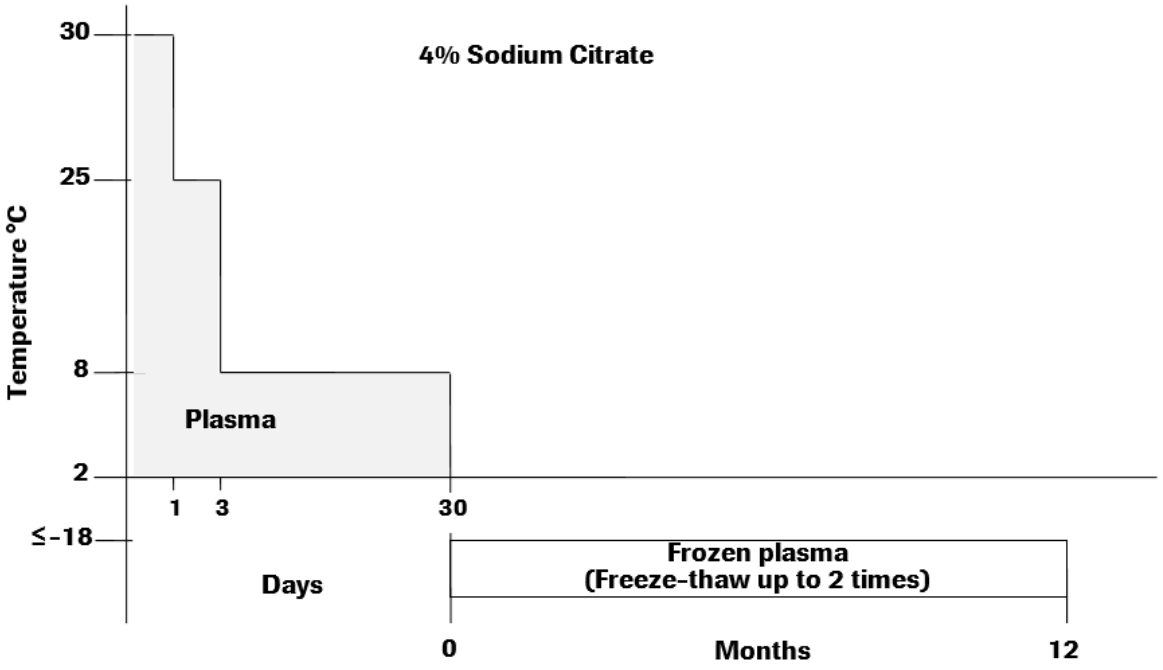
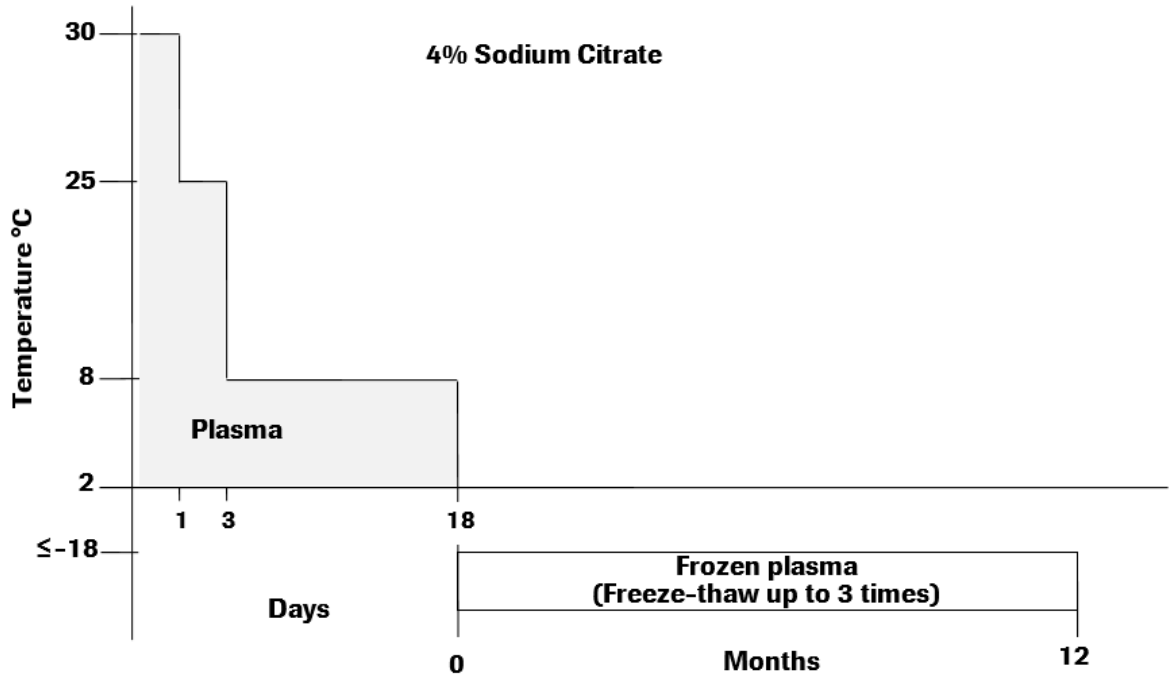


Figure 4 Sample storage conditions for plasma

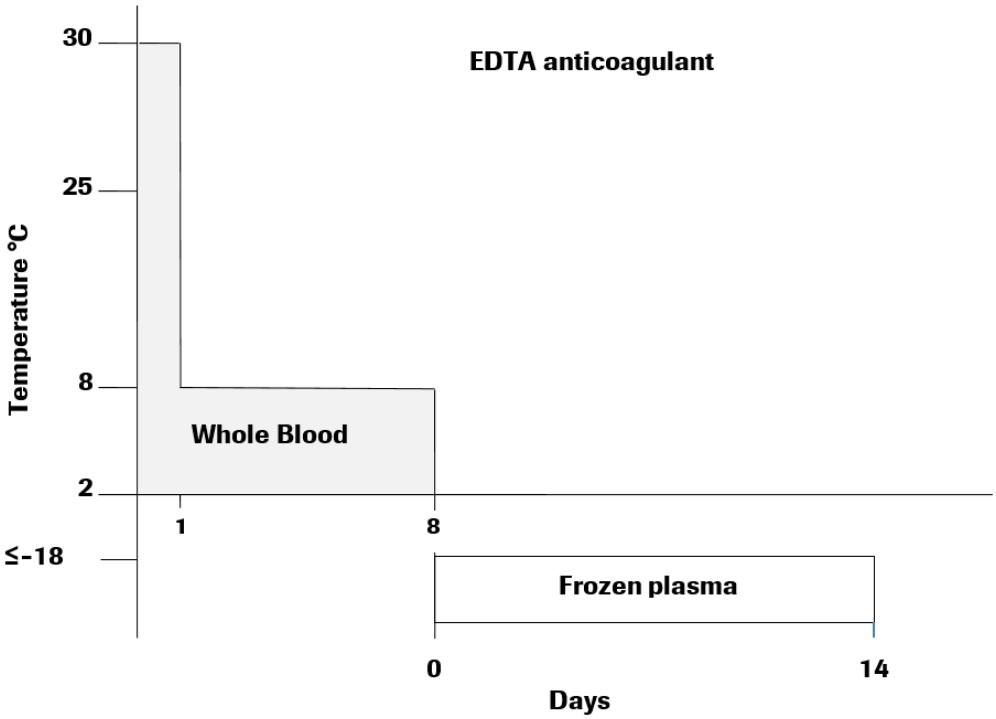


Cadaveric blood samples

- Cadaveric blood samples collected in EDTA anticoagulant tubes and/or in serum clot tubes may be used with the **cobas**® MPX test. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Cadaveric blood collected in EDTA anticoagulant may be stored for up to 8 days at 2-8°C with the following conditions:
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, samples may be stored at up to 30°C, for 24 hours during the 72 hours.

  Other than noted above, cadaveric EDTA plasma separated from the cells may be stored for up to 14 days at ≤-18°C. Refer to Figure 5.

Figure 5 Sample storage conditions for cadaveric samples



- Cadaveric blood collected in serum clot tubes may be stored for up to 5 days on the red blood cells at 2-8°C with the following conditions:
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, samples may be stored at up to 30°C, for 24 hours during the 72 hours.

Other than noted above, cadaveric serum removed from the cells may be stored for up to 14 days at ≤ -18°C. 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 etiological agents.

Instructions for use

Automated sample pipetting and pooling (optional)

Either the **cobas p** 680 instrument, or **cobas**® **Synergy** Core can be used as an optional component of the **cobas**® 6800/8800 Systems used for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample. Refer to the **cobas p** 680 instrument Operator's Manual or to the **cobas**® **Synergy** software User Assistance for more information.

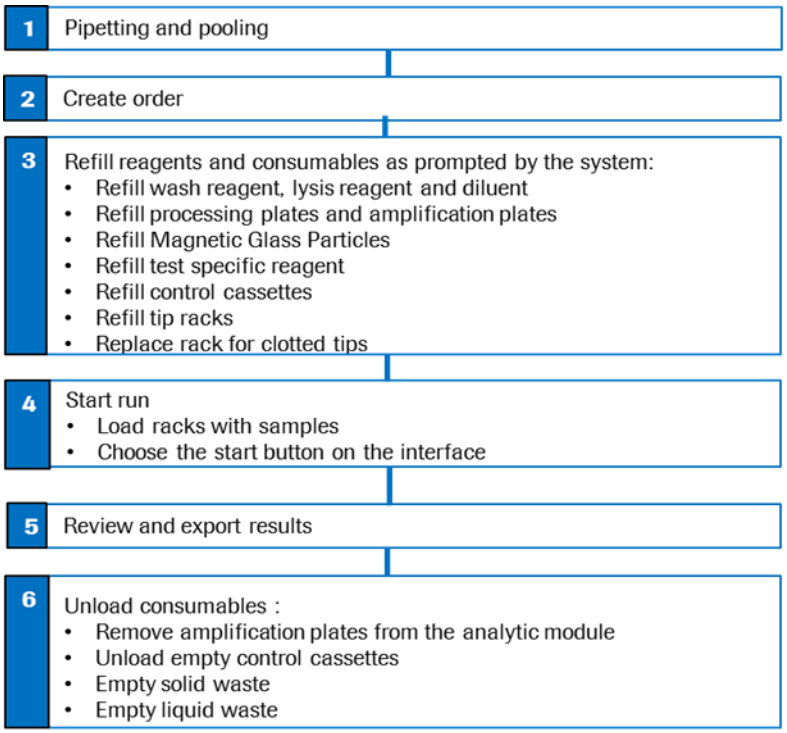
Procedural notes

- Do not use **cobas**® MPX reagents, **cobas**® MPX Control Kit, **cobas**® NHP Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures for proper maintenance of instruments.

Running cobas® MPX

The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual and the **cobas p** 680 instrument Operator's Manual or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures. Figure 6 below summarizes the procedure.

Figure 6 **cobas**® MPX procedure



Results

The **cobas**® 6800/8800 System automatically detects and discriminates HIV RNA, HCV RNA, and HBV DNA simultaneously for the samples and controls.

Quality control and validity of results

- One negative control [(–) C] and three positive controls [MPX M (+) C, MPX O (+) C, and MPX 2 (+) C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all four controls.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags

Table 9 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(-) C	Q02	Invalid	The entire batch is assigned invalid if the result for the (-) C is invalid.
Positive Control	**Flag**	**Result**	**Interpretation**
MPX M (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the MPX M (+) C is invalid.
MPX O (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the MPX O (+) C is invalid.
MPX 2 (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the MPX 2 (+) C is invalid.

If the batch is invalid, repeat testing of the entire batch including samples and controls.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid donor sample results dependent on flags obtained for the individual samples.
- Sample results are valid only if the respective positive controls and the negative control of the corresponding batch are valid.

Four parameters are measured simultaneously for each sample: HIV, HCV, HBV, and the internal control. Final sample results for **cobas**® MPX are reported by the software. In addition to the overall results, individual target results will be displayed in the **cobas**® 6800/8800 software and should be interpreted as follows:

Table 10 Target results for individual target result interpretation

Target results	Interpretation
HIV Non-Reactive	No target signal detected for HIV and IC signal detected.
HIV Reactive	Target signal detected for HIV and IC signal may or may not be detected.
HCV Non-Reactive	No target signal detected for HCV and IC signal detected.
HCV Reactive	Target signal detected for HCV and IC signal may or may not be detected.
HBV Non-Reactive	No target signal detected for HBV and IC signal detected.
HBV Reactive	Target signal detected for HBV and IC signal may or may not be detected.
Invalid	Target and internal control signal not detected.

Repeat testing of individual sample(s)

Sample tubes with a final result of Invalid for one target require repeat testing regardless of valid results for the other targets.

Procedural limitations

- **cobas**® MPX has been evaluated only for use in combination with the **cobas**® MPX Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Do not use heparinized plasma with this test because heparin has been shown to inhibit PCR.
- 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.
- 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 sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® MPX, may affect primers and/or probe binding resulting in the failure to detect presence of virus.
- 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 for method correlation.

Non-clinical performance evaluation

Key performance characteristics – Living donor samples

Limit of Detection (LoD)

WHO International Standards/Roche Primary Standards

The limits of detection (LoD) of **cobas**® MPX 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:

- WHO 3rd International Standard for HIV-1 Group M RNA (NIBSC code 10/152)
- WHO International Standard for HIV-2 RNA (NIBSC code 08/150)[40]
- Roche Primary Standards for HIV-1 Group O RNA
- WHO 2nd International Standard for HCV RNA (NIBSC code 96/798)
- WHO 3rd International Standard for HBV DNA (NIBSC 10/264)

No international standard is currently available for HIV-1 Group O RNA. The Roche HIV-1 Group O RNA Standard is traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot 01. The Roche Primary Standards for HIV-1 Group O RNA are derived from commercially available cultured virus stocks, P/N 2420 (Cat. No. 500493, SeraCare Life Sciences).

For these studies of LoD determination, HIV-1 Group M, HCV, and HBV standards were co-formulated, while HIV-1 Group O and HIV-2 standards were individually formulated. For each formulated standard, three independent dilution series were prepared with normal, HIV, HBV and HCV negative human EDTA-plasma. Each dilution series was tested using three different lots of **cobas**® MPX kits with approximately 63 replicates per lot, for a total of approximately 189 replicates per concentration. For the WHO International HIV-2 Standard, 33 replicates per lot from three independent dilutions and three reagent lots were tested for a total of 99 replicates per concentration. For each virus, PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the LoD, along with the lower and upper limit of the 95% confidence interval (Table 11). The reactivity rates observed in the LoD studies for each virus are summarized in Table 12 to Table 16.

Table 11 Results of PROBIT analysis on LoD data collected with viral standards in EDTA plasma and serum

Matrices	Analyte	Measuring units	LoD	Lower 95% confidence limit	Upper 95% confidence limit
EDTA Plasma	HIV-1 Group M	IU/mL	25.7	21.1	32.8
EDTA Plasma	HIV-1 Group O	copies/mL	8.2	7.0	10.0
EDTA Plasma	HIV-2	IU/mL	4.0	3.3	5.2
EDTA Plasma	HCV	IU/mL	7.0	5.9	8.6
EDTA Plasma	HBV	IU/mL	1.4	1.2	1.7
Serum	HIV-1 Group M	IU/mL	23.7	20.0	29.1
Serum	HIV-1 Group O	copies/mL	12.2	10.3	14.9
Serum	HIV-2	IU/mL	4.4	3.5	5.8
Serum	HCV	IU/mL	8.1	6.8	10.1
Serum	HBV	IU/mL	1.3	1.1	1.5

Table 12 Reactivity rates summary for HIV-1 Group M in EDTA plasma and serum

Matrices	HIV-1 Group M RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
EDTA Plasma	30	186	188	98.9%	96.7%
EDTA Plasma	15	170	189	89.9%	85.6%
EDTA Plasma	7.5	124	189	65.6%	59.5%
EDTA Plasma	4.5	96	189	50.8%	44.6%
EDTA Plasma	1.5	50	189	26.5%	21.2%
Serum	30	186	189	98.4%	95.9%
Serum	15	170	189	89.9 %	85.6%
Serum	7.5	123	189	65.1%	59.0%
Serum	4.5	85	189	45.0%	38.8%
Serum	1.5	31	189	16.4%	12.1%

Table 13 Reactivity rates summary for HIV-1 Group O in EDTA plasma and serum

Matrices	HIV-1 Group O RNA concentration (copies/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
EDTA Plasma	18	187	187	100.0%	98.4%
EDTA Plasma	9	181	187	96.8%	93.8%
EDTA Plasma	4.5	162	189	85.7%	80.8%
EDTA Plasma	2.7	117	189	61.9%	55.7%
EDTA Plasma	0.9	57	189	30.2%	24.7%
Serum	18	186	187	99.5%	97.5%
Serum	9	173	188	92.0%	88.0%
Serum	4.5	142	189	75.1%	69.4%
Serum	2.7	79	189	41.8%	35.8%
Serum	0.9	39	189	20.6%	15.9%

Table 14 Reactivity rates summary for HIV-2 in EDTA plasma and serum

Matrices	HIV-2 RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
EDTA Plasma	10	98	98	100.0%	97.0%
EDTA Plasma	5	98	99	99.0%	95.3 %
EDTA Plasma	2.5	80	98	81.6%	74.0%
EDTA Plasma	1.5	71	99	71.7%	63.3%
EDTA Plasma	0.5	26	99	26.3%	19.1%
Serum	10	98	98	100.0%	97.0%
Serum	5	98	99	99.0%	95.3%
Serum	2.5	81	99	81.8%	74.2%
Serum	1.5	63	98	64.3%	55.6%
Serum	0.5	28	98	28.6%	21.1%

Table 15 Reactivity rates summary for HCV in EDTA plasma and serum

Matrices	HCV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
EDTA Plasma	12	187	188	99.5 %	97.5 %
EDTA Plasma	6	178	189	94.2 %	90.6 %
EDTA Plasma	3	148	189	78.3 %	72.8 %
EDTA Plasma	1.8	112	189	59.3 %	53.0 %
EDTA Plasma	0.6	50	189	26.5 %	21.2 %
Serum	12	186	189	98.4 %	95.9 %
Serum	6	173	189	91.5 %	87.4 %
Serum	3	139	189	73.5 %	67.7 %
Serum	1.8	112	189	59.3 %	53.0 %
Serum	0.6	41	189	21.7 %	16.9 %

Table 16 Reactivity rates summary for HBV in EDTA plasma and serum

Matrices	HBV DNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
EDTA plasma	3.40	188	188	100.0 %	98.4 %
EDTA plasma	1.70	184	189	97.4 %	94.5 %
EDTA plasma	0.85	165	189	87.3 %	82.6 %
EDTA plasma	0.51	126	189	66.7 %	60.6 %
EDTA plasma	0.17	58	189	30.7 %	25.2 %
Serum	3.40	189	189	100.0 %	98.4 %
Serum	1.70	184	189	97.4 %	94.5 %
Serum	0.85	166	189	87.8 %	83.2 %
Serum	0.51	140	189	74.1 %	68.3 %
Serum	0.17	52	189	27.5 %	22.2 %

Genotype verification

The performance of **cobas**® MPX to detect subtypes of HIV-1 Group M (A-H, J, K, BF, BG) and circulating recombinant forms (CRF01_AE and CRF02_AG), HIV-1 Group O, HIV-1 Group N, and the subtypes of HIV-2 (A and B), genotypes of HCV (1 - 6) and genotypes of HBV (A-H and precore mutant) was determined by testing unique clinical samples and/or culture isolated for each subtype or genotype listed in Table 17 to Table 21.

HIV-1 Group M

A total of 115 unique HIV-1 Group M clinical samples with known HIV-1 subtype were quantified for HIV-1 concentrations using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0. All 115 samples were tested after dilution with normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma to 5 x LoD of **cobas**® MPX of which 102 samples were also tested neat (undiluted). All 115 clinical samples with known subtypes were detected neat and/or at 5 x LoD (Table 17).

Table 17 HIV-1 Group M clinical samples

Subtype	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to 5 x LoD
A	100.0% (12/12)	100.0% (12/12)
CRF01_ AE	100.0% (12/12)	100.0% (12/12)
CRF02_AG	100.0% (12/12)	100.0% (12/12)
B	100.0% (11/11)	100.0% (11/11)
C	100.0% (12/12)	100.0% (12/12)
D	100.0% (11/11)	100.0% (11/11)
F	100.0% (10/10)	100.0% (10/10)
G	100.0% (12/12)	100.0% (12/12)
H	100.0% (10/10)	100.0% (10/10)
BF	Not tested*	100% (3/3)
BG	Not tested*	100% (4/4)
J	Not tested*	100% (2/2)
K	Not tested*	100% (4/4)

*Insufficient volume to test at neat

HIV-1 Group O and HIV-1 Group N

A total of seven (7) HIV-1 Group O and two (2) HIV-1 Group N culture isolates were tested after log dilutions were prepared in normal, HIV, HCV, and HBV negative human EDTA-plasma.

For HIV-1 Group O isolates, seven isolates (obtained from individual clinical specimens) were tested in 4 replicates at each dilution for a total of 28 replicates per dilution. HIV-1 Group O culture isolates were detected up to a dilution of 1:1.00E+07 (Table 18) with a reactive rate of 71.4% for all replicates tested. At these dilutions the viral concentrations of these isolates were below the estimated LoD of the assay. For HIV-1 Group N isolates, a total of 4 replicates were tested for one cultured isolate from dilution 1:1.00E+02 to 1:1.00E+07 with a reactive rate of 100% up to 1:1.00E+03. Another HIV-1 Group N clinical specimen was only tested at one dilution (1:1.00E+04) with one replicate and was reactive by the **cobas**® MPX test. The reactive result at 1:1.00E+04 was not included in Table 18 (see footnote).

Table 18 HIV-1 Group O and HIV-1 Group N culture isolates

Sample Dilution	% Reactive (reactive/valid replicates tested) HIV-1 Group O	% Reactive (reactive/valid replicates tested) HIV-1 Group N
1:1.00E+02	100.0% (28/28)	100.0% (4/4)
1:1.00E+03	100.0% (28/28)	100.0% (4/4)
1:1.00E+04	89.3% (25/28)	0.0% (0/4)*
1:1.00E+05	71.4% (20/28)	0.0% (0/4)
1:1.00E+06	71.4% (20/28)	0.0% (0/4)
1:1.00E+07	71.4% (20/28)	0.0% (0/4)

*One HIV-1 Group N clinical specimen was tested at only one dilution (1:1.00E+04), with only one replicate. The reactive result was not included in the calculation in this table.

HIV-2

A total of five HIV-2 subtype A (four) and B (one) cultured isolates were tested after log dilutions were prepared in normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma. For subtype A, a total of 16 replicates across four isolates were tested for each dilution. For one isolate of subtype B, four total replicates were tested for each dilution. A total of 11 HIV-2 subtype A (five) and B (six) clinical samples were also tested after log dilutions were prepared in normal, virus-negative human EDTA-plasma. For subtype A, 20 total replicates across five clinical samples and for subtype B, 24 total replicates across six clinical samples were tested using four replicates for each dilution. All cultured isolates were detected by **cobas**® MPX. Clinical samples were detected by **cobas**® MPX at up to dilutions of 1:1.00E+03 for subtypes A and B. The overall results are summarized in Table 19. The dilutions that reached the viral concentration below the estimated LoD of the assay (Table 11) were not detected by **cobas**® MPX.

Table 19 HIV-2 culture isolates and clinical samples

Sample Dilution	% Reactive (reactive/valid replicates tested) Culture isolate Subtype A	% Reactive (reactive/valid replicates tested) Culture isolate Subtype B	% Reactive (reactive/valid replicates tested) Clinical sample Subtype A	% Reactive (reactive/valid replicates tested) Clinical sample Subtype B
1:1.00E+02	100.0% (16/16)	100.0% (4/4)	100.0% (20/20)	100.0% (24/24)
1:1.00E+03	100.0% (16/16)	100.0% (4/4)	65.0% (13/20)	50.0% (12/24)
1:1.00E+04	100.0% (15/15)	100.0% (4/4)	25.0% (5/20)	0.0% (0/24)
1:1.00E+05	100.0% (16/16)	100.0% (4/4)	5.0% (1/20)	0.0% (0/24)
1:1.00E+06	100.0% (16/16)	100.0% (4/4)	0.0% (0/20)	0.0% (0/24)
1:1.00E+07	81.2% (13/16)	0.0% (0/4)	0.0% (0/20)	0.0% (0/24)

HCV

A total of 107 unique HCV clinical samples with known HCV genotype were quantified for HCV concentrations using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 or **cobas**® HCV test. All HCV clinical samples with known genotypes were tested after dilution with normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma (106) or serum (1) to 5 x LoD of **cobas**® MPX. Of those, 105 samples were also tested neat. All samples were tested in single replicate. All 107 HCV-positive clinical samples were detected neat and/or diluted as summarized in Table 20.

Table 20 HCV clinical samples

Genotype	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to 5 x LoD
1a	100.0% (9/9)	100.0% (9/9)
1b	100.0% (12/12)	100.0% (12/12)
1	100.0% (12/12)	100.0% (12/12)
2b*	100.0% (11/11)	100.0% (12/12)
2	100.0% (13/13)	100.0% (13/13)
3a	100.0% (12/12)	100.0% (12/12)
3	100.0% (1/1)	100.0% (1/1)
4	100.0% (13/13)	100.0% (13/13)
5a	100.0% (10/10)	100.0% (10/10)
5	100.0% (2/2)	100.0% (2/2)
6*	100.0% (10/10)	100.0% (11/11)

* One HCV 2b serum and one HCV 6 plasma samples were tested only diluted

HBV

A total of 94 unique HBV clinical samples with known HBV genotype and pre-core mutants were quantified for HBV concentrations using the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test. All 94 HBV clinical samples with known genotypes were tested neat and/or diluted with normal, virus-negative (HIV, HCV and HBV) EDTA-plasma to 5 x LoD of **cobas**® MPX. All samples were tested with single replicates. All 94 HBV-positive clinical samples tested at 1:6 dilution and 93 HBV-positive clinical samples tested as neat were detected by **cobas**® MPX as summarized in Table 21.

Table 21 HBV clinical samples

Genotype	% Reactive (reactive/samples tested) neat	% Reactive (reactive/ samples tested) diluted to 5 x LoD
A	100.0% (15/15)	100.0% (15/15)
B	100.0% (12/12)	100.0% (11/11)
C	100.0% (10/10)	100.0% (9/9)
D	100.0% (12/12)	100.0% (11/11)
E	100.0% (12/12)	100.0% (11/11)
F	100.0% (12/12)	100.0% (12/12)
G	Not tested*	100.0% (1/1)
H	100.0% (8/8)	100.0% (8/8)
Pre core Mutant	100.0% (12/12)	100.0% (12/12)

*Insufficient volume to test at neat

Seroconversion panels

The performance of **cobas**® MPX was evaluated using commercially available seroconversion panels for HIV-1 Group M, HCV, and HBV. The results of **cobas**® MPX were compared to results for the same panels tested using the FDA licensed **cobas**® TaqScreen MPX Test on the **cobas s** 201 system. In addition, a comparison was performed between **cobas**® MPX and CE-Marked IVD and FDA approved serology tests for each target.

HIV-1 Group M seroconversion panels

Twenty commercially available seroconversion panels were used. Each panel member was tested neat and diluted 1:6 and 1:96 to simulate testing in pools with **cobas**® MPX and an FDA licensed multiplex donor screening NAT. The **cobas**® MPX test results were compared to the results obtained with a FDA licensed multiplex NAT. The **cobas**® MPX test results obtained at each dilution were also compared to the results of two serology tests obtained by testing the neat panel members. The two serology tests used are a CE-Marked HIV Ag/Ab Combo assay and an FDA approved diagnostic HIV Ag/Ab combo assay.

Table 22 Performance of **cobas**® MPX on HIV seroconversion panels

HIV Seroconversion panels	Days earlier detection by cobas® MPX than by HIV Antibody/Antigen or by Licensed HIV NAT								
	FDA Approved HIV Ag/Ab Combo Test			CE-Marked HIV Ab/Ag Combo Test			FDA Licensed HIV NAT		
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	7	7	7	0	0	0	-4	0	-4
2	7	7	3	7	7	3	0	0	0
3	3	3	3	3	3	3	0	0	0
4	8	4	4	8	4	4	4	0	4
5	15	8	2	15	8	2	13	6	2
6	7	2	2	7	2	2	5	0	0
7	7	5	5	7	5	5	2	0	0
8	15	15	8	15	15	8	0	0	0
9	12	7	7	12	7	7	5	0	0
10	2	2	0	2	2	0	0	0	-2
11	6	0	0	6	0	0	6	0	0
12	8	8	6	8	8	6	-5	2	0
13	7	7	7	7	7	7	0	0	2
14	10	3	3	10	3	3	2	0	0
15	12	7	7	12	7	7	0	-5	0
16	9	9	7	9	9	7	0	0	0
17	11	11	9	11	11	9	0	0	0
18	2	2	2	2	2	2	0	0	0
19	7	7	7	7	7	7	0	0	2
20	7	7	5	7	7	5	0	2	0
Minimum	2	0	0	0	0	0	-5	-5	-4
Average	8.1	6.1	4.7	7.8	5.7	4.4	1.4	0.3	0.2
Maximum	15	15	9	15	15	9	13	6	4

As demonstrated in Table 22, the **cobas**® MPX test was able to detect HIV-1 RNA several bleeds earlier than the CE-Marked IVD and FDA approved serology combo tests for HIV antigens/antibodies. There was no significant difference between the **cobas**® MPX test and the FDA licensed multiplex NAT in testing seroconversion panels for HIV-1.

HCV seroconversion panels

Twenty-five HCV seroconversion panels were tested using the **cobas**® MPX test. Each panel member was tested neat and at 1:6 and 1:96 dilutions to simulate testing in pools with the **cobas**® MPX test and a FDA licensed multiplex NAT. The **cobas**® MPX test results obtained at each dilution were also compared to the results of two serology tests obtained by testing the neat panel members. The serology tests used are a CE-Marked IVD and a FDA licensed anti-HCV assay.

Table 23 Performance of **cobas**® MPX on HCV seroconversion panels

HCV Seroconversion panels	Days earlier detection than HCV Antibody/Antigen or HCV RNA								
	FDA Licensed Anti-HCV Test			CE Marked Anti-HCV Test			FDA Licensed HCV NAT		
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	13	13	13	7	7	7	0	0	0
2*	20	20	20	20	20	20	0	0	0
3	23	23	23	23	23	23	0	0	0
4*	23	23	23	19	19	19	0	0	0
5	33	33	33	33	33	33	-6	0	0
6*	39	39	39	37	37	37	0	0	0
7	32	32	32	32	32	32	0	0	0
8	38	38	38	38	38	38	-24**	0	0
9*	34	34	32	34	34	32	0	0	0
10*	32	32	29	32	32	29	0	3	0
11	34	34	34	34	34	34	0	0	0
12*	11	11	11	11	11	11	0	0	0
13*	10	10	10	10	10	10	0	0	0
14*	12	12	-2	12	12	-2	0	0	1
15	65	65	65	65	65	65	0	0	0
16	3	3	3	3	3	3	0	0	0
17*	13	13	13	16	16	16	0	0	0
18*	21	21	21	21	21	21	0	0	0
19	34	4	34	34	4	34	0	0	30
20	75	75	75	75	75	75	0	0	0
21	46	42	42	49	45	45	4	0	7
22	35	35	35	35	35	35	0	0	0
23	38	38	25	38	38	25	0	6	0
24	39	39	35	39	39	35	0	7	3
25	2	2	2	0	0	0	0	0	0
Minimum	2	2	-2	0	0	-2	-24	0	0
Average with exclusions*	34.0	31.7	30.4	33.7	31.4	30.1	-1.7	0.9	2.7
Maximum	75	75	75	75	75	75	4	7	30

* Panels were reactive on the first draw when tested with **cobas**® MPX or did not show seroconversion. These panels were excluded from the summary calculations for the minimum, average and maximum number of days earlier detection than HCV antibody or RNA for each dilution. One of the panels was used in 1:96 summary calculations versus serology only (panel 14).

** 24 day interval between adjacent draws.

As shown in Table 23, the **cobas**® MPX test was able to detect HCV RNA several bleeds earlier than the CE-IVD and FDA licensed tests for HCV antibodies. There was no significant difference between the **cobas**® MPX test and the FDA licensed multiplex NAT assay in testing seroconversion panels for HCV.

HBV seroconversion panels

Twenty-one commercially available HBV seroconversion panels were tested using the **cobas**® MPX test. Each panel member was tested neat and at 1:6 and 1:96 dilutions to simulate testing in pools with the **cobas**® MPX test and compared to a FDA licensed multiplex NAT. The **cobas**® MPX test results observed at each dilution were also compared to the results for neat panel members tested by two HBsAg serology tests: a CE-Marked IVD and a FDA licensed HBsAg.

Table 24 Performance of **cobas**® MPX on HBV seroconversion panels

HBV Seroconversion panels	Days earlier detection than HBsAg or HBV DNA								
	FDA Licensed HBsAg Test			CE-Marked HBsAg Test			FDA Licensed HBV NAT		
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	29	12	0	29	12	0	17	0	0
2	19	11	7	15	7	3	0	-3	0
3**	9	9	2	-14	-14	-21	0	0	0
4	38	27	19	38	27	19	4	0	2
5	22	0	0	22	0	0	0	-13	0
6	24	24	0	24	24	0	-7	7	0
7	21	18	7	21	18	7	3	4	0
8	21	14	11	21	14	11	3	0	7
9	19	12	5	19	12	5	0	5	5
10*	12	12	7	19	19	14	0	0	0
11**	17	17	0	0	0	-17	0	0	0
12	28	28	7	28	28	7	0	0	7
13	18	18	7	18	18	7	-8	4	10
14	18	15	7	11	8	0	9	0	5
15**	30	28	14	0	-2	-16	2	12	0
16	17	17	6	17	17	6	0	2	6
17	29	33	18	29	33	18	-4	15	3
18	22	10	0	22	10	0	12	0	0
19	18	14	3	18	14	3	4	0	0
20	28	28	0	28	28	0	-5	14	0
21	22	20	5	17	15	0	2	7	0
Minimum	9	0	0	-14	-14	-21	-8	-13	0
Average	22.5	17.8	6.0	18.2	13.5	2.2	1.6	2.7	2.1
Maximum	38	33	19	38	33	19	17	15	10

* Panel was consistently reactive with **cobas**® MPX, beginning on the first bleed and was excluded from the neat and 1:6 summary calculations for the minimum, average and maximum number of days earlier detection than HBV antibody.

** Low concentrations of HBV DNA were present in diluted panel members which were detected later by **cobas**® MPX than by serology; 1.7 IU/mL in Panel 3 at 1:96, 2.0 IU/mL in Panel 11 at 1:96, and 0.5 IU/mL in Panel 15 at 1:96.

As shown in Table 24, the **cobas**® MPX test generally detected HBV DNA earlier than the FDA licensed serology assays for HBV surface antigen (HBsAg). However, some panels with low viral titers (i.e., panels #3, 11 and 15) were not detected earlier than CE-Marked HBsAg assay. Although there was no significant difference between the **cobas**® MPX test and FDA licensed multiplex NAT in testing seroconversion panels for HBV, there were variations among individual panels in detection of HBV by the **cobas**® MPX test and the FDA licensed multiplex NAT that could be due to the difference in the intervals between bleeds for each panel.

Analytical specificity

The analytical specificity of **cobas**® MPX was evaluated for cross-reactivity with 25 microorganisms at 10^6 particles, copies, or PFU/mL, which included 18 viral isolates, six bacterial strains and one yeast isolate (Table 25). The microorganisms were added to normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma and tested with and without HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 virus added to a concentration of approximately 3 x LoD of **cobas**® MPX for each virus. The tested microorganisms do not cross-react or interfere with **cobas**® MPX.

Table 25 Microorganisms tested for analytical specificity

Viruses	**Flavivirus**	**Bacteria**	**Yeast**
Adenovirus 5	West Nile Virus	*Escherichia coli*	*Candida albicans*
Cytomegalovirus	Dengue Virus type 1	*Propionibacterium acnes*	-
Epstein-Barr Virus	Usutu Virus	*Staphylococcus aureus*	-
Herpes Simplex Virus type 1	-	*Staphylococcus epidermidis*	-
Herpes Simplex Virus type 2	-	*Streptococcus viridans*	-
Hepatitis A Virus	-	*Staphylococcus haemolyticus*	-
Hepatitis E Virus	-	-	-
Hepatitis G Virus	-	-	-
Human T-cell lymphotropic Virus type I	-	-	-
Human T-cell lymphotropic Virus type II	-	-	-
Human Herpes Virus 6	-	-	-
Influenza Virus A	-	-	-
Parvovirus B19	-	-	-
Chikungunya Virus	-	-	-
Varicella Zoster Virus	-	-	-

Plasma samples from each of the disease states (Table 26) were tested with and without HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 added to a concentration of approximately 3 x LoD of **cobas**® MPX for each virus. These disease states did not cross-react or interfere with **cobas**® MPX.

Table 26 Analytical specificity - known virus-positive samples tested for cross reactivity

Disease state	Disease state	Disease state
Adenovirus type 5	Herpes Simplex Virus type1	Human T-cell lymphotropic Virus type I
Cytomegalovirus	Herpes Simplex Virus type 2	Human T-cell lymphotropic Virus type II
Dengue Virus	Hepatitis A Virus	Parvovirus B19
Epstein-Barr Virus	Hepatitis E Virus	West Nile Virus

Analytical specificity – interfering substances

Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 33.2 g/L), hemoglobin (up to 2 g/L), unconjugated bilirubin (up to 0.236 g/L), albumin (up to 60 g/L), and human DNA (up to 0.002 g/L) were tested with and without HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 virus added to a concentration of 3 x LoD of **cobas**® MPX. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® MPX.

Exogenous interference substances

Normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 27) were tested with and without HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O and HIV-2 added to a concentration of 3 x LoD of **cobas**® MPX for each virus. These exogenous substances did not interfere with the sensitivity or specificity of **cobas**® MPX.

Table 27 Concentrations of the drugs added into EDTA-plasma

Name of drug tested	Concentration
Acetaminophen	1324 µmol/L
Acetylsalicylic Acid	3620 µmol /L
Ascorbic Acid	342 µmol/L
Atorvastatin	600 µg Eq/L
Fluoxetine	11.2 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Phenylephrine HCL	491 µmol/L
Sertraline	1.96 µmol/L

Whole system failure

The whole system failure rate for **cobas**® MPX was determined by testing 100 replicates of EDTA plasma spiked with either HIV-1 Group M, HCV, HBV (co-formulated), HIV-1 Group O, and HIV-2, for a total of 300 replicates. These samples were tested at a target concentration of approximately 3 x LoD and were run in pools of 1 (undiluted). The study was performed using the **cobas**® 8800 System with **cobas p** 680 instrument (pipetting and pooling).

The results of this study determined that all replicates were reactive for each target, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.22% for the upper bound [0%: 1.22%].

Cross contamination

The cross-contamination rate for **cobas**® MPX was determined by testing 240 replicates of a normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma sample and 220 replicates of a high titer HBV sample at 1.00E+08 IU/mL. The study was performed using the **cobas**® 8800 System. In total, 5 runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were non-reactive, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.53% for the upper bound [0%: 1.53%].

FDA/CBER panel evaluation

The sensitivity of **cobas**® MPX was determined by testing FDA/CBER Lot Release panels for HIV-1 Group M, HIV-1 Group O, HIV-2, HBV and HCV. Panel members were tested undiluted in replicates across the reagent lots (one replicate per panel member per reagent lot).

HIV-1 Group M

cobas® MPX is able to detect all levels of the FDA/CBER Lot Release Panel with concentrations of 10 cp/mL to 500 cp/mL and the negative panel member was non-reactive as summarized in Table 28.

Table 28 Summary of FDA/CBER HIV-1 Group M RNA lot release panel results

Panel Member	Concentration (cp/mL)	Expected Reactivity for HIV*	Observed HIV Result Reagent Lot 1**	Observed HIV Result Reagent Lot 2	Observed HIV Result Reagent Lot 3
B4	0	NR	NT	NR	NR
B2	10	+/-	NT	R	R
B6	50	+/-	NT	R	R
B5	100	+/-	NT	R	R
B9	500	R	NT	R	R

* Reactivity: NR = non-reactive; +/- = may be reactive or non-reactive and were for information purposes only; R= reactive

** NT = Not Tested

HIV-1 Group O

cobas® MPX is able to detect all levels of the FDA/CBER Lot Release Panel with concentrations of 10 cp/mL to 1000 cp/mL as summarized in Table 29.

Table 29 Summary of FDA/CBER HIV-1 Group O RNA lot release panel results

Panel Member	Concentration (cp/mL)	Expected Reactivity for HIV*	Observed HIV Result Reagent Lot 1	Observed HIV Result Reagent Lot 2	Observed HIV Result Reagent Lot 3
HIV-1 (O) #NC1	0	NR	Not applicable**	Not applicable**	Not applicable**
HIV-1 (O) #03	10	+/-	R	R	R
HIV-1 (O) #02	100	+/-	R	R	R
HIV-1 (O) #01	1000	R	R	R	R

* Reactivity: NR = non-reactive; +/- = may be reactive or non-reactive and were for information purposes only; R= reactive

** Due to non-availability of panel member NC1 (negative panel member), this panel member could not be tested.

HIV-2

cobas® MPX is able to detect all levels of the FDA/CBER Lot Release Panel with concentrations of 5 cp/mL to 100 cp/mL and the negative panel member was non-reactive as summarized in Table 30.

Table 30 Summary of FDA/CBER HIV-2 RNA lot release panel results

Panel Member	Concentration (cp/mL)	Expected Reactivity for HIV*	Observed HIV Result Reagent Lot 1	Observed HIV Result Reagent Lot 2	Observed HIV Result Reagent Lot 3
HIV-2 #1	0	NR	NR	NR	NR
HIV-2 #2	5	+/-	R	R	R
HIV-2 #3	10	+/-	R	R	R
HIV-2 #4	50	R	R	R	R
HIV-2 #5	100	R	R	R	R

* Reactivity: NR = non-reactive; +/- = may be reactive or non-reactive and were for information purposes only; R= reactive

HBV

cobas® MPX is able to detect all levels of the FDA/CBER Lot Release Panel with concentrations of 10 cp/mL to 500 cp/mL and the negative panel member was non-reactive as summarized in Table 31.

Table 31 Summary of FDA/CBER HBV DNA lot release panel results

Panel Member	Concentration (cp/mL)	Expected Reactivity for HBV*	Observed HBV Result Reagent Lot 1	Observed HBV Result Reagent Lot 2	Observed HBV Result Reagent Lot 3
1	0	NR	NR	NR	NR
2	10	+/-	R	R	R
3	100	R	R	R	R
4	50	+/-	R	R	R
5	500	R	R	R	R

* Reactivity: NR = non-reactive; +/- = may be reactive or non-reactive and were for information purposes only; R= reactive

HCV

cobas® MPX is able to detect all levels of the FDA/CBER Lot Release Panel with concentrations of 5 cp/mL to 500 cp/mL and the negative panel member was non-reactive as summarized in Table 32.

Table 32 Summary of FDA/CBER HCV RNA lot release panel results

Panel Member	Concentration (cp/mL)	Expected Reactivity for HCV*	Observed HCV Result Reagent Lot 1	Observed HCV Result Reagent Lot 2	Observed HCV Result Reagent Lot 3
2001 (#2)	0	NR	NR	NR	NR
2002 (#10)	5	+/-	R	NR	R
2003 (#9)	10	+/-	R	R	NR
2004 (#8)	50	+/-	R	R	R
2005 (#7)	100	R	R	R	R
2006 (#6)	500	R	R	R	R

* Reactivity: NR = non-reactive; +/- = may be reactive or non-reactive and were for information purposes only; R= reactive

Key performance characteristics – Cadaveric samples

Analytical sensitivity

The analytical sensitivity of the **cobas**® MPX test for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2, RNA, HCV RNA and HBV DNA in cadaveric EDTA samples was assessed by using the following standards:

- Roche Secondary Standards for HIV-1 Group M RNA, HCV RNA and HBV DNA (traceable to the WHO standards).
- Roche Primary Standard for HIV-1 Group O RNA (traceable to the 2008 FDA/CBER Panel).
- Roche Primary Standard for HIV-2 RNA (traceable to the WHO Standard).

A total of two (2) independent dilution series of 5 concentrations of co-formulated HIV-1 Group M, HBV and HCV, and a blank were prepared in negative pooled moderately hemolyzed and pooled highly hemolyzed cadaveric EDTA plasma samples. HIV-1 Group O and HIV-2 were individually formulated and tested in negative pooled moderately hemolyzed cadaveric EDTA plasma samples. Each dilution series was tested using one of two unique reagent lots of the **cobas**® MPX test. Moderately hemolyzed pools consisted of pools of individual, virus-negative cadaveric EDTA plasma samples having a straw to pink colored appearance. Highly hemolyzed pools consisted of pools of individual, virus-negative cadaveric EDTA plasma samples having a red to brown colored appearance. The results are summarized in Table 33 to Table 40.

Table 33 Analytical sensitivity summary for HIV-1 Group M in moderately-hemolyzed cadaveric EDTA plasma samples

HIV-1 M RNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
750.00	41	41	100.0%	93.0%
375.00	42	42	100.0%	93.1%
187.50	41	41	100.0%	93.0%
93.75	42	34	81.0%	68.2%
46.88	42	29	69.0%	69.1%
0.00	41	0	0.0%	0.0%

Table 34 Analytical sensitivity summary for HIV-1 Group M in highly-hemolyzed cadaveric EDTA plasma samples

HIV-1 M RNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
750.00	42	42	100.0%	93.1%
375.00	42	42	100.0%	93.1%
187.50	42	41	97.6%	89.2%
93.75	42	30	71.4%	57.9%
46.88	42	20	47.6%	34.2%
0.00	42	0	0.0%	0.0%

Table 35 Analytical sensitivity summary for HBV in moderately-hemolyzed cadaveric EDTA plasma samples

HBV DNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
40.00	41	41	100.0%	93.0%
20.00	42	39	92.9%	82.6%
10.00	41	25	61.0%	61.0%
5.00	42	14	33.3%	33.3%
2.50	42	5	11.9%	11.9%
0.00	41	0	0.0%	0.0%

Table 36 Analytical sensitivity summary for HBV in highly-hemolyzed cadaveric EDTA plasma samples

HBV DNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
40.00	42	41	97.6%	89.2%
20.00	42	29	69.0%	55.4%
10.00	42	11	26.2%	15.4%
5.00	41	2	4.9%	0.9%
2.50	42	0	0.0%	0.0%
0.00	42	0	0.0%	0.0%

Table 37 Analytical sensitivity summary for HCV in moderately-hemolyzed cadaveric EDTA plasma samples

HCV RNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
292.00	41	41	100.0%	93.0%
146.00	42	42	100.0%	93.1%
73.00	41	39	95.1%	85.4%
36.50	42	31	73.8%	60.4%
18.20	42	22	52.4%	38.7%
0.00	41	0	0.0%	0.0%

Table 38 Analytical sensitivity summary for HCV in highly-hemolyzed cadaveric EDTA plasma samples

HCV RNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
292.00	42	42	100.0%	93.1%
146.00	42	42	100.0%	93.1%
73.00	42	40	95.2%	85.8%
36.50	42	27	64.3%	50.5%
18.25	42	22	52.4%	38.7%
0.00	42	0	0.0%	0.0%

Table 39 Analytical sensitivity summary for HIV-1 Group O in moderately-hemolyzed cadaveric EDTA plasma samples

HIV-1 O RNA concentration (cp/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
250.00	42	42	100.0%	93.1%
125.00	42	41	97.6%	89.2%
62.50	42	40	95.2%	85.8%
31.20	42	30	71.4%	57.9%
15.60	42	16	38.1%	25. 6%
0.00	41	0	0.0%	0.0%

Table 40 Analytical sensitivity summary for HIV-2 in moderately-hemolyzed cadaveric EDTA plasma samples

HIV-2 RNA concentration (IU/mL)	Number of Valid Replicates	Number of Reactives	% Reactive	95% lower confidence bound (one-sided)
200.00	42	42	100.0%	93.1%
100.00	42	42	100.0%	93.1%
50.00	42	42	100.0%	93.1%
25.00	42	39	92.9%	82. 6%
12.50	42	24	57.1%	43.3%
0.00	41	0	0.0%	0.0%

Sensitivity using clinical specimens

The clinical sensitivity of the **cobas**® MPX test for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2, RNA HCV RNA and HBV DNA was evaluated by testing a total of 60 individual virus-negative cadaveric EDTA plasma samples, of those 35 individual samples were classified as moderately hemolyzed (straw to pink colored) and 25 individual samples were classified as highly hemolyzed (red to brown colored). In addition a total of 60 individual virus-negative living donor samples were tested. All cadaveric and living donor samples were divided evenly across 3 reagent lots, 5 clinical samples spiking groups (for HIV-1 M, HCV and HBV) with 12 samples per group. Each cadaveric and living donor sample was spiked with a co-formulation of three unique clinical samples (HIV-1 Group M, HCV and HBV), or Roche Primary Standards (individually formulated HIV-1 Group O and HIV-2) at approximately 5 x LoD of respective sample types. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure.

All of the cadaveric and the living-donor samples had a reactive rate of 100% (95% confidence interval: 94.0 – 100%). The clinical sensitivity observed in cadaveric sample was equivalent to the sensitivity observed in living donor samples as determined by Fisher's Exact Test and summarized in Table 41.

Table 41 Summary of reactivity rate in cadaveric and living donor samples in EDTA plasma

Analyte	Cadaveric sample % Reactive (Number of reactive /Number of samples tested)	Living donor sample % Reactive (Number of reactive/Number of samples tested)
HIV-1 Group M	100% (60/60)	100% (60/60)
HIV-1 Group O	100% (60/60)	100% (60/60)
HIV-2	100% (60/60)	100% (60/60)
HCV	100% (60/60)	100% (60/60)
HBV	100% (60/60)	100% (60/60)
Fisher's Exact Test, p-value (α=0.05)	No significant differences in reactive rates (p=1.000)	No significant differences in reactive rates (p=1.000)

Specificity

The specificity of the **cobas**® MPX test in cadaveric EDTA plasma and serum samples was evaluated and compared with the specificity in living donor samples by testing single replicates of 60 individual cadaveric EDTA plasma samples, of those 37 individual donor samples were classified as moderately hemolyzed (straw to pink colored) and 23 individual samples were classified as highly hemolyzed (red to brown colored), 61 individual cadaveric serum samples of those 42 individual samples were classified as moderately hemolyzed and 19 individual donor samples were classified as highly hemolyzed, 60 individual sero-negative living-donor plasma samples and 60 individual sero-negative living-donor serum samples.

The studies were performed with 3 independent **cobas**® MPX reagent lots. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure. All the cadaveric and living donor EDTA plasma and serum samples were non-reactive for 100% specificity. The specificity observed for cadaveric samples was equal to the specificity observed for living-donor samples as determined by the Fisher's Exact Test (α=0.05) as summarized in Table 42.

Table 42 Summary of specificity in cadaveric and living donor samples in EDTA plasma

Matrices	Sample type	Number of non-reactive	Number of samples tested	% Non-reactive	Two-sided 95% Confidence Interval
EDTA plasma	Cadaveric donor	60	60	100%	94.0% - 100%
EDTA plasma	Living donor	60	60	100%	94.0% - 100%
Serum	Cadaveric donor	61	61	100%	94.1% - 100%
Serum	Living donor	60	60	100%	94.0% - 100%
-	Overall results using Fisher's Exact Test (α=0.05)	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000

Reproducibility

The reproducibility of the **cobas**® MPX test on the **cobas**® 6800/8800 Systems was determined using 20 cadaveric EDTA plasma samples (moderately and highly hemolyzed) spiked with HIV-1 M, HBV and HCV clinical samples, and Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA to approximately 5 x LoD of the **cobas**® MPX test. The results were compared to the reproducibility obtained with 20 living donor samples spiked with the Roche Primary and Secondary Standards to approximately 5 x LoD of the **cobas**® MPX test.

Testing was performed for the following variable components:

- day-to-day variability over 6 days
- lot-to-lot variability using 3 different reagent lots of the **cobas**® MPX test

One replicate was tested with each of the 3 reagent lots over 6 days for up to 18 replicates per cadaveric and living donor sample. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure. All valid reproducibility data were evaluated by comparing the reactive rates of living donors and cadaveric EDTA plasma samples (two-sided 95% Confidence Intervals) across all variable components. The Fisher's exact p value was calculated for the test of statistical significance of the difference between proportions of reactives observed with cadaveric and living donor samples. No significant differences were observed.

cobas® MPX test is reproducible over multiple days and reagent lots for cadaveric and living donor samples. The results from reagent lot-to-lot variability are summarized in Table 43.

Table 43 cobas® MPX test reagent lot-to-lot reproducibility summary for cadaveric and living donor samples

Analyte	Reagent lot	Sample type	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval	Significant difference using Fisher's Exact Test p-value (α=0.05)
HIV-1 Group M	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group M	1	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group M	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group M	2	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group M	3	Cadaveric	100.0% (118/118)	96.9%	100.0%	p =1.0000
HIV-1 Group M	3	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group O	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group O	1	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group O	2	Cadaveric	100.0% (117/117)	96.9%	100.0%	p =1.0000
HIV-1 Group O	2	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-1 Group O	3	Cadaveric	99.2% (118/119)	95.4%	100.0%	p =0.4979
HIV-1 Group O	3	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.4979
HIV-2	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-2	1	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HIV-2	2	Cadaveric	98.3% (118/120)	94.1%	99.8%	p =0.4979
HIV-2	2	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.4979
HIV-2	3	Cadaveric	99.2% (118/119)	95.4%	100.0%	p =0.4979
HIV-2	3	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.4979
HCV	1	Cadaveric	98.3% (118/120)	94.1%	99.8%	p =0.4979
HCV	1	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.4979
HCV	2	Cadaveric	98.3% (118/120)	94.1%	99.8%	p =0.4979
HCV	2	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.4979
HCV	3	Cadaveric	97.5% (115/118)	92.7%	99.5%	p =0.1203
HCV	3	Living donor	100.0% (120/120)	97.0%	100.0%	p =0.1203
HBV	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HBV	1	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HBV	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p =1.0000
HBV	2	Living donor	100.0% (120/120)	97.0%	100.0%	p =1.0000
HBV	3	Cadaveric	100.0% (118/118)	96.9%	100.0%	p =1.0000
HBV	3	Living donor	99.2% (119/120)	95.4%	100.0%	p =1.0000

Matrix equivalency

The matrix equivalency of the **cobas**® MPX test on the **cobas**® 6800/8800 Systems was evaluated between cadaveric EDTA plasma and cadaveric serum specimens to determine whether there is a sample matrix effect on the sensitivity or reproducibility of the test. The study was conducted using 20 pairs of cadaveric samples, with each set consisting of one cadaveric EDTA plasma sample and one cadaveric serum sample from a single donor. Of those, 15 donor sets were moderately-hemolyzed (straw-to pink-colored), and five of the donor sets were highly-hemolyzed (red-to brown-colored). Each pair of cadaveric serum and plasma samples were spiked with approximately 3 x LoD of either HIV-1 Group M, HIV-1 Group O, or HIV-2, and then co-formulated with approximately 3 x LoD of HBV and approximately 3 x LoD of HCV analyte before testing (10 replicates per sample) with the **cobas**® MPX test.

The observed **cobas**® MPX reactive rates for all analytes are statistically equivalent in either cadaveric plasma or cadaveric serum in both highly-hemolyzed and moderately-hemolyzed samples. The results from cadaveric matrix equivalency are summarized in Table 44.

Table 44 cobas® MPX test matrix equivalency for cadaveric samples

Analyte	Hemolysis Level	Sample Type	Number of sample tested	Number of reactives	Reactive rate	p-value
HIV-1 Group M	Moderately Hemolyzed	Plasma	100	100	100.0%	p = 1.0000
HIV-1 Group M	Moderately Hemolyzed	Serum	100	100	100.0%	p = 1.0000
HIV-1 Group M	Highly Hemolyzed	Plasma	20	20	100.0%	p = 1.0000
HIV-1 Group M	Highly Hemolyzed	Serum	20	20	100.0%	p = 1.0000
HIV-1 Group O	Moderately Hemolyzed	Plasma	30	29	96.7%	p = 0.5279
HIV-1 Group O	Moderately Hemolyzed	Serum	30	30	100.0%	p = 0.5279
HIV-1 Group O	Highly Hemolyzed	Plasma	10	10	100.0%	p = 1.0000
HIV-1 Group O	Highly Hemolyzed	Serum	10	10	100.0%	p = 1.0000
HIV-2	Moderately Hemolyzed	Plasma	20	20	100.0%	p = 0.5259
HIV-2	Moderately Hemolyzed	Serum	20	19	95.0%	p = 0.5259
HIV-2	Highly Hemolyzed	Plasma	20	19	95.0%	p = 1.0000
HIV-2	Highly Hemolyzed	Serum	20	19	95.0%	p = 1.0000
HBV	Moderately Hemolyzed	Plasma	150	150	100.0%	p = 1.0000
HBV	Moderately Hemolyzed	Serum	150	150	100.0%	p = 1.0000
HBV	Highly Hemolyzed	Plasma	50	50	100.0%	p = 0.2087
HBV	Highly Hemolyzed	Serum	50	48	96.0%	p = 0.2087
HCV	Moderately Hemolyzed	Plasma	150	149	99.3%	p = 0.5294
HCV	Moderately Hemolyzed	Serum	150	150	100.0%	p = 0.5294
HCV	Highly Hemolyzed	Plasma	50	49	98.0%	p = 1.0000
HCV	Highly Hemolyzed	Serum	50	49	98.0%	p = 1.0000

Clinical performance evaluation

Reproducibility

The reproducibility of **cobas**® MPX for use on the **cobas**® 6800/8800 Systems was established by testing panel members containing HIV-1 Group M, Group O, HIV-2, HCV, and/or HBV at three different concentrations for each virus across lot, site/instrument, day and batch.

Operators at each **cobas**® MPX test site performed five days of testing, using three lots of **cobas**® MPX reagents to obtain two valid batches per day.

Table 45 presents percent agreement by site/instrument, lot, day, and batch from valid test results for positive panel members. This study demonstrated that **cobas**® MPX for use on the **cobas**® 6800/8800 Systems shows reproducible performance across the variables assessed (lot, site/instrument, day and batch) and for the five analytes tested.

Table 45 Test results summarized by site/instrument, lot, day, and batch (positive panel members)

Viral Target	Viral Concen-tration	Site/ Instrument ID	Site/ Instrument % Positive Results	Lot ID	Lot % Positive Results	Day ID	Day % Positive Results	Batch ID	Batch % Positive Results
HIV-1 Group M	~0.5 x LoD	1	81.7% (49/60)	1	81.7% (49/60)	1	91.7% (33/36)	1	84.3% (75/89)
HIV-1 Group M	~0.5 x LoD	2	84.7% (50/59)	2	88.3% (53/60)	2	77.1% (27/35)	2	81.1% (73/90)
HIV-1 Group M	~0.5 x LoD	3	81.7% (49/60)	3	78.0% (46/59)	3	83.3% (30/36)	-	-
HIV-1 Group M	~0.5 x LoD	-	-	-	-	4	83.3% (30/36)	-	-
HIV-1 Group M	~0.5 x LoD	-	-	-	-	5	77.8% (28/36)	-	-
HIV-1 Group M	~1 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
HIV-1 Group M	~1 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	97.2% (35/36)	2	97.8% (88/90)
HIV-1 Group M	~1 x LoD	3	96.7% (58/60)	3	96.7% (58/60)	3	100.0% (36/36)	-	-
HIV-1 Group M	~1 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-1 Group M	~1 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HIV-1 Group M	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HIV-1 Group M	~3 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
HIV-1 Group M	~3 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)	-	-
HIV-1 Group M	~3 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-1 Group M	~3 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HIV-1 Group O	~0.5 x LoD	1	78.3% (47/60)	1	83.3% (50/60)	1	72.2% (26/36)	1	73.3% (66/90)
HIV-1 Group O	~0.5 x LoD	2	76.7% (46/60)	2	78.3% (47/60)	2	77.8% (28/36)	2	86.7% (78/90)
HIV-1 Group O	~0.5 x LoD	3	85.0% (51/60)	3	78.3% (47/60)	3	77.8% (28/36)	-	-
HIV-1 Group O	~0.5 x LoD	-	-	-	-	4	86.1% (31/36)	-	-
HIV-1 Group O	~0.5 x LoD	-	-	-	-	5	86.1% (31/36)	-	-
HIV-1 Group O	~1 x LoD	1	98.3% (59/60)	1	98.3% (59/60)	1	94.4% (34/36)	1	95.6% (86/90)
HIV-1 Group O	~1 x LoD	2	100.0% (60/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	98.9% (89/90)
HIV-1 Group O	~1 x LoD	3	93.3% (56/60)	3	96.7% (58/60)	3	97.2% (35/36)	-	-
HIV-1 Group O	~1 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-1 Group O	~1 x LoD	-	-	-	-	5	94.4% (34/36)	-	-
HIV-1 Group O	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HIV-1 Group O	~3 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
HIV-1 Group O	~3 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)	-	-
HIV-1 Group O	~3 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-1 Group O	~3 x LoD	-	-	-	-	5	100.0% (36/36)	-	-

Viral Target	Viral Concen-tration	Site/ Instrument ID	Site/ Instrument % Positive Results	Lot ID	Lot % Positive Results	Day ID	Day % Positive Results	Batch ID	Batch % Positive Results
HIV-2	~0.5 x LoD	1	74.1% (43/58)	1	73.3% (44/60)	1	77.8% (28/36)	1	69.7% (62/89)
HIV-2	~0.5 x LoD	2	76.7% (46/60)	2	79.7% (47/59)	2	69.4% (25/36)	2	79.8% (71/89)
HIV-2	~0.5 x LoD	3	73.3% (44/60)	3	71.2% (42/59)	3	75.0% (27/36)	-	-
HIV-2	~0.5 x LoD	-	-	-	-	4	71.4% (25/35)	-	-
HIV-2	~0.5 x LoD	-	-	-	-	5	80.0% (28/35)	-	-
HIV-2	~1 x LoD	1	96.7% (58/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
HIV-2	~1 x LoD	2	98.3% (59/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	96.7% (87/90)
HIV-2	~1 x LoD	3	100.0% (60/60)	3	98.3% (59/60)	3	97.2% (35/36)	-	-
HIV-2	~1 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-2	~1 x LoD	-	-	-	-	5	97.2% (35/36)	-	-
HIV-2	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HIV-2	~3 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
HIV-2	~3 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)	-	-
HIV-2	~3 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HIV-2	~3 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HCV	~0.5 x LoD	1	75.0% (45/60)	1	80.0% (48/60)	1	66.7% (24/36)	1	79.8% (71/89)
HCV	~0.5 x LoD	2	70.7% (41/58)	2	76.7% (46/60)	2	77.8% (28/36)	2	74.2% (66/89)
HCV	~0.5 x LoD	3	85.0% (51/60)	3	74.1% (43/58)	3	69.4% (25/36)	-	-
HCV	~0.5 x LoD	-	-	-	-	4	91.2% (31/34)	-	-
HCV	~0.5 x LoD	-	-	-	-	5	80.6% (29/36)	-	-
HCV	~1 x LoD	1	100.0% (60/60)	1	98.3% (59/60)	1	97.2% (35/36)	1	100.0% (90/90)
HCV	~1 x LoD	2	96.7% (58/60)	2	98.3% (59/60)	2	100.0% (36/36)	2	97.8% (88/90)
HCV	~1 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	97.2% (35/36)	-	-
HCV	~1 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HCV	~1 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HCV	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HCV	~3 x LoD	2	100.0% (59/59)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (89/89)
HCV	~3 x LoD	3	100.0% (60/60)	3	100.0% (59/59)	3	100.0% (36/36)	-	-
HCV	~3 x LoD	-	-	-	-	4	100.0% (35/35)	-	-
HCV	~3 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HBV	~0.5 x LoD	1	80.0% (48/60)	1	80.0% (48/60)	1	80.6% (29/36)	1	72.2% (65/90)
HBV	~0.5 x LoD	2	78.3% (47/60)	2	73.3% (44/60)	2	80.6% (29/36)	2	82.2% (74/90)
HBV	~0.5 x LoD	3	73.3% (44/60)	3	78.3% (47/60)	3	75.0% (27/36)	-	-
HBV	~0.5 x LoD	-	-	-	-	4	77.8% (28/36)	-	-
HBV	~0.5 x LoD	-	-	-	-	5	72.2% (26/36)	-	-
HBV	~1 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HBV	~1 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
HBV	~1 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)	-	-
HBV	~1 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HBV	~1 x LoD	-	-	-	-	5	100.0% (36/36)	-	-
HBV	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
HBV	~3 x LoD	2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
HBV	~3 x LoD	3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)	-	-
HBV	~3 x LoD	-	-	-	-	4	100.0% (36/36)	-	-
HBV	~3 x LoD	-	-	-	-	5	100.0% (36/36)	-	-

Clinical specificity

Reactivity in blood donor population

Samples were collected from consented blood donors recruited from four test sites. Testing with **cobas**® MPX was done according to two testing algorithms: one for individual donation testing, which required a single level of testing; and one for pools of six testing, which required a single level of testing for primary pools that were non-reactive and two levels of testing (primary pool and individual donation resolution testing for primary pools that were reactive) (Table 46). The pool specificity was 99.91% (10,524/10,534; 99.83%-99.95%) (Table 47). Ten reactive pools contained all status negative donations. The clinical specificity for individual donation testing was 99.95% (95% CI: 99.88% to 99.98%). The invalid batch rate for the **cobas**® MPX test was 3.5% (18/509) for initial testing donations in pools of six and for individual donations was 6.8% (16/219). Two HCV-positive NAT yield cases were identified during this study.

Table 46 Clinical specificity of **cobas**® MPX – overall

Pool Size	Frequency (n/N)	Estimate in Percent (95% Clopper Pearson Exact Confidence Interval)
Individual (Plasma)	5,523 / 5,528	99.91% (99.79% to 99.986%)
Individual (Serum)	5,669 / 5,670	99.98% (99.90% to 100.00%)
Individual (Plasma/Serum)	11,192 / 11,198	99.95% (99.88% to 99.98%)
Pools of 6 (Plasma)	62,982 / 62,982	100.00% (99.99% to 100.00%)

N = Total number of status negative donations; n = **cobas**® MPX non-reactive donations

Table 47 Pool reactivity of **cobas**® MPX in volunteer blood donors

Category	No. of Pools	Percentage of Pools Tested
Pools Tested	10,563	100
Non-Reactive pools	10,524	99.63
Reactive pools	39	0.37
Reactive pools with donor status positive	29	0.27
Reactive pools with donor status negative (false positive)*	10	0.10

* Of the 10 false reactive pools, one pool was HIV false reactive, four pools were HCV false reactive, and five pools were HBV false reactive.

Reactivity in source plasma donor population

A total of 108,306 evaluable donations from 24,514 unique donors were tested in pools of 96 with both **cobas**® MPX and an FDA licensed multiplex NAT. One hundred eight thousand two hundred ninety-seven donations tested negative for anti-HIV, anti-HCV, and HBsAg (Table 48). Donation status was assigned based on the concordance of two virus-specific tests (e.g., two NAT results or NAT and serology) on the index donation or the results of follow-up testing. A total of 1,106 evaluable pools were tested with **cobas**® MPX, of which 1,092 (98.7%) were non-reactive and 14 (1.3%) were reactive. Of the 1,092 non-reactive pools, 1,090 pools contained all status-negative donations, and two pools contained at least one status-positive donation. Of the 1,106 pools tested, there were two non-reactive pools with at least one status-positive donation and seven reactive pools with at least one status-positive donation (Table 49).

Table 48 Clinical specificity of the **cobas**® MPX – donation level

Parameter	Total Number of Status-Negative Donations	cobas® MPX Result Reactive	cobas® MPX Result Non-Reactive	Estimate in Percent (95% Exact CI)
Clinical Specificity	108,297	6	108,291	99.99 (99.99, 100.00)
HIV Clinical Specificity	108,297	3	108,294	100.00 (99.99, 100.00)
HCV Clinical Specificity	108,297	1	108,296	100.00 (100.00, 100.00)
HBV Clinical Specificity	108,297	2	108,295	100.00 (99.99, 100.00)

Table 49 Pool reactivity in source plasma donations

Category	Number of Pools	Percentage of Pools Tested
Total Pools of 96[a] tested:	1,106	100
Non-Reactive pools[b]	1,092	98.7
Non-reactive pools with all donations status negative	1,090	98.6 (1,090/1,106)
Non-reactive pools with at least one status-positive donation	2[c]	0.2 (2/1,106)
Reactive pools[b]	14	1.3
Reactive pools with at least one status-positive donation	7	0.6 (7/1,106)
Reactive pools with donation status-negative (false reactive pools)	7	0.6 (7/1,106)

[a] 479/1106 pools had < 96 donations

[b] Donation status was assigned based on the concordance of two virus-specific tests (e.g., two NAT results or NAT and serology) on the index donation, or the results of follow-up testing.

[c] These two non-reactive pools contained donations from an HBV-positive donor. The donor's index donation was HBV-positive on **cobas**® MPX but negative on **cobas**® TaqScreen MPX Test and was confirmed HBV-positive by alternative high-sensitivity NAT. This donor made three subsequent donations that were nonreactive on both NAT screening assays. One of these donations was contained within an HCV-positive pool.

Eleven unique donors contributed 12 reactive donations (six HCV, six HIV, and three HBV). Seven donors completed follow-up testing: three of these donors did not show evidence of infection on follow-up; four donors were confirmed to have infection on follow-up, of whom two seroconverted (HCV) during follow-up (Table 50). One of the three HBV donors was determined to be a NAT HBV yield case.

Table 50 Observed testing reactivity patterns from initial testing on evaluable donations

cobas® MPX Result	**Donation Status[a]**	**Number of Donations**
HCV+	Positive	5
HBV+	Negative	2
HBV+	Positive	4[b]
HCV+	Negative	1
HIV+	Negative	3
Non-Reactive	Negative	108,291
-	Total	108,306

[a] Donation Status was assigned based on the test reactivity pattern (concordance" of two virus-specific tests (e.g., two NAT results or NAT and serology) on the index donation or results of follow-up testing).

[b] These donations are all from the same donor whose index donation was HBV+ and whose subsequent three donations were classified as status positive even though **cobas**® MPX was non-reactive for HBV.

Note: Only evaluable donations are included in this summary table; + = Reactive/Positive

The clinical specificity of **cobas**® MPX for source plasma pools was determined by the analysis of 108,306 evaluable donations from 24,514 unique donors. Evaluable donations had valid **cobas**® MPX, **cobas**® TaqScreen MPX Test and CAS results from testing pools, and valid serology results (across analytes) from testing of individual donations. Of these 108,306 evaluable donations, 108,297 were assigned a donation status of negative, of which 108,291 were **cobas**® MPX non-reactive, for a clinical specificity of 99.99% (95% Clopper Pearson Exact CI: 99.99% to 100.00%) (Table 48). Seven false **cobas**® MPX reactive pools of 96 resolved to contain all status-negative donations. Of the 24,514 unique donors tested, 24,509 contributed only status-negative donations, of which 24,503 were non-reactive on **cobas**® MPX and six had false-reactive results, resulting in specificity (at the donor level) of 99.98% (95% Clopper Pearson Exact CI: 99.95% to 100.00%).

Studies in high risk populations

A total of 511 specimens from individuals at high risk for infection with HIV, HCV, or HBV were distributed among 4 study sites. Specimens were tested neat and diluted (1:6) to simulate pools of 6 with the **cobas**® MPX test for use on the **cobas**® 8800 System (**cobas**® MPX) and the **cobas**® TaqScreen MPX Test for use on the **cobas s** 201 system (**cobas**® TaqScreen MPX Test), incorporating COBAS® AmpliScreen (CAS) HIV-1, HCV, and HBV Tests for viral target resolution. Three **cobas**® MPX reagent and control lots were used for testing. The contributors of these 511 specimens responded to a questionnaire in which they self-reported certain factors associated with a high risk for HIV, HCV, or HBV infection. Two samples had invalid **cobas**® MPX results and were not included in analyses of neat specimens. Overall reactivity was similar between **cobas**® MPX (71/509; 13.9%) and **cobas**® TaqScreen MPX Test (70/509; 13.8%) in neat specimens, as well as in diluted specimens, with 12.9% (66/511) for **cobas**® MPX and 11.9% (61/511) for **cobas**® TaqScreen MPX Test.

Table 51 presents the overall performance of the **cobas**® MPX test in identifying the type of virus in 509 neat samples with valid results for **cobas**® MPX.

Table 51 Overall neat -- Correct versus incorrect identification of virus

cobas® **MPX Result***	**Number**	**%**	**Total**
True reactives	**62**	**97.8%**	**498**
True non-reactives	**436**	**97.8%**	**498**
False reactives	9	1.8%	11
False non-reactives	2	0.4%	11
Total	509	100%	509

* Final status (as compared with **cobas**® TaqScreen MPX Test plus CAS or alternative NAT [NGI testing] results)
Note: Correct identification = True reactive and true non-reactive results (shown in bold type).
Note: Two samples with invalid **cobas**® MPX results were not included in the analyses presented in this table.
CAS = COBAS® AmpliScreen HIV-1, HCV, HBV Tests; NAT = nucleic acid testing; NGI = National Genetics Institute.

Table 52 shows the overall performance of **cobas**® MPX in identifying the type of virus in 511 diluted samples.

Table 52 Overall diluted -- correct versus incorrect identification of virus

cobas® **MPX Result***	**Number**	**%**	**Total**
True reactives	**59**	**97.5**	**498**
True non-reactives	**439**	**97.5**	**498**
False reactives	7	1.37	13
False non-reactives	6	1.17	13
Total	511	100.0	511

* Final status (as compared with **cobas**® TaqScreen MPX Test plus CAS or alternative NAT [NGI testing] results)
Note: Correct identification = True reactive and true non-reactive results (shown in bold type).
CAS = COBAS® AmpliScreen HIV-1, HCV, HBV Tests; NAT = nucleic acid testing; NGI = National Genetics Institute.

Studies in NAT positive populations

A total of 2,569 HIV, HCV, and HBV NAT-positive samples were tested across four test sites with **cobas**® MPX and the **cobas**® TaqScreen MPX Test incorporating CAS. Three lots of **cobas**® MPX reagents were used. The 2,569 samples known to be NAT-positive consisted of 1,015 HIV-positive samples, 1,016 HCV-positive samples, and 538 HBV-positive samples. Each of these samples were tested both neat and dilute (1:6) with **cobas**® MPX and the **cobas**® TaqScreen MPX Test.

HIV NAT positive population

Six HIV specimens did not meet the HIV viral load inclusion criteria in the protocol of $\geq$ 18 x LoD for the **cobas**® TaqScreen MPX Test and those specimens were excluded from the statistical comparison analysis. The remaining 1,009 HIV-positive neat samples generated 1,006 evaluable test results with **cobas**® MPX and 1,008 evaluable test results with the **cobas**® TaqScreen MPX Test incorporating CAS. One thousand and nine HIV diluted samples produced 1,006 evaluable test results with cobas® MPX and 1,009 evaluable test results with the **cobas**® TaqScreen MPX Test (CAS was not performed on dilute samples).

cobas® MPX was reactive for 1,006 of 1,006 (100.00%) HIV neat and 1:6 diluted samples. The **cobas**® TaqScreen MPX Test incorporating CAS was reactive for 1,007 of 1,008 (99.90 %) for HIV neat samples. The **cobas**® TaqScreen MPX Test (no CAS performed) was reactive for 1,005 of 1,009 (99.60%) for HIV diluted samples.

HCV NAT positive population

cobas® MPX was reactive for 1,015 of 1,015 (100.0%) HCV neat samples and 1,016 of 1,016 (100.0%) HCV diluted samples. The **cobas**® TaqScreen MPX Test incorporating CAS was also reactive for 1,014 of 1,014 (100.0 %) for neat samples. The **cobas**® TaqScreen MPX Test (no CAS performed) was reactive for 1,016 of 1,016 (100.0%) for dilute samples.

HBV NAT positive population

The 538 HBV-positive neat samples generated 528 evaluable test results with **cobas**® MPX and 502 evaluable test results with the **cobas**® TaqScreen MPX Test incorporating CAS. The 538 HBV dilute samples produced 533 evaluable test results with **cobas**® MPX, and 538 evaluable test results with the **cobas**® TaqScreen MPX Test (CAS was not performed on dilute samples).

cobas® MPX was reactive for 528 of 528 (100.00%) HBV-positive neat samples and 533 of 533 (100.00%) HBV-positive dilute samples. The **cobas**® TaqScreen MPX Test incorporating CAS was reactive for 502 of 502 (100.0%) for HBV neat samples. The **cobas**® TaqScreen MPX Test (no CAS performed) was reactive for 538 of 538 (100.0%) for HBV diluted samples.

Table 53 compares the sensitivities of **cobas**® MPX and **cobas**® TaqScreen MPX Test Results for HIV, HCV, and HBV known positive samples for samples that had valid results on both tests.

The overall clinical sensitivity of the **cobas**® MPX test was 100.00% (2,429/2,429) for neat known positive samples and 100.00% (2,555/2,555) for diluted (1:6) known positive samples with paired results. The overall clinical sensitivity of the **cobas**® TaqScreen MPX Test was 99.96% (2,523/2,524) for neat known positive samples and 99.84% (2,559/2,563) for diluted (1:6) known positive samples with paired results (Table 53).

Table 53 Comparison of the sensitivities of **cobas**® MPX and **cobas**® TaqScreen MPX Test results for HIV, HCV, and HBV known positive samples for paired samples with valid results

Dilution	Target Virus	Sensitivity in Known Positive Samples[a] cobas® MPX Result	Sensitivity in Known Positive Samples[a] cobas® TaqScreen MPX Test
Neat	**Overall**	100.00% (2,549/2,549)	99.96% (2,523/2,524)
Neat	**HIV**	100.00% (1006/1006)	99.90% (1,007/1,008)
Neat	**HCV**	100.00% (1,015/1,015)	100.00% (1,014/1,014)
Neat	**HBV**	100.00% (528/528)	100.00% (502/502)
1:6	**Overall**	100.00% (2,555/2,555)	99.84% (2,559/2,563)
1:6	**HIV**	100.00% (1006/1006)	99.60% (1,005/1,009)
1:6	**HCV**	100.00% (1016/1016)	100.00% (1016/1016)
1:6	**HBV**	100.00% (533/533)	100.00% (538/538)

[a] Only known positive samples with valid results on both tests were included in the sensitivity analysis

Clinical sensitivity for HIV-1 Group O and HIV-2 seropositive population

HIV-1 Group O seropositive population

A total of 12 HIV-1 Group O seropositive samples were tested after 1:6 dilution using **cobas**® MPX and **cobas**® TaqScreen MPX Test. The samples were tested after 1:6 dilution due to limited volume. All of the HIV-1 Group O samples were reactive for HIV when tested with **cobas**® MPX after a 1:6 dilution as summarized in Table 54.

Table 54 Comparison of overall reactivity for HIV-1 Group O seropositive samples (1:6 dilution)

cobas® TaqScreen MPX Test (1:6 Dilution)	**cobas® MPX (1:6 Dilution) Reactive**	**cobas® MPX (1:6 Dilution) Non-Reactive**	**Total**
Reactive	11	0	11
Non-Reactive	1	0	1
Total	12	0	12

HIV-2 seropositive population

A total of 319 HIV-2 seropositive samples were tested using the **cobas**® MPX test and **cobas**® TaqScreen MPX Test. Out of the 319 seropositive samples, 184 were tested neat and after 1:6 dilution with **cobas**® MPX and **cobas**® TaqScreen MPX Test whereas the remaining 135 were only tested after 1:6 dilution due to limited volume.

A total of 137 samples of the 184 neat tested samples was reactive as summarized in Table 55, for a clinical sensitivity of 74.5% relative to serology using **cobas**® MPX. Comparable sensitivity of **cobas**® MPX towards HIV-2 was also demonstrated when samples were diluted 1:6 prior to testing with both methods. A total of 198 samples of the 319 1:6 diluted samples were reactive with **cobas**® MPX as summarized in Table 56.

Table 55 Comparison of overall reactivity for HIV-2 seropositive samples (neat)

cobas® TaqScreen MPX Test (Neat)	**cobas® MPX (Neat) Reactive**	**cobas® MPX (Neat) Non-Reactive**	**Total**
Reactive	118	7	125
Non-Reactive	19	40	59
Total	137	47	184

Table 56 Comparison of overall reactivity for HIV-2 seropositive samples (1:6 dilution)

cobas® TaqScreen MPX Test (1:6 Dilution)	**cobas® MPX (1:6 Dilution) Reactive**	**cobas® MPX (1:6 Dilution) Non-Reactive**	**Total**
Reactive	173	33	206
Non-Reactive	25	88	113
Total	198	121	319

Additional information

Key test features

Sample type	Plasma and Serum
Minimum amount of sample required for living donor	1000 µL
Amount of sample processed for living donor	850 µL
Minimum amount of sample required for cadaveric donor	300 µL
Amount of sample processed for cadaveric donor	150 µL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 57 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	# Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
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Manufacturer and distributor

Table 58 Manufacturer and distributor

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Document revision

Document Revision Information	
Doc Rev. 9.0 08/2022	Updated title page and **Reagents and Materials** section to include additional **cobas**® NHP Negative Control Kit P/N. Updated the harmonized symbol page. Updated to current economic operators. Added **Technical support** section. Updated **Trademarks and patents** section, including the link. Please contact your local Roche Representative if you have any questions.