cobas® HIV-1

Quantitative nucleic acid test for use on the cobas® 5800/6800/8800 systems

For in vitro diagnostic use

cobas® HIV-1	P/N: 09040803190
For use on the cobas® 5800 system	
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 09051554190
For use on the cobas® 6800/8800 systems	
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 06997767190 or P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 07002220190 or P/N: 09051554190

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Intended use

cobas® HIV

cobas®HIV-1 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) in EDTA plasma or from a **cobas**®Plasma Separation Card (PSC) dried plasma spot of HIV-1-infected individuals.

This test is intended for use in conjunction with clinical presentation and other laboratory markers for the clinical management of HIV-1-infected patients. This test can be used for confirmation of HIV-1 infection in antibody reactive individuals and to assess patient prognosis by measuring the baseline HIV-1 level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

cobas® HBV/HCV/HIV-1 Control Kit

cobas®HBV/HCV/HIV-1 Control Kit is intended for use as a positive run/batch control on the **cobas**® 5800/6800/8800 systems with the **cobas**®HBV, **cobas**®HCV, and **cobas**®HIV-1 tests.

Summary and explanation of the test

Background

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). After seroconversion, infected individuals typically enter a clinically stable, relatively asymptomatic phase that can last for years. The asymptomatic period is characterized by persistent plasma viremia at set points determined by host genetics and a gradual depletion of CD4+ T lymphocytes. Although virus levels in the peripheral blood are relatively low during the asymptomatic phase of the infection, virus replication and clearance appear to be dynamic processes in which high rates of virus production and infection of CD4+ cells are balanced by high rates of virus clearance, death of infected cells and replenishment of CD4+ cells, resulting in relatively stable levels of both plasma viremia and CD4+ cells for approximately 8 years in the average person living with HIV.

Quantitative measurements of HIV viremia in the plasma have shown that higher virus levels are correlated with more rapid clinical progression of HIV disease.[1,2] Furthermore, nearly two decades of clinical research have established that reductions in plasma virus levels with the use of antiretroviral therapy (ART) significantly decrease the risk of clinical progression, including death, development of AIDS, opportunistic infections, and HIV-associated morbidity.[3] HIV viral load is also predictive of the risk of transmission of HIV, and randomized controlled clinical trials have established that early initiation of ART with suppression of the viral load reduces HIV transmission by 96%.[4]

Rationale for HIV-1 testing

At the moment, a great number of antiretroviral drugs are available, targeting the viral protease, integrase, envelope and reverse transcriptase. Genotypic analyses of viruses of different clades have shown naturally-occurring and drug-induced nucleotide changes, polymorphisms and secondary mutations within reverse transcriptase, integrase and protease regions of the HIV-1 pol gene. Resistance testing has become an important

diagnostic tool in the management of HIV-1 infections and is initiated once a patient's viral load has risen to a level detectable by sequencing assays. Viral load monitoring has been shown to reduce the risk of drug resistance, and viral load is clinically considered to be a sentinel indicator of active viral replication heralding viral evolution in patients on therapy.[5,6] Multiple national and international guidelines therefore recommend that HIV-1 viral load should be measured.[3,7-9]

For a number of years the guidelines indicated that a key goal of treatment is suppression of the HIV-1 viral load below the limit of detection of a test (e.g., 50 copies/mL). In 2011, the United States guidelines began to indicate that viral load results of up to 200 copies/mL in patients on ART may not be indicative of treatment failure as some patients may experience low level viremia without virological breakthrough.[3] In Europe, the guidelines continue to state that viral load results should be suppressed below 50 copies/mL.[7] Low-end differences between viral load tests may lead to important differences in the clinical interpretation of viral load results when monitoring treatment response,[10,11] as the goal of treatment is suppression of virus to a level below which drug resistance is least likely to emerge, a level that has not been fully defined.[12] In July 2013, the WHO also began recommending the use of viral load testing in resource-limited settings, defining 1000 copies/mL as the threshold for defining virological failure requiring treatment management decisions. WHO also now recommends the use of dried spot specimens to expand the reach of viral load testing in resource limited settings without ready access to phlebotomy services or robust EDTA plasma sample transportation capabilities.[8] A PSC dried plasma spot, which also stabilizes the HIV RNA in dried plasma can improve viral load testing coverage in these settings by enabling sample transportation over longer distances and harsher environmental conditions than EDTA plasma.

In addition to monitoring response to therapy, guidelines recommend the use of viral load assessment for determining whether a patient whose CD4+ cell count is > 500 cells/mm^3 (viral load > 100,000 copies/mL) should initiate ART and for ensuring that drug resistance sequencing will be successful in appropriate patients (patients with a viral load > 1,000 copies/mL or suboptimal viral load response to ART). The use of viral load assessment should be performed in the prenatal setting to determine whether Caesarean section delivery is needed to prevent mother-to-child transmission of HIV infection (for pregnant women with a viral load > 1,000 copies/mL).

Explanation of the test

cobas®HIV-1 is a quantitative test performed on the **cobas**®5800 system, **cobas**®6800 system, or **cobas**®8800 system. **cobas**®HIV-1 enables the detection and quantitation of HIV-1 RNA in EDTA plasma or from a PSC dried plasma spot of infected patients. Two probes are used to detect and quantify, but not discriminate group M subtypes of HIV-1 and of HIV-1 group O and group N. The viral load is quantified against a non-HIV-1 armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample processing. The RNA-QS functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to HIV-1 2nd WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HIV-1 2nd WHO International Standard (NIBSC code 97/650).

Principles of the procedure

cobas®HIV-1 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**®5800 system is designed as one integrated instrument. The **cobas**®6800/8800 systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**®5800 or **cobas**®6800/8800 system softwares which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HIV-1 RNA detected, a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples, external controls and added armored RNA (RNA-QS) molecules is simultaneously extracted. In summary, 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 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 sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HIV-1 genome. The HIV-1 gag gene and the HIV-1 LTR region (dual target) are amplified by **cobas**®HIV-1. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[13-15] Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**®HIV-1 master mix contains two detection probes specific for the HIV-1 target sequences and one for the RNA-QS. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target and RNA-QS in two different target channels.[16,17] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease 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 increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS, respectively.

Reagents and materials

cobas® HIV-1 reagents and controls

The materials provided for **cobas®**HIV-1 can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 5, Table 13 to Table 15.

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 5.

Table 1 cobas® HIV-1

cobas® HIV-1 Store at 2-8°C 192 test cassette (P/N 09040803190)		
Kit components	**Reagent ingredients**	**Quantity per kit 192 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.	22.3 mL
RNA Quantitation Standard (**RNA-QS**)	Tris buffer, < 0.05% EDTA, <0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), <0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HIV-1 Master Mix Reagent 2 (HIV-1 MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the HIV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 **cobas**® HBV/HCV/HIV-1 Control Kit

cobas® **HBV/HCV/HIV-1 Control Kit** Store at 2–8°C For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09040773190) For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 06997767190 and P/N 09040773190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+)C)	< 0.001% HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein armored, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV 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**	5.2 mL (8 x 0.65mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C)	< 0.001% high titered synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV 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**	5.2 mL (8 x 0.65mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (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 For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09051554190) For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 07002220190 and P/N 09051554190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV- 1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/ vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

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)	43% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes serious 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

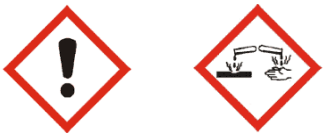
* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas® Specimen Pre-Extraction Reagent

Note: This reagent is optional and should only to be used in conjunction with the PSC to generate dried plasma spot samples. See PSC Method Sheet ms_09411763190.

Table 5 **cobas**® Specimen Pre-Extraction Reagent

cobas® Specimen Pre-Extraction Reagent Store at 2-8°C (P/N 08064695190)			
Reagent	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
cobas® Specimen Pre-Extraction Reagent (SPER)	28% (w/w) guanidine thiocyanate**, 6% (w/v) polydocanol**, 1% (w/v) dithiothreitol**, dihydro sodium citrate	600 mL (15 x 40 mL)	 **DANGER** H302: Harmful if swallowed. 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. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. 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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 6, Table 7 and Table 8. **cobas**®Specimen Pre-Extraction Reagent, used in the PSC dried plasma spot workflow, shall be stored and handled as specified in Table 10 and Table 11. PSC storage and handling requirements are specified in the **PSC Method Sheet ms_09411763190.**

When reagents are not loaded on the **cobas**®5800 and **cobas**®6800/8800 systems, store them at the corresponding temperature specified in Table 6.

Table 6 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® HIV-1	2–8°C
cobas® HBV/HCV/HIV-1 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

Reagent handling requirements for cobas® 5800 system and cobas® 6800/8800 systems

Reagents loaded onto the **cobas**®5800 system or **cobas**®6800/8800 systems are stored at appropriate temperatures, their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 7, Table 8 and Table 9 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit uses information for assay specific reagents is accessible through the system user interface.

Table 7 Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

Reagent	Open-kit stability	Number of kit uses	On-board stability
cobas® HIV-1	90 days from first usage	40	36 days from loading
cobas® HBV/HCV/HIV-1 Control Kit	single use vial	8	36 days from loading
cobas® NHP Negative Control Kit	single use vial	16	36 days from loading

Table 8 Reagent expiry conditions monitored and enforced by the **cobas®** 6800/8800 systems

Reagent	Open-kit stability	Number of kit uses	On-board stability (outside on board refrigerator)
cobas® HIV-1	90 days from first usage	40	40 hours from loading
cobas® HBV/HCV/HIV-1 Control Kit	single use vial	8	8 hours from loading
cobas® NHP Negative Control Kit	single use vial	16	10 hours from loading

Table 9 shows the open-kit stability of the **cobas®omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

Table 9 cobas® omni reagent expiry condition enforced by the **cobas®** 5800/6800/8800 systems

Reagent	Open-kit stability
cobas® omni Lysis Reagent	30 days from loading
cobas® omni MGP Reagent	30 days from first usage
cobas® omni Specimen Diluent	30 days from loading
cobas® omni Wash Reagent	30 days from loading

Store **cobas®**Specimen Pre-Extraction Reagent (used in PSC workflow) at the corresponding temperature specified in Table 10.

Table 10 cobas® Specimen Pre-Extraction Reagent storage

Reagent	Storage temperature
cobas® Specimen Pre-Extraction Reagent	2–8°C

cobas®Specimen Pre-Extraction Reagent is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days when stored at 2-8°C including cumulative 13 hours at 30°C or until expiration date, whichever comes first, as specified in Table 11.

Table 11 cobas® Specimen Pre-Extraction Reagent expiry conditions

Reagent	Open-kit stability	Stability at 30°C outside refrigerator (cumulative time)
cobas® Specimen Pre-Extraction Reagent	30 days from first usage	13 hours

Additional materials required for cobas® 5800/6800/8800 systems

Table 12 Material for use on **cobas**® 5800/6800/8800 systems

Material	P/N
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190

Table 13 Consumables for use on **cobas**® 5800 system*

Material
cobas® **omni** Processing Plate 24
cobas® **omni** Liquid Waste Plate 24
cobas® **omni** Amplification Plate 24
Tip CORE TIPS with Filter, 1ml
Tip CORE TIPS with Filter, 300µL
cobas® **omni** Liquid Waste Container
Solid Waste Bag or Solid Waste Bag With insert

Table 14 Consumables for use on **cobas**® 6800/8800 systems*

Material
cobas® **omni** Processing Plate
cobas® **omni** Amplification Plate
cobas® **omni** Pipette Tips
cobas® **omni** Liquid Waste Container
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer

*For Part Numbers please refer to the **cobas**® 6800/8800 systems User Assistance.

Table 15 Other materials and consumables required for PSC dried plasma spot application only

Materials
cobas® Plasma Separation Card (P/N 09411763190)*
Sterile or disposable forceps or tweezers**
3 x 140 µL EDTA coated capillaries with dispenser
Single Use lancing device (e.g. Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)*
Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams) (for PSC storage and delivery, see **PSC Method Sheet ms_09411763190** for more information)
Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)
Pipette (e.g., Multistepper pipette)
Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Greiner Bio-one Cryo.s™) with caps

* See **PSC Method Sheet ms_09411763190** for more information about the PSC sample collection.

**To prevent cross-contamination, use only one pair of forceps for each patient! The usage of metal forceps that are autoclaved after single use is recommended.

Instrumentation and software required

The **cobas**®5800 software, the **cobas**®6800/8800 systems software, **cobas**®HIV-1 analysis package (ASAP) and dried PSC plasma spot application analysis package (**cobas**®HIV-PSC ASAP) for the **cobas**®5800/6800/8800 systems shall be installed.

For **cobas**®5800 and **cobas**®6800/8800 systems with software 2.0 or higher, the x800 Data Manager software and PC (or server) will be provided with the system.

For the **cobas**® 6800/8800 systems with software version 1.4, the Instrument Gateway (IG) server will be provided with the systems.

Table 16 Instrumentation

Equipment	**P/N**
cobas® 5800 system	08707464001
cobas® 6800 system	05524245001 and 09575154001
cobas® 8800 system	05412722001 and 09575146001
Sample Supply Module for **cobas**® 6800/8800 systems	06301037001 and 09936882001

Refer to the **cobas**®5800 System or **cobas**®6800/8800 Systems User Assistance for additional information.

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.
- **cobas**®HIV-1 has not been evaluated for use as a screening test for the presence of HIV-1 in blood or blood products.
- All patient 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.[18,19] Only personnel proficient in handling infectious materials and the use of **cobas**®HIV-1 and **cobas**®5800/6800/8800 systems 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 or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
    - If spillage of PSC dried plasma spot samples in **cobas**®Specimen Pre-Extraction Reagent (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- **cobas**®HBV/HCV/HIV-1 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.
- Refer to **PSC Method Sheet ms_09411763190** for additional warnings and precautions.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- **cobas**®Specimen Pre-Extraction Reagent is light sensitive and shipped in light protective bottles.
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- 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.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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®**HIV-1 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 or **cobas®**Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium or potassium hypochlorite solution. This mixture can produce a highly toxic gas.
- 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®**HIV-1 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 or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas®**5800 or **cobas®**6800/8800 instrument, follow the instructions in the **cobas®** 5800 or **cobas®**6800/8800 systems User Assistance to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

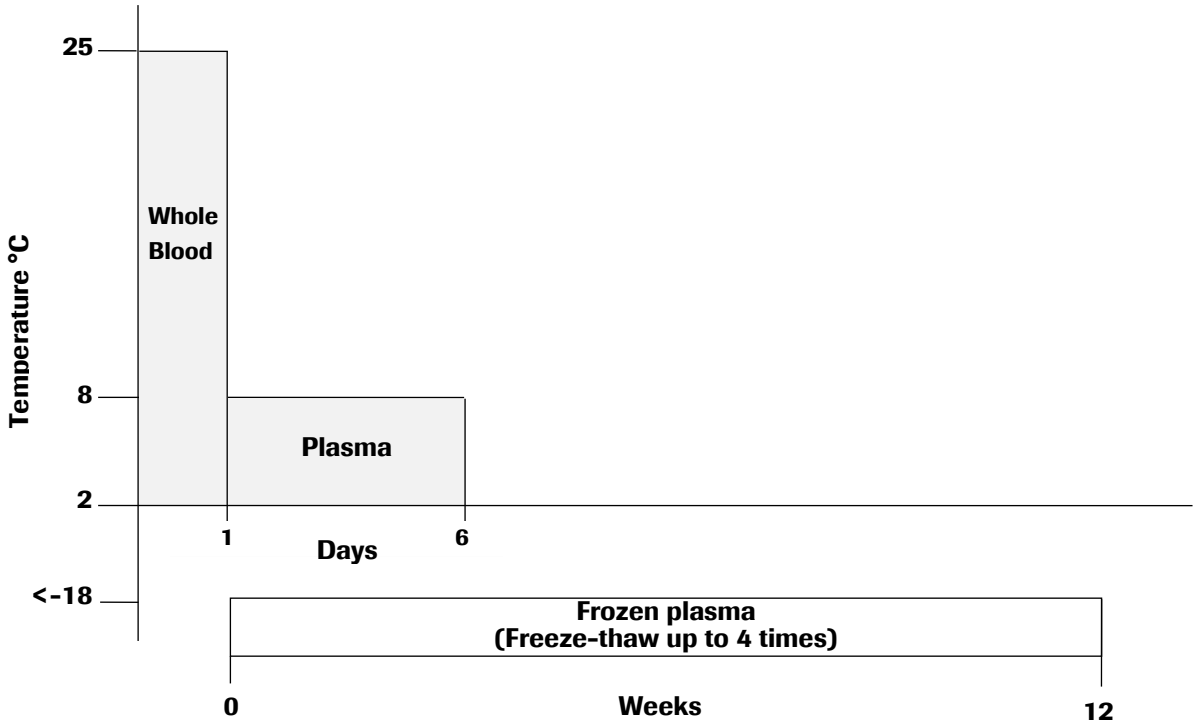
If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then briefly mix (e.g., vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

Note: After centrifugation, if there is potential that cells have re-suspended into the plasma, consider re-centrifugation before processing on the instrument.

EDTA plasma samples

- Whole blood should be collected in BD Vacutainer®PPT Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in EDTA tubes may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation EDTA plasma samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. Long-term storage for up to 6 months at -75°C (+/- 15°C) has been evaluated.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 EDTA plasma sample storage conditions



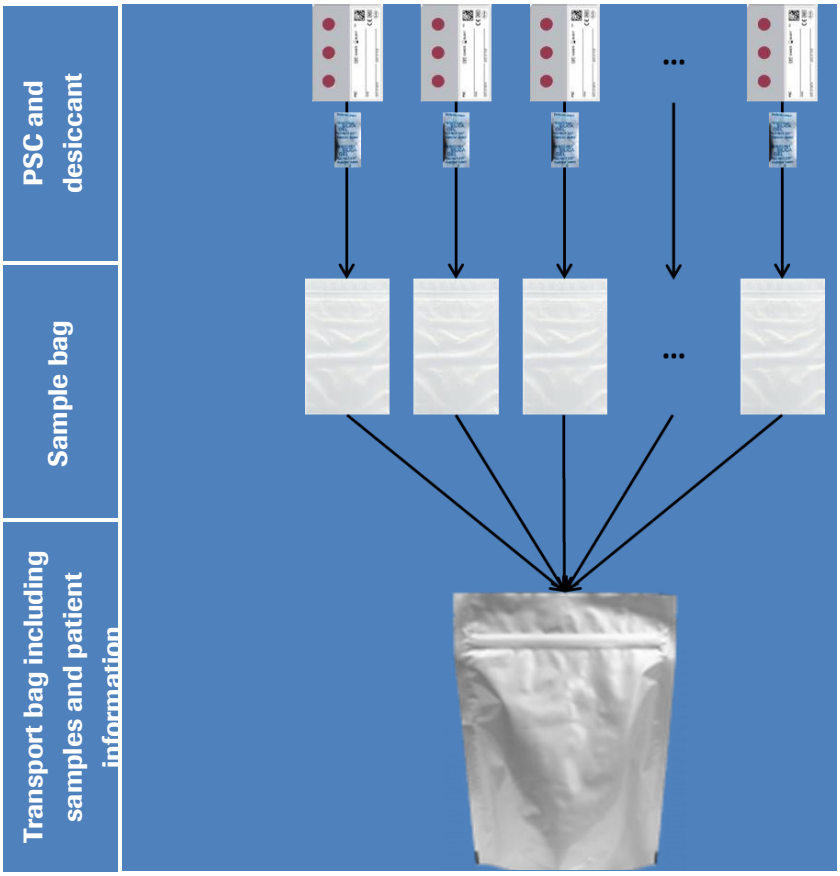
If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

PSC dried plasma spot samples

- Collect PSC dried plasma spot samples using appropriate clinical procedures (refer to **PSC Method Sheet ms_09411763190**).
- Check the expiry date of the PSC and proceed only if the PSC has not expired yet.
- Make sure that the bag in which the PSC is sealed is completely closed and intact.

- Label the PSC with patient's name, date of birth, date and time of sample collection.
- Apply 140 µL of whole blood on each circle of the PSC membrane delineated by the spotting layer using an appropriate capillary and a dispenser. It is recommended to fill all three spots on the PSC, in order to allow retesting.
- Do not apply samples from more than one patient on the same PSC.
- Do not allow the membranes to get in contact with gloves, tools or any potentially contaminated surfaces during this process.
- Ensure that BOTH sides of the PSC spots (front: membrane with blood; back: spot with plasma) are saturated after 5 minutes. Check the back side through the transparent back layer.
- Allow the PSC to dry at room temperature for at least 4 hours (to maximum overnight), protecting it from direct sunlight.
- Do not remove the spotting layer. This will be done at the laboratory, prior to sample extraction.
- After drying, store the PSC in an individual sample bag with 4 grams of desiccant and seal the bag. The collected sample bags must be packed in a transport bag together with their relative Patient Information Sheet. It is recommended to pack a maximum number of 25 PSCs per transport bag (see Figure 2 for an overview).

Figure 2 Overview of the packaging concept for PSC transport



- If 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. PSCs may be transported for a period of 28 days before being analyzed at 18-45°C and up to 85% humidity. PSCs in individual sample bags with 4 grams of desiccant, within a transport bag may be stored after transportation at room temperature (18-30°C), at 2-8°C or at ≤ -10°C for up to 56 days (with and without layer separation).

Instructions for use

Procedural notes

- Do not use **cobas**®HIV-1 reagents, **cobas**®HBV/HCV/HIV-1 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.
- **cobas**®HIV-1 can be run with two minimum required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). Figure 14 and Figure 15 below summarizes the procedure.
- **cobas**®HIV-1 can be run with 1300 µL **cobas**®Specimen Pre-Extraction Reagent (for the 850 µL PSC sample workflow). Please note, **cobas**®HIV-1 PSC workflow cannot run in mixed batch mode with plasma or serum samples, but with **cobas**®HIV-1/ HIV-2 Qualitative Dried Blood Spot on **cobas**® 6800/8800 System Software 1.4.
  Mixed batch testing is working on **cobas**®5800 system and **cobas**®6800/8800 systems with software version 2.0 or higher. Figure 14 and Figure 15 summarizes the procedure also for PSC dried plasma spot samples.

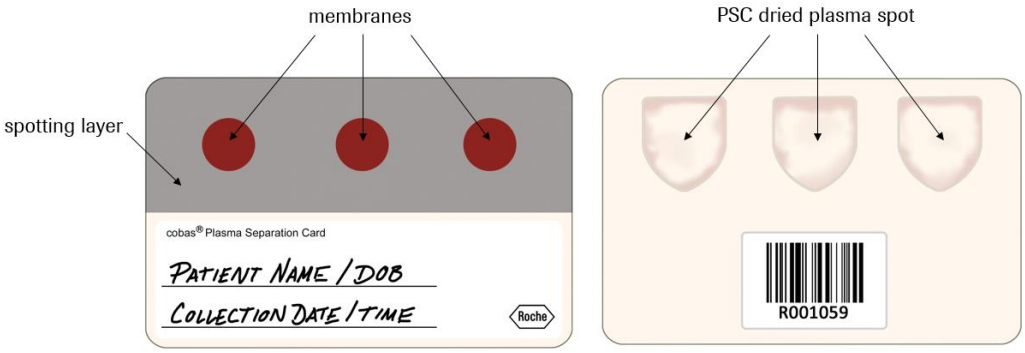
Running cobas® HIV-1 on cobas® 5800/6800/8800 systems

- The operation of the instruments is described in detail in the **cobas**®5800 system or **cobas**®6800/8800 systems User Assistance.
- Refer to the **cobas**®5800 system or **cobas**®6800/8800 systems User Assistance for proper maintenance of instruments.
- Verify that the correct sample type (**PSC**) and the correct ASAP is used (**cobas**®HIV-PSC ASAP) before starting the test procedure.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of RD5 or MPA sample racks. Refer to the **cobas**®5800 system or **cobas**®6800/8800 systems User Assistance for proper barcode specifications and additional information on loading sample tubes.

PSC dried plasma spot sample preparation and pre-analytic procedure

- Check the integrity of the transport bag before opening. Proceed only if the transport bag is completely sealed.
- Open the transport bag and, for each sample bag, proceed only if:
    - The laboratory request form is completely filled out.
    - The barcode of the laboratory request form and the PSC match.
    - The sample bag is completely closed and contains a PSC with 4 grams of desiccant.
    - The sample collection date is available, and the sample collection occurred in the past 28 days, and before the expiration date of the PSC (see Figure 3).
    - The PSC is not expired.
    - The PSC dried plasma spot looks homogeneous on the front side and looks completely covered with plasma when observed from the backside (see Figure 3).

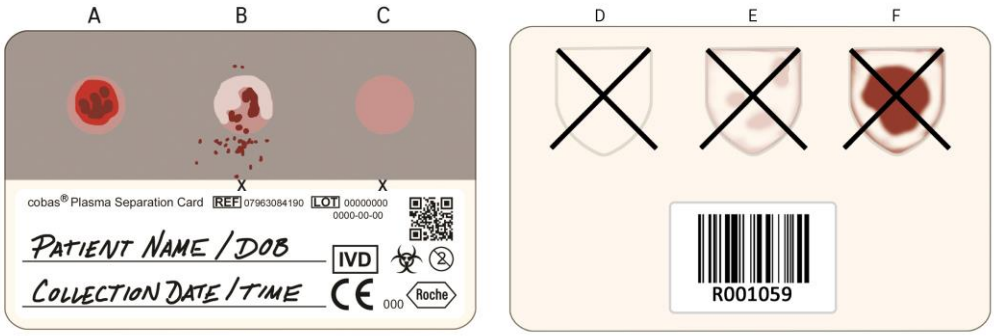
Figure 3 PSC dried plasma spots to be processed (left: front side. right: back side)



    - Acceptability of PSC dried plasma spots:
        - accept circles with small blood coagulates/clots (Figure 4A)
        - reject if blood spills are visible (Figure 4B & 4E)
        - reject if only an incomplete amount of blood was applied to spot due to air bubbles in capillary or blood spills (Figure 4C & 4D)
        - reject if the membrane is damaged and the card shows dark red-brownish back side (Figure 4F)

Note: The three spots are meant for retesting. A PSC could contain a bad spot, but still provide a good sample for testing. Properly mark the bad spots, in order to be recognizable. Avoid marking them on the spotting layer. Always compare the three spots between each other to evaluate their quality.

Figure 4 Acceptance criteria for PSC spots (left: front side; right: back side). Spots with small blood coagulates/clots (A) are accepted. Spots with spills (B&E), with incomplete amount of blood applied (C&D) or visibly damaged membranes (F) should not be processed. Spots should be clearly marked when rejected.

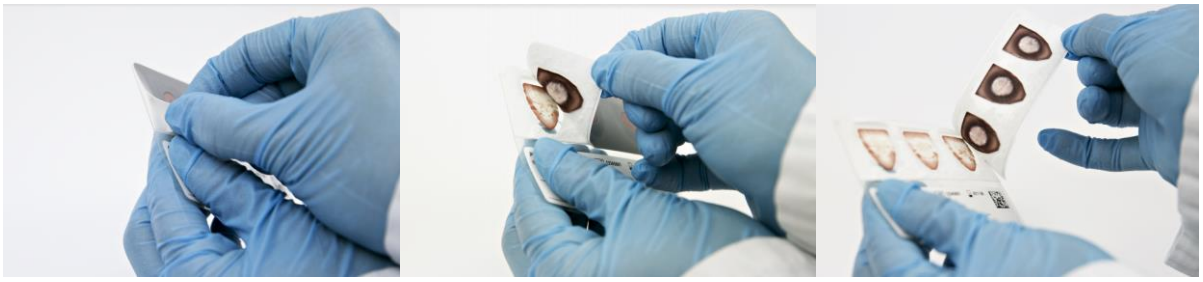


Label a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Greiner Bio-one Cryo.s™]) for each PSC with its corresponding barcode of the laboratory request form (Figure 5) and place them into a rack. Transfer tubes into a laminar flow hood together with the sample bags containing the PSCs. Refer to the **cobas®** 5800 and **cobas®** 6800/8800 systems User Assistance for proper barcode labeling.

Figure 5 Labeling of the tube with the corresponding barcode of the PSC

- Uncap the tubes within the laminar flow hood.
- For the PSC selected, open the sample bag and remove the spotting layer (Figure 6).

Figure 6 Removal of spotting layer



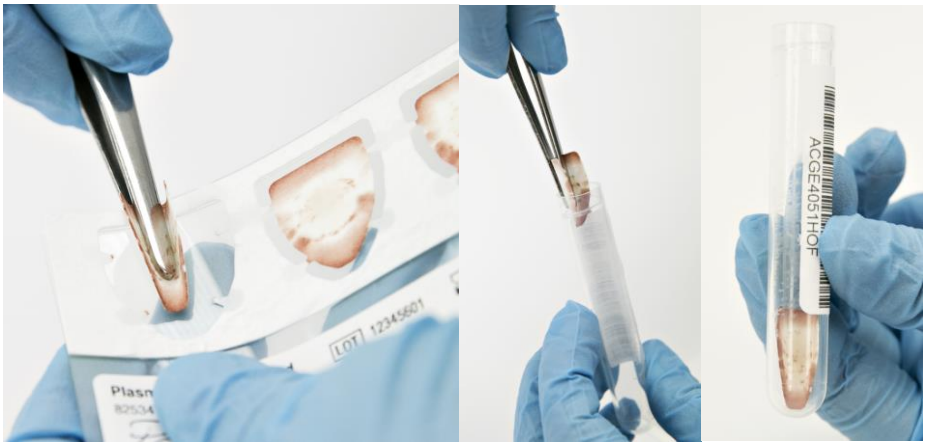
- Slightly bend the PSC and remove one dried plasma spot with sterile forceps or tweezers by pulling it up. Bend the removed dried plasma spot on the PSC to facilitate tube insertion (Figure 7). Use one pair of forceps or tweezers per patient.

Note: Dried plasma spots may become brittle upon storage. See storage requirements for PSC. Handle them carefully while inserting into the tube.

Figure 7 Removal of the PSC dried plasma spot and bending

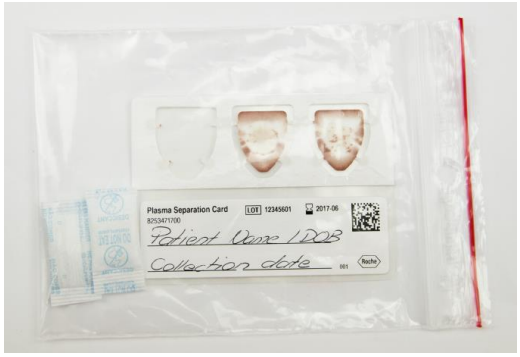
- Transfer one pre-bent PSC dried plasma spot into the corresponding tube so that the lowest tip of the dried PSC dried plasma spot reaches the bottom of the tube and is attached to the tube wall to prevent pipetting errors (Figure 8). Adjust the position of the PSC dried plasma spot with a sterile pipette tip, if necessary. Ensure the tube and the PSC of the transferred PSC dried plasma spot have the same barcode.

Figure 8 Transfer of the PSC dried plasma spot into the tube



- Place PSC with remaining dried plasma spots back to its original sample bag containing 4 grams of fresh desiccant for retesting, if required (Figure 9). PSCs can be stored for a period of 56 days after transport with and without spotting layer separation (at 18-30°C, or at 2-8°C, or at ≤ -10°C).

Figure 9 PSC in sample bag for potential retest

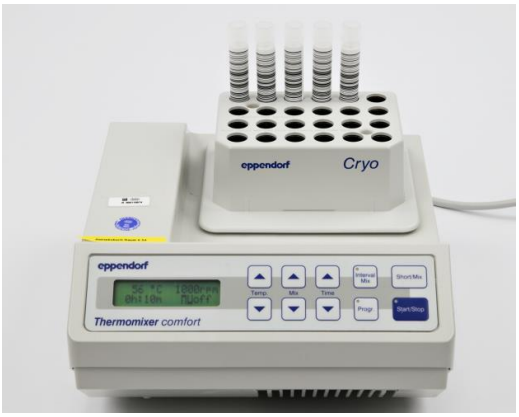


Allow **cobas®** Specimen Pre-Extraction Reagent (SPER) to equilibrate to ambient temperature before use. Pipette 1300 µL of SPER into the tubes containing the PSC dried plasma spots (Figure 10) and cap the tubes. Make sure the tubes are properly capped to prevent evaporation.

Figure 10 1300 µL SPER addition

- Place tubes in each of the positions 1 - 24 on a preheated Eppendorf Thermomixer®(e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes and incubate for 10 minutes, at 56°C and 1000 rpm to extract the virus from the dried plasma (Figure 11). Start the incubation right after the addition of SPER.

Figure 11 Incubation

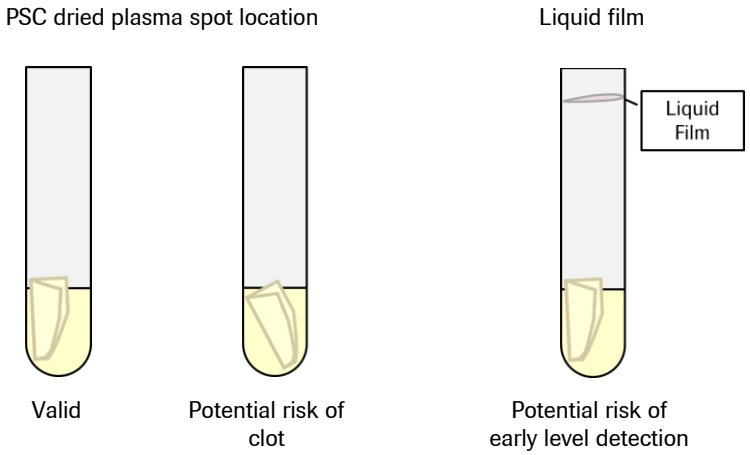


- Transfer the tubes onto a sample rack and uncap the tubes one by one to minimize cross-contamination (Figure 12). Change gloves after removing the caps.

Figure 12 Uncapping of the tubes

- Ensure the PSC dried plasma spot is correctly placed along the tube walls (Figure 13) to avoid sample clots. Adjust the position of the PSC dried plasma spot with a sterile pipette tip, if necessary.
- Eliminate any potential liquid film located above the liquid level using a sterile pipette tip (to avoid early level detection).
- Load the tubes onto the **cobas**®5800 system or **cobas**®6800/8800 systems.

Figure 13 PSC dried plasma spot preparation before the analytic workflow



Note: Please be sure to remove any liquid film created during the process.

Figure 14 cobas® HIV-1 test procedure on **cobas**® 5800 system

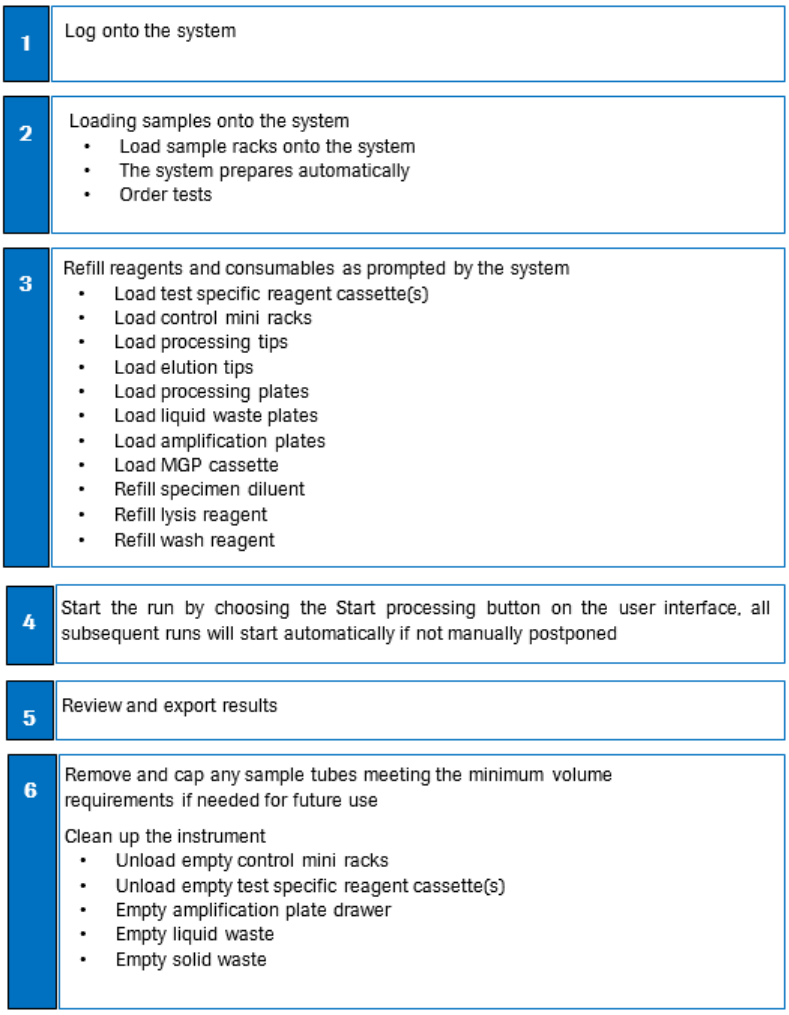
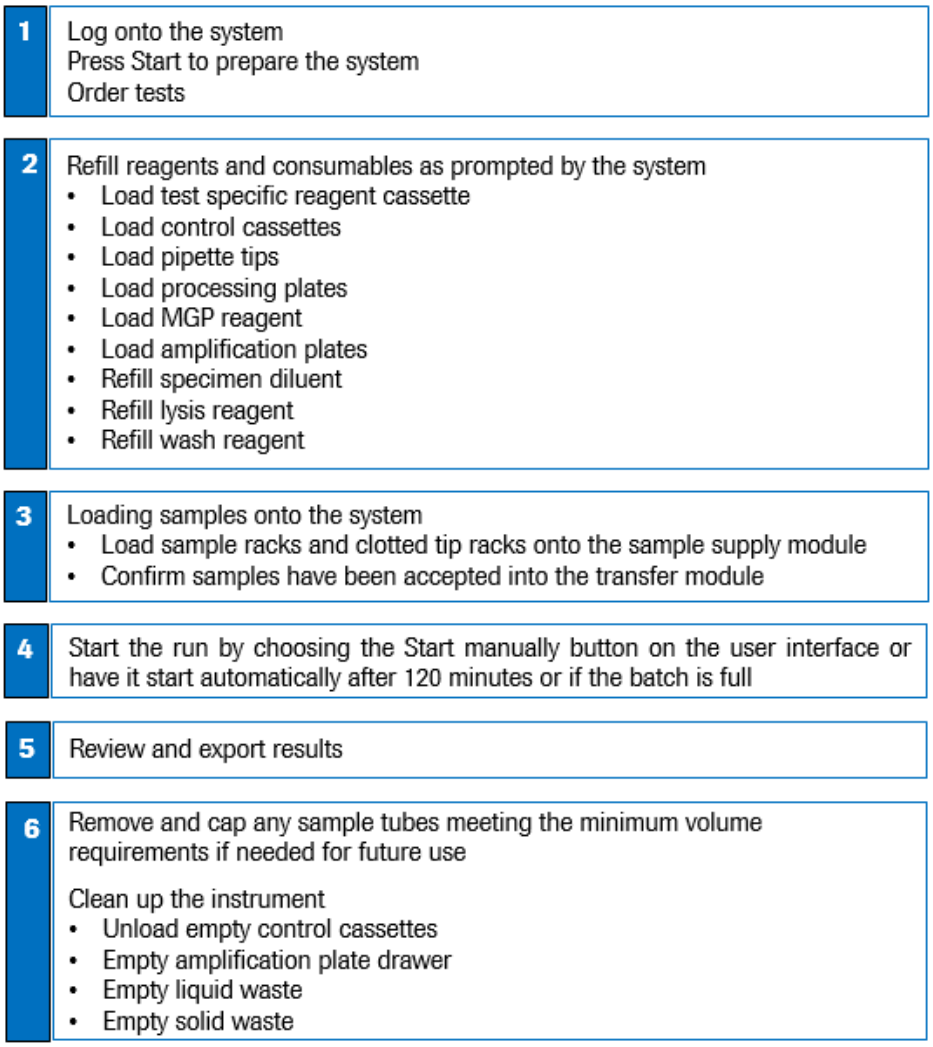


Figure 15 cobas® HIV-1 test procedure on **cobas**® 6800/8800 systems



Results

The **cobas**®5800 system and **cobas**®6800/8800 systems automatically determine the HIV-1 RNA concentration for the samples and controls. The HIV-1 RNA concentration is expressed in copies per milliliter (cp/mL) or International Units per milliliter (IU/mL). The conversion factor for **cobas**®HIV-1 is 0.6 cp/IU.

Quality control and validity of results on cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas**®NHP Negative Control [(–) C] and two **cobas**®HBV/HCV/HIV Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV H (+) C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the software and/or report, check for flags and their associated results to ensure the batch validity (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- The results of the controls are shown in the "Controls" app of the software.
- Controls are marked with "Valid" in the column "Control result" if the respective target of the controls are reported valid. Controls are marked with 'Invalid' in the column "Control result" if the respective Targets of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information will be shown in the detail view.
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Validation of results is performed automatically by the instrument software based on control results.

NOTE: The **cobas**®5800 system and the **cobas**®6800/8800 systems with software version 2.0 or higher will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Quality control and validity of results on the cobas® 6800/8800 systems with software version 1.4

- One **cobas**®NHP Negative Control [(–) C] and two **cobas**®HBV/HCV/HIV Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV H (+) C], are processed with each batch.
- In the software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**®6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based control results.

Control flags on cobas® 6800/8800 systems with software version 1.4

Table 17 Control flags for negative and positive controls

Negative Control	Flag	Result	Interpretation
(–) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Flag**	**Result**	**Interpretation**
HxV L (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
HxV H (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

Interpretation of results for cobas® 5800/6800/8800 systems

For a valid control batch, check each individual sample for flags in the **cobas**®5800 system and **cobas**®6800/8800 systems software and/or reports. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 18 Target results for individual target result interpretation

Results	Interpretation
Target Not Detected	HIV RNA not detected. Report results as “HIV not detected.”
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “HIV detected, less than (Titer Min).” Titer min = 20 cp/mL and 33 IU/mL (500 µL plasma) Titer min = 50 cp/mL and 83 IU/mL (200 µL plasma) Titer min = 790 cp/mL and 1317 IU/mL (PSC)
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as “(Titer) of HIV-1 detected”.
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “HIV detected, greater than (Titer Max).” Titer max = 1.00E+07 cp/mL and 1.67E+07 IU/mL (500 µL, 200 µL and PSC)

[a] Sample result > Titer Max refers to HIV-1 positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with HIV-1 negative EDTA plasma, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

The results of the samples are shown in the in the "Results" app of the software.

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

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D: Result validation failure because of an invalid positive control
    - Q06D: Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 18 above.
- If one or more sample targets are marked with "Invalid" the software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 systems with software version 1.4

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

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 18 above.

Procedural limitations

- **cobas**®HIV-1 plasma workflow has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 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**®5800/6800/8800 systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**®HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the 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.
- **cobas**®HIV-1 is not intended for use as a screening test for the presence of HIV-1 in blood or blood products.

Non-clinical performance evaluation

System equivalency

System equivalency of the **cobas**®5800, **cobas**®6800 and **cobas**®8800 systems was demonstrated via performance studies. The data presented in this Instructions for Use support equivalent performance for all systems.

Key performance characteristics for EDTA plasma samples

Limit of Detection (LoD)

WHO International Standard

The limit of detection of **cobas**®HIV-1 was determined by analysis of serial dilutions of the WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays (2nd WHO International Standard, NIBSC code 97/650) group M subtype B obtained from NIBSC (National Institute for Biological Standards and Control), in HIV-negative human EDTA plasma using sample processing volumes of 500 µL and 200 µL. Panels of five concentration levels plus a negative were tested over three lots of **cobas**®HIV-1 test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma from both sample processing volumes are shown in Table 19 and Table 20. The study demonstrates that **cobas**®HIV-1 detected HIV-1 RNA at a concentration of 13.2 cp/mL (22.0 IU/mL) as determined by Probit with a hit rate of 95% for the 500 µL sample processing volume and at a concentration of 35.5 cp/mL (59.2 IU/mL) or greater as determined by Probit with a hit rate of 95% for the 200 µL sample processing volume.

Table 19 Limit of detection in EDTA plasma (500 µL)

Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
40.0	66.7	189	189	100.0%
20.0	33.3	189	186	98.4%
10.0	16.7	189	171	90.5%
5.0	8.3	189	125	66.1%
2.5	4.2	189	67	35.4%
0.0	0.0	189	0	0.0%
LoD by PROBIT at 95% hit rate		13.2 cp/mL; 95% confidence range: 11.4–15.9 cp/mL 22.0 IU/mL; 95% confidence range: 19.0–26.5 IU/mL		

Table 20 Limit of detection in EDTA plasma (200 µL)

Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
200.0	333.3	189	189	100.0%
100.0	166.7	188	188	100.0%
50.0	83.3	189	186	98.4%
25.0	41.7	189	164	86.8%
12.5	20.8	189	112	59.3%
0.0	0.0	188	0	0.0%
LoD by PROBIT at 95% hit rate		35.5 cp/mL; 95% confidence range: 30.8–43.2 cp/mL 59.2 IU/mL; 95% confidence range: 51.3–72.0 IU/mL		

Linear range

The linearity study of **cobas**®HIV-1 was performed with a dilution series consisting of 12 panel members (500 µL sample processing volume) and 11 panel members (200 µL sample processing volume) spanning the linear range for the predominant HIV-1 group M subtype B. Panel members were prepared from a high titer HIV-1 RNA positive cell culture supernatant specimen. The evaluation was performed according to CLSI Guideline EP06-A.[20] Three reagent lots were analyzed on three **cobas**®6800/8800 systems, three operators and in total 16 replicates per panel member and lot across four testing days (four replicates per kit lot and day).

With 500 µL processing volume, **cobas**®HIV-1 is linear from 20 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 $\log_{10}$ in human EDTA plasma (see Figure 16). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

With 200 µL processing volume, **cobas**®HIV-1 is linear from 50 cp/mL to 1.00E+07 cp/mL (83.3 IU/mL to 1.67E+07 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 $\log_{10}$ in human EDTA plasma (see Figure 17). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

Figure 16 Linear range determination in EDTA plasma (500 µL)

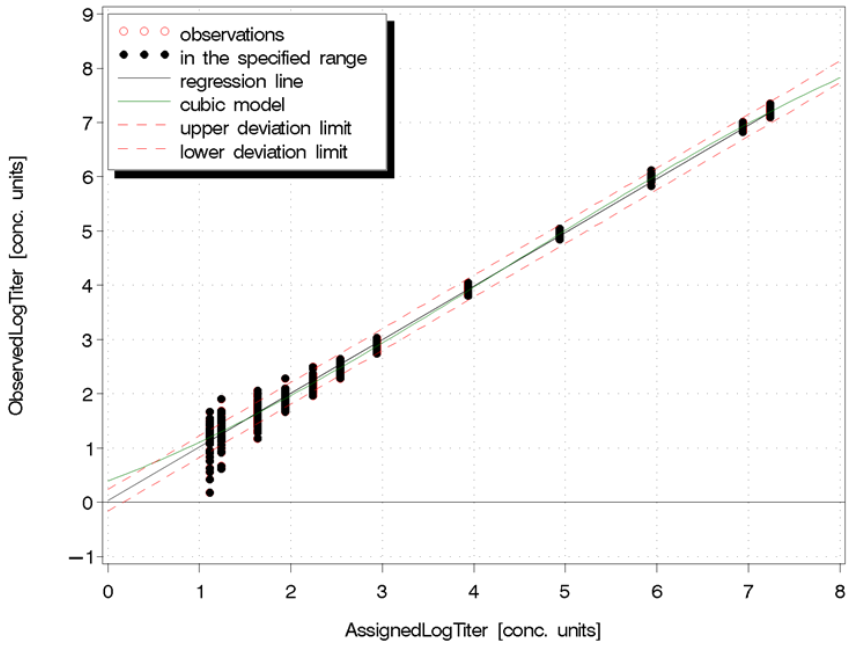
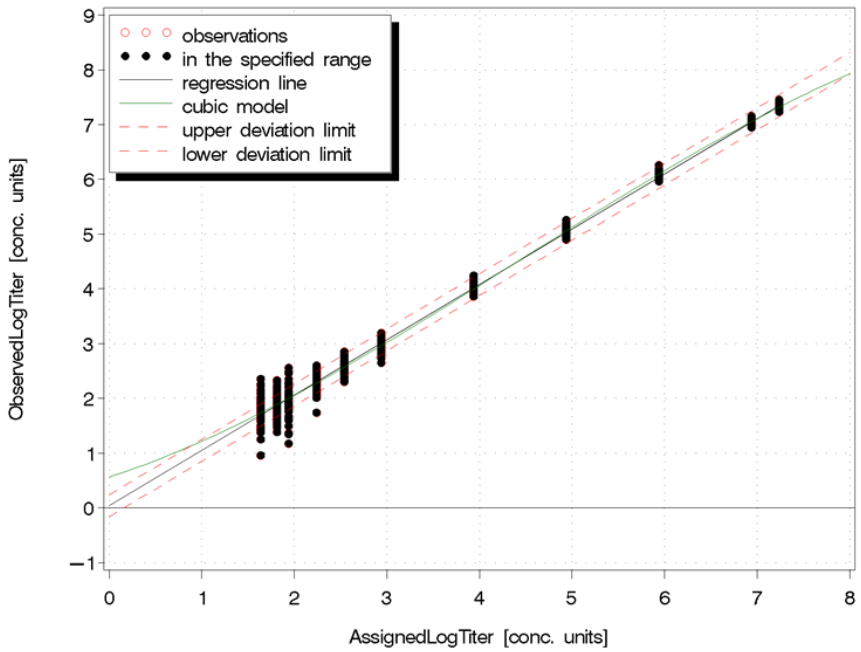


Figure 17 Linear range determination in EDTA plasma (200 µL)



Precision – within laboratory

Precision of **cobas**®HIV-1 was determined by analysis of serial dilutions of an HIV-1 high positive sample (Group M Subtype B; cultured virus) in HIV negative EDTA plasma. Eight dilution levels (500 µL sample processing volume) and seven dilution levels (200 µL sample processing volume) were tested in 48 replicates for each level and processing volume across three lots of **cobas**®HIV-1 test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**®HIV-1 test procedure on the fully automated **cobas**® 6800/8800 system. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 21 and Table 22.

cobas®HIV-1 showed high precision for three lots of reagents tested across a concentration range of 1.00E+02 cp/mL to 1.00E+07 cp/mL with 500 µL sample processing volume and 2.00E+02 cp/mL to 1.00E+07 cp/mL with 200 µL sample processing volume.

Table 21 Within laboratory precision of **cobas**® HIV-1 (EDTA plasma samples – processing volume of 500 µL)*

Nominal concentration (cp/mL)	Assigned concentration (cp/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	8.67E+06	Cell Culture	0.04	0.06	0.03	0.05
1.00E+06	8.67E+05	Cell Culture	0.06	0.05	0.04	0.05
1.00E+05	8.67E+04	Cell Culture	0.05	0.07	0.04	0.05
1.00E+04	8.67E+03	Cell Culture	0.06	0.06	0.04	0.05
1.00E+03	8.67E+02	Cell Culture	0.07	0.06	0.07	0.07
4.00E+02	3.47E+02	Cell Culture	0.09	0.10	0.09	0.09
2.00E+02	1.73E+02	Cell Culture	0.11	0.08	0.14	0.11
1.00E+02	8.67E+01	Cell Culture	0.15	0.11	0.10	0.12

*Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 22 Within laboratory precision of **cobas**® HIV-1 (EDTA plasma samples – processing volume of 200 µL)*

Nominal concentration (cp/mL)	Assigned concentration (cp/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	8.67E+06	Cultured Virus	0.04	0.05	0.04	0.04
1.00E+06	8.67E+05	Cultured Virus	0.07	0.05	0.05	0.06
1.00E+05	8.67E+04	Cultured Virus	0.07	0.07	0.06	0.07
1.00E+04	8.67E+03	Cultured Virus	0.08	0.08	0.06	0.08
1.00E+03	8.67E+02	Cultured Virus	0.12	0.12	0.08	0.11
4.00E+02	3.47E+02	Cultured Virus	0.11	0.13	0.09	0.11
2.00E+02	1.73E+02	Cultured Virus	0.20	0.12	0.15	0.16

*Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Subtype verification

The performance of **cobas**®HIV-1 on HIV-1 group M subtypes, group O and group N was evaluated by:

- Verification of the limit of detection for group M subtypes, group O and group N
- Verification of the linearity for group M subtypes, group O and group N
- Titer assignment was performed using **cobas**®HIV-1

Verification of limit of detection for group M subtypes, group O and group N

Cultured HIV-1 samples for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1O, HIV-1N were diluted to three different concentration levels in EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with 1 lot of **cobas**®HIV-1 reagents. The results from EDTA plasma using 500 µL are shown in Table 23. These results verify that **cobas**®HIV-1 detected HIV for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1O, and HIV-1N at the claimed concentration of 20 cp/mL or below with an upper one-sided 95% confidence interval being equal to or greater to the expected hit rate of 95%.

Table 23 LoD verification of HIV-1 group M subtypes, group O, and group N in 500 µL EDTA plasma

		10 cp/mL			20 cp/mL			40 cp/mL		
Group	Subtype	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
M	A	63	59	93.7% (97.8%)	63	63	100% (100%)	63	63	100% (100%)
	C	63	51	81.0% (88.6%)	63	61	96.8% (99.4%)	63	63	100% (100%)
	D	63	48	76.2% (84.7%)	62	60	96.8% (99.4%)	63	63	100% (100%)
	F	63	59	93.7% (97.8%)	63	63	100% (100%)	63	63	100% (100%)
	G	63	54	85.7% (92.3%)	63	63	100% (100%)	63	63	100% (100%)
	H	63	52	82.5% (89.9%)	63	63	100% (100%)	63	63	100% (100%)
	CRF01_AE	63	52	82.5% (89.9%)	63	62	98.4% (99.9%)	63	63	100% (100%)
	CRF02_AG	63	56	88.9% (94.7%)	63	62	98.4% (99.9%)	63	63	100% (100%)
O		63	49	77.8% (86.0%)	63	57	90.5% (95.8%)	63	63	100% (100%)
N		63	57	90.5% (95.8%)	63	63	100% (100%)	63	63	100% (100%)

* Upper one-sided 95% confidence interval

Similarly, the limit of detection was verified for the 10 subtypes tested with 200 µL input volume. The data are summarized in Table 24. These results verify that **cobas**®HIV-1 detected HIV RNA for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), and HIV-1N at the claimed concentration of 50 cp/mL or below with an upper one-sided 95% confidence interval being equal to or greater to the expected hit rate of 95%. HIV-1O was verified at 100 cp/mL.

Table 24 LoD verification of HIV-1 group M subtypes, group O and group N in 200 µL EDTA plasma

		25 cp/mL			50 cp/mL			100 cp/mL		
Group	Subtype	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
M	A	63	54	85.7% (92.3%)	63	60	95.2% (98.7%)	63	63	100% (100%)
	C	63	50	79.4% (87.3%)	63	62	98.4% (99.9%)	63	63	100% (100%)
	D	63	51	81.0% (88.6%)	63	63	100% (100%)	63	63	100% (100%)
	F	63	56	88.9% (94.7%)	63	62	98.4% (99.9%)	63	63	100% (100%)
	G	63	52	82.5% (89.9%)	63	62	98.4% (99.9%)	63	63	100% (100%)
	H	63	61	96.8% (99.4%)	63	63	100% (100%)	63	63	100% (100%)
	CRF01_AE	63	53	84.1% (91.1%)	63	57	90.5% (95.8%)	63	63	100% (100%)
	CRF02_AG	63	49	77.8% (86.0%)	63	63	100% (100%)	63	63	100% (100%)
O		63	44	69.8% (79.3%)	63	56	88.9% (94.7%)	63	63	100% (100%)
N		63	55	87.3% (93.5%)	63	63	100% (100%)	63	63	100% (100%)

* Upper one-sided 95% confidence interval

Verification of linear range for group M subtypes, group O and group N

The dilution series used in the verification of subtypes linearity study of **cobas**®HIV-1 consists of seven panel members (500 µL sample processing volume) and six panel members (200 µL sample processing volume) spanning the linear range. Panel members were prepared from high titer HIV-1 RNA positive cell culture supernatant specimens of the respective subtype. Testing was conducted with two lots of **cobas**®HIV-1 reagent; 14 replicates per level were tested in EDTA plasma.

The linear range of **cobas**®HIV-1 was verified for group M subtypes, group O and group N. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.2 $\log_{10}$.

Specificity

The specificity of **cobas**®HIV-1 was determined by analyzing HIV negative EDTA plasma samples from individual donors. Six hundred individual EDTA plasma samples were tested with two lots of **cobas**®HIV-1 reagents. All samples tested negative for HIV-1 RNA. In the test panel the specificity of **cobas**®HIV-1 was 100% (95% confidence limit: ≥ 99.5%).

Analytical specificity

The analytical specificity of **cobas**®HIV-1 was evaluated by diluting a panel of microorganisms with HIV RNA positive and HIV RNA negative EDTA plasma. The microorganisms were added to negative human EDTA plasma and tested with and without HIV RNA. None of the non-HIV pathogens interfered with test performance. Negative results were obtained with **cobas**®HIV-1 for all microorganism samples without HIV-1 target and positive results were obtained on all of the microorganism samples with HIV-1 target. The mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 25 Microorganisms tested for cross-reactivity

Viruses		Bacteria	Yeast
Adenovirus type 5	Varicella-Zoster Virus	Propionibacterium acnes	Candida albicans
Cytomegalovirus	West Nile Virus	Staphylococcus aureus	-
Epstein-Barr Virus	St. Louis encephalitis Virus	-	-
Hepatitis A Virus	Murray Valley encephalitis Virus	-	-
Hepatitis B Virus	Dengue virus types 1, 2, 3, and 4	-	-
Hepatitis C Virus	FSME Virus (strain HYPR)	-	-
Hepatitis D Virus	Influenza Virus A	-	-
Human T-Cell Lymphotropic Virus types 1 and 2	Zika Virus	-	-
Human Herpes Virus Type-6	Human Papillomavirus	-	-
Herpes Simplex Virus Type 1 and 2	Yellow Fever Virus	-	-

Analytical specificity – interfering substances

Elevated levels of triglycerides (up to 34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples as well as the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA) have been tested in the presence and absence of HIV RNA.

In addition, drug compounds listed in Table 26 were tested at three times the C_{max} in presence and absence of HIV RNA.

All potentially interfering substances show no interference with the test performance. Negative results were obtained with **cobas**®HIV-1 for all samples without HIV target and positive results were obtained on all of the samples with HIV-1 target. The mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially

interfering substances was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 26 Drug compounds tested for interference with the quantitation of HIV RNA by **cobas®** HIV-1

Class of drug	Generic drug name	
Immune Modulators	Peginterferon α-2a	Peginterferon α-2b
	Ribavirin	-
HIV Entry Inhibitor	Maraviroc	
HIV Integrase Inhibitors	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitors	Efavirenz	Nevirapine
	Etravirine	Rilpivirine
HIV Protease Inhibitors	Atazanavir	Lopinavir
	Tipranavir	Nelfinavir
	Darunavir	Ritonavir
	Fosamprenavir	Saquinavir
HCV Protease Inhibitors	Boceprevir	Telaprevir
	Simeprevir	-
Reverse Transcriptase or DNA Polymerase Inhibitors	Abacavir	Tenofovir
	Emtricitabine	Adefovir dipivoxil
	Entecavir	Telbivudine
	Foscarnet	Zidovudine
	Cidofovir	Aciclovir
	Lamivudine	Valganciclovir
	Ganciclovir	Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin Clarithromycin Ethambutol Fluconazole Isoniazid	Pyrazinamide Rifabutin Rifampicin Sulfamethoxazole Trimethoprim

Method correlation

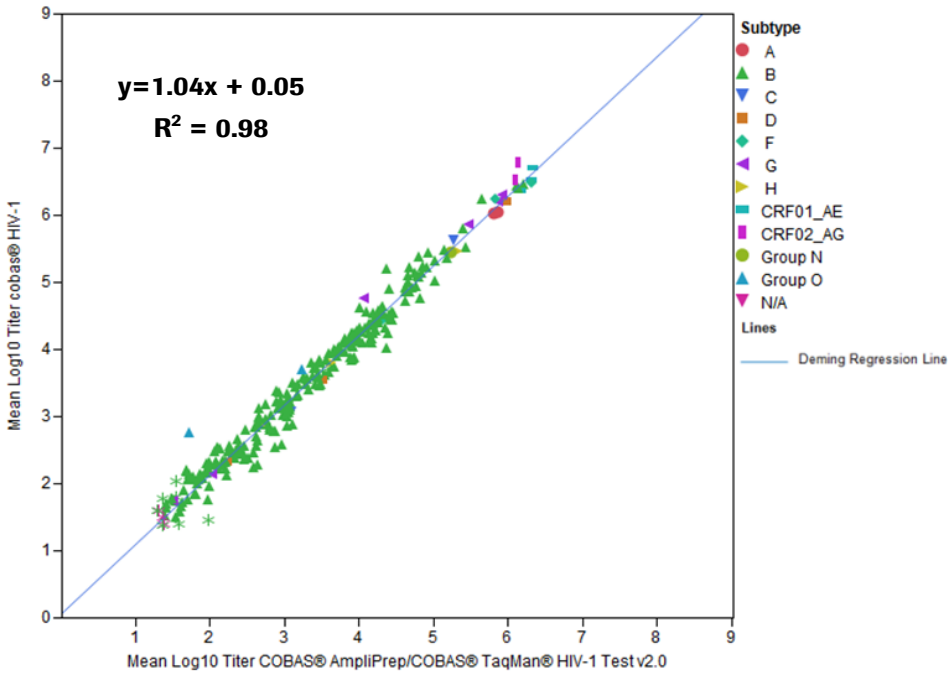
Performance evaluation of cobas® HIV-1 compared to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

The performance of **cobas®** HIV-1 and the COBAS®AmpliPrep/COBAS®TaqMan®HIV-1 Test v2.0 (TaqMan® HIV-1 Test, v2.0) were compared by analysis of 251 EDTA plasma specimens from patients infected with HIV-1 and by analysis of 16 diluted cell culture supernatants. The specimens comprised of HIV-1 M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1 O and HIV-1N and were tested in duplicate at an external site. Titers below the quantitation range (< 20 cp/mL) were generated for one of the two replicates for five samples on both the COBAS®AmpliPrep/COBAS®TaqMan®System and the **cobas®** 6800/8800 systems, and for five samples on the COBAS®AmpliPrep/COBAS®TaqMan®System only. One sample on COBAS®AmpliPrep/COBAS®TaqMan®

generated one valid aliquot only. Titers below the quantitation range were excluded from analysis. The Deming regression was performed considering log-transformed titers. For samples where both replicates were within the quantitation range, the mean log_{10} titer was used for analysis.

The Deming regression results are shown in Figure 18. The symbol ✳ in Figure 18 shows single determination.

Figure 18 Regression analysis of **cobas**® HIV-1 vs TaqMan® HIV-1 Test, v2.0, EDTA plasma samples



Whole system failure

The whole system failure rate for **cobas**®HIV-1 was determined by testing 100 replicates of EDTA plasma spiked with HIV-1 group M subtype B. These samples were tested at a target concentration of approximately 3 x LoD.

The results of this study determined that all replicates were valid and positive for the HIV-1 target, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.6% for the upper bound [0%: 3.6%].

Cross contamination

The cross-contamination rate for **cobas**®HIV-1 was determined by testing 240 replicates of HIV negative human EDTA-plasma sample and 225 replicates of a high titer HIV-1 sample at 4.00E+06 cp/mL. The study was performed using the **cobas**®6800 System. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.5% for the upper bound [0%: 1.5%].

Key performance characteristics for PSC dried plasma spot samples

Limit of Detection (LoD) using the PSC

The plasma limit of detection of **cobas**®HIV-1 in combination with the PSC was determined by analysis of plasma titers assigned to serial dilutions of HIV-1 group M cell culture supernatant, in HIV-negative human whole blood. Panels of five concentration levels plus a negative were tested over three lots of PSCs and three lots of **cobas**®HIV-1 test reagents, multiple runs, days, operators, and instruments. Samples of the highest panel member were centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas**®HIV-1 with the 3rd HIV-1 WHO International Standard (NIBSC code 10/152), HIV-1 group M, subtype B for preparation of the high and low calibrators.

The combined results from three PSC lots, negative donors and dilution series with individual plasma titer assignments (CBM) are shown in Table 27. The study demonstrates that **cobas**®HIV-1 in combination with the PSC detected HIV-1 RNA at a concentration of 790.2 cp/mL with a hit rate of 95% as determined by Probit analysis.

Table 27 Limit of detection in combination with the PSC

Assigned plasma titer concentration (HIV-1 RNA cp/mL)	Number of valid replicates	Number of positives	Hit rate in %
1971.1	62	62	100.00
1925.3	63	63	100.00
1358.1	62	62	100.00
985.5	63	62	98.41
962.6	63	62	98.41
679	63	59	93.65
657	63	59	93.65
641.8	63	57	90.48
452.7	63	52	82.54
328.5	63	47	74.60
320.9	63	46	73.02
226.3	62	49	79.03
164.3	63	36	57.14
160.4	63	35	55.56
113.2	63	38	60.32
0	189	0	0.00

Assigned plasma titer concentration (HIV-1 RNA cp/mL)	Number of valid replicates	Number of positives	Hit rate in %
LoD by PROBIT at 95% hit rate	790.2 cp/mL; 95% confidence range: 658.9 – 1003.6 cp/mL 1317 IU//mL; 95% confidence range: (1098.2 – 1672.7 IU/mL)		

Estimation of LoD in whole blood

The corresponding titers in whole blood of the same sample cannot be exactly determined, since whole blood is not a sample type for viral load testing. Whole blood titers based on the amount of HIV-1 RNA spiked into the whole blood samples were not used for LoD estimation because the amount of spiked RNA does not necessarily correspond to the amount of RNA in plasma. Even after centrifugation, RNA can remain in the buffy coat or associated with the cellular fraction of whole blood. However, since other technologies like dried blood spots have used spiked whole blood titers to estimate their limit of detection in whole blood, an estimate for the PSC whole blood LoD can be provided based on an empirical factor which is assumed to be related to the average hematocrit content (45%) of the samples (Table 28).

Whole blood LoD values are estimates based on the assumption:

Whole blood LoD estimate = PSC plasma LoD / 1.8

Table 28 Whole blood LoD estimate

LoD by PROBIT analysis (95% Hit Rate)	439.0 cp/mL
95% confidence interval	366.1 – 557.6 cp/mL

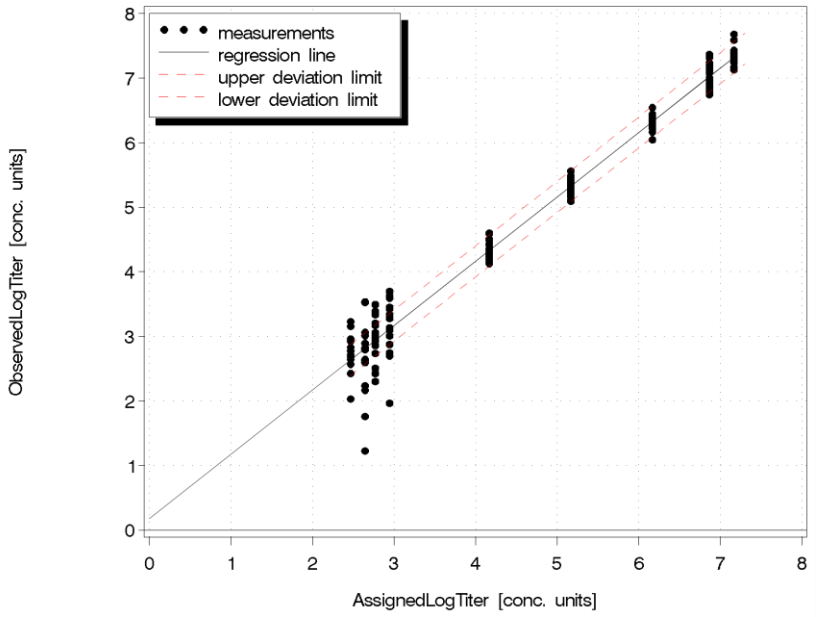
Linear range using the PSC

The linearity study of **cobas**®HIV-1 in combination with the PSC was performed with a dilution series consisting of 9 panel members spanning the linear range for the predominant HIV-1 group M subtype B. Panel members were prepared from a high titer HIV-1 RNA positive cell culture supernatant specimen. The evaluation was performed according to CLSI Guideline EP06-A.[20] Two PSC lots and two reagent lots were analyzed on two **cobas**®6800/8800 systems, by three operators and in total 20 replicates per panel member and reagent/PSC lot.

Samples of one panel member were centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas**®HIV-1 with the 3rd HIV-1 WHO International Standard (NIBSC code 10/152), HIV-1 group M, subtype B for preparation of the high and low calibrator.

In combination with the PSC, **cobas**®HIV-1 is linear from 790 cp/mL to 1.00E+07 cp/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.24 $\log_{10}$ with the PSC (see Figure 19). Across the linear range, the accuracy of the test was within ± 0.3 $\log_{10}$ deviation from the linear regression fit.

Figure 19 Linear range determination using the PSC



Precision – within laboratory using the PSC

Precision of **cobas**®HIV-1 in combination with the PSC was determined by analysis of serial dilutions of an HIV-1 high positive sample (high titer HIV-1 RNA positive cell culture supernatant specimen) in HIV negative EDTA whole blood. Five dilution levels were tested in 48 replicates for each level and processing volume across two lots of PSC and two lots of **cobas**®HIV-1 test reagents using two instruments and two operators over 12 days. Each sample was carried through the entire PSC workflow and **cobas**®HIV-1 procedure on the fully automated **cobas**® 6800/8800 systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 29.

cobas®HIV-1 in combination with the PSC showed high precision for two lots of PSC and reagents tested across a concentration range of 2.00E+03 cp/mL to 1.00E+07 cp/mL.

Table 29 Within laboratory precision of **cobas**® HIV-1 in combination with the PSC

Measured concentration (cp/mL in centrifuged EDTA plasma)	Source material	Pooled SD
1.02E+07	Cell Culture	0.08
9.12E+05	Cell Culture	0.08
8.32E+04	Cell Culture	0.09
7.94E+03	Cell Culture	0.13
2.00E+03	Cell Culture	0.23

Subtype verification using the PSC

Although HIV subtype should not affect PSC performance, cultured HIV-1 samples for common HIV-1M subtypes (A, C and D) were diluted to one concentration level in whole blood. The precision and accuracy determination was performed with 12 replicates for each sample. Testing was conducted with 1 lot of PSC and 1 lot of **cobas**®HIV-1 reagents.

Each whole blood sample was centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using **cobas**®HIV-1 with the 3rd HIV-1 WHO International Standard (NIBSC code 10/152), HIV-1 group M, subtype B for preparation of the high and low calibrator.

The results are shown in Table 30. These results confirm that **cobas**®HIV-1 in combination with the PSC detected and correctly quantified HIV-1M subtypes A, C and D.

Table 30 Verification of HIV-1 group M subtypes A, C and D

HIV-1 M Subtype	Number of valid replicates	Accuracy	Precision	Plasma vs. PSC-plasma Equivalency
Subtype A	12	-0.13	0.14	-0.20
Subtype C	12	-0.08	0.12	-0.10
Subtype D	12	-0.04	0.11	-0.10

Specificity using the PSC

The specificity of **cobas**®HIV-1 in combination with the PSC was determined by analyzing HIV-negative EDTA whole blood samples from individual donors. In total 159 individual EDTA whole blood samples were tested with two lots of PSC and two lots of **cobas**®HIV-1 reagents. All samples tested negative for HIV-1 RNA. In the test panel the specificity of **cobas**®HIV-1 was 100% (95% confidence limit: ≥ 98.13%).

Whole system failure using the PSC

The whole system failure rate for **cobas**®HIV-1 in combination with the PSC was determined by testing 100 replicates of EDTA whole blood spiked with HIV-1 group M subtype B. These samples were tested at a target concentration of approximately 3 x LoD.

The results of this study determined that all replicates were valid and positive for the HIV-1 target, resulting in a whole system failure rate of 0%.

Performance of PSC dried plasma spot samples compared to plasma samples

The performance of PSC dried plasma spot samples were compared to centrifuged EDTA plasma samples by analysis of 132 specimens from patients infected with HIV-1 (FP= prospective collection, VL=viral load testing, CD=CD4 count leftover samples). The specimens were tested one replicate each (PSC dried plasma spot and EDTA plasma) at an external site. Titers below the quantitation range were excluded from analysis. The Deming regression was performed considering log-transformed titers.

The Deming regression results are shown in Figure 20. The symbols ✳ and ● in Figure 20 show single determinations.

Figure 20 Deming regression

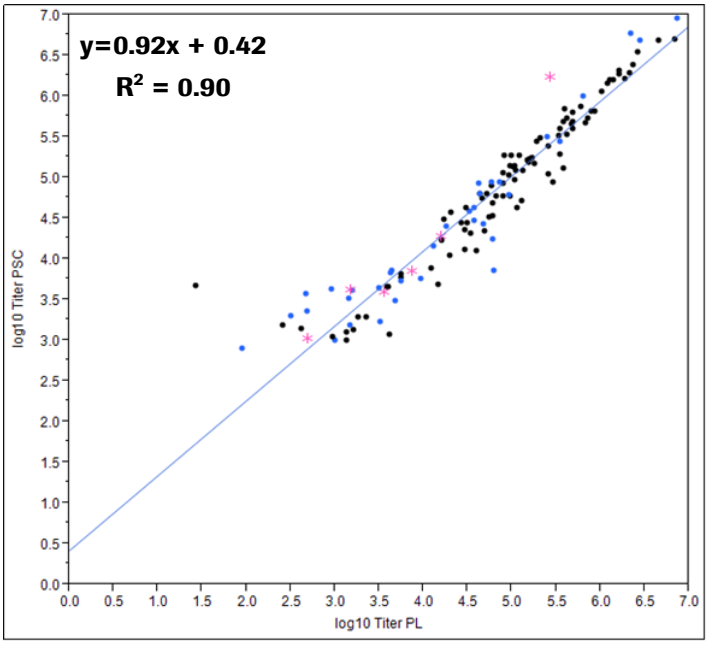


Table 31 Summary of statistical data

Matrix (Plasma Type) Equivalency	Number of specimens with valid titer	Bland Altman Analysis		Deming Regression Analysis		
		Mean Log_{10} difference	95% CI [lower/upper]	Slope	Intercept	R-squared
PSC vs liquid plasma	132	0.05	[-0.01; 0.11]	0.92	0.42	0.90

Clinical performance evaluation

Reproducibility

Reproducibility of **cobas**®HIV-1 was evaluated in EDTA plasma using the 500 µL sample processing volume on the **cobas**®6800 system. The study was performed using panels constructed from well characterized HIV-1 group M, subtype B cultured virus stock and from EDTA plasma that was negative for HIV-1 RNA and HIV-1/2 antibodies. The 8-member panel included one negative panel member and 7 positive panel members covering the linear range of **cobas**®HIV-1 as well as key medical decision points for the intended use, supported by the 2015 Department of Health and Human Services Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents.[3] Testing was done with three reagent lots, three sites, two operators per site, two runs per day, 6 days of testing per reagent lot, and three replicate per run. Reproducibility was evaluated using a random effects model including lot, site, operator, day, run, and within-run. Table 32 shows the total variance, total precision SDs, and lognormal CVs for **cobas**®HIV-1 as determined by analysis of variance. The within-run component contributed the most variability for the majority of the panel members.

Table 32 Attributable percentage of total variance, total precision standard deviation, and lognormal CV of HIV RNA quantitation by positive panel member on the **cobas**® 6800 system (reproducibility)

HIV RNA Concentration (log_{10} copies/mL)			Percent of Total Variance (CV(%))						
Expected	Mean[a] (SD)[b]	No. of Tests[c]	Lot	Site	Operator	Day	Run	Within-Run	Total Variance CV(%)[d]
1.70	1.69 (0.191)	323	17% (18.23)	0% (0.00)	0% (0.00)	1% (3.42)	5% (9.66)	78% (40.32)	46.25
2.30	2.22 (0.116)	321	32% (15.15)	0% (0.00)	1% (2.26)	4% (5.60)	0% (0.00)	63% (21.55)	27.27
2.60	2.48 (0.102)	323	34% (13.84)	4% (4.85)	3% (3.75)	0% (0.00)	1% (2.74)	58% (17.99)	23.86
3.00	2.84 (0.092)	324	39% (13.30)	0% (0.00)	1% (2.01)	0% (0.00)	7% (5.67)	52% (15.37)	21.33
4.00	3.86 (0.081)	324	43% (12.33)	1% (1.94)	3% (3.34)	10% (5.82)	6% (4.54)	37% (11.39)	18.85
5.00	4.92 (0.084)	324	43% (12.64)	0% (0.00)	3% (3.56)	6% (4.65)	6% (4.52)	42% (12.60)	19.44
6.70	6.63 (0.087)	324	45% (13.60)	0% (0.00)	2% (3.00)	3% (3.42)	0% (0.00)	50% (14.23)	20.32

Note: This table only includes results with detectable viral load.

[a] Calculated using SAS MIXED procedure based on $\log_{10}$ transformed measurements.

[b] Calculated using the total variability from the SAS MIXED procedure based on $\log_{10}$ transformed measurements.

[c] Number of valid tests with detectable viral load.

[d] Lognormal model used for CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

CV(%) = percent coefficient of variation; HIV = human immunodeficiency virus; No. = number; RNA = ribonucleic acid; SD = standard deviation; sqrt = square root.

In Table 33 below, the negative percent agreement (NPA) for the **cobas**®6800 system using all valid negative panel member tests was 100%.

Table 33 Negative percent agreement using the negative panel member

Expected HIV RNA Concentration	No. of Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	322	0	322	100.00	(98.86, 100.00)

[a] NPA = (number of negative results / total number of valid tests in negative panel member) * 100.

[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.

CI = confidence interval; HIV = human immunodeficiency virus; No. = number; NPA = negative percent agreement; RNA = ribonucleic acid.

Validation of viral load quantitation

The performance of **cobas**®HIV-1 on the **cobas**®6800 system was compared to that of the FDA-approved COBAS® AmpliPrep/COBAS®TaqMan®HIV-1 Test v2.0 (TaqMan®HIV-1 Test, v2.0) by analysis of paired EDTA plasma specimens from 410 subjects with HIV-1 viral loads spanning the linear range of both tests. Demographic characteristics of the subjects are shown in Table 34.

Table 34 Summary of demographic characteristics

Demographic Characteristics	Statistics
	(N=410)
Age (years)	
Mean (SD)	41.8 (11)
Median	43
Range	19 – 72
Sex, n (%)	
Male	321 (78.3%)
Female	89 (21.7%)
Race, n (%)	
Asian	5 (1.2%)
Black	163 (39.8%)
Latino	17 (4.1%)
White	94 (22.9%)
Other	91 (22.2%)
Unknown	40 (9.8%)
Ethnicity, n (%)	
Hispanic	101 (24.6%)
Non-Hispanic	231 (56.3%)
Unknown	78 (19.0%)
Antiviral Medication, n (%)	
Yes	208 (50.7%)

Demographic Characteristics	Statistics
No	137 (33.4%)
Unknown	65 (15.9%)
CD4 Cell Count (cells/µL), n (%)	
N	391
Mean (SD)	438.1 (267.7)
Median	401
Range	0 – 1548

SD = standard deviation.

Of 410 paired samples tested, 305 paired samples had viral load measurements within the linear range of both assays. Table 35 shows the mean paired viral load difference between **cobas®**HIV-1 and the TaqMan®HIV-1 Test, v2.0.

Table 35 Mean of paired viral load difference between **cobas®** HIV-1 and the TaqMan® HIV-1 Test, v2.0

Number of Paired Samples	Mean of Paired Difference (log_{10} copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
305	0.112	0.013	(0.086, 0.137)

CI = confidence interval.

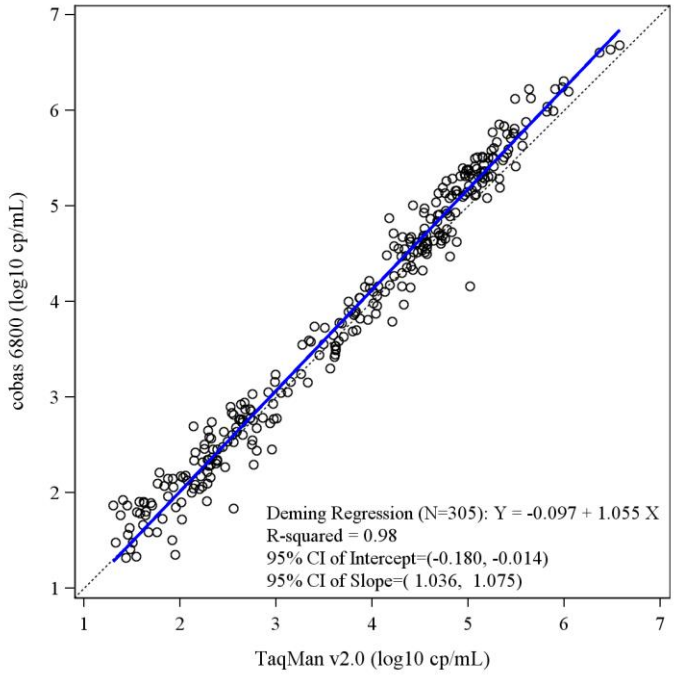
The results of Deming regression analysis between **cobas®**HIV-1 and the TaqMan®HIV-1 Test, v2.0 are tabulated in Table 36 and shown graphically in Figure 21. The dashed line indicates perfect agreement between the two test methods.

Table 36 Parameter estimates of Deming regression analysis between **cobas®** HIV-1 on the **cobas®** 6800 system and the TaqMan® HIV-1 Test, v2.0

Number of Paired Samples = 305				
Parameter	Parameter Estimate (log_{10} copies/mL)	Standard Error	95% CI	R^2
Intercept	-0.097	0.042	(-0.180, -0.014)	0.98
Slope	1.055	0.010	(1.036, 1.075)	

CI = confidence interval.

Figure 21 Deming regression analysis between **cobas**® HIV-1 on the **cobas**® 6800 system and the TaqMan® HIV-1 Test, v2.0



Subsets of paired samples were also tested to compare **cobas**®HIV-1 results using the 200 µL and 500 µL sample processing volumes on both the **cobas**®6800 and **cobas**®8800 systems. All comparisons showed a mean of paired difference of less than 0.095 $\log_{10}$ copies/mL.

For the comparison across sample volumes, Table 37 shows the mean paired difference between **cobas**®HIV-1 results using the 200 µL and 500 µL sample processing volumes on the **cobas**®6800 system.

Table 37 Mean of paired viral load difference between the 200 µL and 500 µL sample processing volumes on the **cobas**® 6800 system

Number of Paired Samples	Mean of Paired Difference ($\log_{10}$ copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
111	0.094	0.014	(0.067, 0.121)

CI = confidence interval.

Table 38 shows the mean paired difference between **cobas**®HIV-1 results using the 200 µL and 500 µL sample processing volumes on the **cobas**®8800 system.

Table 38 Mean of paired viral load difference between the 200 µL and 500 µL sample processing volumes on the **cobas**® 8800 system

Number of Paired Samples	Mean of Paired Difference ($\log_{10}$ copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
111	0.080	0.012	(0.056, 0.105)

CI = confidence interval.

For the comparison across systems, Table 39 shows the mean paired difference between **cobas**®HIV-1 results on the **cobas**®6800 system and the **cobas**®8800 system using the 200 µL sample processing volume.

Table 39 Mean of paired viral load difference between the **cobas**® 6800 system and **cobas**® 8800 system using the 200 µL sample processing volume

Number of Paired Samples	Mean of Paired Difference ($\log_{10}$ copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
109	0.011	0.013	(-0.014, 0.036)

CI = confidence interval.

Table 40 shows the mean paired difference between **cobas**®HIV-1 results on the **cobas**®6800 system and the **cobas**®8800 system using the 500 µL sample processing volume.

Table 40 Mean of paired viral load difference between the **cobas**® 6800 system and **cobas**® 8800 system using the 500 µL sample processing volume

Number of Paired Samples	Mean of Paired Difference ($\log_{10}$ copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
123	-0.001	0.012	(-0.024, 0.022)

CI = confidence interval.

A subset of paired samples spanning the linear range of the assay was also tested to compare **cobas**®HIV-1 results from BD Vacutainer®PPT Plasma Preparation Tubes for Molecular Diagnostic Test Methods and EDTA plasma tubes without a gel separator. This comparison was done using the **cobas**®6800 system. The results are shown in Table 41.

Table 41 Mean of paired viral load difference between BD Vacutainer® PPT™ Plasma Preparation Tubes and EDTA plasma tubes

Number of Paired Samples	Mean of Paired Difference (log_{10} copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
42	0.026	0.027	(-0.029, 0.081)

CI = confidence interval.

Clinical evaluation

The use of **cobas**®HIV-1 in monitoring HIV-1-infected subjects on antiretroviral treatment was examined by testing specimens from participants in a phase III clinical trial completed by Boehringer Ingelheim Pharmaceuticals, Inc. (BI) trial number BI 1100.1486 (VERxVE trial) using the 500 µL sample processing volume on the **cobas**®6800 system. Subjects were included if they had sufficient sample volume up to 144 weeks of follow-up for **cobas**®HIV-1 and had not discontinued the VERxVE trial due to adverse events. Viral load results after 24 and 48 weeks of treatment using a 50 copies/mL threshold and a 200 copies/mL threshold were compared to a virological definition of treatment failure. Virological failure was defined as a viral load of greater than or equal to 50 copies/mL at the subject's last trial visit after at least 48 weeks of treatment.

Table 42 shows the demographic characteristics of the 355 subjects included in the study.

Table 42 Demographics characteristics

Demographic Characteristics	Statistics
	(N=355)
Age (years)	-
Mean (SD)	38 (9.3)
Median	38
Range	19 – 68
Sex, n (%)	-
Male	322 (90.7%)
Female	33 (9.3%)
Race/Ethnicity, n (%)	-
Asian	5 (1.4%)
Black / African-American	45 (12.7%)
White / Caucasian	303 (85.4%)
Other	2 (0.6%)
CD4 Count at Screening (cells/µL), n (%)	-
50 to < 200	117 (33.0%)
200 to < 350	209 (58.9%)
350 to < 400	17 (4.8%)
≥ 400	9 (2.5%)
Unknown	3 (0.8%)

SD = standard deviation.

The results of comparisons using the 50 copies/mL and 200 copies/mL thresholds at 24 and 48 weeks of treatment with virological failure are shown in Table 43 and Table 44Table .

The analyses of the 50 copies/mL virological threshold with virological failure (at Week 24 and Week 48) are shown in Table 43. At Week 24, the PPV was 15.6% (10/64, 95% CI: 7.8%, 26.9%), and, at Week 48, the PPV was 25.7% (9/35, 95% CI: 12.5%, 43.3%). At Week 24, the NPV was 90.9% (251/276, 95% CI: 86.9%, 94.1%), and, at Week 48, the NPV was 91.1% (285/313, 95% CI: 87.3%, 94%). At Week 24, the OR was 1.86 (95% CI: 0.75, 4.29), which was not statistically significant (p = 0.191). At Week 48, the OR was 3.51 (95% CI: 1.31, 8.71) which was statistically significant (p = 0.012).

Table 43 Comparison of a 50 copies/mL virological threshold with virological failure

-		Virological Failure[a]		-
On-Treatment Visit	**Virological Threshold**	**Yes**	**No**	**Total**
Week 24	≥ 50 cp/mL	10	54	64
	< 50 cp/mL	25	251	276
	Total	35	305	340[+]
Week 48	≥ 50 cp/mL	9	26	35
	< 50 cp/mL	28	285	313
	Total	37	311	348[+]

[+] Valid results obtained by **cobas®**HIV-1.

[a]Virological Failure is classified as 'Yes' if the viral load of a specimen was greater than or equal to 50 cp/mL at Week 144 or at the final visit if there was no Week 144 visit. Final visit had to be at Week 48 or later.

When 200 cp/mL thresholds were used to define virological failure as shown in, at Week 24, the PPV was 22.2% (2/9, 95% CI: 2.8%, 60%), and, at Week 48, the PPV increased to 100% (2/2, 95% CI: 15.8%, 100%). At Week 24, the NPV was 90.0% (298/331, 95% CI: 86.3%, 93%), and, at Week 48, the NPV was 89.9% (311/346, 95% CI: 86.2%, 92.9%). At Week 24, the OR was 2.57 (95% CI: 0.25, 14.27), which was not statistically significant (p = 0.469). At Week 48, the OR was 20.76 (95% CI: 2.46, Not Calculable) which was statistically significant (p = 0.022).

Table 44 Comparison of a 200 copies/mL virological threshold with virological failure

-		Virological Failure[a]		-
On-Treatment Visit	**Virological Threshold**	**Yes**	**No**	**Total**
Week 24	≥ 200 cp/mL	2	7	9
	< 200 cp/mL	33	298	331
	Total	35	305	340[+]
Week 48	≥ 200 cp/mL	2	0	2
	< 200 cp/mL	35	311	346
	Total	37	311	348[+]

[+] Valid results obtained by **cobas®** HIV-1.

[a] Virological Failure is classified as 'Yes' if the viral load of a specimen was greater than or equal to 50 cp/mL at Week 144 or at the final visit if there was no Week 144 visit. Final visit had to be at Week 48 or later.

All odds ratios were above 1 and increased between 24 to 48 weeks of treatment. Statistically significant odds

ratios were seen for both thresholds at Week 48. At both thresholds, the high NPV demonstrates the ability of the test to predict which patients are not failing treatment at each timepoint.

Analysis of odds ratios also demonstrated that viral load measurements of patients on treatment that are greater than the given thresholds have higher likelihood of correlation with subsequent virological failure (positive predictive value, or PPV). However, the small number of treatment failures in the study limited the statistical analysis of PPV for virologic failure.

Additionally, the clinical performance of **cobas**®HIV-1 with the **cobas**®Plasma Separation Card (PSC) sample collection device has been evaluated in external studies which supports the use of the PSC for accurate, feasible, and reliable HIV-1 viral load monitoring.[21]

Conclusion

cobas®HIV-1 can reliably quantitate HIV-1 and monitor response to antiretroviral treatment. The results of these studies support the utility of the test in the clinical management of HIV-1-infected patients.

Additional information

Key test features for EDTA plasma samples

Sample type	EDTA plasma
Minimum amount of sample required	650 µL or 350 µL
Sample processing volume	500 µL or 200 µL
Analytical sensitivity	13.2 cp/mL (500 µL) 35.5 cp/mL (200 µL)
Linear range	500 µL: 20 cp/mL – 1.0E+07 cp/mL
	200 µL: 50 cp/mL – 1.0E+07 cp/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%)
Genotypes detected	HIV-1M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1O, HIV-1N

Key test features for PSC dried plasma spot samples

Sample type	Dried plasma spot coming from Plasma Separation Card
Minimum amount of sample required	140 µL whole blood
Sample processing volume	850 µL
Analytical sensitivity	790.2 cp/mL
Linear range	790 cp/mL – 1.0E+07 cp/mL
Specificity	100% (one-sided 95% confidence interval: 98.3%)

Symbols

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

Table 45 Symbols used in labeling for Roche PCR diagnostics products

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

Technical support

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

Manufacturer and importer

Table 46 Manufacturer and importer

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876, USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

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EC REP Roche Diagnostics GmbH
Sandhofer Str. 116
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CE 2797

IVD

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Document revision

Document Revision Information	
Doc Rev. 4.1 12/2024	Added system software version 2.0 information for **cobas**® 6800/8800 systems. Please contact your local Roche Representative if you have any questions.
Doc Rev. 5.0 05/2025	Revised to comply with IVDR, including use of EU Importer and summary of safety and performance. Removed Rx Only from front page. Updated HxV Control Kits hazard information. Clarify long term storage in **Plasma sample collection, transport, and storage** section. Corrected typographical errors. Updated the harmonized symbol page. Added **cobas**® 5800 specific information. Added intended use for **cobas**® HBV/HCV/HIV-1 Control Kit. Updated **cobas**® branding. Added system software version 2.0 information for **cobas**® 6800/8800 systems P/Ns of consumables removed, detailed information on consumables are referenced in the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance Updated **Figure 4** including explanatory text for **Figure 4**. Updated the harmonized symbol page. Added IVD symbol. Please contact your local Roche Representative if you have any questions.
07/2025	Increment SAP material number to 06. Please contact your local Roche Representative if you have any questions.

The summary of the safety and performance report can be found using the following link:
https://ec.europa.eu/tools/eudamed