Rx Only

cobas® HIV-1

Quantitative nucleic acid test

for use on the cobas® 4800 System

For in vitro diagnostic use

cobas® HIV-1	120 Tests	P/N: 08792992190
cobas® HBV/HCV/HIV-1 Control Kit	10 Sets	P/N: 06979572190
cobas® 4800 System Sample Preparation Kit 2	240 Tests 960 Tests	P/N: 06979513190 P/N: 06979521190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Specimen Diluent 2	240 Tests	P/N: 06979556190
cobas® 4800 System Lysis Kit 2	240 Tests 960 Tests	P/N: 06979530190 P/N: 06979548190

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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.

This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progression for the clinical management of HIV-1-infected patients. This 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.

Summary and explanation of the test

Background

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. Most importantly, viral load monitoring has been shown to reduce the risk of drug resistance and 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 HIV treatment 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.[3] European guidelines continue to recommend the use of 50 copies/mL as the threshold for determining treatment failure.[7] The correct threshold to use has not been determined in a rigorous clinical trial. 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.

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 nucleic acid test performed on the **cobas**® 4800 System. **cobas**® HIV-1 enables the detection and quantitation of HIV 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 armored RNA quantitation standard (RNA QS), which is introduced into each sample 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**® 4800 System consists of the **cobas**® x 480 sample preparation instrument and the **cobas**® z 480 real-time PCR analyzer. Automated data management is performed by the **cobas**® 4800 software which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HIV 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 acids from patient samples, external controls and RNA QS molecules are simultaneously extracted. In summary, viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acids from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HIV. 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 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 deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[12-14] 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, any newly formed amplicon is not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

cobas® HIV-1 master mix contains two detection probes specific for the HIV-1 target sequences and one for 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 detection channels.[15,16] When not bound to the target sequence, the fluorescent signals of the intact probes are 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.

Materials and reagents

Reagents

All unopened reagents and controls shall be stored as recommended in the Reagent storage and handling requirements table.

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HIV-1 120 Tests (P/N: 08792992190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 x 1.75 mL	N/A
	HIV-1 MMX R2 (**cobas**® **HIV-1** Master Mix Reagent 2) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A
	RNA QS (**cobas**® RNA Quantitation Standard) 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	10 x 1.75 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HBV/HCV/HIV-1 Control Kit 10 Sets (P/N: 06979572190)	**HBV/HCV/HIV-1 L(+)C** (**cobas**® HBV/HCV/HIV-1 Low Positive Control) < 0.001% 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 HIV 1/2, antibody to HCV, 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	10 x 0.75 mL	 **WARNING** H317 May cause an allergic skin reaction. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P333 + P313 If skin irritation or rash occurs: Get medical advice/attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/container to an approved waste disposal plant. 55965-84-9 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)
	HBV/HCV/HIV-1 H(+)C (**cobas**® HBV/HCV/HIV-1 High Positive Control) < 0.001% 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 HIV 1/2, antibody to HCV, 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	10 x 0.75 mL	
	(–) C (**cobas**® Negative Control) Normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, 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	10 x 0.75 mL	

Kit		Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Sample Preparation Kit 2 240 Tests (P/N: 06979513190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 x 8 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 x 17 mL	
cobas® 4800 System Sample Preparation Kit 2 960 Tests (P/N: 06979521190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 x 16 mL	
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 x 17 mL	
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 x 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 x 200 mL	N/A
cobas® 4800 System Specimen Diluent 2 240 Tests (P/N: 06979556190)	**SD 2** Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 x 8 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 x 1.0 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing mist or vapours. P280: Wear protective gloves. P284: Wear respiratory protection. Response: P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01 -6 Proteinase, Tritirachium album serine

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 x 27 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing 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. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 x 1.0 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing mist or vapours. P280: Wear protective gloves. P284: Wear respiratory protection. P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01 -6 Proteinase, Tritirachium album serine

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 x 84 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing 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. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

[a] Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagent	Storage Temperature	Storage Time
cobas® HIV-1	2–8°C	Stable until the expiration date indicated
cobas® HBV/HCV/HIV-1 Control Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Sample Preparation Kit 2	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiration date indicated
cobas® 4800 System Specimen Diluent 2	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 2	2–8°C	Stable until the expiration date indicated

Do not freeze reagents.

Additional materials required

Materials	P/N
cobas® 4800 System Extraction (deepwell) Plate 2.0 mL	06884008001
cobas® 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Lab gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas**® x 480 instrument **cobas**® z 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.2.0 or higher
cobas® 4800 System **cobas**® HIV-1 AP v1.1.0 or higher

Note: Contact your local Roche representative for a detailed order list for sample racks, tip racks, reagent racks and plate carriers accepted on the instruments.

Supported sample tubes

The test accepts commonly used primary and secondary tubes.

The following sample tubes are supported:

Primary tubes

Nominal Diameter (mm)	Sample input volume – processed (centrifuged) whole blood		Tube Additive
	400 µL processing volume	200 µL processing volume	EDTA Plasma
11-14	1800 µL or more	1000 µL or more	With or without gel
14.5-16	More than 4000 µL	More than 4000 µL	With or without gel

For specific sample tube order information, and minimum sample input volumes for specific primary tubes, contact your local Roche representative.

Secondary tubes

Nominal Diameter (mm)	Sample input volume	
	400 µL processing volume	200 µL processing volume
11-16	1000 µL or more (specific secondary tubes have a minimum input volume of less than 1000 µL)	750 µL or more (specific secondary tubes have a minimum input volume of less than 750 µL)

For specific sample tube order information, and minimum sample input volumes for specific secondary tubes, contact your local Roche representative.

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 analytical sensitivity of this test, care should be taken to keep reagents, samples 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.[17,18] Only personnel proficient in handling biohazardous materials and the use of **cobas**® HIV-1 and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
- **cobas**® HBV/HCV/HIV-1 Control Kit contains plasma derived from human blood. The source material has been tested by a licensed antibody test and found to be non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg and antibody to HBc. Testing by PCR methods showed no detectable HIV-1 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.
- Prevent exposure of MGP to sources of magnetic field.
- **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.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas**® x 480 instrument or **cobas**® z 480 analyzer, consult the **cobas**® 4800 System – User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the appropriate **cobas**® 4800 System – User Assistance.

Note: ***For specific instructions, see "Sample collection, transport, and storage".***

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the lab gloves.
- Wear eye protection, lab coats and disposable lab gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- 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.
- Maintain a consistent temperature in the laboratory that conforms to the environmental specifications of the system, as provided in the **cobas**® 4800 System - User Assistance.

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 bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 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, **cobas**® 4800 Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 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**® 4800 Lysis Buffer 2, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

- Lab gloves must be worn and must be changed between handling samples and **cobas**® HIV-1 reagents to prevent contamination. Avoid contaminating lab gloves when handling samples and controls. Wear lab gloves, lab coats, and eye protection when handling samples and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of samples is not prevented during sample handling.

Integrity

- Do not use kits after their expiry dates.
- Do not pool reagents.
- Do not use disposable items after their expiry dates.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® HIV-1, **cobas**® 4800 System Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 contain sodium azide (see "**Warnings and precautions**"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the cobas® 4800 System - User Assistance.

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® x 480 instrument, follow the instructions in the appropriate **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas**® x 480 instrument or the **cobas**® z 480 analyzer. Clean the **cobas**® x 480 instrument or the **cobas**® z 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - User Assistance.

Sample collection, transport, and storage

Note: Handle all samples 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.

Sample collection

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.

Note: The user must follow the guidance provided by the tube manufacturer for plasma preparation.

Sample transport storage and stability

- Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation and subsequent testing.
- Plasma samples may be stored in secondary tubes for up to 24 hours at 2°C to 30°C, up to 72 hours at 2°C to 8°C or up to 6 weeks at ≤ -18°C. Separated plasma samples in secondary tubes are stable for up to three freeze/thaw cycles when stored frozen at ≤ -18°C.
- 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

Running the test

Sample processing volume

The default sample processing volume for **cobas**® HIV-1 is 400 µL. For low volume samples, a sample processing volume of 200 µL may be chosen. Only in this case, **cobas**® 4800 System Specimen Diluent 2 as an additional reagent has to be loaded onto the system. The user will be prompted to do so by the software wizard if the sample type "Diluted Plasma" was chosen during the work order creation.

Figure 1: cobas® HIV-1 workflow

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Start run
5	Scan parameter cards
6	Load samples
7	With LIS: confirm work order Without LIS: create work order
8	Load consumables (deepwell plate, microwell plate, tip racks)
9	Load reagents
10	Start sample preparation run
11	Unload and seal microwell plate
12	Load microwell plate into analyzer
13	Remove samples, used reagents, and deepwell plate
14	Review results
15	With LIS: send results to LIS
16	Unload analyzer

Note: Refer to the cobas® 4800 System - User Assistance for cobas® HIV-1 for detailed operating instructions.

Run Size

The generic sample preparation reagents (**cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit) are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and samples to be run. **cobas**® HIV-1 is available in a single kit size sufficient to test up to 120 (10×12) samples, including controls and samples. For each test batch, one HBV/HCV/HIV-1 Low Positive Control, one HBV/HCV/HIV-1 High Positive Control and one Negative Control must be used. For a single test run, the maximum number of samples allowed is 93 specimens and 3 controls. Figure 1 summarizes the procedure.

Note: For optimal use of reagents, the generic sample preparation reagents can be used for a run containing 1-21 total samples (10×24 test kit size) or 1-93 total samples (10×96 test kit size). However, different kit sizes of the cobas® 4800 System Wash Buffer Kit, cobas® 4800 System Sample Preparation Kit 2 and cobas® 4800 System Lysis Kit 2 cannot be combined. For example, if a 96-test Wash Buffer reagent bottle is scanned at the start of the run, 96-test size reagents from the other sample preparation reagent kits must also be used.

Workflow

cobas® HIV-1 is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas**® x 480 instrument followed by amplification/detection on the **cobas**® z 480 analyzer. **cobas**® HIV-1 may be performed alone, or in mixed-batch mode with tests that share the same automated sample extraction process and PCR profile for amplification and detection. At the test selection step the software will display tests that are compatible with **cobas**® HIV-1 for mixed-batch mode. Refer to the **cobas**® 4800 System - User Assistance for details.

1. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System - User Assistance.
2. Perform maintenance actions by following the instructions in the **cobas**® 4800 System - User Assistance.
3. Collect all reagents and consumables needed. All reagents except HIV-1 MMX R2 and MMX R1 must be at ambient temperature prior to loading on the **cobas**® x 480 instrument. The HIV-1 MMX R2 and MMX R1 reagents may be taken directly from 2-8°C storage as they equilibrate to ambient temperature on board the **cobas**® x 480 instrument by the time they are used in the process.

Note: All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.

4. Start a new run and select the workflow type as HIV-1. To perform a mixed batch run, select other applicable workflow types (i.e. CMV, HCV or HCV GT) in addition to HIV-1.
5. Follow the software wizard guide and scan the barcode on the control ranges and calibration coefficients parameter cards.

Note: Scan parameter cards from unexpired reagents. The software does not check reagent expiry dates in parameter cards. Check the expiry date printed in the parameter card or in the reagent kits before scanning the corresponding barcode ID.

6. Load the samples. Primary or secondary sample tubes can be loaded and minimum sample volume depends on the tube type and size. Refer to the supported sample tubes section for more details.
7. Create the work order. There are three ways to create a work order:
   - By using the sample editor before sample rack is loaded into **cobas**® x 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary. When selecting the requested results, select "HIV-1".
   - By following the software wizard for the new run and loading samples into **cobas**® x 480 instrument when prompted. The sample barcodes will be automatically scanned, and the requested results for each specimen must be defined. When selecting the requested results, select"HIV-1".
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System - User Assistance for more details. Load samples and define/select work order or use LIS as appropriate.
8. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.

9. Load the reagents.

   Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:

   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once LYS 2 or WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial immediately prior to dispensing into the reagent reservoir.

10. Load amplification/detection reagent vials (HIV-1 MMX R2, MMX R1 and RNA QS), control vials [HBV/HCV/HIV-1 L(+)C, HBV/HCV/HIV-1 H(+)C and (–) C] and generic reagent vials (P2 and SD2 as required) directly onto the reagent carrier.

Note: In order to prevent unnecessary run aborts and contamination, it is required to flick down the reagent vials to avoid formation of bubbles/liquid films. Controls should be opened starting with the ones closest to you (from position 24 to 1). Change lab gloves after handling positive controls.

11. Start sample preparation run. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carriers. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System - User Assistance.
12. After unloading the microwell plate follow the instructions in the **cobas**® 4800 System - User Assistance for sealing and transferring the plate to the **cobas**® z 480 analyzer.
13. Load the microwell plate into the analyzer and start the amplification and detection run as instructed in the **cobas**® 4800 System - User Assistance.

Note: The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 40 minutes after the end of the cobas® x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system aborts the run if the timer has expired.

14. Remove samples, used reagents and deepwell plate as instructed in the **cobas**® 4800 System - User Assistance**.**
15. After the amplification and detection run is completed, follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.
16. If working with LIS, send results to the LIS.
17. Follow the instructions in the **cobas**® 4800 System - User Assistance to unload the microwell plate from the **cobas**® z 480 analyzer.

Results

The **cobas**® 4800 System automatically determines 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 the **cobas**® HIV-1 Test is 0.6 cp/IU.

Quality control and validity of results

- One negative control (–) C and two positive controls, a low positive control HBV/HCV/HIV-1 L(+)C and a high positive control HBV/HCV/HIV-1 H(+)C, are processed with each batch.
- In the **cobas**® 4800 Software and/or report, check for batch validity.
- Invalidation of results is performed automatically by the **cobas**® 4800 Software based on negative and positive control failures.

Control result interpretation

Table 1: Control result interpretation for negative and positive controls

Negative Control	Result	Interpretation
(–) C	Target Not Detected	Control is valid. HIV-1 RNA not detected.
	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Result**	**Interpretation**
HBV/HCV/HIV-1 L(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
HBV/HCV/HIV-1 H(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

Interpretation of results

Note: All assay and batch validation is determined by the cobas® 4800 Software.

Note: A valid batch may include both valid and invalid sample results.

For a valid batch, sample results are interpreted as shown in Table 2.

Table 2: Target results for individual target result interpretation

cobas® HIV-1	**Result Report and 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 = 2.00E+01 cp/mL and 3.33E+01 IU/mL (400 µL) Titer min = 6.00E+01 cp/mL and 1.00E+02 IU/mL (200 µL)
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 (400 µL and 200 µL)

[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 and the test should be repeated. Multiply the reported result by the dilution factor.

List of result flags

The following table lists all flags which are relevant for result interpretation.

Table 3: List of flags

Flag code	Description	Recommended action
R4800	The target is invalid due to calculation failure.	The target is invalid due to calculation failure. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4801	The quantitation standard is invalid.	The quantitation standard is invalid for a sample. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4802	An external control is invalid.	An external control is invalid.[a] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4803	The quantitation standard is invalid.	The quantitation standard is invalid for an external control. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4804	The external control is out of range.	The external control is out of range.[b] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
X3	Error: Clot was detected Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

[a] This is a sample flag and it occurs when an external control in the run is called invalid.

[b] This flag includes all scenarios in which the external control is invalid (target calling or titer).

Note: For all system flags refer to the cobas® 4800 System - User Assistance.

Procedural limitations

1. **cobas**® HIV-1 has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2, **cobas**® 4800 System Wash Buffer Kit and **cobas**® 4800 System Specimen Diluent 2.
2. Reliable results are dependent on adequate sample collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and **cobas**® 4800 System - User Assistance.
3. This test has been validated only for use with EDTA plasma. Testing of other sample types may result in inaccurate results.
4. 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.
5. 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.
6. The predictive value of an assay depends on the prevalence of the disease in any particular population.
7. The addition of AmpErase enzyme into the **cobas**® HIV-1 Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
8. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
9. Only the **cobas**® x 480 instrument and **cobas**® z 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
10. 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.
11. Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of **cobas**® HIV-1 has been determined in a non-clinical study to be 0.0%. Run to run cross-contamination has not been observed.
12. **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

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 EDTA plasma using sample processing volumes of 400 µL and 200 µL. Panels of six concentration levels plus a negative were tested over three lots of **cobas**® HIV-1 reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma from both sample processing volumes are shown in Table 4 and Table 5. The study demonstrates that **cobas**® HIV-1 detected HIV-1 RNA with a hit rate of ≥ 95%, as determined by PROBIT, at a concentration of 14.2 cp/mL (23.7 IU/mL) for the 400 µL sample processing volume and at a concentration of 43.9 cp/mL (73.4 IU/mL) for the 200 µL sample processing volume.

Table 4: Limit of detection in EDTA plasma (400 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
60.0	100.0	252	252	100.0%
30.0	50.0	251	251	100.0%
20.0	33.3	252	247	98.0%
10.0	16.7	252	227	90.1%
5.0	8.3	252	160	63.5%
2.0	3.3	252	86	34.1%
0.0	0.0	71	0	0.0%
LoD by PROBIT at 95% hit rate		14.2 cp/mL; 95% confidence range: 12.5–16.6 cp/mL 23.7 IU/mL; 95% confidence range: 20.8–27.7 IU/mL		

Table 5: 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
100.0	166.7	251	251	100.0%
60.0	100.0	251	249	99.2%
30.0	50.0	251	227	90.4%
15.0	25.0	250	172	68.8%
7.0	11.7	250	110	44.0%
3.5	5.8	250	83	33.2%
0.0	0.0	68	0	0.0%
LoD by PROBIT at 95% hit rate		43.9 cp/mL; 95% confidence range: 37.7–52.7 cp/mL 73.4 IU/mL; 95% confidence range: 62.9–88.1 IU/mL		

Linear range

Linearity of **cobas**® HIV-1 was determined by analysis with a dilution series consisting of 12 panel members (400 µL sample processing volume) and 11 panel members (200 µL sample processing volume) with the predominant HIV-1 group M subtype B spanning the assay linear range. Panel members were prepared from a high titer HIV-1 RNA positive cell culture supernatant specimen.

With the 400 µL sample processing volume, **cobas**® HIV-1 was linear from 20.0 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) and showed a maximal deviation from the better fitting non-linear regression of less than ±0.07 $\log_{10}$ (see Figure 2). Across the linear range, the accuracy of the test was within ±0.18 $\log_{10}$.

With the 200 µL sample processing volume, **cobas**® HIV-1 was linear from 60.0 cp/mL to 1.00E+07 cp/mL (100.0 IU/mL to 1.67E+07 IU/mL) and showed a maximal deviation from the better fitting non-linear regression of less than ±0.08 $\log_{10}$ (see Figure 3). Across the linear range, the accuracy of the test was within ±0.19 $\log_{10}$.

Figure 2: Linear range determination in EDTA plasma (400 µL)

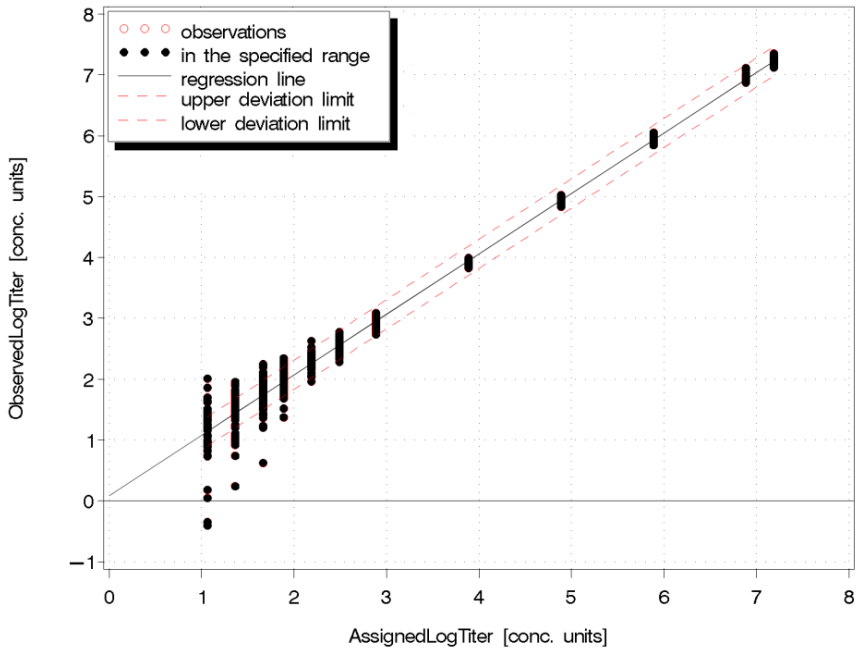
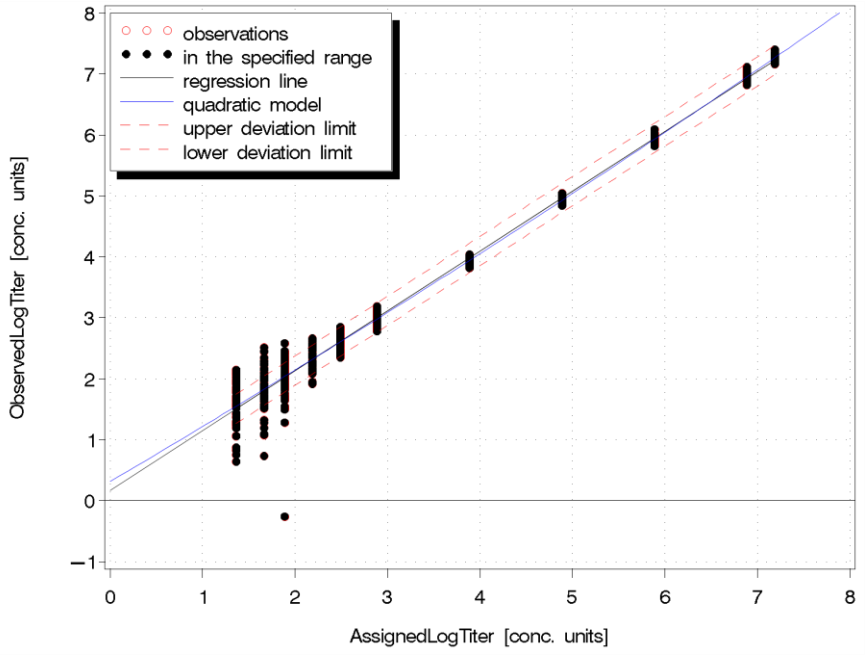


Figure 3: Linear range determination in EDTA plasma (200 µL)



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. Seven dilution levels (400 µL sample processing volume) and five dilution levels (200 µL sample processing volume) were tested in 16 replicates for each level and sample processing volume across three lots of **cobas**® HIV-1 reagents using three instruments and four operators over 15 days. The results are shown in Table 6 and Table 7.

cobas® HIV-1 showed excellent precision for three lots of reagents tested across a concentration range of 1.0E+02 cp/mL to 2.00E+07 cp/mL with 400 µL sample processing volume and 1.00E+04 cp/mL to 2.00E+07 cp/mL with 200 µL sample processing volume.

Table 6: Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – sample processing volume of 400 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
2.0E+07	1.54E+07	Cell Culture	0.05	0.05	0.04	0.05
1.0E+06	7.70E+05	Cell Culture	0.05	0.05	0.05	0.05
1.0E+05	7.70E+04	Cell Culture	0.05	0.05	0.05	0.05
1.0E+04	7.70E+03	Cell Culture	0.04	0.06	0.07	0.06
1.0E+03	7.70E+02	Cell Culture	0.09	0.10	0.07	0.09
4.0E+02	3.08E+02	Cell Culture	0.08	0.10	0.09	0.09
1.0E+02	7.70E+01	Cell Culture	0.18	0.24	0.15	0.19

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

Table 7: 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
2.0E+07	1.54E+07	Cultured Virus	0.04	0.04	0.04	0.04
1.0E+07	7.70E+06	Cultured Virus	0.04	0.04	0.04	0.04
1.0E+06	7.70E+05	Cultured Virus	0.03	0.04	0.05	0.04
1.0E+05	7.70E+04	Cultured Virus	0.04	0.05	0.03	0.04
1.0E+04	7.70E+03	Cultured Virus	0.05	0.04	0.04	0.04

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

Group/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-1 M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1O and HIV-1N were diluted in EDTA plasma to the LoD concentration of the predominant group/subtype (HIV-1 M subtype B) based on 95% Hit Rate LoD analysis (20.0 cp/mL). Hit rate analysis was performed with 42 replicates for each group/subtype. These results verify that **cobas**® HIV-1 detected HIV for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1 O and HIV-1N at the concentration of 20 cp/mL with an upper one-sided 95% confidence interval being greater to the expected hit rate of 95%.

Table 8: LoD verification of HIV-1 group M subtypes, group O, and group N in 400 µL EDTA plasma

Group	Subtype	Tested concentration: 20.0 cp/mL		
		Number of positive replicates	Number of valid replicates	Hit rate in % (upper one sided 95% CI)
M	A	39	42	92.86% (98.02%)
C		42	42	100% (100%)
D		41	42	97.62% (99.88%)
F		39	42	92.86% (98.02%)
G		38	42	90.48% (96.68%)
H		41	42	97.62% (99.88%)
CRF01_AE		40	42	95.24% (99.15%)
CRF02_AG		41	42	97.62% (99.88%)
O		39	42	92.86% (98.02%)
N		41	42	97.62% (99.88%)

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. Panel members were prepared from high titer HIV-1 RNA positive cell culture supernatant specimens of the respective group/subtype. The tested linear range of **cobas**® HIV-1 spanned from the LLoQ (20.0 cp/mL for a sample processing volume of 400 µL) to the ULoQ (1.0E+07 cp/mL) and included at least two medical decision points. Twelve 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 maximal deviation between the linear regression and the better fitting non-linear regression was less than 0.12 $\log_{10}$.

Specificity

The specificity of **cobas**® HIV-1 was determined by analyzing HIV negative EDTA plasma samples from individual donors. Six hundred fourteen individual EDTA plasma samples were tested with three lots of the **cobas**® HIV-1 reagents. All samples tested negative for HIV-1 RNA. In the test panel the specificity of **cobas**® HIV-1 was 100.0% [the lower bound of 95% one sided confidence interval (Clopper Pearson) was 99.5%].

Analytical specificity

The analytical specificity of **cobas**® HIV-1 was evaluated by diluting a panel of pathogens with HIV RNA positive and HIV RNA negative EDTA plasma (Table 9). The pathogens were added to negative 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 pathogen samples without HIV-1 target and positive results were obtained on all of the pathogen samples with HIV-1 target. Furthermore, the mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.32 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 9: Pathogens tested for cross-reactivity

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

Analytical specificity – interfering substances

Elevated levels of triglycerides (27.9 - 29.0 g/L), conjugated bilirubin (0.18 - 0.22 g/L), unconjugated bilirubin (0.19 - 0.2 g/L), albumin (57.8 - 60.6 g/L), hemoglobin (1.8 - 2.3 g/L) and human DNA (2 mg/L) in samples were tested in presence and absence of HIV RNA. The tested substances were shown not to interfere with the test performance of **cobas**® HIV-1. Moreover, the presence of markers for the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody (ANA) were tested.

In addition, drug compounds listed in Table 10 were tested at three times the C_{max} in presence and absence of HIV RNA.

All potentially interfering substances have been shown to not interfere 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. Furthermore, the mean $\log_{10}$ titer of each of the positive HIV-1 samples containing potentially interfering substances was within ± 0.20 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 10: 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	Nelfinavir
	Darunavir	Ritonavir
	Fosamprenavir	Saquinavir
	Lopinavir	Tipranavir
HCV Protease Inhibitors	Boceprevir	Telaprevir
	Simeprevir	
Reverse Transcriptase or DNA Polymerase Inhibitors	Abacavir	Ganciclovir
	Aciclovir	Lamivudine
	Adefovir dipivoxil	Sofosbuvir
	Cidofovir	Telbivudine
	Emtricitabine	Tenofovir
	Entecavir	Valganciclovir
	Foscarnet	Zidovudine
Compounds for Treatment of Opportunistic Infections	Azithromycin	Pyrazinamide
	Clarithromycin	Rifabutin
	Ethambutol	Rifampicin
	Fluconazole	Sulfamethoxazole
	Isoniazid	Trimethoprim

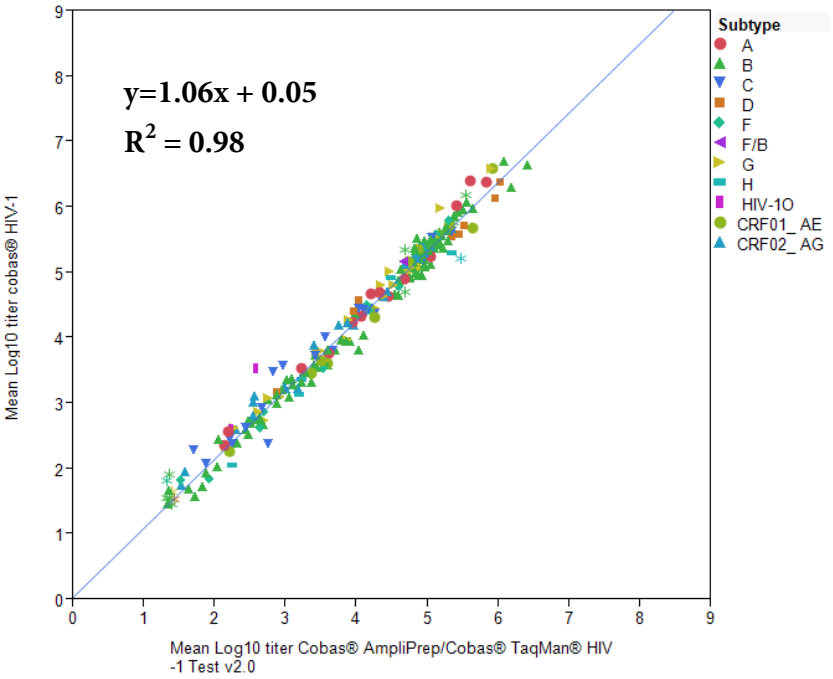
Method correlation

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 243 EDTA plasma samples from patients infected with HIV-1. The samples comprised of HIV-1 M (A–D, F–H, F/B, CRF01_AE, CRF02_AG) and HIV-1 O were tested in duplicate at an external site. The Deming regression was performed considering log-transformed titers.

The Deming regression results are shown in Figure 4. The symbol * in the figures shows single determination. The color represents the subtype.

Figure 4: 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 0.0%. The two-sided 95% exact confidence interval was 0.0% 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 230 replicates of HIV negative EDTA-plasma samples and 235 replicates of a high titer HIV-1 sample at 1.9E+07 cp/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 230 replicates of the negative samples were negative, resulting in a cross-contamination rate of 0.0% with a one-sided upper 95% confidence interval of 1.3%.

Additional information

Key assay features

Sample type	EDTA plasma
Sample processing volume	400 µL or 200 µL
Analytical sensitivity	14.2 cp/mL (400µL) 43.9 cp/mL (200 µL)
Linear range	400 µL: 20.0 cp/mL – 1.0E+07 cp/mL 200 µL: 60.0 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

Symbols

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

Table 11: Symbols used in labeling for Roche PCR diagnostic products

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

Technical Support

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

Manufacturer

Table 12: Manufacturer

Manufactured in the United States

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

Made in USA

Trademarks and patents

https://diagnostics.roche.com/us/en/about-us/patents

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Document revision

Document Revision Information	
Doc. Rev 5.0 02/2024	Updated Lysis Kits 2 hazard information. Updated the harmonized symbol page. Updated to current economic operators. Added **Technical support** section. Updated **Trademarks and patents** section, including the link. Updated **cobas**® branding. Please contact your local Roche Representative if you have any questions.