Rx Only

cobas® MPX

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

For in vitro diagnostic use

cobas® MPX – 96	P/N: 06997708190
cobas® MPX – 480	P/N: 06997716190
cobas® MPX Control Kit	P/N: 06997724190
cobas® NHP Negative Control Kit	P/N: 07002220190
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

Table of contents

Intended use

The **cobas**® MPX test, for use on **cobas**® 6800 and **cobas**® 8800 Systems is a qualitative in vitro test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA, and Hepatitis B Virus (HBV) DNA in human plasma and serum.

This test is intended for use to screen donor samples for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA in plasma and serum samples from individual human donors, including donors of whole blood, blood components, 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 and in testing of 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 aliquots of individual samples. For donations from cadaveric (non-heart beating) organ and tissue donors, samples may only be screened as individual sample.

For an individual sample, results are simultaneously detected and discriminated for HIV, HCV, and HBV.

The **cobas**® MPX test can be considered a supplemental test that confirms HIV infection for samples that are repeatedly reactive on a CE-IVD test for antibodies to HIV and reactive on the **cobas**® MPX test.

The **cobas**® MPX test can be considered a supplemental test that confirms HCV infection for samples that are repeatedly reactive on a CE-IVD test for antibodies to HCV and reactive on the **cobas**® MPX test.

The **cobas**® MPX test can be considered a supplemental test that confirms HBV infection for samples that are repeatedly reactive on a CE-IVD test for Hepatitis B surface antigen and reactive on the **cobas**® MPX test.

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

Summary and explanation of the test

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 Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), Hepatitis C Virus (HCV), and Hepatitis B Virus (HBV). These agents are primarily transmitted by exposure to contaminated blood or blood and plasma products, exposure to certain body tissues or fluids, by sexual contact, or by an infected mother to 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]

HCV is considered to be the principal etiologic agent responsible for 90% to 95% of post transfusion non-A and non-B

hepatitis cases.[12-14] The reported prevalence of HCV varies from 0.5 to 2.0% in Western Europe [15] and between 6% and 40% in Egypt.[16]

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.[17-19] 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.[20]

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).[21,22] 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.[23] 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.[24,25] 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.[26,27] 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.[24] 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,[28] and in vaccinated donors with a breakthrough, subclinical infection.[29-31]

Explanation of the test

The **cobas**® MPX test is a qualitative multiplex test that is run on the **cobas**® 6800 System and **cobas**® 8800 System. The **cobas**® MPX test 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

The **cobas**® MPX test 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) molecules (which serve as the sample preparation and amplification/detection process control) is simultaneously extracted. In addition the test utilizes four external 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).[32-34] Any contaminating amplicons 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 amplicons 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.[35,36] 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 test

cobas® MPX test
Store at 2-8°C
96 test cassette (P/N 06997708190)
480 test cassette (P/N 06997716190)

Kit components	Reagent ingredients	Quantity per kit	
		96 tests	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

cobas® MPX Control Kit

Store at 2-8°C
(P/N 06997724190)

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. 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 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/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 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)

Table 2 cobas® MPX Control Kit

cobas® MPX Control Kit

Store at 2-8°C
(P/N 06997724190)

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/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 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

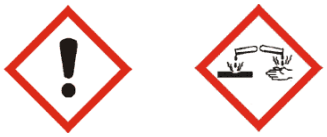
cobas® NHP Negative Control Kit Store at 2-8°C (P/N 07002220190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, 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/ 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 Mixture 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 875mL	 **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

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	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable	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.

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

cobas® 6800 / 8800 Systems	**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 (Optional)	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.[37,38] Only personnel proficient in handling infectious materials and the use of the **cobas**® MPX test, the **cobas**® 6800/8800 Systems, and optionally the **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.
- 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 test 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 test kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- 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 Systems 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.

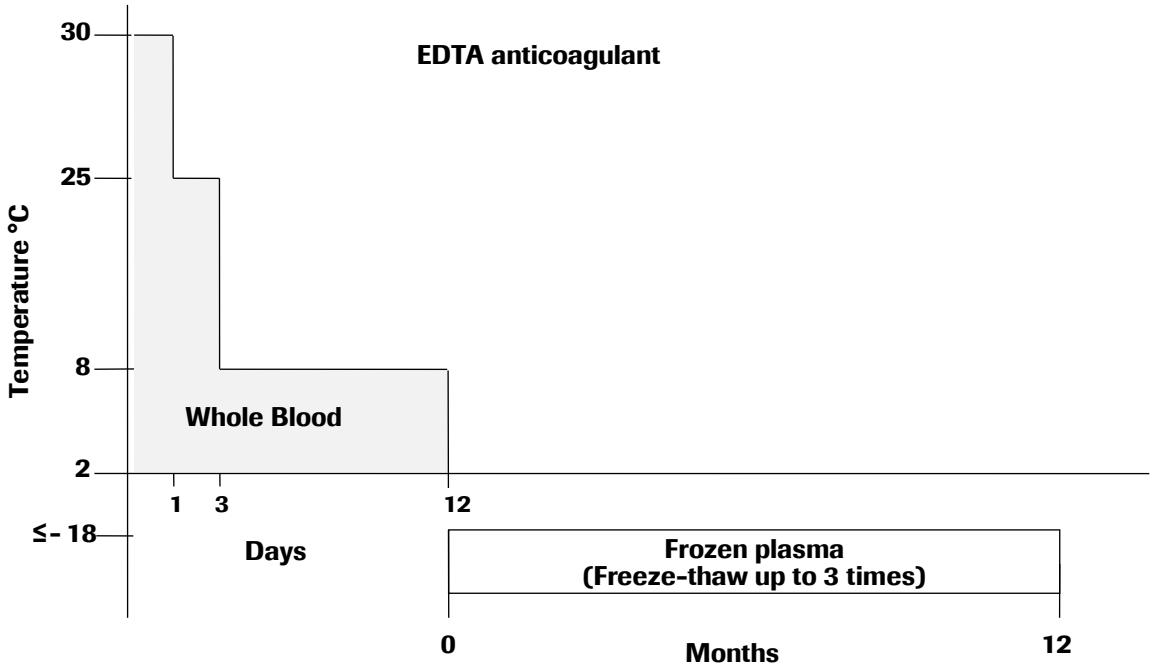
- It is recommended that serum samples are tested within 8 hours of centrifugation at 1600 x g for 20 minutes or are tested within 24 hours of high-speed centrifugation (e.g., 2600 x g for 20 minutes).

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 the **cobas**® MPX test. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- 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, 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 12 months at ≤ -18°C with three freeze/thaw cycles. Refer to Figure 1.

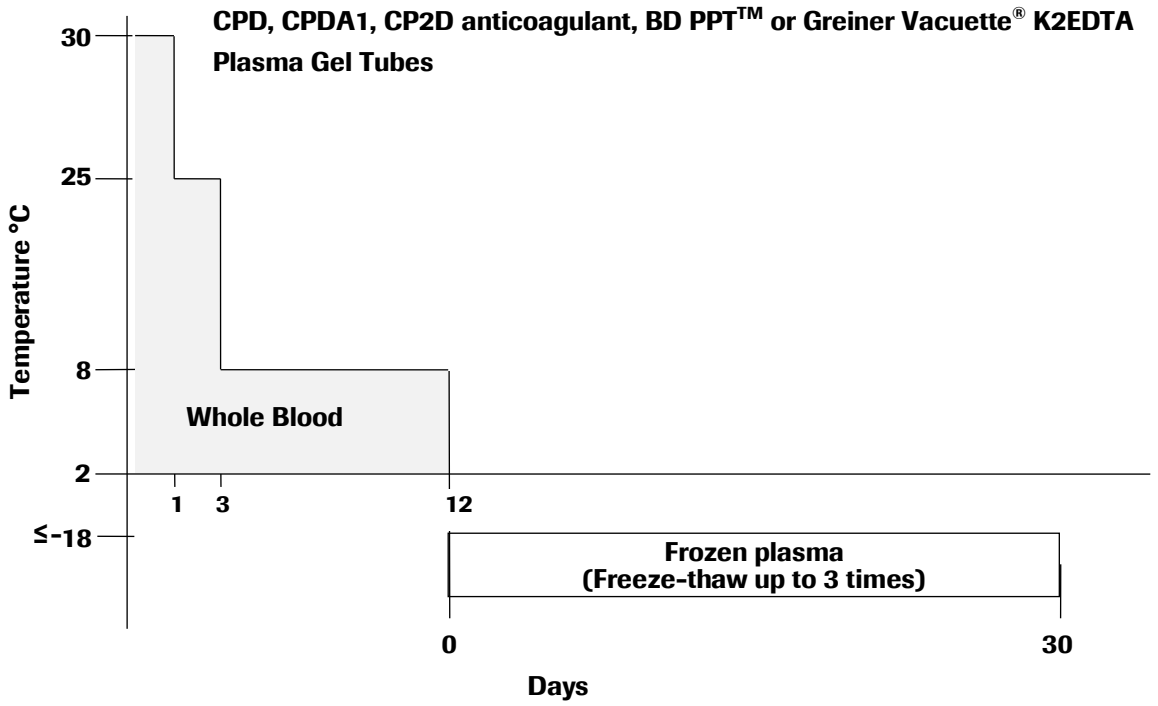
Figure 1 Sample storage conditions for living donor sample in EDTA anticoagulant



- 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 2.

Figure 2 Sample storage 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 collected in 4% sodium citrate anticoagulant may be stored for up to 30 days at 2-8°C with the following conditions:
    - 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.

  In addition, plasma collected in 4% sodium citrate anticoagulant may be stored for up to 12 months at ≤ -18°C with two freeze/thaw cycles or
- Plasma collected in 4% sodium citrate anticoagulant may be stored for up to 18 days at 2-8°C with the following conditions:
    - 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.

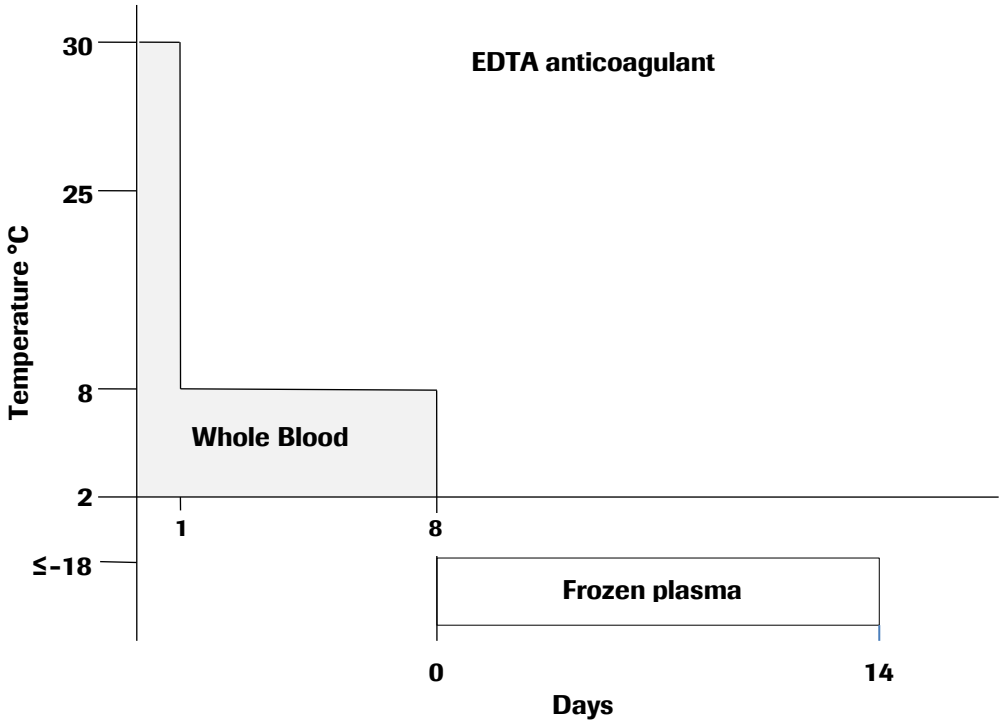
  In addition, plasma collected in 4% sodium citrate anticoagulant may be stored for up to 12 months at ≤ -18°C with three freeze/thaw cycles.

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 3.

Figure 3 Sample storage conditions for cadaveric sample



- Cadaveric blood samples collected in serum clot tubes may be stored for up to 5 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 24 hours at up to 30°C, during the 72 hours.

- If living donor and/or cadaveric samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic 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 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.

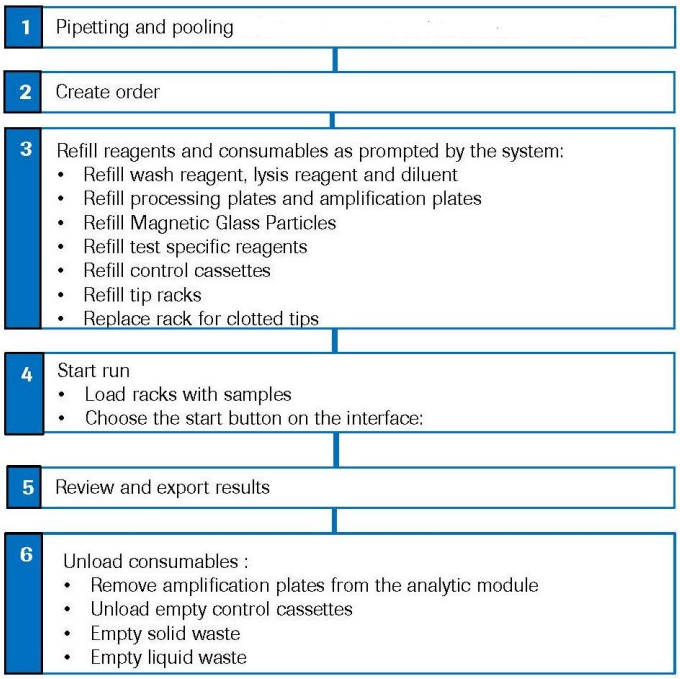
Procedural notes

- Do not use **cobas**® MPX test 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 for proper maintenance of instruments.

Running the cobas® MPX test

The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual; refer to 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 4 below summarizes the procedure.

Figure 4 **cobas**® MPX test procedure



Results

The **cobas**® 6800/8800 Systems automatically detect and discriminate 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] is 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 the **cobas**® MPX test 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 be 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 be 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 be 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

- The **cobas**® MPX test 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.
- 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 the **cobas**® MPX test, 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.

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 the **cobas**® MPX test for HIV-1 Group M RNA HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA were determined using the following standards:

- 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)[39]
- 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 the WHO International HIV-1 Group M, HCV and HBV, HIV-2, and Roche primary HIV-1 Group O standards, 3 independent dilution series of each viral standard co-formulated for HIV-1 Group M, HCV, HBV members and individually formulated HIV-1 Group O, and HIV-2 were prepared with normal, virus-negative (HIV, HBV and HCV) human EDTA-plasma. Each dilution series was tested using 3 different lots of the **cobas**® MPX test 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 3 independent dilutions and 3 reagent lots were tested for a total of 99 replicates per concentration. For each virus, 95% PROBIT analysis (Table 11) and 50% PROBIT analysis (Table 12) 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 intervals. The reactivity rates observed in the LoD studies for each virus are summarized in Table 13 to Table 17.

Table 11 Results of 95% 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
	HIV-1 Group O	copies/mL	8.2	7.0	10.0
	HIV-2	IU/mL	4.0	3.3	5.2
	HCV	IU/mL	7.0	5.9	8.6
	HBV	IU/mL	1.4	1.2	1.7
Serum	HIV-1 Group M	IU/mL	23.7	20.0	29.1
	HIV-1 Group O	copies/mL	12.2	10.3	14.9
	HIV-2	IU/mL	4.4	3.5	5.8
	HCV	IU/mL	8.1	6.8	10.1
	HBV	IU/mL	1.3	1.1	1.5

Table 12 Results of 50% 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	3.8	3.4	4.3
	HIV-1 Group O	copies/mL	1.7	1.5	1.9
	HIV-2	IU/mL	0.9	0.8	1.1
	HCV	IU/mL	1.3	1.1	1.4
	HBV	IU/mL	0.3	0.3	0.3
Serum	HIV-1 Group M	IU/mL	4.6	4.1	5.1
	HIV-1 Group O	copies/mL	2.5	2.2	2.7
	HIV-2	IU/mL	0.9	0.8	1.1
	HCV	IU/mL	1.4	1.3	1.6
	HBV	IU/mL	0.3	0.3	0.3

Table 13 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%
	15	170	189	89.9%	85.6%
	7.5	124	189	65.6%	59.5%
	4.5	96	189	50.8%	44.6%
	1.5	50	189	26.5%	21.2%
Serum	30	186	189	98.4%	95.9%
	15	170	189	89.9%	85.6%
	7.5	123	189	65.1%	59.0%
	4.5	85	189	45.0%	38.8%
	1.5	31	189	16.4%	12.1%

Table 14 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%
	9	181	187	96.8%	93.8%
	4.5	162	189	85.7%	80.8%
	2.7	117	189	61.9%	55.7%
	0.9	57	189	30.2%	24.7%
Serum	18	186	187	99.5%	97.5%
	9	173	188	92.0%	88.0%
	4.5	142	189	75.1%	69.4%
	2.7	79	189	41.8%	35.8%
	0.9	39	189	20.6%	15.9%

Table 15 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%
	5	98	99	99.0%	95.3%
	2.5	80	98	81.6%	74.0%
	1.5	71	99	71.7%	63.3%
	0.5	26	99	26.3%	19.1%
Serum	10	98	98	100.0%	97.0%
	5	98	99	99.0%	95.3%
	2.5	81	99	81.8%	74.2%
	1.5	63	98	64.3%	55.6%
	0.5	28	98	28.6%	21.1%

Table 16 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%
	6	178	189	94.2%	90.6%
	3	148	189	78.3%	72.8%
	1.8	112	189	59.3%	53.0%
	0.6	50	189	26.5%	21.2%
Serum	12	186	189	98.4%	95.9%
	6	173	189	91.5%	87.4%
	3	139	189	73.5%	67.7%
	1.8	112	189	59.3%	53.0%
	0.6	41	189	21.7%	16.9%

Table 17 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%
	1.70	184	189	97.4%	94.5%
	0.85	165	189	87.3%	82.6%
	0.51	126	189	66.7%	60.6%
	0.17	58	189	30.7%	25.2%
Serum	3.40	189	189	100.0%	98.4%
	1.70	184	189	97.4%	94.5%
	0.85	166	189	87.8%	83.2%
	0.51	140	189	74.1%	68.3%
	0.17	52	189	27.5%	22.2%

Reproducibility

The reproducibility of the **cobas**® MPX test on the **cobas**® 6800/8800 Systems was determined using the following standards:

- Roche Secondary Standards for HIV-1 Group M, HCV, and HBV
- Roche Primary Standards for HIV-1 Group O and HIV-2

This study consisted of testing 3 panels of co-formulated HIV-1 Group M, HCV, and HBV members and individually formulated HIV-1 Group O, and HIV-2 members at concentrations of approximately 0.5 x, 1 x and 2 x the LoD of the **cobas**® MPX test for each virus. Testing was performed for the following variability components:

- day-to-day variability over 3 days
- lot-to-lot variability using 3 different reagent lots of the **cobas**® MPX test
- instrument-to-instrument variability using 3 different **cobas**® 8800 Systems

Approximately 21 replicates were tested with each of the 3 panels for total of 63 replicates with each reagent lot. All valid reproducibility data were evaluated by calculating the percentage of reactive test results for each concentration level across all variable components.

The limits of two-sided 95% Confidence Intervals for each Reactive Rate were calculated for each of the three levels of HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV tested across 3 days, 3 reagent lots, and 3 **cobas**® 8800 Systems. The **cobas**® MPX test is reproducible over multiple days, reagent lots and multiple instruments. The results from reagent lot-to-lot variability are summarized in Table 18.

Table 18 cobas® MPX test reagent lot-to-lot reproducibility summary

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
HIV-1 Group M	2 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	1 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	98.4% (62/63)	91.5%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	0.5 x LoD	1	85.7% (54/63)	74.6%	93.3%
		2	95.2% (60/63)	86.7%	99.0%
		3	92.1% (58/63)	82.4%	97.4%
HIV-1 Group O	2 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	1 x LoD	1	92.1% (58/63)	82.4%	97.4%
		2	93.7% (59/63)	84.5%	98.2%
		3	93.7% (59/63)	84.5%	98.2%
	0.5 x LoD	1	74.6% (47/63)	62.1%	84.7%
		2	76.2% (48/63)	63.8%	86.0%
		3	74.6% (47/63)	62.1%	84.7%

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
HIV-2	2 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	98.4% (62/63)	91.5%	100.0%
	1 x LoD	1	82.5% (52/63)	70.9%	90.9%
		2	93.7% (59/63)	84.5%	98.2%
		3	87.3% (55/63)	76.5%	94.4%
	0.5 x LoD	1	74.6% (47/63)	62.1%	84.7%
		2	71.4% (45/63)	58.7%	82.1%
		3	73.0% (46/63)	60.3%	83.4%
HCV	2 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	1 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	98.4% (62/63)	91.5%	100.0%
	0.5 x LoD	1	77.8% (49/63)	65.5%	87.3%
		2	98.4% (62/63)	91.5%	100.0%
		3	93.7% (59/63)	84.5%	98.2%
HBV	2 x LoD	1	100.0% (63/63)	94.3%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	1 x LoD	1	90.5% (57/63)	80.4%	96.4%
		2	90.5% (57/63)	80.4%	96.4%
		3	93.7% (59/63)	84.5%	98.2%
	0.5 x LoD	1	84.1% (53/63)	72.7%	92.1%
		2	76.2% (48/63)	63.8%	86.0%
		3	77.8% (49/63)	65.5%	87.3%

Genotype verification

The performance of the **cobas**® MPX test 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 19 to Table 23.

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 the **cobas**® MPX test 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 19).

Table 19 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 7 HIV-1 Group O and 2 HIV-1 Group N cultured isolates were tested after log dilutions were prepared in normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma. For HIV-1 Group O isolates, 28 total replicates across 7 isolates were tested using 4 replicates of each dilution. For HIV-1 Group N isolates, two isolates were tested. A total of 4 replicates were tested for one isolate from dilution 1:1.00E+02 to 1:1.00E+03 and 1 replicate was tested for the second isolate at dilution of 1:1.00E+04. HIV-1 Group O culture isolates were detected up to dilution of 1:1.00E+07 and Group N culture isolates were detected up to dilution of 1:1.00E+04 (Table 20).

Table 20 HIV-1 Group O and HIV-1 Group N cultured isolates

Sample Dilution	% Reactive (reactive/valid replicates tested)	
	HIV-1 Group O	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)	20% (1/5)
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)

HIV-2

A total of 5 HIV-2 subtype A (4) and B (1) 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 4 isolates were tested for each dilution. For 1 isolate of subtype B, 4 total replicates were tested for each dilution. A total of 11 HIV-2 subtype A (5) and B (6) clinical samples were also tested after log dilutions were prepared in normal, virus-negative human EDTA-plasma. For subtype A, 20 total replicates across 5 clinical samples and for subtype B, 24 total replicates across 6 clinical samples were tested using 4 replicates for each dilution. All cultured isolates were detected by the **cobas**® MPX test. Clinical samples were detected by the **cobas**® MPX test at up to dilutions of 1:1.00E+03 for subtypes A and B. The overall results are summarized in Table 21.

Table 21 HIV-2 cultured isolates and clinical samples

Sample Dilution	% Reactive (reactive/valid replicates tested)			
	Cultured isolate		Clinical sample	
	Subtype A	Subtype B	Subtype A	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% (0/4)	0.0% (0/20)	0.0% (0/24)

HCV

A total of 96 unique HCV clinical samples with known HCV genotype were quantified for HCV concentrations using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0. All 96 HCV clinical samples with known genotypes were tested after dilution with normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma to 5 x LoD of the **cobas**® MPX test. Of those, 95 samples were also tested neat. All samples were tested in single replicate. All 96 HCV-positive clinical samples were detected neat and/or diluted as summarized in Table 22.

Table 22 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% (1/1)	100.0% (1/1)
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)

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 the **cobas**® MPX test. All samples were tested with single replicates. All 94 HBV-positive clinical samples were detected both at neat and/or diluted as summarized in Table 23.

Table 23 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% (12/12)
E	100.0% (12/12)	100.0% (11/11)
F	100.0% (12/12)	100.0% (12/12)
G	Not tested*	100% (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 the **cobas**® MPX test was evaluated using commercially available seroconversion panels for HIV-1 Group M, HCV, and HBV. The results of the **cobas**® MPX test 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 the **cobas**® MPX test and CE-IVD and FDA licensed serology tests for each target.

HIV-1 Group M Seroconversion panels

Ten commercially available seroconversion panels were used. Each panel member was tested neat and diluted 1:6 and 1:96 to simulate testing in pools for testing with **cobas**® MPX and **cobas**® TaqScreen MPX Test. The **cobas**® MPX test results were compared to the results obtained with the **cobas**® TaqScreen MPX Test and with results with the CE-IVD and FDA licensed HIV serology tests tested neat. The overall performance results are shown in Table 24.

Table 24 Performance of **cobas**® MPX test on HIV Seroconversion panels

HIV Seroconversion panels	Days earlier detection than HIV Antibody/Antigen or HIV RNA								
	Abbott ARCHITECT HIV Ag/Ab Combo: Neat			Abbott PRISM HIV Ag/Ab Combo: Neat			cobas® TaqScreen MPX Test: Neat, 1:6, 1:96		
	Days earlier detection by the cobas® MPX								
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	3	3	3	3	3	3	0	0	0
2	7	2	2	12	7	7	5	0	0
3	7	5	5	7	5	5	2	0	0
4	15	15	8	15	15	8	0	0	0
5	7	7	7	7	7	7	0	0	2
6	10	3	3	10	3	3	2	0	0
7	9	9	7	9	9	7	0	0	0
8	11	11	9	11	11	9	0	0	0
9	2	2	2	2	2	2	0	0	0
10	7	7	7	7	7	7	0	0	2
Minimum	2	2	2	2	2	2	0	0	0
Average	7.8	6.4	5.3	8.3	6.9	5.8	0.9	0	0.4
Maximum	15	15	15	15	15	9	5	0	2

HCV Seroconversion panels

Ten commercially available seroconversion panels were used. Each panel member was tested neat and diluted 1:6 and 1:96 to simulate testing in pools for testing with the **cobas**® MPX and **cobas**® TaqScreen MPX tests. The **cobas**® MPX results were compared to the results obtained with the **cobas**® TaqScreen MPX Test and with results with the CE-IVD and FDA licensed HCV serology tests tested neat. The overall performance results are shown in Table 25.

Table 25 Performance of **cobas**® MPX test on HCV Seroconversion panels

HCV Seroconversion panels	Days earlier detection than HCV Antibody/Antigen or HCV RNA								
	ORTHO HCV Version 3.0 ELISA Test System: Neat			Abbott PRISM HCV: Neat			cobas® TaqScreen MPX Test: Neat, 1:6, 1:96		
	Days earlier detection by the cobas® MPX								
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	13	13	13	13	13	13	0	0	0
2	23	23	23	23	23	23	0	0	0
3	33	33	33	33	33	33	-6	0	0
4	32	32	32	32	32	32	0	0	0
5	38	38	38	38	38	38	-24**	0	0
6	34	34	34	34	34	34	0	0	0
7*	11	11	11	11	11	11	0	0	0
8	65	65	65	65	65	65	0	0	0
9*	13	13	13	16	16	16	0	0	0
10*	21	21	21	21	21	21	0	0	0
Minimum	13	13	13	13	13	13	-24	0	0
Average with exclusions*	34	34.	34	34	34	34	-3	0	0
Maximum	65	65	65	65	65	65	0	0	0

* Panels that were consistently reactive with the **cobas**® MPX test, beginning on the first bleed, were excluded from the summary calculations for the minimum, average and maximum number of days earlier detection than HCV antibody.

** 24 day interval between adjacent draws.

HBV Seroconversion panels

Ten commercially available seroconversion panels were used. Each panel member was tested neat and diluted 1:6 and 1:96 to simulate testing in pools for testing with the **cobas**® MPX and **cobas**® TaqScreen MPX tests. The **cobas**® MPX results were compared to the results obtained with the **cobas**® TaqScreen MPX Test and with results with the CE-IVD and FDA licensed HBV serology tests tested neat. The overall performance results are shown in Table 26.

Table 26 Performance of **cobas**® MPX test on HBV Seroconversion panels

HBV Seroconversion panels	Days earlier detection than HBsAg or HBV DNA								
	ORTHO HBSAg ELISA Test System 3: Neat			Abbott PRISM HBsAg: Neat			cobas® TaqScreen MPX Test: Neat, 1:6, 1:96		
	Days earlier detection by the cobas® MPX								
	Neat	1:6	1:96	Neat	1:6	1:96	Neat	1:6	1:96
1	36	19	7	29	12	0	17	0	0
2	19	11	7	8	0	-4*	0	-3	0
3	24	24	0	24	24	0	-7	7	0
4	17	17	0	0	0	-17*	0	0	0
5	30	30	9	28	28	7	0	0	7
6	28	28	17	18	18	7	-8	4	10
7	16	13	5	11	8	0	9	0	5
8	30	28	14	0	-2*	-16*	2	12	0
9	24	24	13	17	17	6	0	2	6
10	38	42	27	29	33	18	-4	15	3
Minimum	16	11	0	0	-2	-17	-8	-3	0
Average	26.2	23.6	9.9	16.4	13.8	0.1	0.9	3.7	3.1
Maximum	38	42	27	29	33	18	17	15	10

* Low concentrations of HBV DNA were present in diluted panel members which were detected later by the **cobas**® MPX test than by serology; 0.6 IU/mL in Panel 2 at 1:96, 2.0 IU/mL in Panel 4 at 1:96 (plus abnormally early but low S/Co serology result), not detected in Panel 8 at 1:6, and 0.5 IU/mL in Panel 8 at 1:96, in the draw showing **cobas**® MPX test NAT conversion, using alternate NAT quantitation.

Analytical specificity

The analytical specificity of the **cobas**® MPX test was evaluated for cross-reactivity with 25 microorganisms at 10^6 particles, copies, or PFU/mL, which included 18 viral isolates, 6 bacterial strains and 1 yeast isolate (Table 27). 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 the **cobas**® MPX test for each virus. The tested microorganisms do not cross-react or interfere with the **cobas**® MPX test.

Table 27 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 28) 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 the **cobas**® MPX test for each virus. These disease states do not cross-react or interfere with the **cobas**® MPX test.

Table 28 Disease states samples tested for analytical specificity

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 the **cobas**® MPX test. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of the **cobas**® MPX test.

Exogenous interference substances

Normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 29) 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 the **cobas**® MPX test for each virus. These exogenous substances did not interfere with the sensitivity or specificity of the **cobas**® MPX test.

Table 29 Clinical samples tested with drugs

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

Correlation

Performance evaluation of the cobas® MPX test compared to the cobas® TaqScreen MPX Test, v2.0

The performance of the **cobas**® MPX test and the **cobas**® TaqScreen MPX Test, v2.0 were compared using 100 individual seropositive plasma samples each for HIV-1 Group M, HCV and HBV, which were tested neat and diluted to 1:6. For HIV-2, 48 seropositive samples were tested neat and 99 samples were tested diluted 1:6, and for HIV-1 Group O, 13 seropositive samples were tested diluted 1:6. In addition, 103 seronegative plasma samples were tested neat with both methods.

The seronegative samples demonstrated 100% specificity by generating 103 out of 103 non-reactive results with both methods.

For HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV positive samples, both methods were in agreement based on the McNemars's test, demonstrating that the performance of **cobas**® MPX test and **cobas**® TaqScreen MPX Test, v2.0 are equivalent (Table 30 and Table 31).

Table 30 Correlation of seropositive samples (Neat)

Methods		Individual viral target results			
cobas® TaqScreen MPX Test, v2.0	**cobas® MPX**	**HIV-1 Group M**	**HBV**	**HCV**	**HIV-2**
Non-reactive	Non-reactive	0	0	0	4
Reactive	Non-Reactive	0	0	0	4*
Non-reactive	Reactive	0	0	0	7
Reactive	Reactive	100	100	100	33
Total		100	100	100	48
McNemar's Test, p-value (two-sided, α=0.05)		1.0	1.0	1.0	0.55

* Four discordant samples that were non-reactive with the **cobas**® MPX test at neat had titers below the limit of quantification for the HIV-2 Quant PCR assay (< 100 copies/mL, Hopital Bichat-Claude Bernard) and were non-reactive on both assays at 1:6 dilution.

Table 31 Correlation of seropositive samples (1:6 dilution)

Methods		Individual viral target results				
cobas® TaqScreen MPX Test, v2.0	**cobas® MPX**	**HIV-1 M**	**HBV**	**HCV**	**HIV-2**	**HIV-1 O**
Non-reactive	Non-reactive	0	0	0	39	0
Reactive	Non-Reactive	0	0	0	6*	0
Non-reactive	Reactive	0	0	0	8	0
Reactive	Reactive	100	100	100	46	13
Total		100	100	100	99	13
McNemar's Test, p-value (two-sided, α=0.05)		1.0	1.0	1.0	0.79	1.0

* Six discordant samples generated non-reactive by **cobas**® MPX test. Three of the six discordant specimens that were non-reactive with the **cobas**® MPX test at 1:6 dilutions were below the limit of quantification (<100 copies/mL) for the HIV-2 Quant PCR assay (Hopital Bichat-Claude Bernard). The 3 remaining specimens also had low titers (27.7 IU/mL, below level of quantification for HIV-2 RNA LDT and 150 copies/mL for the HIV-2 Quant PCR assay) and all 3 of these samples were reactive on both assays at neat.

Whole system failure

The whole system failure rate for the **cobas**® MPX test 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 the **cobas**® MPX test 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%].

Cadaveric samples

Sensitivity

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 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 32.

Table 32 Summary of reactivity rate in cadaveric and living donor samples in EDTA plasma

Analyte	Cadaveric sample	Living donor sample
	% Reactive (Number of reactive /Number of samples tested)	**% 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 ($\alpha = 0.05$)	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 sample 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 and 60 individual serum samples. The study was 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 samples from EDTA plasma and serum 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 ($\alpha = 0.05$) as summarized in Table 33.

Table 33 Summary of specificity in cadaveric and living donor samples in EDTA plasma and serum

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%
	Living donor	60	60	100%	94.0% - 100%
Serum	Cadaveric donor	61	61	100%	94.1% - 100%
	Living donor	60	60	100%	94.0% - 100%
Overall results using Fisher's Exact Test ($\alpha = 0.05$)		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 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 a total of 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 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.

The **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 34.

Table 34 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 (α=0.05)
HIV-1 Group M	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	100.0% (118/118)	96.9%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
HIV-1 Group O	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	100.0% (117/117)	96.9%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	99.2% (118/119)	95.4%	100.0%	p-value=0.4979
		Living donor	100.0% (120/120)	97.0%	100.0%	
HIV-2	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	98.3% (118/120)	94.1%	99.8%	p-value=0.4979
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	99.2% (118/119)	95.4%	100.0%	p-value=0.4979
		Living donor	100.0% (120/120)	97.0%	100.0%	

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 (α=0.05)
HCV	1	Cadaveric	98.3% (118/120)	94.1%	99.8%	p-value=0.4979
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	98.3% (118/120)	94.1%	99.8%	p-value=0.4979
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	97.5% (115/118)	92.7%	99.5%	p-value=0.1203
		Living donor	100.0% (120/120)	97.0%	100.0%	
HBV	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value=1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	100.0% (118/118)	96.9%	100.0%	p-value=1.0000
		Living donor	99.2% (119/120)	95.4%	100.0%	

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 35 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 35 Test results summarized by site/instrument, lot, day, and batch (positive panel members)

		Site/Instrument		Lot		Day		Batch	
Viral Target	**Viral Concen-tration**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% 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)
		2	84.7% (50/59)	2	88.3% (53/60)	2	77.1% (27/35)	2	81.1% (73/90)
		3	81.7% (49/60)	3	78.0% (46/59)	3	83.3% (30/36)		
						4	83.3% (30/36)		
						5	77.8% (28/36)		
	~1 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	97.2% (35/36)	2	97.8% (88/90)
		3	96.7% (58/60)	3	96.7% (58/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						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)
		2	76.7% (46/60)	2	78.3% (47/60)	2	77.8% (28/36)	2	86.7% (78/90)
		3	85.0% (51/60)	3	78.3% (47/60)	3	77.8% (28/36)		
						4	86.1% (31/36)		
						5	86.1% (31/36)		
	~1 x LoD	1	98.3% (59/60)	1	98.3% (59/60)	1	94.4% (34/36)	1	95.6% (86/90)
		2	100.0% (60/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	98.9% (89/90)
		3	93.3% (56/60)	3	96.7% (58/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	94.4% (34/36)		
	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
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)
		2	76.7% (46/60)	2	79.7% (47/59)	2	69.4% (25/36)	2	79.8% (71/89)
		3	73.3% (44/60)	3	71.2% (42/59)	3	75.0% (27/36)		
						4	71.4% (25/35)		
						5	80.0% (28/35)		
	~1 x LoD	1	96.7% (58/60)	1	100.0% (60/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	98.3% (59/60)	2	96.7% (58/60)	2	100.0% (36/36)	2	96.7% (87/90)
		3	100.0% (60/60)	3	98.3% (59/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	97.2% (35/36)		
	~3 x LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						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)
		2	70.7% (41/58)	2	76.7% (46/60)	2	77.8% (28/36)	2	74.2% (66/89)
		3	85.0% (51/60)	3	74.1% (43/58)	3	69.4% (25/36)		
						4	91.2% (31/34)		
						5	80.6% (29/36)		
	~1 × LoD	1	100.0% (60/60)	1	98.3% (59/60)	1	97.2% (35/36)	1	100.0% (90/90)
		2	96.7% (58/60)	2	98.3% (59/60)	2	100.0% (36/36)	2	97.8% (88/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	97.2% (35/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3 × LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (59/59)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (89/89)
		3	100.0% (60/60)	3	100.0% (59/59)	3	100.0% (36/36)		
						4	100.0% (35/35)		
						5	100.0% (36/36)		
HBV	~0.5 × LoD	1	80.0% (48/60)	1	80.0% (48/60)	1	80.6% (29/36)	1	72.2% (65/90)
		2	78.3% (47/60)	2	73.3% (44/60)	2	80.6% (29/36)	2	82.2% (74/90)
		3	73.3% (44/60)	3	78.3% (47/60)	3	75.0% (27/36)		
						4	77.8% (28/36)		
						5	72.2% (26/36)		
	~1 × LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						5	100.0% (36/36)		
	~3 × LoD	1	100.0% (60/60)	1	100.0% (60/60)	1	100.0% (36/36)	1	100.0% (90/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	100.0% (36/36)		
						4	100.0% (36/36)		
						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 36). The pool specificity was 99.91% (10,524/10,534; 99.83%-99.95%) (Table 37). 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 36 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 37 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 38). 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 39).

Table 38 Clinical specificity of the **cobas**® MPX – donation level

Parameter	Total Number of Status-Negative Donations	cobas® MPX Result		Estimate in Percent (95% Exact CI)
		Reactive	Non-Reactive	
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 39 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 40). One of the three HBV donors was determined to be a NAT HBV yield case.

Table 40 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 test 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 99.994% (95% Confidence Interval: 99.988% to 99.997%). 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 99.976% (95% Confidence Interval: 99.947% to 99.989%).

Studies in high risk populations

Third-party vendors collected samples from individuals at high risk for infection with HIV, HCV, or HBV. High-risk factors were included, but were not limited to, a history of incarceration; history of a diagnosis of a sexually-transmitted disease; history of multiple sex partners; use of injection drugs; diagnosed with or treated for HIV; and diagnosed with or treated for hepatitis. Some sample contributors indicated more than one risk factor. A total of 510 samples from a high risk population were distributed approximately evenly across three test sites and tested with **cobas**® MPX and **cobas**® TaqScreen MPX incorporating CAS.

All samples were prepared as panels. The diluted samples were manually diluted with pooled human plasma confirmed to be negative for HIV-1/2, HCV, and HBV. At the testing sites, samples were tested neat with both **cobas**® MPX and **cobas**® TaqScreen incorporating CAS (for target resolution), as per the Standard Specimen Processing Procedure recommended in the **cobas**® TaqScreen MPX Test Package Insert. Samples were also tested dilute to simulate pools of six with both **cobas**® MPX and **cobas**® TaqScreen. CAS was not performed on dilute samples.

The 510 neat samples generated results from **cobas**® MPX and the **cobas**® TaqScreen MPX Test which included 179 samples reactive (for one or more targets) on **cobas**® MPX (35.1%); and 181 samples that were reactive on **cobas**® TaqScreen MPX Test (35.5%). 488 (95.7%) samples that showed results concordant between **cobas**® MPX and **cobas**® TaqScreen MPX Test, while 22 (4.3%) of samples produced results that were discordant between **cobas**® MPX and **cobas**® TaqScreen MPX Test.

For the 510 high-risk neat samples, **cobas**® MPX correctly identified the presence or absence of viral target 97.0%

(495/510) of the time, compared to CAS or alternative NAT (NGI; National Genetics Institute) test results. For the 3% of samples for which **cobas**® MPX did not correctly identify the presence or absence of viral target, **cobas**® MPX incorrectly detected a viral target in samples that did not contain a viral target 1.8% (9/510) of time (false reactive result) and failed to detect a viral target in samples that contained a target 1.2% (6/510) of the time (false non-reactive result). These results are summarized in Table 41.

Table 41 Correct versus incorrect identification of virus – neat

	cobas® MPX Result[a]	**%**	**Total Correct**
True reactives	**170**	**97.0%**	**495**
True non-reactives	**325**		
False reactives	9	1.8%	15
False non-reactives	6	1.2%	
Total	510	100.0%	510

a Final status (as compared with CAS or alternative NAT [NGI testing] results).

Note: Correct identification = True reactive and true non-reactive results (shown in bold type).

Of 510 dilute samples tested, 153 samples were reactive on **cobas**® MPX (30.0%), compared to 151 samples that were reactive on **cobas**® TaqScreen MPX Test (29.6%). Of the 510 dilute samples, 484 (94.9%) samples showed results concordant between **cobas**® MPX and **cobas**® TaqScreen MPX Test; and 26 (5.1%) samples showed results discordant between **cobas**® MPX and **cobas**® TaqScreen MPX Test.

cobas® MPX correctly identified the viral target 96.7% (492/509) of the time (509 dilute samples excludes one sample for which no NGI result was obtained). For the 3.4% of samples for which **cobas**® MPX did not correctly identify the viral target, **cobas**® MPX incorrectly detected a viral target in samples that did not contain a viral target 1.2% (6/509) of time (false reactive result) and failed to detect a viral target in samples that contained a target 2.2% (11/509) of the time (false non-reactive result). These results are summarized in Table 42.

Table 42 Correct versus incorrect identification of virus – dilute

	cobas® MPX Result[a]	**%**	**Total correct**
True reactives	**147**	**96.7**	**492**
True non-reactives	**345**		
False reactives	6	1.2	17
False non-reactives	11	2.2	
Total	509[b]	100	509[b]

[a] Final status (as compared with CAS or alternative NAT [NGI testing] results), which was performed on neat aliquot.

[b] Excludes one sample for which no NGI result was obtained.

Note: Correct identification = True reactive and true non-reactive results (shown in bold type).

Clinical sensitivity

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. Four 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. Only neat, not dilute, samples, were tested with the licensed CAS Tests per the Standard Specimen Processing Procedure recommended in the **cobas**® TaqScreen MPX Test Package Insert. Table 43 compares the sensitivities of **cobas**® MPX and **cobas**® TaqScreen Test Results for HIV, HCV, and HBV Known Positive Samples.

The overall clinical sensitivity of the **cobas**® MPX was 100.0% (2,549/2,549) for neat known positive samples and 100.0% (2,555/2,555) for dilute (1:6) known positive samples. The overall clinical sensitivity of the **cobas**® TaqScreen MPX Test was 99.9% (2,523/2,524) for neat known positive samples and 99.8% (2,559/2,563) for dilute (1:6) known positive samples. The overall positive percent agreement (PPA) across all known positive samples in this study between **cobas**® MPX and the **cobas**® TaqScreen MPX Test was 100.0% for both neat and dilute samples (Table 43).

Table 43 Comparison of the sensitivities of **cobas**® MPX and **cobas**® TaqScreen Test results for HIV, HCV, and HBV known positive samples

		Sensitivity in Known Positive Samples[a]		Difference (cobas® MPX Result − cobas® TaqScreen MPX Test)	
Dilution	**Target Virus**	**cobas® MPX Result**	**cobas® TaqScreen MPX Test**	**Estimate**	**95% Confidence Interval**
Neat	**Overall**	100.00% (2,549/2,549)	99.96% (2,523/2,524)	0.04%	(-0.04%, 0.12%)
	HIV	100.00% (1,006/1,006)	99.90% (1,007/1,008)	0.10%	(-0.10%, 0.29%)
	HCV	100.00% (1,015/1,015)	100.00% (1,014/1,014)	0.00%	Not applicable
	HBV	100.00% (528/528)	100.00% (502/502)	0.00%	Not applicable
1:6	**Overall**	100.00% (2,555/2,555)	99.84% (2,559/2,563)	0.16%	(0.00%, 0.31%)
	HIV	100.00% (1,006/1,006)	99.60% (1,005/1,009)	0.40%	(0.01%, 0.78%)
	HCV	100.00% (1,016/1016)	100.00% (1,016/1,016)	0.00%	Not applicable
	HBV	100.00% (533/533)	100.00% (538/538)	0.00%	Not applicable

[a] Only known positive samples with valid test results were included in the sensitivity analysis.

HIV NAT-positive population

The 1,015 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 fifteen HIV dilute 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 (100.0%) HIV neat samples and 1,006 of 1,006 (100.0%) HIV dilute 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 dilute samples. The PPA between **cobas**® MPX and the **cobas**® TaqScreen MPX Test for neat and dilute HIV samples was 100.0% and 100.0% respectively.

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 dilute 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. The PPA between **cobas**® MPX and the **cobas**® TaqScreen MPX Test for neat and dilute HCV samples was 100.0% and 100.0% respectively.

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 (100.0%) HBV-positive neat samples and 533 of 533 (100.0%) 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 dilute samples. The PPA between **cobas**® MPX and the **cobas**® TaqScreen MPX Test for neat and dilute HBV samples was 100.0% and 100.0% respectively.

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 44, for a clinical sensitivity of 100.0% relative to serology.

Table 44 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)**		**Total**
	Reactive	**Non-Reactive**	
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 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 45, 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 46.

Table 45 Comparison of overall reactivity for HIV-2 seropositive samples (neat)

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

Table 46 Comparison of overall reactivity for HIV-2 seropositive samples (1:6 dilution)

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

Confirmation of serology results

Data from the Known Positive Study included 2,555 known-positive samples, each with nucleic acid test (NAT)-confirmed infection with either HIV, HCV, or HBV and serology test results. Supplemental serology test results were also known for 1,771 (69.3%) samples. The correct **cobas**® MPX result, defined as reactive for the viral target for which the specimen was known to be positive (e.g., HIV, HCV, or HBV), was compared to the supplemental serology results. The percentages of correct results (sensitivity estimate) for **cobas**® MPX were calculated for each target virus and overall, with associated 95% confidence intervals (CI). **cobas**® MPX correctly identified 1,771 of 1,771 (100.0%) of specimens with reactive serology and supplemental serology results. Table 47 shows the reactivity of **cobas**® MPX for each viral target analyte, compared to the known viral target serology and supplemental serology test result, as well as an estimate of sensitivity and 95% CI overall and for each viral target.

Table 47 Sensitivity of the **cobas**® MPX for neat known positive specimens with confirmatory serology results

Dilution	Test	Target Virus	Total Known Positive Specimens*	Number Reactive By Test	Sensitivity Estimate	95% Score CI
Neat	**MPX8800**	**Overall**	1,771	1,771	100.00%	(99.78%, 100.00%)
Neat	**MPX8800**	**HIV**	496	496	100.00%	(99.23%, 100.00%)
Neat	**MPX8800**	**HCV**	747	747	100.00%	(99.49%, 100.00%)
Neat	**MPX8800**	**HBV**	528	528	100.00%	(99.28%, 100.00%)

* Only known positive specimens with valid **cobas**® MPX results from neat samples and confirmatory serology results are included in this sensitivity analysis.

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 48 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)	D Distributed by	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	IVD In vitro diagnostic medical device	Temperature limit
REF Catalogue number	LLR Lower Limit of Assigned Range	TDF Test Definition File
CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	Male	This way up
	Manufacturer	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	CONTROL − Negative control	UDI Unique Device Identification
Consult instructions for use	NON STERILE Non sterile	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	Patient Name	Urine Fill Line Urine Fill Line
CONTENT Contents of kit	# Patient number	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Peel here	Use-by date
Date of manufacture	CONTROL + Positive control	
Device for near-patient testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	
Device for self-testing		

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributors

Table 49 Manufacturer and distributors

	Manufactured in the United States	
	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany www.roche.com	
	Made in USA	
Distributed by	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-800-5973)

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, and foreign equivalent patents of each.

COBAS, COBAS OMNI, COBAS P, AMPERASE, AMPLIPREP, TAQMAN, and TAQSCREEN are trademarks of Roche.

The trademark "Armored RNA®" is owned by Asuragen, Inc. and Cenetron Diagnostics, Ltd.

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

Certain components of this product are covered by one or more US Patents and their foreign equivalents issued to Novartis Vaccines and Diagnostics, Inc. and licensed to Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd.

See http://www.roche-diagnostics.us/patents

Copyright

CE 0123

IVD

References

1. Global report. UNAIDS Report on the global AIDS epidemic. 2012.
2. Tebit DM, Arts EJ. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. Lancet Infect Dis. 2011;11:45-56.
3. Papathanasopoulos MA, Hunt GM, Tiemessen CT. Evolution and diversity of HIV-1 in Africa--a review. Virus Genes. 2003;26:151-163.
4. McCutchan FE. Global epidemiology of HIV. J Med Virol. 2006;78 Suppl 1:S7-S12.
5. Barin F, M'Boup S, Denis F, et al. Serological evidence for virus related to simian T-lymphotropic retrovirus III in residents of west Africa. Lancet. 1985;2(8469-70):1387-1389.
6. Clavel F, Guétard D, Brun-Vézinet F, Sinka K. Isolation of a new human retrovirus from West African patients with AIDS. Science. 1986;233(4761):343-346.
7. Dougan S, Patel B, Tosswill JH, et al. Diagnoses of HIV-1 and HIV-2 in England, Wales, and Northern Ireland associated with west Africa. Sex Transm Infect. 2005;81:338-341.
8. Matheron S, Mendoza-Sassi G, Simon F, Olivares R, Coulaud JP, Brun-Vezinet F. HIV-1 and HIV-2 AIDS in African patients living in Paris. AIDS. 1997;11:934-936.
9. Valadas E, Franc L, Sousa S, Antunes F. 20 years of HIV-2 infection in Portugal: trends and changes in epidemiology. Clin Infect Dis. 2009;48:1166-1167.
10. Dietrich U, Maniar JK, Rüubsamen-Waigmann H. The epidemiology of HIV in India. Trends Microbiol. 1995;3:17-21.
11. Solomon S, Kumarasamy N, Ganesh AK, et al. Prevalence and risk factors of HIV-1 and HIV-2 infection in urban and rural areas in Tamil Nadu, India. Int J STD AIDS. 1998;9:98-103.
12. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989;244(4902):359-362.
13. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57:1333-1342.
14. Averhoff FM, Glass N, Holtzman D. Global burden of hepatitis C: considerations for healthcare providers in the United States. Clin Infect Dis. 2012;55 Suppl 1:S10-S15.
15. Trepo C, Pradat P. Hepatitis C virus infection in Western Europe. J Hepatol. 1999:31 Suppl 1: 80-83.
16. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J Viral Hepat. 2009;16:650-658.
17. Chisari FV, Ferrari C. Viral Hepatitis. In: Nathanson N, editor. Viral Pathogenesis. 1st ed. Philadelphia:Lippincott - Williams & Wilkins, 1997; pp. 745-778.
18. Hollinger FB, Liang TJ. Hepatitis B Virus. In: Knipe DM, Howley PM, Griffin DE, et al., editors. Fields' Virology, vol. 1. 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2001: pp. 2971-3036.
19. WHO, Global prevalence of Hepatitis B Virus Infection. 2013; www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/index1.html

20. WHO campaigns, World Hepatitis Day, More must be done to stop this silent killer. Available at http://www.who.int/campaigns/hepatitis-day/2013/en/index.html

21. Perkins HA, Busch MP. Transfusion-associated infections: 50 years of relentless challenges and remarkable progress. Transfusion. 2010;50:2080-2099.

22. Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepatitis C and HIV transfusion-transmitted infections in the 21st century. Vox Sang. 2011;100:92-98.

23. Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion. 2009;49:2454-2489.

24. Hourfar MK, Jork C, Schottstedt V, et al.; German Red Cross NAT Study Group. Experience of German Red Cross blood donor services with nucleic acid testing: results of screening more than 30 million blood donations for human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus. Transfusion. 2008;48:1558-1566.

25. Roth WK, Busch P, Schuller A, et al. International survey on NAT testing of blood donations: expanding implementation and yield from 1999 to 2009. Vox Sang. 2012;102:82-90.

26. Zou S, Stramer SL, Notari EP, et al. Current incidence and residual risk of hepatitis B infection among blood donors in the United States. Transfusion. 2009;49:1609-1620.

27. Zou S, Dorsey KA, Notari EP, et al. Prevalence, incidence, and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing. Transfusion. 2010;50:1495-1504.

28. Raimondo G, Allain JP, Brunetto MR, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008;49:652-657.

29. Linauts S, Saldanha J, Strong DM. PRISM hepatitis B surface antigen detection of hepatitis B virus minipool nucleic acid testing yield samples. Transfusion. 2008;48:1376-1382.

30. Phikulsod S, Oota S, Tirawatnapong T, et al.; Working Group for NAT Study in Thai Blood Donations. One-year experience of nucleic acid technology testing for human immunodeficiency virus Type 1, hepatitis C virus, and hepatitis B virus in Thai blood donations. Transfusion. 2009;49:1126-1135.

31. Stramer SL, Wend U, Candotti D, et al. Nucleic acid testing to detect HBV infection in blood donors. N Engl J Med. 2011;364:236-247.

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

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

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

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

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

37. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.

38. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

39. HIV-2 RNA International Standard (NIBSC code 08/150): http://www.nibsc.ac.uk/products/biological_reference_materials/product_catalogue/detail_page.aspx?catid=08/150.

Document revision

Document Revision Information	
Doc Rev. 5.0 (Mfg-US) 05/2021	Updated hazard warnings. Updated **trademarks and patents** section. Updated distributors addresses. Inserted Rx Only symbol on first page. Added Made in statement. Updated the harmonized symbol page. Added **Technical support** section. Please contact your local Roche Representative if you have any questions.