Rx Only

cobas® HIV-1/HIV-2 Qualitative

Nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® HIV-1/HIV-2 Qualitative	P/N: 09040528190
For use on the cobas® 5800 System:	
cobas® HIV-1/HIV-2 Qualitative Control Kit	P/N: 09040536190
cobas® NHP Negative Control Kit	P/N: 09051554190
For use on the cobas® 6800/8800 Systems:	
cobas® HIV-1/HIV-2 Qualitative Control Kit	P/N: 07862091190 or P/N: 09040536190
cobas® NHP Negative Control Kit	P/N: 07002220190 or P/N: 09051554190

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Intended use

cobas® HIV-1/HIV-2 Qualitative for use on the **cobas®** 5800/6800/8800 Systems is an in vitro nucleic acid amplification test for the qualitative detection and differentiation of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) RNA in human serum and plasma.

The test is intended to be used as an aid in diagnosis of HIV-1/HIV-2 infection. Detection of HIV-1 or HIV-2 nucleic acid is indicative of HIV-1 or HIV-2 infection, respectively. The presence of HIV-1 or HIV-2 nucleic acid in the plasma or serum of individuals without antibodies to HIV-1 or HIV-2 is indicative of acute or primary infection**.** The **cobas®** HIV-1/HIV-2 Qualitative may also be used as an additional test to confirm the presence of HIV-1 or HIV-2 infection in an individual with specimens reactive for HIV-1 or HIV-2 antibodies or antigens. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects and pregnant women.

This assay is not intended to be used for monitoring patient status, or for screening donors of blood, plasma, or human cells, tissues, and cellular and tissue-based products (HCT/Ps) for HIV.

Summary and explanation of the test

Background

HIV is the etiologic agent of acquired immunodeficiency syndrome (AIDS).[1] HIV-1 is the predominant cause of AIDS worldwide, with over 35 million people infected.[2] After infection, infected individuals typically enter a clinically stable, relatively asymptomatic phase that can last for years. Without antiretroviral treatment, individuals typically progress to AIDS, which is marked by immune system depletion of CD4+ cells, susceptibility to opportunistic infections, and eventual death.[3] HIV-2, mainly found in West Africa, can also cause AIDS. Between 1 and 2 million people are thought to be infected with HIV-2 worldwide.

The distinction between HIV-1 and HIV-2 is important for several reasons: (1) HIV-2 appears less virulent than HIV-1, with lower viral loads, a slower rate of CD4+ cell loss, and a slower progression to opportunistic infections; (2) HIV-2 viral loads may be incorrectly quantified by HIV-1 viral load tests; and (3) some HIV-1 medications, particularly non-nucleoside reverse transcriptase inhibitors, are not effective against HIV-2.[4] Co-infection with both HIV-1 and HIV-2 is also possible. Co-infection has no obvious effect on the rate of individuals' progression to AIDS, but does complicate viral load monitoring and antiretroviral treatment.[4] Due to the importance of distinguishing between HIV-1 and HIV-2 infection, national and international guidelines have included the identification and differentiation of HIV-1 and HIV-2 as a requirement for the proper diagnosis of HIV infection.[5,6]

Rationale for PCR testing

Historically, HIV testing has been based on the antibody response that patients make to the virus. Although these antibodies are ineffective at combating the virus, they are found in almost all chronically infected patients. The major limitation of antibody testing is the several week "window period" during acute infection before the onset of a detectable antibody response. This window period has been decreased by "fourth generation" HIV immunoassay tests, which detect HIV p24 antigen as well as antibody.[7] However, nucleic acid amplification tests have the potential to reduce the window period of fourth generation immunoassay tests, in detecting HIV infection even further, because of the sensitivity of polymerase chain reaction (PCR) methods over protein methods.[7]

Depending on the risk of HIV infection in the population being tested, the reduction in window period from nucleic acid testing can be important for both the individual and the community.[8] For an individual, diagnosis of HIV during acute infection offers the opportunity for immediate treatment, which may potentially delay disease progression by preventing immune system damage and by preserving anti-HIV cellular immune responses. Early treatment may also limit the size and genetic diversity of the viral reservoir which is established, making it easier to achieve a functional cure in patients treated during acute infection. For the community, acutely infected patients play a major role in HIV transmission, because these patients typically have very high viral loads and are unaware of their infection status. Identifying and treating these patients may play a critical role in stopping the spread of HIV epidemics.[9,10] PCR is already the standard of care for the diagnosis of HIV in infants, and the high sensitivity and specificity of PCR would not only allow for the detection of acute infection in individuals of all ages, but also the confirmation of HIV diagnosis in seropositive or serology-indeterminate individuals.[11,12]

Explanation of the test

cobas® HIV-1/HIV-2 Qualitative is a qualitative test performed on the **cobas®**5800 System, **cobas®** 6800 System and **cobas®** 8800 System. **cobas®** HIV-1/HIV-2 Qualitative enables the simultaneous detection and differentiation of HIV-1 and HIV-2 nucleic acid in ethylenediaminetetraacetic acid (EDTA) plasma, and serum of infected patients. Two probes are used to detect HIV-1, but not to discriminate HIV-1 group M subtypes and HIV-1 group O and group N. A third probe is used to detect HIV-2, but not to discriminate HIV-2 group A and group B.

Principles of the procedure

cobas® HIV-1/HIV-2 Qualitative is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas®** 5800 System is designed as one integrated instrument. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 5800 System or **cobas®** 6800/8800 Systems software, which assigns test results for all tests as non-reactive, reactive, or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added armored ribonucleic acid (RNA) internal control (IC) molecules (which serve as the sample preparation and amplification/detection process control) is simultaneously extracted. In addition, the test utilizes three external controls: two positive and one negative control. 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 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 and HIV-2 genomes. The HIV-1 gag gene, HIV-1 LTR region (dual target for HIV-1), and HIV-2 LTR region are amplified by **cobas®** HIV-1/HIV-2 Qualitative.

Selective amplification of IC is achieved by the use of sequence-specific forward and reverse primers, which are selected to have no homology with the HIV-1 or HIV-2 genomes. A thermostable deoxyribonucleic acid (DNA) polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and IC sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into

the newly synthesized DNA (amplicon).[13-15] Any contaminating 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.

cobas® HIV-1/HIV-2 Qualitative master mix contains two detection probes specific for the HIV-1 target sequences, one for HIV-2 target sequences and one for the IC. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target, HIV-2 target and IC in three different target channels.[16,17] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and IC, respectively.

Reagents and materials

cobas® HIV-1/HIV-2 Qualitative reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4. Materials required, but not provided can be found Table 2, Table 3, Table 4, Table 8 and Table 9. Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

Table 1 cobas® HIV-1/HIV-2 Qualitative

(HIV-1/HIV-2)

Store at 2-8°C
192 test cassette (P/N 09040528190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HIV-1/HIV-2 Master Mix Reagent 2 (HIV-1/HIV-2 MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, Tween 20, EDTA, < 0.06% dATP, dCTP, dGTP, < 0.14% dUTP, < 0.01% upstream and downstream HIV-1, HIV-2 and internal control primers, < 0.01% fluorescent-labeled HIV-1 and HIV-2 probes, < 0.01% fluorescent-labeled internal control probe, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® HIV-1/HIV-2 Qualitative Control Kit

(HIV-1/HIV-2 (+)C)

Store at 2–8°C
For use on the **cobas**® 5800 System (P/N 09040536190)
For use on the **cobas**® 6800/8800 Systems (P/N 07862091190 or P/N 09040536190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HIV-1M/HIV-2 Positive Control (HIV-1M/HIV-2 (+)C)	< 0.001% Synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% Synthetic (armored) HIV-2 RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, non-reactive by licensed tests for antibody to HIV-1/2; HIV-1 RNA and HIV-2 RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 × 0.65 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1)
HIV-1O Positive Control (HIV-1O (+)C)	< 0.001% Synthetic (armored) HIV-1 Group O RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, non-reactive by licensed tests for antibody to HIV-1/2; HIV-1 RNA and HIV-2 RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 × 0.65 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Table 3 cobas® NHP Negative Control Kit

(NHP-NC)

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051554190)

For use on the **cobas**® 6800/8800 Systems (P/N 07002220190 or P/N 09051554190)

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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas omni reagents for sample preparation

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

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

* These reagents are not included in the **cobas**® HIV-1/HIV-2 Qualitative kit. See listing of additional materials required (Table 8 and Table 9).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7. When reagents are not loaded on the **cobas**® 5800 or **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/HIV-2 Qualitative	2–8°C
cobas® HIV-1/HIV-2 Qualitative Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the cobas® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® HIV-1/HIV-2 Qualitative	Date not passed	90 days from first usage	Max 40 runs	Max 36 days[b]
cobas® HIV-1/HIV-2 Qualitative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas omni Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a] Single use reagents

[b] Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System.

Reagent handling requirements for the cobas® 6800/8800 Systems

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 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas®** 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® HIV-1/HIV-2 Qualitative	Date not passed[a]	90 days from first usage	Max 40 runs	Max 40 hours
cobas® HIV-1/HIV-2 Qualitative Control Kit	Date not passed[a]	Not applicable[b]	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed[a]	Not applicable[b]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable

[a] Reagents are not expired

[b] Single use reagents

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

Additional materials required for the cobas® 5800 System

Table 8 Material and consumables for use on the **cobas®** 5800 System

Material	P/N
cobas omni Processing Plate	08413975001
cobas omni Amplification Plate	08499853001
cobas omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300uL	07345607001
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 or Solid Waste Bag With Insert	07435967001 or 08030073001

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **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 and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® HIV-1/2 Qual-Serum/Plasma analysis package for the **cobas**® 5800 System shall be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 software and **cobas**® HIV-1/2Qual-Serum/Plasma analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 10 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module (**cobas**® 6800/8800 Systems only)	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.
Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use only.
- This test is not intended for use in screening blood or plasma donors. All samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[18,19] Only personnel proficient in handling infectious materials and the use of **cobas**® HIV-1/HIV-2 Qualitative and **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect following appropriate site procedures.
- **cobas**® HIV-1/HIV-2 Qualitative 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 HIV-1/2. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA and HIV-2 RNA. 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/HIV-2 Qualitative kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of these 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/HIV-2 Qualitative 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**® 5800 instrument, follow the instructions in the **cobas**® 5800 System User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide to properly clean and decontaminate the surface of the instrument(s).

Sample collection, transport, and storage

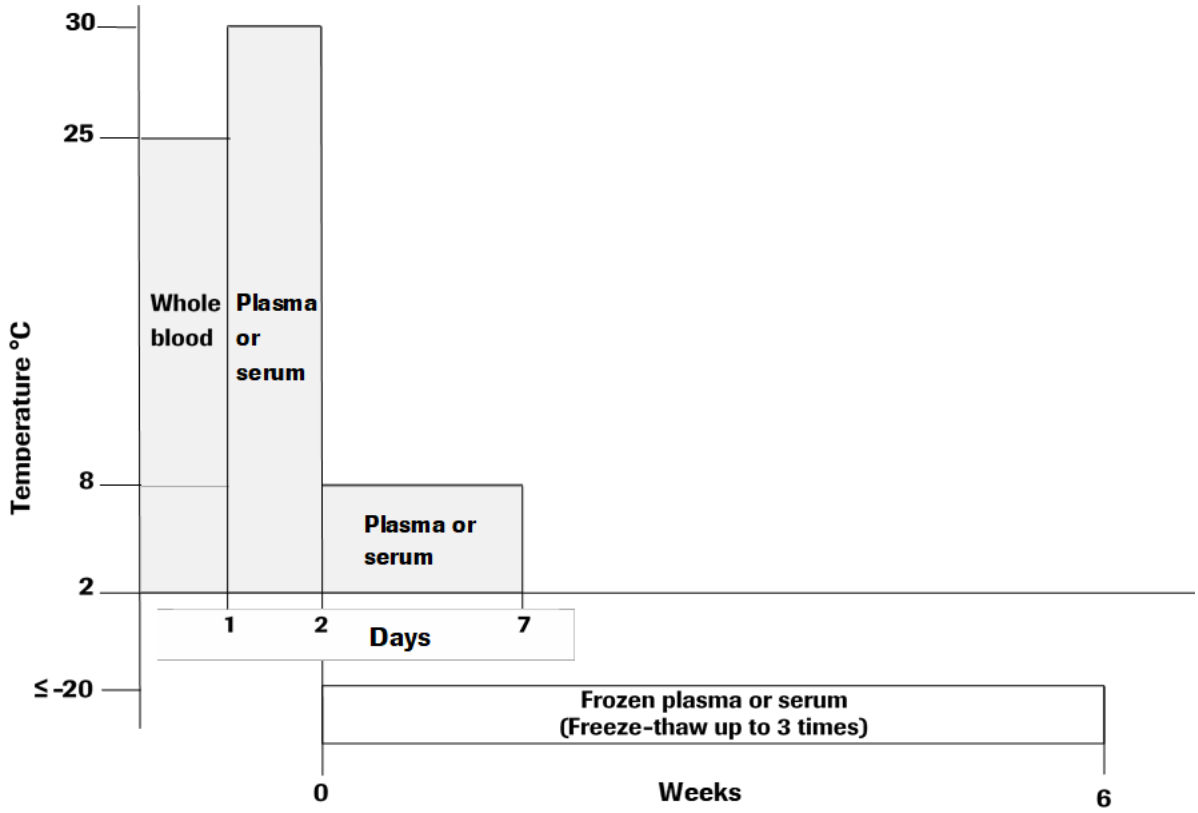
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely 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.

Samples

EDTA plasma and serum samples

- Whole Blood should be collected in SST™ Serum Separation Tubes, 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 SST™ Serum Separation Tubes, 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 or serum preparation. Centrifugation should be performed according to manufacturer's instructions.
- Upon separation EDTA plasma or serum samples may be stored in secondary tubes for up to 24 hours at 30°C followed by up to 5 days at 2°C to 8°C or up to 6 weeks at ≤ -20°C. For long-term storage, temperatures at ≤ -60°C are recommended.
- Plasma and serum samples are stable for up to three freeze/thaw cycles when frozen at ≤ -20°C.
- Refer to Figure 1 for sample storage conditions.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Figure 1 Whole blood, plasma and serum sample storage conditions



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

Instructions for use

Procedural notes

- Do not use **cobas**® HIV-1/HIV-2 Qualitative reagents, **cobas**® HIV-1/HIV-2 Qualitative 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**® 5800 or **cobas**® 6800/8800 Systems User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® HIV-1/HIV-2 Qualitative on the cobas® 5800 System

cobas® HIV-1/HIV-2 Qualitative can be run with required sample volumes of 675 µL (for the 500µL sample workflow). The test procedure is described in detail in the **cobas**® 5800 System User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

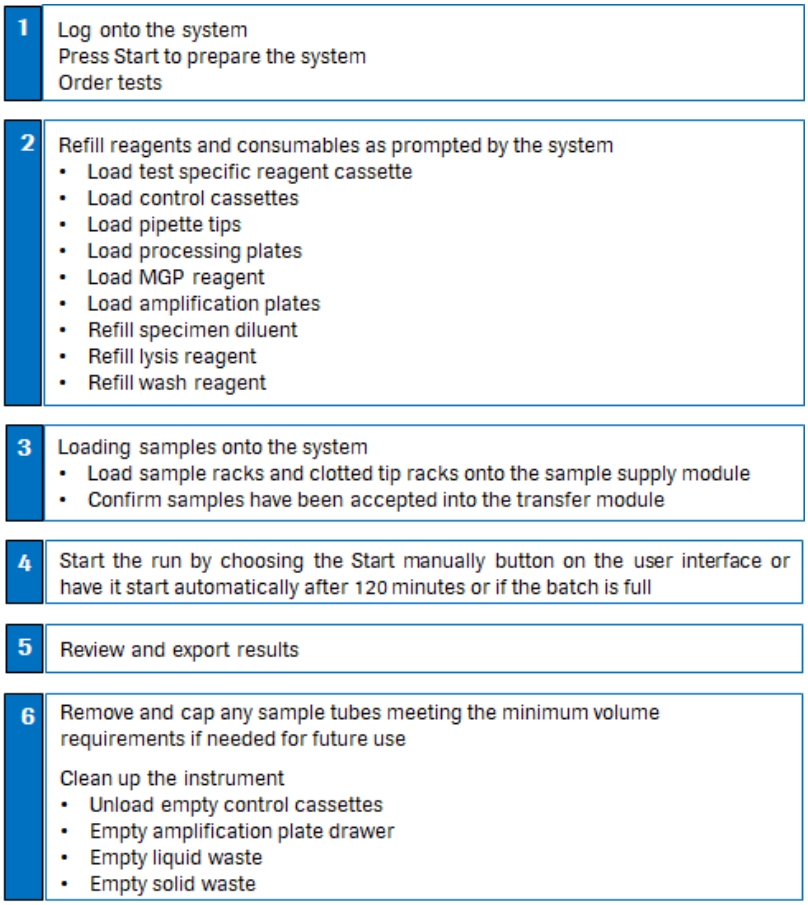
Figure 2 cobas® HIV-1/HIV-2 Qualitative test procedure on the **cobas**® 5800 System

1	Log onto the system
2	Loading samples onto the system • Load sample racks onto the system • The system prepares automatically • Order tests
3	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette(s) • Load control mini racks • Load processing tips • Load elution tips • Load processing plates • Load liquid waste plates • Load amplification plates • Load MGP cassette • Refill specimen diluent • Refill lysis reagent • Refill wash reagent
4	Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up the instrument • Unload empty control mini racks • Unload empty test specific reagent cassette(s) • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Running cobas® HIV-1/HIV-2 Qualitative on the cobas® 6800/8800 Systems

cobas® HIV-1/HIV-2 Qualitative can be run with a minimum required sample volume of 675 µL (for the 500µL sample workflow). The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide. Figure 3 below summarizes the procedure.

Figure 3 cobas® HIV-1/HIV-2 Qualitative test procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 Systems automatically detect and discriminate HIV-1 and HIV-2 simultaneously in samples and controls, displaying test validity, overall results, as well as individual target results.

Quality control and validity of results on the cobas® 5800 System

- One Normal Human Plasma Negative Control [(–) C] and two positive controls, [HIV-1 M/HIV-2 (+)C] and [HIV-1 O (+)C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 System software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

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

Control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the positive controls is invalid, repeat testing of the all positive controls and all associated samples.
- If the negative control is invalid, repeat testing of all controls and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One Normal Human Plasma Negative Control [(–) C] and two positive controls [HIV-1 M/HIV-2 (+)C] and [HIV-1 O (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls.

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

Control flags on the cobas® 6800/8800 Systems

Table 11 Control flags for negative and positive controls

Negative Control	Flag	Result	Interpretation
(–) C	Q02 (Control batch failed)	Invalid	The entire batch is assigned invalid if the result for the (–) C is invalid.
Positive Control	**Flag**	**Result**	**Interpretation**
HIV-1 M/HIV-2 (+) C	Q02 (Control batch failed)	Invalid	The entire batch is assigned invalid if the result for the HIV-1 M/HIV-2 (+) C is invalid.
HIV-1 O (+) C	Q02 (Control batch failed)	Invalid	The entire batch is assigned invalid if the result for the HIV-1 O (+) C is invalid.

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

(-) C stands for NHP negative control, HIV-1 M/HIV-2 (+) C stands for **cobas**® HIV-1 M/HIV-2 positive control and HIV-1 O (+) C stands for **cobas**® HIV-1 O positive control in the **cobas**® 6800/8800 software.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 System software in the "Results" app.
For a valid control batch, check each individual sample for flags in the **cobas**® 5800 System software and/or report. The result interpretation should be as follows:

- A valid control batch may include both valid and invalid sample results.
- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D : Result validation failure because of an invalid positive control
    - Q06D :Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 12 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Results and their corresponding interpretation for detecting HIV-1 and HIV-2 are shown below in Table 12.

Table 12 Results for individual target result interpretation

Result		Interpretation
HIV-1 Reactive	HIV-2 Reactive	All requested results were valid. Target signal detected for HIV-1 and HIV-2.
HIV-1 Reactive	HIV-2 Non-Reactive	All requested results were valid. Target signal detected for HIV-1. No target signal detected for HIV-2.
HIV-1 Non-Reactive	HIV-2 Reactive	All requested results were valid. No target signal detected for HIV-1. Target signal detected for HIV-2.
HIV-1 Non-Reactive	HIV-2 Non-Reactive	All requested results were valid. No target signal detected for HIV-1 or HIV-2.
Invalid	Invalid	Both HIV-1 and HIV-2 results are invalid. Original specimen should be re-tested to obtain valid HIV-1 and HIV-2 results. If the results are still invalid, a new specimen should be obtained.

Interpretation of results on the cobas® 6800/8800 Systems

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.
- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values in "Overall Result" column for individual samples should be interpreted as follows:
  - Reactive – All requested results are reactive or one of the requested results is reactive and the other non-reactive
  - Non-Reactive – All requested results are non-reactive
  - Invalid – At least one requested result is invalid
- Reported target results for individual samples are valid unless indicated otherwise.

Results and their corresponding interpretation for detecting HIV-1 and HIV-2 are shown below in Table 13.

Table 13 Target results for individual target result interpretation

Valid	Overall Result	Target 1	Target 2	Interpretation
Yes	**Reactive**	HIV-1 Reactive	HIV-2 Reactive	All requested results were valid. Target signal detected for HIV-1 and HIV-2.
Yes	**Reactive**	HIV-1 Reactive	HIV-2 Non-Reactive	All requested results were valid. Target signal detected for HIV-1. No target signal detected for HIV-2.
Yes	**Reactive**	HIV-1 Non-Reactive	HIV-2 Reactive	All requested results were valid. No target signal detected for HIV-1. Target signal detected for HIV-2.
Yes	**Non-Reactive**	HIV-1 Non-Reactive	HIV-2 Non-Reactive	All requested results were valid. No target signal detected for HIV-1 or HIV-2.
No	**Invalid**	Invalid	Invalid	Both HIV-1 and HIV-2 results are invalid. Original specimen should be re-tested to obtain valid HIV-1 and HIV-2 results. If the results are still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® HIV-1/HIV-2 Qualitative has been evaluated only for use in combination with the **cobas**® HIV-1/HIV-2 Qualitative Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent and **cobas omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 Systems.
- Reliable results depend on proper sample type (EDTA plasma or serum) and sample collection (venipuncture), as well as storage and handling procedures. Use of the assay with other types of specimens may not yield accurate results.
- Detection of HIV-1 and HIV-2 nucleic acid is dependent on the number of virus particles present in the sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- False negative (non-reactive) results may be obtained when testing individuals undergoing anti-retroviral therapy (ART) or taking pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP).
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HIV-1/HIV-2 Qualitative may affect primers and/or probe binding resulting in the failure to detect the presence of virus.
- Invalid test results could occur due to interference with triglycerides at concentrations higher than 25 g/L and with human DNA at concentrations higher than 1.5 mg/L.
- Non-reactive test result does not exclude the possibility of infection with HIV. A comprehensive risk history and clinical judgement should be considered before concluding that an individual is not infected with HIV
- 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.
- The device is intended to be used as an aid in diagnosis and should not be used in isolation but in conjunction with clinical status, history and risk factors of individuals being tested. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
- **cobas**® HIV-1/HIV-2 Qualitative is not intended for use as a screening test for the presence of HIV-1/HIV-2 in blood or blood products.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

WHO International Standards/Roche Primary Standards

The limit of detection of the **cobas**® HIV-1/HIV-2 Qualitative was determined by using the following standards:

- WHO 3rd International Standard for HIV-1 group M RNA (NIBSC code 10/152)
- WHO International Standard for HIV-2 RNA (NIBSC code 08/150)
- Roche Primary Standards for HIV-1 group O RNA

No international standard is currently available for HIV-1 group O RNA. The Roche HIV-1 group O RNA Standard is traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot 01. The Roche Primary Standards for HIV-1 group O RNA are derived from commercially available cultured virus stocks, P/N 2420 (Cat. No. 500493, SeraCare Life Sciences). One copy of HIV-1 RNA is equivalent to 1.7 International Unit (IU) and one copy of HIV-2 RNA is equivalent to 0.2 International Unit (IU).

Serial dilutions of the standards in HIV-negative human EDTA plasma and serum were prepared. Panels of five or six concentration levels plus a negative were tested over three lots of **cobas**® HIV-1/HIV-2 Qualitative reagents, multiple runs, days, operators, and instruments.

For each virus, 95% PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the LoD, along with the lower and upper limit of the 95% confidence interval (Table 14). The reactivity rates observed in the LoD studies for each virus are summarized in Table 15 to Table 17.

Table 14 Results of 95% PROBIT analysis on LoD data collected with viral standards in EDTA plasma and serum

Matrices	Analyte	Measuring units	Limit of detection	Lower 95% confidence limit	Upper 95% confidence limit
EDTA plasma	HIV-1 group M	copies/mL	12.8	10.2	18.0
EDTA plasma	HIV-1 group O	copies/mL	15.4	11.9	22.2
EDTA plasma	HIV-2	copies/mL	35.4	24.1	72.3
Serum	HIV-1 group M	copies/mL	12.8	10.1	18.2
Serum	HIV-1 group O	copies/mL	13.3	10.6	18.7
Serum	HIV-2	copies/mL	26.3	20.1	40.8

Table 15 Reactivity rates summary for HIV-1 group M in EDTA plasma and serum

Matrices	HIV-1 group M RNA concentration (cp/mL)	Number of reactive	Number of valid replicates	% Reactive
EDTA plasma	40	189	189	100%
EDTA plasma	30	189	189	100%
EDTA plasma	20	187	189	99%
EDTA plasma	10	174	189	92%
EDTA plasma	5	124	189	66%
EDTA plasma	2.5	91	189	48%
EDTA plasma	0	0	189	0%
Serum	40	189	189	100%
Serum	30	189	189	100%
Serum	20	187	189	99%
Serum	10	176	189	93%
Serum	5	126	189	67%
Serum	2.5	86	189	46%
Serum	0	0	189	0%

Table 16 Reactivity rates summary for HIV-1 group O in EDTA plasma and serum

Matrices	HIV-1 group O RNA concentration (cp/mL)	Number of reactive	Number of valid replicates	% Reactive
EDTA plasma	40	189	189	100%
EDTA plasma	30	189	189	100%
EDTA plasma	20	185	188	98%
EDTA plasma	10	163	189	86%
EDTA plasma	5	117	189	62%
EDTA plasma	2.5	78	189	41%
EDTA plasma	0	0	189	0%
Serum	40	189	189	100%
Serum	30	189	189	100%
Serum	20	186	189	98%
Serum	10	173	189	92%
Serum	5	132	189	70%
Serum	2.5	91	189	48%
Serum	0	0	189	0%

Table 17 Reactivity rates summary for HIV-2 in EDTA plasma and serum

Matrices	HIV-2 RNA concentration (cp/mL)	Number of reactive	Number of valid replicates	% Reactive
EDTA plasma	80	126	126	100%
EDTA plasma	40	124	126	98%
EDTA plasma	20	115	126	91%
EDTA plasma	10	81	126	64%
EDTA plasma	5	61	126	48%
EDTA plasma	0	0	189	0%
Serum	80	126	126	100%
Serum	40	125	126	99%
Serum	20	114	126	90%
Serum	10	96	126	76%
Serum	5	49	126	39%
Serum	0	0	189	0%

Precision – within laboratory

The precision of **cobas**® HIV-1/HIV-2 Qualitative was determined using the following standards:

- Roche Secondary Standard for HIV-1 group M
- Roche Primary Standard for HIV-2

Two panels of individually formulated HIV-1 group M and HIV-2 target, each comprising 3 panel members at concentrations of approximately 0.6 ×, 1 ×, and 3 × of the LoD of **cobas**® HIV-1/HIV-2 Qualitative were tested in this study. Testing was performed for the following variability components:

- day-to-day variability over 4 days
- lot-to-lot variability using 3 different reagent lots of **cobas**® HIV-1/HIV-2 Qualitative
- instrument-to-instrument variability using 3 different **cobas**® 6800/8800 Systems

Approximately 84 replicates were tested with each of the 3 panel members for each reagent lot for a total of 252 replicates over all reagent lots per target. Precision results were evaluated by calculating the percentage of reactive test results at each concentration level for each of the variability components analyzed.

The limits of two-sided 95% confidence intervals for each reactive rate were calculated for each of the three levels of HIV-1 group M and HIV-2 tested across 4 days, 3 reagent lots, and 3 **cobas**® 6800/8800 Systems. **cobas**® HIV-1/HIV-2 Qualitative is reproducible over multiple days, reagent lots and multiple instruments. The results from reagent lot-to-lot variability are summarized in Table 18 and Table 19.

Table 18 cobas® HIV-1/HIV-2 Qualitative reagent lot-to-lot precision summary (EDTA plasma)

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
HIV-1 group M	~0.6 × LoD	1	77.4% (65/84)	67.0%	85.8%
HIV-1 group M	~0.6 × LoD	2	76.2% (64/84)	65.7%	84.8%
HIV-1 group M	~0.6 × LoD	3	82.1% (69/84)	72.3%	89.6%
HIV-1 group M	~1 × LoD	1	98.8% (83/84)	93.5%	100%
HIV-1 group M	~1 × LoD	2	98.8% (83/84)	93.5%	100%
HIV-1 group M	~1 × LoD	3	100% (84/84)	95.7%	100%
HIV-1 group M	~3 × LoD	1	100% (84/84)	95.7%	100%
HIV-1 group M	~3 × LoD	2	100% (84/84)	95.7%	100%
HIV-1 group M	~3 × LoD	3	100% (84/84)	95.7%	100%
HIV-2	~0.6 × LoD	1	90.5% (76/84)	82.1%	95.8%
HIV-2	~0.6 × LoD	2	85.7% (72/84)	76.4%	92.4%
HIV-2	~0.6 × LoD	3	86.9% (73/84)	77.8%	93.3%
HIV-2	~1 × LoD	1	97.6% (82/84)	91.7%	99.7%
HIV-2	~1 × LoD	2	96.4% (81/84)	89.9%	99.3%
HIV-2	~1 × LoD	3	97.6% (82/84)	91.7%	99.7%
HIV-2	~3 × LoD	1	100% (84/84)	95.7%	100%
HIV-2	~3 × LoD	2	100% (84/84)	95.7%	100%
HIV-2	~3 × LoD	3	100% (84/84)	95.7%	100%

Table 19 cobas® HIV-1/HIV-2 Qualitative reagent lot-to-lot precision summary (serum)

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
HIV-1 group M	~0.6 × LoD	1	85.7% (72/84)	76.4%	92.4%
HIV-1 group M	~0.6 × LoD	2	70.2% (59/84)	59.3%	79.7%
HIV-1 group M	~0.6 × LoD	3	78.6% (66/84)	68.3%	86.8%
HIV-1 group M	~1 × LoD	1	98.8% (82/83)	93.5%	100%
HIV-1 group M	~1 × LoD	2	98.8% (83/84)	93.5%	100%
HIV-1 group M	~1 × LoD	3	98.8% (83/84)	93.5%	100%
HIV-1 group M	~3 × LoD	1	100% (84/84)	95.7%	100%
HIV-1 group M	~3 × LoD	2	100% (84/84)	95.7%	100%
HIV-1 group M	~3 × LoD	3	100% (84/84)	95.7%	100%
HIV-2	~0.6 × LoD	1	81.0% (68/84)	70.9%	88.7%
HIV-2	~0.6 × LoD	2	81.0% (68/84)	70.9%	88.7%
HIV-2	~0.6 × LoD	3	85.7% (72/84)	76.4%	92.4%
HIV-2	~1 × LoD	1	95.2% (79/83)	88.1%	98.7%
HIV-2	~1 × LoD	2	98.8% (82/83)	93.5%	100%
HIV-2	~1 × LoD	3	97.6% (82/84)	91.7%	99.7%
HIV-2	~3 × LoD	1	100% (84/84)	95.7%	100%
HIV-2	~3 × LoD	2	100% (84/84)	95.7%	100%
HIV-2	~3 × LoD	3	100% (84/84)	95.7%	100%

Group/subtype verification and inclusivity

The performance of **cobas®** HIV-1/HIV-2 Qualitative on HIV-1 group M subtypes, group O, group N and HIV-2 group B was evaluated by:

- Verification of the limit of detection for HIV-1 group M subtypes, group N and HIV-2 group B
- Verification of the inclusivity for HIV-1 group M subtypes, group O, group N and HIV-2 group A and group B

Verification of limit of detection for HIV-1 group M subtypes, group N and HIV-2 group B

Clinical or cultured HIV samples for HIV-1 group M (A, C, D, F, G, H) and circulating recombinant forms (CRF01_AE, CRF02_AG), HIV-1 group N and HIV-2 group B were diluted in EDTA plasma and serum to the LoD concentration of the predominant group/subtype (HIV-1 group M subtype B or HIV-2 group A). The reactive rate determination was performed with 42 replicates. Testing was conducted with 1 lot of **cobas®** HIV-1/HIV-2 Qualitative reagents. The results from HIV-1 are shown in Table 20 and the results from HIV-2 are shown in Table 21. These results verify that **cobas®** HIV-1/HIV-2 Qualitative detected HIV for HIV-1 group M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1 group N and HIV-2 group B at the claimed concentration for each matrix or below with an upper 95% confidence interval being equal to or greater to the expected reactivity rate of 95%.

Table 20 LoD verification of HIV-1 group M subtypes and group N in EDTA plasma and serum

Group	Subtype	Plasma: 12.6 cp/mL Number of valid replicates	Plasma: 12.6 cp/mL Number of valid reactive	% Reactive (95% CI*)	Serum: 12.1 cp/mL Number of valid replicates	Serum: 12.1 cp/mL Number of valid reactive	% Reactive (95% CI*)
M	A	42	40	95% (99%)	42	40	95% (99%)
M	C	42	41	98% (100%)	42	42	100% (99.4%)
M	D	42	37	88% (96%)	42	37	88% (96%)
M	F	42	38	90% (97%)	42	38	90% (97%)
M	G	42	40	95% (99%)	42	39	93% (99%)
M	H	42	38	90% (97%)	42	41	98% (100%)
M	CRF01_AE	42	38	90% (97%)	42	38	90% (97%)
M	CRF02_AG	42	36	86% (95%)	42	39	93% (99%)
N	N/A	42	39	93% (99%)	42	37	88% (96%)

* Upper 95% confidence interval

Table 21 LoD verification of HIV-2 group B in EDTA plasma and serum

Group	Plasma: 27.9 cp/mL Number of valid replicates	Plasma: 27.9 cp/mL Number of valid reactive	% Reactive (95% CI*)	Serum: 23.4 cp/mL Number of valid replicates	Serum: 23.4 cp/mL Number of valid reactive	% Reactive (95% CI*)
B	42	42	100% (100%)	42	42	100% (100%)

* Upper 95% confidence interval

Verification of inclusivity for HIV-1 group M subtypes, group O, group N and HIV-2 group A and group B

The performance of **cobas**® HIV-1/HIV-2 Qualitative to detect subtypes of HIV-1 group M (A, C, D, F, G, H, J, K) and circulating recombinant forms (CRF01_AE, CRF02_AG, CRF12_BF, CRF14_BG), HIV-1 group O, HIV-1 group N, HIV-2 group A and HIV-2 group B was determined by testing unique clinical samples and/or culture isolated for each group or subtype in EDTA plasma or serum.

HIV-1 group M

A total of 105 unique HIV-1 group M clinical samples with known HIV-1 subtype were tested neat (undiluted) and after dilution to ~5 × LoD of **cobas**® HIV-1/HIV-2 Qualitative. All 105 clinical samples with known subtypes were detected neat and at ~5 × LoD (Table 22).

In addition, four HIV-1 group M subtype CRF12_BF and one HIV-1 group M subtype CRF14_BG clinical sample was tested after dilution series were prepared. One replicate of each of the neat samples and one of each dilution from 1:1.0E+01 to 1:5.0E+02 (2-4 dilutions per sample) for HIV-1 group M subtype CRF12_BF and from 1:2.0E+01 to 1:1.2E+02 (4 dilutions) for HIV-1 group M subtype CRF14_BG was tested, all yielding in reactive results. All of the tested clinical samples were detected at ≤ 5 × LoD.

Table 22 HIV-1 group M clinical samples

Subtype / circulating recombinant forms	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to ~5 × LoD
A	100% (10/10)	100% (10/10)
C	100% (10/10)	100% (10/10)
D	100% (10/10)	100% (10/10)
F	100% (10/10)	100% (10/10)
G	100% (10/10)	100% (10/10)
H	100% (10/10)	100% (10/10)
J	100% (5/5)	100% (5/5)
K	100% (9/9)	100% (9/9)
CRF01_ AE	100% (10/10)	100% (10/10)
CRF02_AG	100% (10/10)	100% (10/10)
CRF12_BF	100% (2/2)	100% (2/2)
CRF14_BG	100% (9/9)	100% (9/9)

HIV-1 group O and HIV-1 group N

A total of 10 HIV-1 group O and one HIV-1 group N clinical or cultured sample was tested after dilution series were prepared. Two replicates of each of the neat samples and four of each dilution from 1:1.0E+01 to 1:4.8E+05 (3-5 dilutions per sample) for HIV-1 group O were tested, all yielding in reactive results. Two replicates of neat sample and four of each dilution from 1:1.0E+04 to 1:1.4E+05 (5 dilutions) for HIV-1 group N were tested. The neat sample and the dilutions from 1:1.0E+04 to 1:4.5E+04 yielded in 100% reactive results, while dilution 1:1.4E+05 yielded in a 50% reactive result. All of the tested samples were detected at ≤ 3 × LoD.

HIV-2

A total of 16 unique HIV-2 group A and group B clinical or cultured samples were tested neat (undiluted) and after dilution to ~5 × LoD of **cobas**® HIV-1/HIV-2 Qualitative. All 16 HIV-2 samples were detected neat and at ~5 × LoD (Table 23).

In addition, six HIV-2 group A and four HIV-2 group B clinical samples were tested after dilution series were prepared. One replicate of each of the neat samples and one of each dilution from 1:1.0E+01 to 1:9.0E+02 (2-5 dilutions per sample) for HIV-2 group A and from 1:2.0E+01 to 1:6.0E+01 (2-4 dilutions) for HIV-2 group B was tested, all yielding in reactive results. All of the tested clinical samples were detected at ≤ 3 × LoD.

Table 23 HIV-2 clinical or cultured samples

Subtype	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to ~5 × LoD
A	100% (4/4)	100% (4/4)
B	100% (6/6)	100% (6/6)

Seroconversion panels

The performance of **cobas**® HIV-1/HIV-2 Qualitative was evaluated using commercially available seroconversion panels for HIV-1 group M.

HIV-1 group M Seroconversion panels

Twenty five commercially available seroconversion panels were used. Each panel member was tested neat with **cobas**® HIV-1/HIV-2 Qualitative and the results were compared to the results obtained with an FDA licensed 4th generation HIV Ag/Ab serology test. The overall performance results are shown in Table 24.

Table 24 Performance of **cobas®** HIV-1/HIV-2 Qualitative on HIV Seroconversion panels

HIV Sero-conversion panel	Number of Panel Members tested	Number of Panel Members with reactive result-cobas® HIV-1/HIV-2 Qualitative	Number of Panel Members with reactive result-HIV Ag/Ab Assay	Days to first reactive result-cobas® HIV-1/HIV-2 Qualitative	Days to first reactive result-HIV Ag/Ab Assay	Days earlier detection with cobas® HIV-1/HIV-2 Qualitative-HIV Ag/Ab Assay
HIV6243	10	6	4	18	25	7
HIV9011	11	3	2	30	38	8
HIV9012	8	5	3	9	16	7
HIV9013	7	3	2	18	23	5
HIV9018	10	5	3	21	28	7
HIV9020	21	5	3	83	90	7
HIV9022	9	3	2	23	25	2
HIV9030	16	6	3	40	47	7
HIV9031	19	8	4	120	146	26
HIV9034	13	4	3	41	46	5
HIV9076	9	3	3	66	66	0
HIV9089	6	5	3	7	16	9
HIV12008	13	7	5	21	28	7
PRB954	7	5	2	7	17	10
PRB956	5	4	2	40	47	7
PRB958	6	6	4	0	7	7
PRB961	9	4	2	19	27	8
PRB962	6	4	2	7	14	7
PRB963	7	4	2	9	17	8
PRB967	6	5	3	3	17	14
PRB968	10	6	4	15	26	11
PRB969	10	7	3	53	70	17
PRB973	4	4	2	0	7	7
PRB976	4	4	2	0	7	7
PRB977	4	4	2	0	13	13
Total	230	120	70	-	-	-

Potentially interfering microbial contaminants

The analytical specificity of **cobas®** HIV-1/HIV-2 Qualitative was evaluated by testing a panel of microorganisms at 10^5 or 10^6 particles, copies, or PFU/mL, for viral isolates and bacterial strains/yeast isolates, respectively (Table 25). The microorganisms were added to HIV negative human EDTA plasma and tested with and without HIV-1 and HIV-2 virus added to a concentration of approximately 3 × LoD of **cobas®** HIV-1/HIV-2 Qualitative for each virus. Non-reactive results were obtained with **cobas®** HIV-1/HIV-2 Qualitative for all microorganism samples without HIV-1 and HIV-2 target and reactive results were obtained for all of the microorganism samples with HIV-1 and HIV-2 targets. The tested microorganisms do not cross-react or interfere with **cobas®** HIV-1/HIV-2 Qualitative.

Table 25 Microorganisms tested for potentially interfering microbial contaminants

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

EDTA plasma samples from each of the disease states (one from Adenovirus type 5 and ten from each of the other disease states) listed in Table 26 were tested with and without HIV-1 and HIV-2 added to a concentration of approximately 3 × LoD of **cobas**® HIV-1/HIV-2 Qualitative for each virus. These disease states do not cross-react or interfere with **cobas**® HIV-1/HIV-2 Qualitative.

Table 26 Disease states samples tested for potentially interfering microbial contaminants

Disease state
Adenovirus type 5
Cytomegalovirus
Dengue Virus
Epstein-Barr Virus
Hepatitis B Virus
Hepatitis C Virus
Hepatitis E Virus
Herpes Simplex Virus type 1
Herpes Simplex Virus type 2
Human T-cell lymphotropic Virus type I
Human T-cell lymphotropic Virus type II
West Nile Virus

A potential interference between HIV-1 and HIV-2 was investigated by testing panels consisting of HIV-1 or HIV-2 at a titer of approximately 3× LoD in the presence of potentially interfering high titers of HIV-2 (up to approximately 2E+05 cp/mL) or HIV-1 (up to approximately 2E+07 cp/mL), respectively (Table 27). Furthermore, the reactive rate of single and co-formulated panels for both HIV targets was assessed with approximately 3 × LoD and high titers.

The reactive rate for each of the targets HIV-1 and HIV-2 was 100%, respectively. Single formulated panels were reactive in the target channel and non-reactive in the non-target channel. HIV-1 and HIV-2 showed no cross-reactivity or competitive interference on **cobas**® HIV-1/HIV-2 Qualitative.

Table 27 HIV-1 and HIV-2 tested for potential interference

Co-formulated HIV-1/HIV-2 panel Concentration of HIV-1	Co-formulated HIV-1/HIV-2 panel Concentration of HIV-2	% Reactive for HIV-1 (reactive/samples tested) in HIV-1 channel	% Reactive for HIV-2 (reactive/samples tested) in HIV-2 channel
~3 × LoD	~3 × LoD	100% (11/11)	100% (11/11)
~3 × LoD	-	100% (11/11)	0% (11/11)
-	~3 × LoD	0% (11/11)	100% (11/11)
~3 × LoD	~20 × LoD	100% (11/11)	100% (11/11)
~3 × LoD	~60 × LoD	100% (11/11)	100% (11/11)
~3 × LoD	~2E+04 cp/mL	100% (11/11)	100% (11/11)
~3 × LoD	~2E+05 cp/mL	100% (11/11)	100% (11/11)
~20 × LoD	~3 × LoD	100% (11/11)	100% (11/11)
~60 × LoD	~3 × LoD	100% (11/11)	100% (11/11)
~2E+04 cp/mL	~3 × LoD	100% (11/11)	100% (11/11)
~2E+06 cp/mL	~3 × LoD	100% (11/11)	100% (11/11)
~2E+07 cp/mL	~3 × LoD	100% (11/11)	100% (11/11)
~2E+06 cp/mL	~2E+04 cp/mL	100% (11/11)	100% (11/11)
~2E+07 cp/mL	~2E+05 cp/mL	100% (11/11)	100% (11/11)
negative	negative	0% (11/11)	0% (11/11)
~2E+07 cp/mL	-	100% (11/11)	0% (11/11)
-	~2E+05 cp/mL	0% (11/11)	100% (11/11)

Potentially interfering endogenous and exogenous substances

Elevated levels of triglycerides (33 g/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60 g/L), hemoglobin (2 g/L) and human DNA (2 mg/L) in samples as well as the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and antinuclear antibody (ANA) have been tested in the presence and absence of HIV-1 and HIV-2 RNA.

In addition, drug compounds listed in Table 28 were tested at three times the C_{max} in the presence and absence of HIV-1 and HIV-2 RNA.

All potentially interfering substances show no interference with the test performance with the exception of triglycerides and human DNA. At concentrations higher than 25 g/L triglycerides and 1.5 mg/L human DNA invalid results could occur due to interference. Non-reactive results were obtained with **cobas**® HIV-1/HIV-2 Qualitative for all samples without HIV target and reactive results were obtained on all of the samples with HIV-1 and HIV-2 targets.

Table 28 Drug compounds tested for interference with the **cobas**® HIV-1/HIV-2 Qualitative

Class of drug	Generic drug name
Immune Modulators	Peginterferon α-2a
Immune Modulators	Peginterferon α-2b
Immune Modulators	Ribavirin
HCV Inhibitors	Simeprevir
HCV Inhibitors	Sofosbuvir
Reverse Transcriptase or DNA Polymerase Inhibitors	Emtricitabine
Reverse Transcriptase or DNA Polymerase Inhibitors	Entecavir
Reverse Transcriptase or DNA Polymerase Inhibitors	Foscarnet
Reverse Transcriptase or DNA Polymerase Inhibitors	Cidofovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Lamivudine
Reverse Transcriptase or DNA Polymerase Inhibitors	Ganciclovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Tenofovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Adefovir dipivoxil
Reverse Transcriptase or DNA Polymerase Inhibitors	Telbivudine
Reverse Transcriptase or DNA Polymerase Inhibitors	Aciclovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Valganciclovir
Compounds for Treatment of Opportunistic Infections	Azithromycin
Compounds for Treatment of Opportunistic Infections	Clarithromycin
Compounds for Treatment of Opportunistic Infections	Ethambutol
Compounds for Treatment of Opportunistic Infections	Fluconazole
Compounds for Treatment of Opportunistic Infections	Isoniazid
Compounds for Treatment of Opportunistic Infections	Pyrazinamide
Compounds for Treatment of Opportunistic Infections	Rifabutin
Compounds for Treatment of Opportunistic Infections	Rifampicin
Compounds for Treatment of Opportunistic Infections	Sulfamethoxazole
Compounds for Treatment of Opportunistic Infections	Trimethoprim
Statin	Atorvastatin
Selective Serotonin Reuptake Inhibitor	Fluoxetine
Selective Serotonin Reuptake Inhibitor	Sertraline
Selective Serotonin Reuptake Inhibitor	Paroxetine
Antihistamine	Loratadine
Beta-blocker	Nadolol
Decongestant	Phenylephrine HCl
Nonsteroidal Anti-inflammatory drug	Naproxen
Nonsteroidal Anti-inflammatory drug	Ibuprofen
Pain reliever	Acetaminophen
Pain reliever	Acetylsalicylic Acid
Vitamins	Ascorbic Acid

Cross contamination

The cross-contamination rate for the system was determined by testing 240 replicates of an 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 non-reactive, resulting in a cross-contamination rate of 0%. The 95% confidence interval was 0% for the lower bound and 1.5% for the upper bound.

Clinical performance evaluation

Reproducibility

Reproducibility of **cobas**® HIV-1/HIV-2 Qualitative was evaluated in EDTA plasma across reagent lot, test site/instrument system, operator, days, batch, and within batch. Reproducibility testing was performed in three sites using three reagent lots, two **cobas**® 6800 Systems and one **cobas**® 8800 System, two operators over 6 days; three replicates of each panel member were performed for each batch. Each panel consisted of one negative panel member and six positive panel members. The negative percent agreement was estimated as 100%, with a corresponding 95% exact CI of (98.9%, 100.0%) and the positive percent agreement was 100% for each panel member for both HIV-1 and HIV-2.

For HIV-1 positive panel members, the coefficient of variation (CV(%)) for all panel members was ≤ 1.9%, demonstrating very low variability of **cobas**® HIV-1/HIV-2 Qualitative results across reagent lots, sites/instruments, days, operators, and batches.

Table 29 Attributable percentage of total variance, total precision Standard Deviation and CV(%) of cycle threshold Values from HIV-1 Reactive results with the **cobas**® HIV-1/HIV-2 Qualitative by positive HIV-1 panel member

Panel Member	N[a]	Lot[b]	Site[b]	Operator[b]	Day[b]	Batch[b]	Within-Batch[b]	Total Precision Standard Deviation[c]	Total Precision CV(%)[d]
~3 × LoD (3.78E1) HIV-1, Negative HIV-2	324	0.0% (0.0%)	0.0% (0.0%)	2.2% (0.3%)	6.6% (0.5%)	0.0% (0.0%)	91.1% (1.8%)	0.69	1.9%
>3 × LoD (1.00E5) HIV-1, Negative HIV-2	322	0.0% (0.0%)	15.5% (0.4%)	0.0% (0.0%)	33.1% (0.5%)	4.5% (0.2%)	46.9% (0.6%)	0.24	0.9%
>3 × LoD (1.00E5) HIV-1, ~3 × LoD (8.37E1) HIV-2	323	0.0% (0.0%)	7.8% (0.3%)	0.0% (0.0%)	45.0% (0.6%)	10.9% (0.3%)	36.3% (0.6%)	0.25	1.0%
~3 × LoD (3.78E1) HIV-1, >3 × LoD (1.00E5) HIV-2	323	1.5% (0.2%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	0.0% (0.0%)	98.5% (1.8%)	0.67	1.8%

Note: The table only includes results with detectable analyte.

[a] Number of valid tests with detectable analyte.

[b] Cells within the columns for the lot, site, operator, day, batch, and within-batch components display the percentage of total variance (%)and within parentheses the percent coefficient of variation (CV%) of the component.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] CV(%) = (standard deviation / mean) * 100%.

For HIV-2 positive panel members, the coefficient of variation (CV(%)) for all panel members was ≤ 1.7%, demonstrating very low variability of **cobas**® HIV-1/HIV-2 Qualitative results across reagent lots, sites/instruments, days, operators, and batches.

Table 30 Attributable percentage of total variance, total precision Standard Deviation, and CV(%) of cycle threshold values from HIV-2 reactive results with the **cobas**® HIV-1/HIV-2 Qualitative by positive HIV-2 panel member

Panel Member	N[a]	Lot[b]	Site[b]	Operator[b]	Day[b]	Batch[b]	Within-Batch[b]	Total Precision Standard Deviation[c]	Total Precision CV(%)[d]
Negative HIV-1, ~3 × LoD (8.37E1) HIV-2	324	60.0% (1.3%)	3.3% (0.3%)	0.0% (0.0%)	14.6% (0.7%)	4.5% (0.4%)	17.6% (0.7%)	0.59	1.7%
Negative HIV-1, >3 × LoD (1.00E5) HIV-2	324	31.9% (0.9%)	10.0% (0.5%)	0.0% (0.0%)	32.9% (1.0%)	0.0% (0.0%)	25.1% (0.8%)	0.42	1.7%
>3 × LoD (1.00E5) HIV-1, ~3 × LoD (8.37E1) HIV-2	323	26.0% (0.7%)	4.0% (0.3%)	0.0% (0.0%)	9.9% (0.4%)	4.2% (0.3%)	55.9% (1.0%)	0.46	1.3%
~3 × LoD (3.78E1) HIV-1, >3 × LoD (1.00E5) HIV-2	323	38.2% (0.9%)	8.2% (0.4%)	0.0% (0.0%)	24.7% (0.8%)	0.0% (0.0%)	28.9% (0.8%)	0.39	1.5%

Note: The table only includes results with detectable analyte.

[a] Number of valid tests with detectable analyte.

[b] Cells within the columns for the lot, site, operator, day, batch, and within-batch components display the percentage of total variance (%)and within parentheses the percent coefficient of variation (CV%) of the component.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] CV(%) = (standard deviation / mean) * 100%.

Clinical method comparison

HIV-1 and HIV-2 clinical sensitivity

The performance of **cobas**® HIV-1/HIV-2 Qualitative on the **cobas**® 6800/8800 Systems was compared to an alternative HIV-1 qualitative NAT or HIV-2 NAT in subjects known to be infected with Human Immunodeficiency Virus type 1 (HIV-1) or Human Immunodeficiency Virus type 2 (HIV-2).

Testing of specimens from HIV-1 infected individuals

Overall 1030 specimens were tested from subjects known to be HIV-1 positive with HIV-1 viral loads ≥ 100 copies/mL. Of 1030 specimens evaluable for statistical analysis, 537 (52.1%) were from female subjects, 752 (73.0%) were from African/African American subjects, and the median age of subjects was 37 years (range: 18-81 years). There were 736 HIV-1 B subtype specimens and 294 HIV-1 Non-B subtype specimens.

The HIV-1 sensitivity of **cobas**® HIV-1/HIV-2 Qualitative was 100% (1030/1030, 95% CI: 99.6% to 100%). The sensitivity is for samples with viral RNA concentrations equal to or greater than 100 copies/mL (Table 31). Similar performance was observed between plasma and serum specimens.

Table 31 HIV-1 Sensitivity of **cobas®** HIV-1/HIV-2 Qualitative for the HIV-1 known positive population

Population, Specimen Type	Total Known Positive Specimens	Number Reactive by Test	HIV-1 Sensitivity Estimate	95% Exact CI
HIV-1, Overall	1030	1030	100%	(99.6%, 100%)
HIV-1, Plasma	712	712	100%	(99.5%, 100%)
HIV-1, Serum	318	318	100%	(98.8%, 100%)

Testing of specimens from HIV-2 infected individuals

Overall, 183 specimens from subjects known to be HIV-2 positive with HIV-2 viral loads ≥ 100 copies/mL by HIV-2 Plasma RNA Quantitative Assay were tested. Of the 183 evaluable specimens, 92 (50.3%) were from male subjects, all were African subjects, and the median age of subjects was 50 years (range: 23-77 years).

The HIV-2 sensitivity of **cobas®** HIV-1/HIV-2 Qualitative was 99.5% (182/183, 95% CI: 97.0% to 99.99%) (Table 32). The sensitivity is for samples with viral RNA concentrations equal to or greater than 100 copies/mL. Similar performance was observed between plasma and serum specimens.

Table 32 HIV-2 Sensitivity of **cobas®** HIV-1/HIV-2 Qualitative for the HIV-2 known positive population

Population, Specimen Type	Total Known Positive Specimens	Number Reactive by Test	HIV-2 Sensitivity Estimate	95% Exact CI
HIV-2, Overall	183	182	99.5%	(97.0%, 99.99%)
HIV-2, Plasma	115	115	100.0%	(96.8%, 100%)
HIV-2, Serum	68	67	98.5%	(92.1%, 99.96%)

Clinical specificity

The clinical specificity of **cobas®** HIV-1/HIV-2 Qualitative on the **cobas®** 6800/8800 Systems was determined in comparison to a clinical algorithm using two immunoassays followed by NAT testing to resolve indeterminate results in an HIV low-risk population (US Centers For Disease Control and Prevention HIV Laboratory Testing Algorithm). The HIV low-risk population consisted of 1988 healthy blood donors and 3929 low-risk subjects from an area with less than 1% HIV prevalence. Of 5917 specimens evaluable for statistical analysis, 3301 (55.8%) were from female subjects, 3942 (66.6%) were from white/Caucasian subjects, and the median age of subjects was 36 (range: 17-92 years).

The overall HIV-1 specificity and HIV-2 specificity of **cobas®** HIV-1/HIV-2 Qualitative was 100%, with no difference between plasma and serum specimens (Table 33).

Table 33 Specificity of **cobas®** HIV-1/HIV-2 Qualitative by target analyte in the HIV low-risk population

Target Analyte, Specimen Type	Total cobas® HIV-1/HIV-2 Qualitative Nonreactive Subjects	Status-Negative Subjects by CDC HIV Testing Algorithm	Specificity Estimate	95% Exact CI
HIV-1, Overall[a]	5902	5902	100%	(99.94%, 100%)
HIV-1, Plasma[a]	3608	3608	100%	(99.90%, 100%)
HIV-1, Serum[a]	2294	2294	100%	(99.84%, 100%)
HIV-2, Overall[b]	5914	5914	100%	(99.94%, 100%)
HIV-2, Plasma[b]	3618	3618	100%	(99.90%, 100%)
HIV-2, Serum[b]	2296	2296	100%	(99.84%, 100%)

[a] Fifteen specimens that were HIV-1 positive by the CDC HIV testing algorithm were not included in the specificity analysis for the HIV-1 target (10 were plasma and 5 were serum specimens).

[b] Three serum specimens were not included because the CDC HIV testing algorithm result was not obtained due to insufficient specimen volume for HIV-2 NAT testing.

Prospective study of individuals at high risk for HIV-1 infection

The performance of **cobas®** HIV-1/HIV-2 Qualitative was compared to an alternative HIV-1 qualitative NAT in subjects at high risk for HIV-1 infection. All 519 specimens from subjects at high risk for HIV-1 infection were from subjects with at least 1 risk factor for HIV infection (injection drug user (current or ever), unprotected sex with an HIV-infected person (current or ever), diagnosed with sexually transmitted disease within the last year, multiple sex partners (more than 1 partner in the last 12 months), MSM (current or ever), unprotected sex with a person diagnosed with a sexually transmitted disease within the last year). Of the 519 evaluable specimens, 345 (66.5%) were from male subjects, 289 (55.7%) were from African/African American subjects, and the median age of subjects was 39 years (range: 18-80 years).

The HIV-1 PPA of **cobas®** HIV-1/HIV-2 Qualitative was 100% (5/5), and the HIV-1 NPA was 100% (514/514, 95% CI: 99.3% to 100%). Similar performance was observed for plasma and serum specimens.

Prospective study of individuals at high risk for HIV-2 infection

The performance of **cobas®** HIV-1/HIV-2 Qualitative was compared to a clinical algorithm using two immunoassays followed by NAT testing to resolve indeterminate results in subjects at high risk for HIV-2 infection (U.S. Centers For Disease Control and Prevention HIV Laboratory Testing Algorithm).

All specimens from subjects at high risk for HIV-2 infection were from an HIV-2 endemic area of West Africa (i.e., Guinea-Bissau, Cameroon, Cote d'Ivoire). Of 499 evaluable specimens, 366 (73.3%) were from male subjects, all subjects were African, and the median age of subjects was 28 years (range: 19-66 years).

The PPA of **cobas®** HIV-1/HIV-2 Qualitative for HIV-1 in the HIV-2 High-risk population was 79.0% (79/100; 95% CI: 69.7%-86.5%), and the NPA was 99.5% (395/397; 95% CI: 98.2%-99.9%). The PPA of **cobas®** HIV-1/HIV-2 Qualitative for HIV-2 in the HIV-2 High-risk population was 44.4% (4/9), and the NPA was 100% (490/490; 95% CI: 99.2%-100%). Similar performance was observed for plasma and serum specimens.

Testing of specimens from pregnant women

The performance of **cobas**® HIV-1/HIV-2 Qualitative was compared to a clinical algorithm using two immunoassays followed by NAT testing to resolve indeterminate results in HIV-1 high risk and HIV low-risk pregnant women (U.S. Centers For Disease Control and Prevention HIV Laboratory Testing Algorithm). Overall, 344 specimens were assessed from 309 subjects and 35 contrived specimens from HIV-1 known positive, HIV-1 high risk, and HIV low-risk populations of pregnant women. Of 309 female subjects, 110 (35.6%) were African/African American, 281 (91.0%) were either in their second or third trimester, and the median age was 28 years (range: 18-47 years). All 60 specimens from HIV-1 known positive pregnant women (including 35 contrived specimens) were HIV-1 reactive with **cobas**® HIV-1/HIV-2 Qualitative for an HIV-1 sensitivity of 100% (60/60, 95% CI: 94.0% to 100%). The HIV-1 specificity of **cobas**® HIV-1/HIV-2 Qualitative for HIV low-risk pregnant women was 100% (48/48, 95% CI: 92.6% to 100%). For specimens from HIV-1 high risk pregnant women, percent agreement was calculated between **cobas**® HIV-1/HIV-2 Qualitative results and the CDC HIV testing algorithm results. The HIV-1 PPA was 48.8% (20/41), the HIV-1 NPA was 100% (195/195, 95% CI: 98.1% to 100%), and the HIV-2 NPA was 100% (236/236, 95% CI: 98.4% to 100%). Of the 21 samples with HIV-1 nonreactive results by **cobas**® HIV-1/HIV-2 Qualitative, 19 samples had target not detected HIV-1 NAT results and 2 plasma samples had viral loads of <30 copies/mL by an FDA-approved HIV-1 Quantitative NAT. It is unclear whether pregnancy is an independent predictor of antigen/antibody-positive, NAT-negative discordance or if sample handling or other sample collection issues resulted in these discrepancies.

Testing of specimens from pediatric individuals

The performance of **cobas**® HIV-1/HIV-2 Qualitative was compared to a clinical algorithm using two immunoassays followed by NAT testing to resolve indeterminate results in HIV-1 high risk and HIV low-risk pediatric subjects (U.S. Centers For Disease Control and Prevention HIV Laboratory Testing Algorithm). Overall, 328 specimens were assessed from 302 subjects and 26 contrived specimens from HIV-1 known positive, HIV-1 high risk, and HIV low-risk populations of pediatric subjects. Of 302 subjects, 161 (53.3%) were female and the median age was 18 years (range: 1-21 years). All 76 specimens from HIV-1 known positive pediatric subjects (including 26 contrived specimens) were HIV-1 reactive with **cobas**® HIV-1/HIV-2 Qualitative for an HIV-1 sensitivity of 100% (76/76, 95% CI: 95.3% to 100%). The HIV-1 specificity of **cobas**® HIV-1/HIV-2 Qualitative for the HIV low-risk pediatric population was 100% (52/52, 95% CI: 93.2% to 100%). For specimens from the HIV-1 high risk pediatric population, percent agreement was calculated between **cobas**® HIV-1/HIV-2 Qualitative results and the CDC HIV testing algorithm results. The HIV-1 PPA was 100% (2/2), the HIV-1 NPA was 100% (197/197, 95% CI: 98.1% to 100%) and the HIV-2 NPA was 100% (200/200, 95% CI: 98.2% to 100%).

Sample collection tube equivalency between PPT and EDTA plasma

Paired EDTA and PPT plasma specimens from 54 HIV-1 known positive subjects (26 between 100-250 copies/mL and 28 between 250 and 75,000 copies/mL), 52 HIV-1 high-risk subjects, and 53 HIV low risk subjects were tested to demonstrate equivalency between EDTA plasma and PPT plasma specimens. The HIV-1 OPA for **cobas**® HIV-1/HIV-2 Qualitative results from paired specimens in EDTA plasma compared with PPT plasma was 100% (54/54, 95% CI: 93.4% to 100%) for HIV-1 known positive specimens, 100% (52/52, 95% CI: 93.2% to 100%) for HIV-1 high-risk specimens, and 100% (53/53, 95% CI: 93.3% to 100%) for HIV low risk specimens, respectively.

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies.

The results presented in the Instructions for Use support equivalent performance for all systems

Additional information

Key test features

Sample type	EDTA plasma and serum		
Minimum amount of sample required	675 µL		
Sample process volume	500 µL		
Analytical sensitivity		HIV-1M	HIV-2
	EDTA plasma	12.8 cp/mL	35.4 cp/mL
	Serum	12.8 cp/mL	26.3 cp/mL
Groups/subtypes – inclusivity	HIV-1M (A–D, F–H, J, K, CRF01_AE, CRF02_AG, CRF12_BF, CRF14_BG), HIV-1O, HIV-1N, HIV-2 (A and B)		

Symbols

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

Table 34 Symbols used in labeling for Roche PCR diagnostics products

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

Technical support

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

Manufacturer and distributors

Table 35 Manufacturer and distributors

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

Made in USA

Distributed by

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

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References

1. AAHVM. Fundamentals of HIV Medicine. Washington, DC;2007.
2. UNAIDS. Global AIDS Update. Geneva;2016.
3. Hoover DR, Saah AJ, Bacellar H, et al. Clinical manifestations of AIDS in the era of pneumocystis prophylaxis. N Engl J Med. 1993;329(69):1922-6.
4. Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. Clin Infect Dis. 2011;52(6):780-7.
5. CDC. Laboratory Testing for the Diagnosis of HIV Infection. Atlanta, GA;2014.
6. Gökengin D, Geretti AM, Begovac J, et al. 2014 European Guideline on HIV testing. Int J STD AIDS. 2014;25(10):695-704.
7. Masciotra S, McDougal JS, Feldman J, Sprinkle P, Wesolowski L, Owen SM. Evaluation of an alternative HIV diagnostic algorithm using specimens from seroconversion panels and persons with established HIV infections. J Clin Virol. 2011;52 Suppl 1:S17-22.
8. O'Brien M, Markowitz M. Should we treat acute HIV infection? Current HIV/AIDS Reports. 2012;9(2):101-10.
9. Branson BM, Mermin J. Establishing the diagnosis of HIV infection: new tests and a new algorithm for the United States. J Clin Virol. 2011;52 Suppl 1:S3-4.
10. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection. N Engl J Med. 2011;364(20):1943-54.
11. World Health Organization. Diagnosis of HIV infection in infants and children. Geneva, 2010.
12. Wittek M, Stürmer M, Doerr HW, Berger A. Molecular assays for monitoring HIV infection and antiretroviral therapy. Expert Rev Mol Diagn. 2007;7(3):237-46.
13. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990;93:125-8.
14. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature. 1995;373:487-93.
15. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. Cell. 1995;80:869-78.
16. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY). 1992;10:413-7.
17. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Res. 1996;6:986-94.
18. Center for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
19. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 3.0 09/2022	Updated front page and table 2 and table 3 with separate information for the Positive and Negative Control Kit use. Updated Patent web address. Updated to current economic operators. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 4.0 11/2022	Added **cobas**® 5800 specific information. Increased minimum sample volume. Please contact your local Roche Representative if you have any questions.