cobas® HIV-1/HIV-2 Qualitative

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

For in vitro diagnostic use

cobas® HIV-1/HIV-2 Qualitative	P/N: 07862113190
cobas® HIV-1/HIV-2 Qualitative Control Kit	P/N: 07862091190
cobas® NHP Negative Control Kit	P/N: 07002220190
cobas® Specimen Pre-Extraction Reagent	P/N: 08064695190

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Intended use

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

The test is intended to be used as an aid in diagnosis of HIV-1/HIV-2. 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. In infants born to HIV-infected mothers and who have maternal antibodies to HIV-1 or HIV-2, the presence of HIV nucleic acid is indicative of active infection. **cobas**® HIV-1/HIV-2 Qualitative may also be used to confirm HIV-1 or HIV-2 infection in an individual with specimens reactive for HIV-1 or HIV-2 antibodies or antigens.

Summary and explanation of the test

Background

Human immunodeficiency virus (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 opportunity 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-nucleotide 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 diagnosis 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 even further, because of the sensitivity of 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 infected 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**® 6800 System and **cobas**® 8800 System. **cobas**® HIV-1/HIV-2 Qualitative enables the simultaneous detection and discrimination of HIV-1 and HIV-2 nucleic acid in EDTA plasma, serum and DBS of infected patients. Two probes are used to detect HIV-1, but not to discriminate group M subtypes of HIV-1 and of 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**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for all tests as 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 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 a 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 reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HIV-1 and HIV-2 genomes. The HIV-1 gag gene, the HIV-1 LTR region (dual target for HIV-1) and the HIV-2 LTR region are amplified by **cobas**® HIV-1/HIV-2. 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 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 5.

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

cobas® HIV-1/HIV-2 Qualitative
Store at 2-8°C
96 test cassette (P/N 07862113190)

Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	13 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
HIV-1/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	6 mL

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

cobas® HIV-1/HIV-2 Qualitative Control Kit
Store at 2–8°C
(P/N 07862091190)

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 x 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.
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 x 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.

* Product safety labeling primarily follows EU GHS guidance

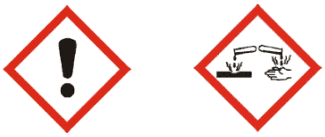
Table 3 **cobas**® NHP Negative Control Kit

cobas® NHP Negative Control Kit Store at 2-8°C (P/N 07002220190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HIV-1/2; HIV-1 RNA and HIV-2 RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant.

* Product safety labeling primarily follows EU GHS guidance

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear eye protection/ face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. 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. P501: Dispose of contents/container to an approved waste disposal plant. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol
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 10 and Table 11).

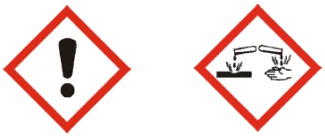
** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

cobas® Specimen Pre-Extraction Reagent

Table 5 cobas® Specimen Pre-Extraction Reagent*

cobas® Specimen Pre-Extraction Reagent
Store at 2-8°C
(P/N 08064695190)

Reagent	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas® Specimen Pre-Extraction Reagent (SPER)	28% (w/w) guanidine thiocyanate, 6% (w/v) polydocanol, 1% (w/v) dithiothreitol, dihydro sodium citrate	600 mL (15 x 40 mL)	 **DANGER** H302: Harmful if swallowed. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product.. P273: Avoid release to the environment. P280: Wear eye protection/face protection. 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. P501: Dispose of contents/ container to an approved waste disposal plant.

* This reagent is not included in the **cobas**® HIV-1/HIV-2 Qualitative kit. See listing of additional materials required (Table 10 and Table 11).

** Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 6 and Table 7. **cobas**® Specimen Pre-Extraction Reagent (SPER), used in DBS workflow, shall be stored and handled as specified in Table 8 and Table 9.

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

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

Reagent	Storage temperature
cobas® HIV-1/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

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	30 days from first usage	Max 10 runs	Max 8 hours
cobas® HIV-1/HIV-2 Qualitative Control Kit	Date not passed	Not applicable	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

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

Store **cobas**® Specimen Pre-Extraction Reagent (used in DBS workflow) at the corresponding temperature specified in Table 8.

Table 8 cobas® Specimen Pre-Extraction Reagent storage

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

cobas® Specimen Pre-Extraction Reagent is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days when stored at 2-8°C including cumulative 13 hours at 30°C or until expiration date, whichever comes first as specified in Table 9.

Table 9 cobas® Specimen Pre-Extraction Reagent expiry conditions

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	Stability at 30°C outside refrigerator (cumulative time)
cobas® Specimen Pre-Extraction Reagent	Date not passed	30 days from first usage	Not applicable	Max 13 hours

Additional materials required

Table 10 Materials and consumables for use on **cobas**® 6800/8800 Systems

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

Table 11 Other materials and consumables required for DBS application only

Materials
Whatman 903® filter card, Munktell Specimen Collection card TFN or equivalent (12-13 mm spot diameter)
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Cryo.s™) with caps
Eppendorf Thermomixer (e.g., model R 5355 or equivalent) with Thermoblock for 24 cryo tubes
Sterile or disposable forceps or tweezers
Resealable bags and desiccant sachets (for DBS storage)

Instrumentation and software required

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

Table 12 Instrumentation

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

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

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

Precautions and handling requirements

Warnings and precautions

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

- For *in vitro* diagnostic use only.
- **cobas**® HIV-1/HIV-2 Qualitative has not been evaluated for use as a screening test for the presence of HIV-1/HIV-2 in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[18,19] Only personnel proficient in handling infectious materials and the use of **cobas**® HIV-1/HIV-2 Qualitative and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect following appropriate site procedures.
    - If spillage of DBS samples in **cobas**® Specimen Pre-Extraction Reagent (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- **cobas**® 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.**
- **cobas**® Specimen Pre-Extraction Reagent is light sensitive and shipped in light protective bottles.
- Use only supplied or specified required consumables to ensure optimal test performance.

- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

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, wash reagent, and **cobas**® Specimen Pre-Extraction Reagent (required for DBS application only) 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 and **cobas**® Specimen Pre-Extraction Reagent contain 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 reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent or **cobas**® Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium 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**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual 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.

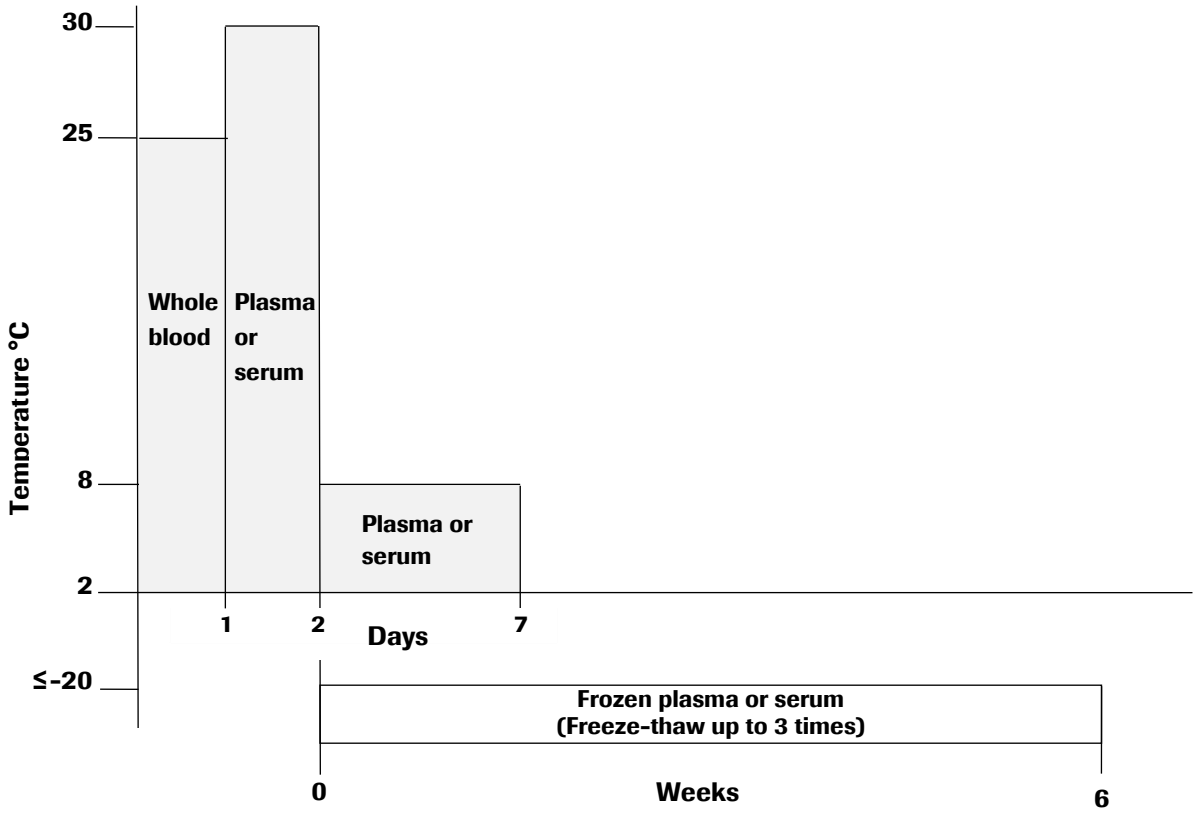
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

Samples

EDTA plasma and serum samples

- 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 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 samples are stable for up to three freeze/thaw cycles when frozen at ≤ -20°C.
- Refer to Figure 1 for sample storage conditions.

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.

Dried blood spots

- Collect DBS samples using appropriate clinical procedures.
- It is recommended to apply a minimum of 70 µL of capillary blood inside each delineated circle on the DBS card.
- Ensure that BOTH sides of the paper are saturated and completely fill the delineated circle.
- Allow DBS to dry at room temperature (18-25°C) for at least 3 hours, protecting the DBS card from direct sunlight.
- For further details consult package insert of filter cards used.
- It is recommended to prepare at least 3 paper disks per patient sample.
- Store DBS in individual resealable bags with a desiccant sachet in each bag.
- DBS may be transported or stored at 15-30°C for up to three months.
- 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

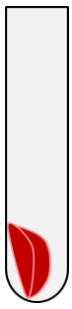
Procedural notes

- Do not use **cobas**® HIV-1/HIV-2 Qualitative reagents, **cobas**® HIV-1/HIV-2 Qualitative Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** reagents, or **cobas**® Specimen Pre-Extraction Reagent after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Dried blood spot sample preparation

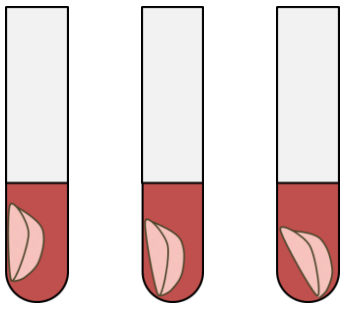
- Allow **cobas**® Specimen Pre-Extraction Reagent (SPER) to equilibrate to ambient temperature before use.
- Excise one DBS from the DBS card.
- Transfer the spot into a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Cryo.s™]) using sterile or disposable forceps or tweezers.
- Ensure the DBS is located at the bottom of the tube as shown in Figure 2.
- Pipette 1150 µL of SPER into the tube containing the DBS and cap the tube.
- Ensure the DBS is completely covered with SPER.
- Place tubes in each of the positions 1 - 24 on a preheated Eppendorf Thermomixer (e.g., model R 5355 or equivalent) with Thermoblock for 24 cryo tubes and incubate for 10 minutes, at 56°C and 1000 rpm to extract the virus from the dried whole blood.
- Decap the tubes and ensure the DBS is attached to the tube wall (Figure 3) to avoid sample clots.
- Eliminate any potential liquid film located above the liquid level (Figure 4) using a sterile pipette tip (to avoid early level detection).
- Transfer the tubes to the **cobas**® 6800/8800 Systems.

Figure 2 DBS in tube



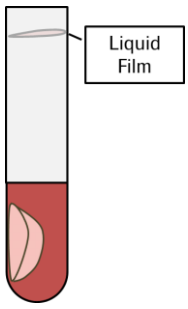
Valid

Figure 3 DBS location



Valid Valid Potential risk of clot

Figure 4 Liquid film

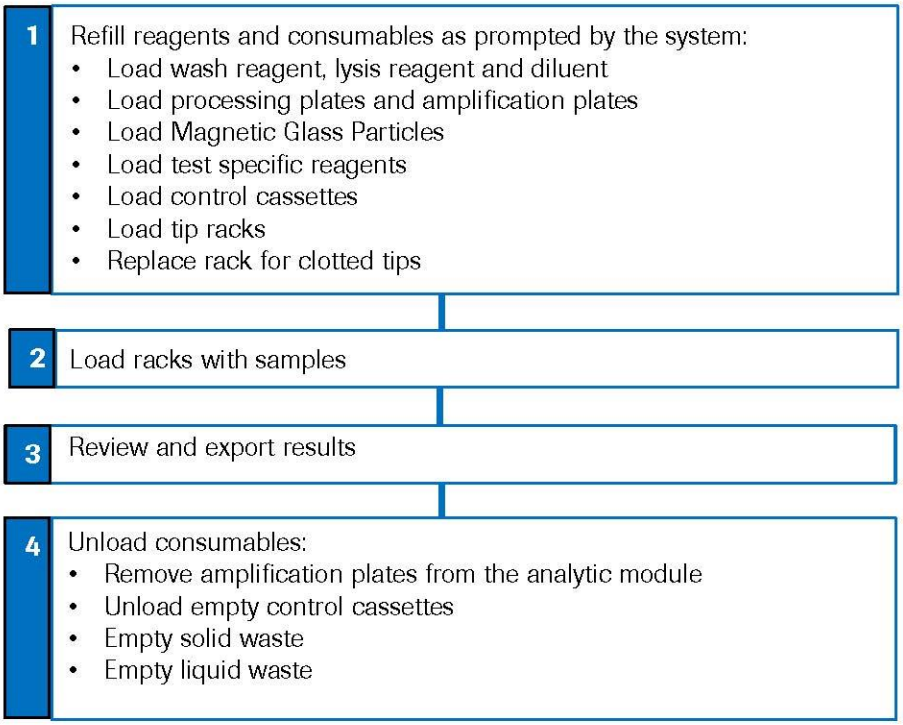


Potential risk of early level detection

Running cobas® HIV-1/HIV-2 Qualitative

cobas® HIV-1/HIV-2 Qualitative can be run with a minimum required sample volume of 650 µL (for the 500 µL plasma or serum sample workflow) or 1150 µL **cobas**® Specimen Pre-Extraction Reagent (for the 850 µL DBS sample workflow). Please note, DBS samples cannot be run in mixed batch mode with plasma or serum samples. The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual. Figure 5 below summarizes the procedure.

Figure 5 cobas® HIV-1/HIV-2 Qualitative test procedure



Results

The **cobas**® 6800/8800 Systems automatically detects and discriminates HIV-1 and HIV-2 simultaneously for the samples and controls, displaying test validity, overall results, as well as individual target results.

Quality control and validity of results

- One Normal Human Plasma Negative Control [(–) C] and two positive controls [HIV-1M/HIV-2 (+)C and HIV-1O (+)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

Table 13 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-1M/HIV-2 (+)C	Q02 (Control batch failed)	Invalid	The entire batch is assigned invalid if the result for the HIV-1M/HIV-2 (+)C is invalid.
HIV-1O (+)C	Q02 (Control batch failed)	Invalid	The entire batch is assigned invalid if the result for the HIV-1O (+)C is invalid.

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

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

Interpretation of results

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

- A valid batch may include both valid and invalid sample results.
- 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 14.

Table 14 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**	HIV-1 Reactive	Invalid	Not all requested results were valid. Target signal detected for HIV-1. HIV-2 result is invalid. Original specimen should be re-tested to obtain valid HIV-2 results. If the result is still invalid, a new specimen should be obtained.
No	**Invalid**	Invalid	HIV-2 Reactive	Not all requested results were valid. Target signal detected for HIV-2. HIV-1 result is invalid. Original specimen should be re-tested to obtain valid HIV-1 results. If the result is still invalid, a new specimen should be obtained.
No	**Invalid**	HIV-1 Non-Reactive	Invalid	Not all requested results were valid. No target signal detected for HIV-1. HIV-2 result is invalid. Original specimen should be re-tested to obtain valid HIV-2 results. If the result is still invalid, a new specimen should be obtained.
No	**Invalid**	Invalid	HIV-2 Non-Reactive	Not all requested results were valid. No target signal detected for HIV-2. HIV-1 result is invalid. Original specimen should be re-tested to obtain valid HIV-1 results. If the result is still invalid, a new specimen should be obtained.
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, **cobas omni** Wash Reagent, and **cobas**® Specimen Pre-Extraction Reagent (used in dried blood spot workflow) for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- 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.
- 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.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- **cobas**® HIV-1/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) for EDTA plasma, serum and DBS samples
- WHO International Standard for HIV-2 RNA (NIBSC code 08/150) for plasma and serum samples
- Roche Primary Standards for HIV-2 RNA for DBS samples
- Roche Primary Standards for HIV-1 group O RNA for EDTA plasma and serum samples

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). The Roche HIV-2 RNA Standard is traceable to the WHO International Standard for HIV-2 RNA (NIBSC code 08/150). The Roche Primary Standards for HIV-2 RNA are derived from commercially available cultured virus stocks, P/N HIV-2 NIH-Z (Cat. No. 10-27-000, Applied Biotechnologies, Inc.). 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 IU.

Serial dilutions of the standards in HIV-negative human EDTA plasma, serum or whole blood for DBS 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 15). The reactivity rates observed in the LoD studies for each virus are summarized in Table 16 to Table 18.

Table 15 Results of 95% PROBIT analysis on LoD data collected with viral standards in EDTA plasma, serum and DBS

Matrices	Analyte	Measuring units	LoD	Lower 95% confidence limit	Upper 95% confidence limit
EDTA plasma	HIV-1 group M	copies/mL	12.6	10.9	15.2
	HIV-1 group O	copies/mL	14.8	12.8	17.7
	HIV-2	copies/mL	27.9	22.9	36.6
Serum	HIV-1 group M	copies/mL	12.1	10.5	14.5
	HIV-1 group O	copies/mL	12.6	10.9	15.2
	HIV-2	copies/mL	23.4	19.6	29.7
DBS	HIV-1 group M	copies/mL	255	224	299
	HIV-2	copies/mL	984	856	1169

Table 16 Reactivity rates summary for HIV-1 group M in EDTA plasma, serum and DBS

Matrices	HIV-1 group M RNA concentration (cp/mL)	Number of reactive	Number of valid replicates	% Reactive
EDTA plasma	40	189	189	100%
	30	189	189	100%
	20	187	189	99%
	10	174	189	92%
	5	124	189	66%
	2.5	91	189	48%
	0	0	189	0%
Serum	40	189	189	100%
	30	189	189	100%
	20	187	189	99%
	10	176	189	93%
	5	126	189	67%
	2.5	86	189	46%
	0	0	189	0%
DBS	750	252	252	100%
	600	252	252	100%
	360	246	250	98%
	180	220	249	88%
	90	163	252	65%
	45	109	250	44%
	0	0	107	0%

Table 17 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%
	30	189	189	100%
	20	185	188	98%
	10	163	189	86%
	5	117	189	62%
	2.5	78	189	41%
	0	0	189	0%
Serum	40	189	189	100%
	30	189	189	100%
	20	186	189	98%
	10	173	189	92%
	5	132	189	70%
	2.5	91	189	48%
	0	0	189	0%

Table 18 Reactivity rates summary for HIV-2 in EDTA plasma, serum and DBS

Matrices	HIV-2 RNA concentration (cp/mL)	Number of reactive	Number of valid replicates	% Reactive
EDTA plasma	80	126	126	100%
	40	124	126	98%
	20	115	126	91%
	10	81	126	64%
	5	61	126	48%
	0	0	189	0%
Serum	80	126	126	100%
	40	125	126	99%
	20	114	126	90%
	10	96	126	76%
	5	49	126	39%
	0	0	189	0%
DBS	3000	252	252	100%
	1450	241	247	98%
	725	226	246	92%
	362	167	248	67%
	181	103	250	41%
	0	0	108	0%

Reproducibility

The reproducibility 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 x, 1 x and 3 x 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. Reproducibility 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 19 and Table 20.

Table 19 cobas® HIV-1/HIV-2 Qualitative reagent lot-to-lot reproducibility 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	~3 x LoD	1	100% (84/84)	95.7%	100%
		2	100% (84/84)	95.7%	100%
		3	100% (84/84)	95.7%	100%
	~1 x LoD	1	98.8% (83/84)	93.5%	100%
		2	98.8% (83/84)	93.5%	100%
		3	100% (84/84)	95.7%	100%
	~0.6 x LoD	1	77.4% (65/84)	67.0%	85.8%
		2	76.2% (64/84)	65.7%	84.8%
		3	82.1% (69/84)	72.3%	89.6%
HIV-2	~3 x LoD	1	98.8% (83/84)	93.5%	100%
		2	96.4% (81/84)	89.9%	99.3%
		3	100% (84/84)	95.7%	100%
	~1 x LoD	1	98.8% (83/84)	93.5%	100%
		2	98.8% (83/84)	93.5%	100%
		3	97.6% (82/84)	91.7%	99.7%
	~0.6 x LoD	1	66.7% (56/84)	55.5%	76.6%
		2	69.0% (58/84)	58.0%	78.7%
		3	69.0% (58/84)	58.0%	78.7%

Table 20 cobas® HIV-1/HIV-2 Qualitative reagent lot-to-lot reproducibility summary (DBS)

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
HIV-1 group M	~3 x LoD	1	100% (84/84)	95.7%	100%
		2	100% (83/83)	95.7%	100%
		3	97.6% (82/84)	91.7%	99.7%
	~1 x LoD	1	96.4% (81/84)	89.9%	99.3%
		2	96.4% (81/84)	89.9%	99.3%
		3	95.2% (80/84)	88.3%	98.7%
	~0.6 x LoD	1	88.0% (73/83)	79.0%	94.1%
		2	83.3% (70/84)	73.6%	90.6%
		3	88.1% (74/84)	79.2%	94.1%
HIV-2	~3 x LoD	1	100% (83/83)	95.7%	100%
		2	100% (84/84)	95.7%	100%
		3	100% (83/83)	95.7%	100%
	~1 x LoD	1	97.6% (82/84)	91.7%	99.7%
		2	97.6% (82/84)	91.7%	99.7%
		3	98.8% (83/84)	93.5%	100%
	~0.6 x LoD	1	88.1% (74/84)	79.2%	94.1%
		2	91.7% (77/84)	83.6%	96.6%
		3	85.7% (72/84)	76.4%	92.4%

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 O (verified by dilution in whole blood for DBS), 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 O, 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, serum or whole blood for DBS, and additionally HIV-1 group O in whole blood for DBS, to the LoD concentration of the predominant group/subtype (HIV-1 group M subtype B or HIV-2 group A) based on the LoD determined with 95% PROBIT analysis over all lots combined. 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 21 and the results from HIV-2 are shown in Table 22.

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 O, 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 21 LoD verification of HIV-1 group M subtypes, group O, and group N in EDTA plasma, serum or whole blood for DBS

		Plasma: 12.6 cp/mL			Serum 12.1 cp/mL			DBS 255 cp/mL		
Group	Subtype	Number of valid replicates	Number of reactive	% Reactive (95% CI*)	Number of valid replicates	Number of reactive	% Reactive (95% CI*)	Number of valid replicates	Number of reactive	% Reactive (95% CI*)
M	A	42	40	95% (99%)	42	40	95% (99%)	41	37	90% (97%)
	C	42	41	98% (100%)	42	42	100% (99.4%)	42	42	100% (100%)
	D	42	37	88% (96%)	42	37	88% (96%)	42	39	93% (99%)
	F	42	38	90% (97%)	42	38	90% (97%)	42	40	95% (99%)
	G	42	40	95% (99%)	42	39	93% (99%)	42	42	100% (100%)
	H	42	38	90% (97%)	42	41	98% (100%)	42	41	98% (100%)
	CRF01_AE	42	38	90% (97%)	42	38	90% (97%)	42	41	98% (100%)
	CRF02_AG	42	36	86% (95%)	42	39	93% (99%)	42	42	100% (100%)
O		N/A	N/A	N/A	N/A	N/A	N/A	41	39	95% (99%)
N		42	39	93% (99%)	42	37	88% (96%)	41	40	98% (100%)

* Upper 95% confidence interval

Table 22 LoD verification of HIV-2 group B in EDTA plasma, serum or whole blood for DBS

	Plasma: 27.9 cp/mL			Serum 23.4 cp/mL			DBS 984 cp/mL		
Group	Number of valid replicates	Number of positives	% Reactive (95% CI*)	Number of valid replicates	Number of positives	% Reactive (95% CI*)	Number of valid replicates	Number of positives	% Reactive (95% CI*)
B	42	42	100% (100%)	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 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 x LoD of **cobas**® HIV-1/HIV-2 Qualitative. All 105 clinical samples with known subtypes were detected neat and at ~5 x LoD (Table 23).

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 x LoD.

Table 23 HIV-1 group M clinical samples

Subtype / circulating recombinant forms	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to ~5 x 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 x 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 x LoD of **cobas**® HIV-1/HIV-2 Qualitative. All 16 HIV-2 samples were detected neat and at ~5 x LoD (Table 24).

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 x LoD.

Table 24 HIV-2 clinical or cultured samples

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

Specificity

The specificity of **cobas**® HIV-1/HIV-2 Qualitative was determined by analyzing HIV negative EDTA plasma, HIV negative serum and HIV negative DBS samples from individual blood donors. A total of 613 individual EDTA plasma and 607 individual serum samples were tested with two lots of **cobas**® HIV-1/HIV-2 Qualitative reagents. In addition, 604 individual DBS samples were tested with three lots of **cobas**® HIV-1/HIV-2 Qualitative reagents. All samples tested were found non-reactive for HIV-1 and HIV-2. In each of the EDTA plasma, serum and DBS samples, the specificity of **cobas**® HIV-1/HIV-2 Qualitative was 100% (95% confidence limit: ≥ 99.5%).

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 and an FDA licensed comparator nucleic acid test tested neat. The overall performance results are shown in Table 25.

Table 25 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			Days to first reactive result			Days earlier detection with cobas® HIV-1/HIV-2 Qualitative	
		cobas® HIV-1/HIV-2 Qualitative	Compara-tor NAT	HIV Ag/Ab Assay	cobas® HIV-1/HIV-2 Qualitative	Compara-tor NAT	HIV Ag/Ab Assay	Compara-tor NAT	HIV Ag/Ab Assay
HIV6243	10	6	6	4	18	18	25	0	7
HIV9011	11	3	3	2	30	30	38	0	8
HIV9012	8	5	5	3	9	7	16	-2	7
HIV9013	7	3	2	2	18	23	23	5	5
HIV9018	10	5	5	3	21	21	28	0	7
HIV9020	21	5	5	3	83	83	90	0	7
HIV9022	9	3	4	2	23	17	25	-6	2
HIV9030	16	6	5	3	40	40	47	0	7
HIV9031	19	8	6	4	120	131	146	11	26
HIV9034	13	4	4	3	41	41	46	0	5
HIV9076	9	3	3	3	66	66	66	0	0
HIV9089	6	5	5	3	7	7	16	0	9
HIV12008	13	7	7	5	21	21	28	0	7
PRB954	7	5	5	2	7	7	17	0	10
PRB956	5	4	4	2	40	40	47	0	7
PRB958	6	6	6	4	0	0	7	0	7
PRB961	9	4	4	2	19	19	27	0	8
PRB962	6	4	4	2	7	7	14	0	7
PRB963	7	4	5	2	9	7	17	-2	8
PRB967	6	5	5	3	3	3	17	0	14
PRB968	10	6	6	4	15	15	26	0	11
PRB969	10	7	6	3	53	53	70	0	17
PRB973	4	4	4	2	0	0	7	0	7
PRB976	4	4	4	2	0	0	7	0	7
PRB977	4	4	2	2	0	-*	13	-*	13
Total	230	120	115	70					

* Invalid result for the comparator NAT for the first panel member of panel PRB977, where a reactive NAT result was expected. The panel (PRB977) was therefore not included in the evaluation of the comparator NAT results.

Analytical specificity

The analytical specificity of **cobas**® HIV-1/HIV-2 Qualitative was evaluated for cross-reactivity with 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 26). 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 x 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 26 Microorganisms tested for cross-reactivity

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

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 27 were tested with and without HIV-1 and HIV-2 added to a concentration of approximately 3 x 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 27 Disease states samples tested for analytical specificity

Disease state		
Adenovirus type 5	Hepatitis B Virus	Herpes Simplex Virus type 2
Cytomegalovirus	Hepatitis C Virus	Human T-cell lymphotropic Virus type I
Dengue Virus	Hepatitis E Virus	Human T-cell lymphotropic Virus type II
Epstein-Barr Virus	Herpes Simplex Virus type1	West Nile Virus

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

Correlation

EDTA plasma and serum

The performance of **cobas**® HIV-1/HIV-2 Qualitative was compared to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (HIV-1 EDTA plasma samples) and to a CE-marked HIV-1/HIV-2 antibody differentiation test (HIV-1 and HIV-2 EDTA plasma and serum samples).

For the correlation to COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0, clinical EDTA plasma samples were analyzed at one external site. For HIV-1 positive and HIV negative clinical EDTA plasma samples, a total percent agreement of 100% between the two tests was shown, demonstrating that the performance of **cobas**® HIV-1/HIV-2 Qualitative and COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is equivalent (Table 29).

Table 29 Summary of results for method correlation for HIV-1 EDTA plasma samples

Method Correlation HIV-1 EDTA plasma samples		**COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0**	
		Reactive	Non-reactive
cobas® HIV-1/HIV-2 Qualitative	Reactive	68	0
	Non-reactive	0	80

For the comparison to a CE-marked HIV-1/HIV-2 antibody differentiation test, clinical EDTA plasma or serum samples were analyzed at one external site. For HIV-1 positive and HIV-1 negative EDTA plasma or serum samples, a total percent agreement of 100% between the two tests was shown. For HIV-2 positive and HIV-2 negative EDTA plasma or serum samples, a total percent agreement of 99.7% between the two tests was shown. This demonstrates that the performance of **cobas**® HIV-1/HIV-2 Qualitative and a CE-marked HIV-1/HIV-2 antibody differentiation test is equivalent (Table 30 and Table 31).

Table 30 Summary of results for method correlation for HIV-1 EDTA plasma and serum samples

Method Correlation HIV-1 EDTA plasma and serum samples		**CE-marked HIV-1/HIV-2 antibody differentiation test**		
		Reactive	Non-reactive	Indeterminate
cobas® HIV-1/HIV-2 Qualitative	Reactive	138	0	0
	Non-reactive	0	164	1*

* The sample which showed a non-reactive result with **cobas**® HIV-1/HIV-2 Qualitative and an indeterminate result with the CE-marked HIV-1/HIV-2 antibody differentiation test was confirmed to be negative with an alternative CE-marked 4th generation HIV Ag/Ab serological test.

Table 31 Summary of results for Method Correlation for HIV-2 EDTA plasma and serum samples

Method Correlation HIV-2 EDTA plasma and serum samples		**CE-marked HIV-1/HIV-2 antibody differentiation test**		
		Reactive	Non-reactive	Indeterminate
cobas® HIV-1/HIV-2 Qualitative	Reactive	14	0	0
	Non-reactive	1	287	1*

* The sample which showed a non-reactive result with **cobas®** HIV-1/HIV-2 Qualitative and an indeterminate result with the CE-marked HIV-1/HIV-2 antibody differentiation test was confirmed to be negative with an alternative CE-marked 4th generation HIV Ag/Ab serological test.

DBS

The performance of **cobas®** HIV-1/HIV-2 Qualitative was compared to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 by analysis of clinical early infant DBS samples at one external site. For HIV-1 positive and HIV-1 negative DBS samples, a total percent agreement of 99.6% between the two tests was shown, demonstrating that the performance of **cobas®** HIV-1/HIV-2 Qualitative and COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is equivalent (Table 32).

Table 32 Summary of results for Method Correlation for HIV-1 DBS samples

Method Correlation HIV-1 DBS samples		**COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0**	
		Reactive	Non-reactive
cobas® HIV-1/HIV-2 Qualitative	Reactive	127	1*
	Non-reactive	0	151

* The sample which showed a reactive result with **cobas®** HIV-1/HIV-2 Qualitative and a non-reactive result with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was confirmed to be HIV-1 positive with nested PCR.

Whole system failure

The Whole System Failure rate for **cobas®** HIV-1/HIV-2 Qualitative was determined by testing 100 replicates of EDTA plasma and 100 replicates of whole blood for DBS spiked with both HIV-1 group M subtype B and HIV-2. 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 and HIV-2 targets, resulting in a Whole System Failure rate of 0% for EDTA plasma and DBS. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.6% for the upper bound for each target and matrix.

Cross contamination

The cross-contamination rate for **cobas®** HIV-1/HIV-2 Qualitative was determined by testing 240 replicates of an HIV negative DBS sample and 225 replicates of a high titer HIV-1 DBS sample at 2.0E+07 cp/mL. The study was performed following the DBS sample preparation workflow. 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.

Additional information

Key test features

Sample type	EDTA plasma, serum and dried blood spot (DBS)
Minimum amount of sample required	650 µL for EDTA plasma and serum samples or one DBS sample (70 µL dried blood per spot) in 1150 µL **cobas**® Specimen Pre-Extraction Reagent (SPER)
Sample process volume	500 µL for EDTA plasma and serum samples or 850 µL for DBS samples

Analytical sensitivity

	HIV-1M	HIV-2
EDTA plasma	12.6 cp/mL	27.9 cp/mL
Serum	12.1 cp/mL	23.4 cp/mL
DBS	255 cp/mL	984 cp/mL

Specificity	100% (one-sided 95% confidence interval: 99.5%) (EDTA plasma/serum) 100% (one-sided 95% confidence interval: 99.5%) (DBS)
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 33 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In Vitro* diagnostic medical device
	Authorized representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD performance evaluation only		Global Trade Item Number
	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.	**Rx Only**	US Only: Federal law restricts this device to sale by or on the order of a physician.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 34 Manufacturer and distributors

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

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

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Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

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Document revision

Document Revision Information	
Doc Rev. 2.0 10/2018	Updated the wording of the description of the required heater shaker model. Updated product safety labeling sentence for P-Code P310 for **cobas**® Specimen Pre-Extraction Reagent (SPER). Minor grammatical and formatting changes. Updated hazard warnings. Updated descriptions of and added Rx Only symbol and description to the harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 01/2019	Updated description of screening tests used for NHP screening in **Table 2, Table 3** and in the **Warnings and precautions** section. Please contact your local Roche Representative if you have any questions.