Rx Only

cobas® HIV-1

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

For in vitro diagnostic use

cobas® HIV-1	P/N:06998836190
cobas® HBV/HCV/HIV-1 Control Kit	P/N:06998887190
cobas® NHP Negative Control Kit	P/N:07002220190

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Intended use

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 of HIV-1-infected individuals using the automated **cobas®** 6800/8800 Systems for specimen processing, amplification and detection. The test can quantitate HIV-1 RNA over the range of 20-10,000,000 copies/mL (33 to 1.67 x 10^7 International Units/mL).

This test is intended for use in conjunction with clinical presentation and other laboratory markers for the clinical management of HIV-1 infected patients. The test can be used 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® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.

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]

Explanation of the test

cobas® HIV-1 is a quantitative test performed on the **cobas®** 6800 System and **cobas®** 8800 System. **cobas®** HIV-1 enables the detection and quantitation of HIV-1 RNA in EDTA plasma of infected patients. Two probes are used to detect and quantify, but not discriminate group M, N and O subtypes. 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.

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®** 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®** 6800/8800 software 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 PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[5-7] Any contaminating amplicons 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 amplicons 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.[8,9] 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

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

Table 1 cobas® HIV-1

cobas® HIV-1
Store at 2-8°C
96 test cassette (P/N 06998836190)

Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	13 mL
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	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 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	6 mL

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

cobas® HBV/HCV/HIV-1 Control Kit
Store at 2–8°C
(P/N 06998887190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+)C) Titer assignment for each analyte is lot specific with the following target concentrations: HBV Target: ~2.3 Log_{10} IU/mL HCV Target: ~2.3 Log_{10} IU/mL HIV-1 Target: ~2.6 Log_{10} cp/mL	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 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, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	5.2 mL (8 x 0.65 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C) Titer assignment for each analyte is lot specific with the following target concentrations: HBV Target: ~6.3 Log_{10} IU/mL HCV Target: ~6.3 Log_{10} IU/mL HIV-1 Target: ~5.3 Log_{10} cp/mL	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 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, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	5.2 mL (8 x 0.65 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.

* Product safety labeling primarily follows EU GHS guidance

Table 3 cobas® NHP Negative Control Kit

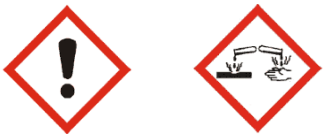
cobas® NHP Negative Control Kit
Store at 2-8°C
(P/N 07002220190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302 + P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

* Product safety labeling primarily follows EU GHS guidance

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 inhaled. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P273: Avoid release to the environment. P280: Wear protective gloves/eye protection/face protection. P305 + P351 + P338: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/Physician if you feel unwell. P330: Rinse mouth
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

***** These reagents are not included in the **cobas®** HIV-1 test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Do not freeze reagents or controls.

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

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

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

Reagent	Storage temperature
cobas® 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

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

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

Reagent	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® HIV-1	30 days from first usage	Max 10 runs	Max 8 hours
cobas® HBV/HCV/HIV-1 Control Kit	Not applicable	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	30 days from loading*	Not applicable	Not applicable

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

Additional materials required (sold separately)

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

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

Instrumentation and software required

The **cobas®** 6800/8800 software and **cobas®** HIV-1 analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas®** 6800/8800 Systems Operator's Manual for additional information for primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination (Refer to CLSI guideline, MM19-A[10]).

- **cobas®** HIV-1 is only intended for quantitation of HIV-1 viral load and is not intended for initial clinical diagnosis of HIV-1 infection.
- **cobas®** HIV-1 is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
- For in vitro diagnostic use only.
- 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.[11,12] Only personnel proficient in handling infectious materials and the use of **cobas®** HIV-1 and **cobas®** 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 hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **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, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- 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.

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, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) 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 hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas®** 6800/8800 instrument, follow the instructions in the **cobas®** 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, 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 (15-30ºC) 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.

This test has been validated for use with only human plasma collected in EDTA anticoagulant. Testing of specimen collected with other anticoagulants may result in inaccurate results.

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.
- 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 collection tube 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. For long-term storage up to 6 months, temperatures at ≤ -60°C are recommended.
- Plasma samples are stable for up to four freeze/thaw cycles when stored frozen at ≤ -18°C.
- Ensure sufficient whole blood collection to allow usage of the processing volume for EDTA plasma of 500 µL (for a total minimum sample requirement of 650 µL) or 200 µL (for a total minimum sample requirement of 350 µL).
- 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.

Instructions for use

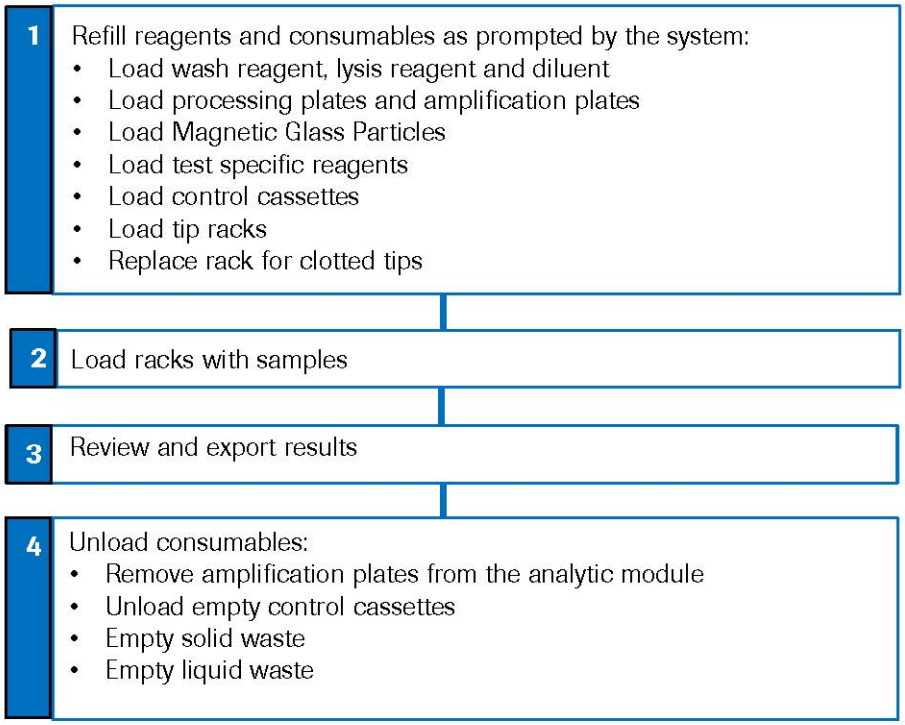
Procedural notes

- 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.
- Refer to the **cobas®** 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Running cobas® HIV-1

cobas® HIV-1 can be run with a minimum required sample volume of 650 µL for the 500 µL sample workflow or 350 µL for the 200 µL sample workflow. The test procedure is described in detail in the **cobas®** 6800/8800 Systems Operator's Manual. Figure 1 below summarizes the procedure.

Figure 1 cobas® HIV-1 test procedure



Results

The **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

- One negative control (–) C and two positive controls, a low positive control HIV-1 L(+)C and a high positive control HIV-1 H(+)C, are processed with each batch.
- The user checks for batch validity either within the **cobas**® 6800/8800 software (monitor) or in a printed report.
- The batch is valid if no flags appear for all three controls, which include one negative control and two positive controls: HIV-1 L(+)C, HIV-1 H(+)C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L(+)C and HxV H(+)C.

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

Control flags

Table 9 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.

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

HxV L(+)C stands for **cobas**® HBV/HCV/HIV-1 low positive control and HxV H(+)C stands for **cobas**® HBV/HCV/HIV-1 high positive control in the **cobas®** 6800/8800 software.

Interpretation of results

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

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

Table 10 Target results for individual target result interpretation

Results	Interpretation
Target Not Detected	HIV-1 RNA not detected. Report results as "HIV-1 not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HIV-1 detected, less than (Titer Min)." Titer min = 20 cp/mL and 33 IU/mL (500 µL sample processing volume) Titer min = 50 cp/mL and 83 IU/mL (200 µL sample processing volume)
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-1 detected, greater than (Titer Max)." Titer max = 1.00E+07 cp/mL and 1.67E+07 IU/mL (for the 500 µL and 200 µL sample processing volumes)

[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.

Procedural limitations

- **cobas®** HIV-1 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®** 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.
- 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.
- The detection rate of HIV-1 group O at 20 cp/mL (claimed LLoQ for the **cobas®** HIV-1 with the 500 µL sample processing volume) was observed to be 90.5%. Similarly, the detection rate of HIV-1 CRF01_AE, HIV-1 Group O at 50 cp/mL (claimed LLoQ for the **cobas®** HIV-1 with the 200 µL sample processing volume) was observed to be 90.5% and 88.9% respectively. Both detection rates are lower than what was observed for all other genotypes for both sample processing volumes.
- 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 donated blood or plasma, or as a diagnostic test to confirm the presence of HIV-1 infection.
- Samples from subjects under 19 years of age were not evaluated.

Non-clinical performance evaluation

Key performance characteristics

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) group M subtype B obtained from NIBSC, 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 11 and Table **12**. The study demonstrates that **cobas®** HIV-1 detected HIV-1 RNA with a detection rate of 95%, as determined by PROBIT, at a concentration of 13.2 cp/mL (22.0 IU/mL) for the 500 µL sample processing volume and at a concentration of 35.5 cp/mL (59.2 IU/mL) for the 200 µL sample processing volume.

Table 11 HIV-1 RNA WHO International Standard 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	Detection 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% detection rate		13.2 cp/mL; 95% confidence interval: 11.4, 15.9 cp/mL 22.0 IU/mL; 95% confidence interval: 19.0, 26.5 IU/mL		

Table 12 HIV-1 RNA WHO International Standard 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 interval: 30.8–43.2 cp/mL 59.2 IU/mL; 95% confidence interval: 51.3–72.0 IU/mL		

Linear range

The linearity of **cobas®** HIV-1 was evaluated using dilution series consisting of 12 panel members for the 500 µL sample processing volume and 11 panel members for 200 µL sample processing volume, spanning the linear range of the assay 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.[13] Three reagent lots were analyzed on three **cobas®** 6800/8800 Systems, three operators for a total of 16 replicates per panel member and lot across four testing days (total of 12 testing days; four replicates per kit lot and day).

With the 500 µL sample processing volume, **cobas®** HIV-1 was demonstrated to be linear from 20 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) (Figure 2).

With the 200 µL sample processing volume, **cobas®** HIV-1 was demonstrated to be linear from 50 cp/mL to 1.00E+07 cp/mL (83.3 IU/mL to 1.67E+07 IU/mL) (Figure 3).

Figure 2 Linear range determination in EDTA plasma (500 µL)

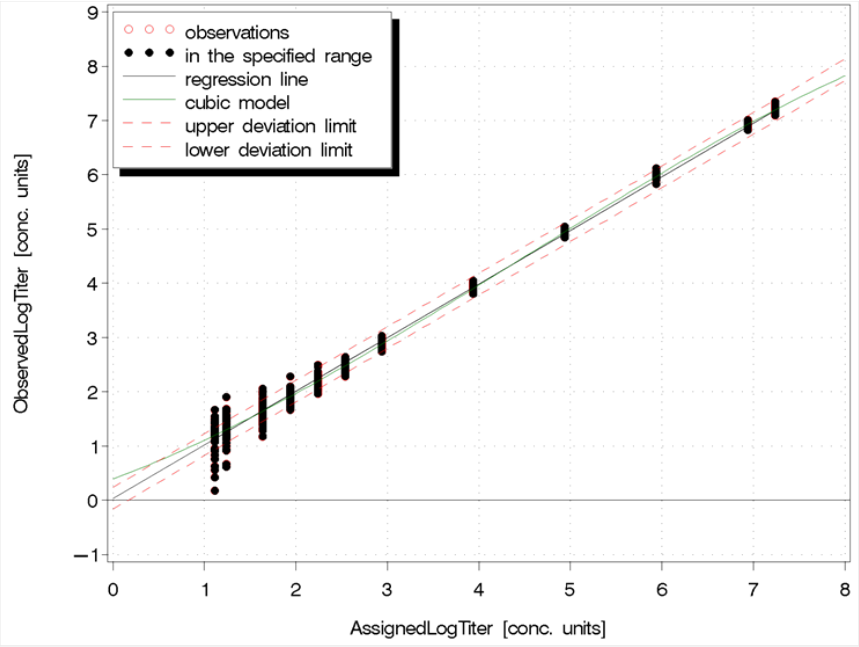
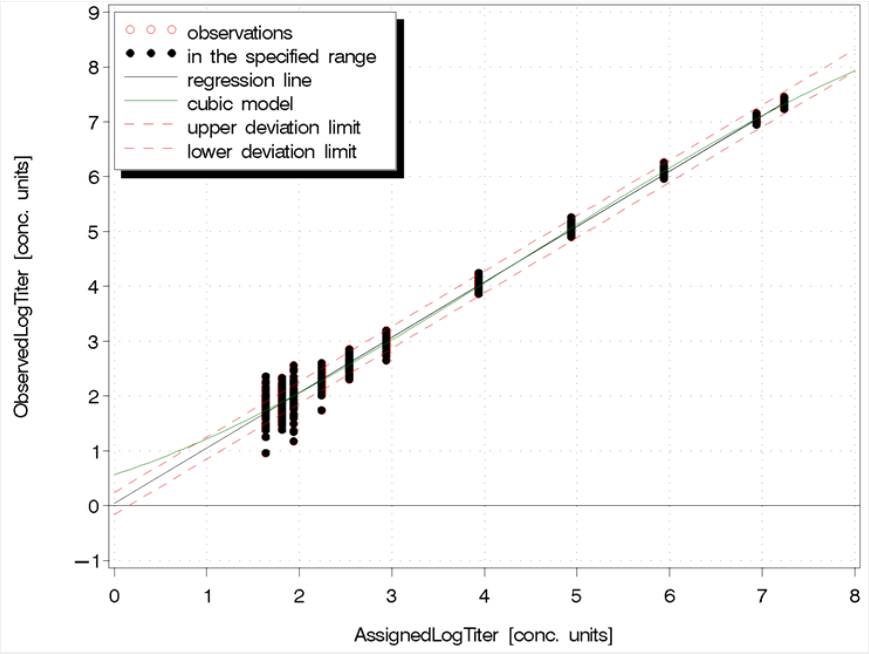


Figure 3 Linear range determination in EDTA plasma (200 µL)



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 across three lots of **cobas®** HIV-1 test reagents using three **cobas®** 6800/8800 Systems and three operators over 12 days. The results are shown in Table 13 and Table 14.

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

Table 13 Within laboratory precision of **cobas®** HIV-1 (EDTA plasma samples – sample 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	Cultured Virus	0.04	0.06	0.03	0.05
1.00E+06	8.67E+05	Cultured Virus	0.06	0.05	0.04	0.05
1.00E+05	8.67E+04	Cultured Virus	0.05	0.07	0.04	0.05
1.00E+04	8.67E+03	Cultured Virus	0.06	0.06	0.04	0.05
1.00E+03	8.67E+02	Cultured Virus	0.07	0.06	0.07	0.07
4.00E+02	3.47E+02	Cultured Virus	0.09	0.10	0.09	0.09
2.00E+02	1.73E+02	Cultured Virus	0.11	0.08	0.14	0.11
1.00E+02	8.67E+01	Cultured Virus	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 refer to the log-transformed titers obtained with each of the three reagent lots and with all lots combined.

Table 14 Within laboratory precision of **cobas®** HIV-1 (EDTA plasma samples – sample 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 deviation (SD) columns refer to the log-transformed titer for each of the three reagent lots and with all lots combined. .

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 detection rate determination was performed with 63 replicates for each level. Testing was conducted with one lot of **cobas®** HIV-1 reagents. The results from EDTA plasma using 500 µL are shown in Table 15. These results verify that **cobas®** HIV-1 detected HIV RNA of HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), and HIV-1N at 20 cp/mL or below with a rate of ≥ 95%. HIV-1O was detected at 20 cp/mL with a rate of 90.5%.

Table 15 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	Detection rate in % (95% CI*)	Number of valid replicates	Number of positives	Detection rate in % (95% CI*)	Number of valid replicates	Number of positives	Detection 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 the 200 µL sample processing volume. The data are summarized in Table 16. These results verify that **cobas®** HIV-1 detected HIV RNA of HIV-1M (A, C, D,

F, G, H, CRF02_AG), and HIV-1N at 50 cp/mL or below with a rate of ≥ 95%. HIV-1 CRF01_AE and HIV-1O were detected at 50 cp/mL with a rate of 90.5 and 88.9% respectively.

Table 16 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 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 spanning the linear range for the 500 µL sample processing volume and six panel members for the 200 µL sample processing volume. 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 $\log_{10}$.

Performance with HIV-1 negative specimens

The performance of **cobas®** HIV-1 was determined by analyzing 600 EDTA plasma samples from healthy HIV negative individuals. Each of these samples was tested with two lots of cobas HIV-1 reagents. All samples tested negative for HIV-1 RNA. In the test panel, the results of all specimens tested with cobas HIV-1 was 100% "Target Not Detected" (95% confidence interval: 99.5, 100%).

Potentially interfering microbial contaminants

The analytical specificity of **cobas®** HIV-1 was evaluated by testing a panel of microorganisms prepared in HIV RNA negative EDTA plasma (Table 17). Potential interference was evaluated by testing the same organisms in EDTA plasma containing low levels of HIV-1 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 $\pm$ 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 17 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	TBE 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		

Potentially interfering endogenous and exogenous substances

Elevated levels of triglycerides (up to 34.5 g/L), conjugated bilirubin (0.252 g/L), unconjugated bilirubin (0.253 g/L), albumin (58.7 g/L), hemoglobin (up to 2.85 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 presence and absence of HIV-1 RNA.

In addition, drug compounds listed in Table 18 were tested at three times the C_{max} in presence and absence of HIV-1 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 $\pm$ 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 18 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	Ribavirin
	Peginterferon α-2b	
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	Acyclovir
	Lamivudine	Valganciclovir
	Ganciclovir	Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin	Pyrazinamide
	Clarithromycin	Rifabutin
	Ethambutol	Rifampicin
	Fluconazole	Sulfamethoxazole
	Isoniazid	Trimethoprim

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%].

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 replicates per run. Reproducibility was evaluated using a random effects model including lot, site, operator, day, run, and within-run. Table 19 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 19 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 log10 transformed measurements.
[b] Calculated using the total variability from the SAS MIXED procedure based on log10 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 20 below, the negative percent agreement (NPA) for the **cobas**® 6800 System using all valid negative panel member tests was 100%.

Table 20 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 21.

Table 21 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%)
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 22 shows the mean paired viral load difference between cobas® HIV-1 and the TaqMan® HIV-1 Test, v2.0.

Table 22 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.

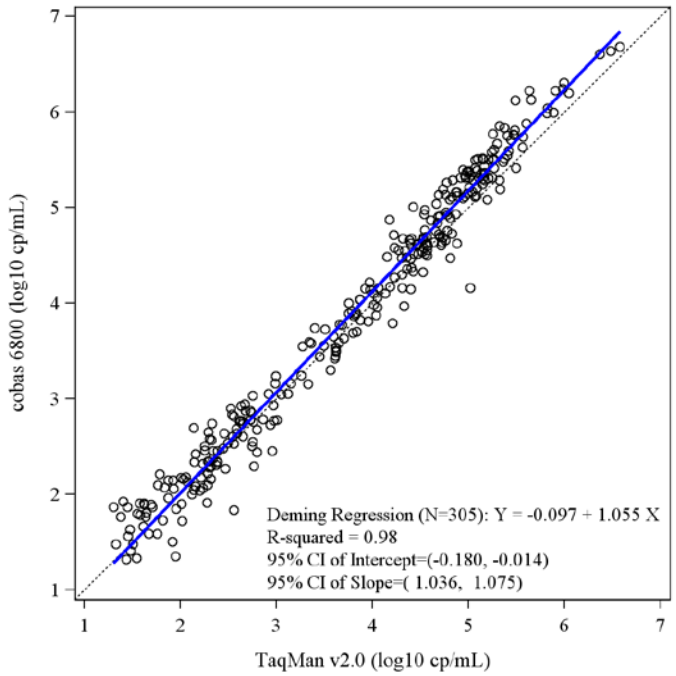
The results of Deming regression analysis between cobas® HIV-1 and the TaqMan® HIV-1 Test, v2.0 are tabulated in Table 23 and shown graphically in Figure 4. The dashed line indicates perfect agreement between the two test methods.

Table 23 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 4 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 24 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 24 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 25 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 25 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 26 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 26 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 27 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 27 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 28.

Table 28 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 29 shows the demographic characteristics of the 355 subjects included in the study.

Table 29 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 virological failure are shown in Table 30 and Table 31.

The analyses of the 50 copies/mL virological threshold with virological failure (at Week 24 and Week 48) are shown in Table 30. 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 30 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 Table 31, 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 31 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.

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

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	20 cp/mL – 1.0E+07 cp/mL (500 µL) 50 cp/mL – 1.0E+07 cp/mL (200 µL)
Performance with HIV-1 negative specimens	100% (two sided 95% confidence interval: 99.5%, 100%)
Genotypes detected	HIV-1M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1O, HIV-1N

Symbols

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

Table 32 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In Vitro* Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains Sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number
	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 33 Manufacturer and distributors

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

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

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Document revision

Document Revision Information	
Doc Rev. 1.0 01/2016	First Publishing.

Doc Rev. 2.0 11/2016	Added mixing step to Specimen collection, transport, and storage section. Clarified storage of EDTA plasma sample storage in secondary tubes upon separation in Samples section. Added Roche web address www.roche.com. Please contact your local Roche Representative if you have any questions.
Doc Rev. X.0 XX/201X	Throughout IFU, added 200 µL claim and references to 500 µL sample processing volume. Added Roche web address www.roche.com Please contact your local Roche Representative if you have any questions. Updated hazard code for lysis reagen in Reagent and Materials section.