

WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT

Product: cobas HIV-1 Quantitative nucleic acid test for use on the cobas 5800/6800/8800 Systems¹ WHO reference number: PQDx 0365-046-00

cobas HIV-1 Quantitative nucleic acid test for use on the **cobas** 5800/6800/8800 Systems with product codes 07000995190 and 09040803190, manufactured by Roche Molecular Systems, Inc., CE-market regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 13 October 2020.

Summary of WHO prequalification assessment for cobas HIV-1 Quantitative nucleic acid test for use on the cobas 5800/6800/8800 Systems

	Date	Outcome
Prequalification listing	13 October 2020	listed
Dossier review	abridged	N/A
Onsite Assessment of quality management system	11 July 2022	MR
Product performance evaluation	9 July 2018 to 17 September 2018 24 June 2019 to 17 January 2020	MR

MR: Meet Requirements, N/A: Not Applicable

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which the WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Correction of some typographical errors and updating items required but not provided.	26 October 2020
3.0	1. Extension of claimed shelf-life from 18 months to 24 months from the date of manufacture for the cobas HIV-1 96T kit and cobas HBV/HCV/HIV-1 Control kit.	12 July 2023

¹ The product was formerly prequalified as cobas HIV-1 Quantitative nucleic acid test for use on the cobas 6800/8800 Systems and amended after addition of the 5800 system to the prequalified product.

	<p>2. The changes apply to Product code 07000995190 cobas HIV-1 test for use on the cobas 6800/8800 Systems and Product code 06997767190 cobas HBV/HCV/HIV-1 Control Kit for use on the cobas 6800/8800 Systems.</p> <p>3. Change of Legal Manufacturer</p> <p>4. Change of Notified Body</p> <p>5. Addition of the 192T Kit size</p> <p>6. Addition of cobas 5800 Instrument.</p>	
4.0	Added a footnote to clarify the name of the product from the time it was initially prequalified to when the 5800 system was added. Update on the manufacturing site inspection with the most recent inspection.	4 September 2023

Intended use:

According to the claim of intended use from Roche Diagnostics GmbH, “cobas HIV-1 is an *in vitro* nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) in EDTA plasma or from a cobas Plasma Separation Card (PSC) dried plasma spot of HIV-1-infected individuals.

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

Assay description:

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

Nucleic acid from patient samples, external controls and added armored RNA (RNA-QS) molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HIV-1 genome. The HIV-1 gag gene and the HIV-1 LTR region (dual target) are amplified by cobas HIV-1. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).

Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

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

Test kit contents:

cobas HIV-1 (96 test cassette)	Product code 07000995190
Proteinase Solution (PASE)	13 mL vessel x 1
RNA Quantitation Standard (RNA-QS)	13 mL vessel x 1
Elution Buffer (EB)	13 mL vessel x 1
Master Mix Reagent 1 (MMX-R1)	5.5 mL vessel x 1
HIV-1 Master Mix Reagent 2 (HIV-1 MMX-R2)	6 mL vessel x 1
cobas HIV-1 (192 test cassette)	Product code 09040803190
Proteinase Solution (PASE)	22.3 mL vessel x 1
RNA Quantitation Standard (RNA-QS)	21.2 mL vessel x 1

Elution Buffer (EB)	21.2 mL vessel x 1
Master Mix Reagent 1 (MMX-R1)	7.5 mL vessel x 1
HIV-1 Master Mix Reagent 2 (HIV-1 MMX-R2)	9.7 mL vessel x 1

Items required but not provided:

cobas HBV/HCV/HIV-1 Control Kit	Product code 06997767190 and 09040773190
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L (+) C)	0.65 mL x 8 vials
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H (+) C)	0.65 mL x 8 vials
cobas NHP Negative Control Kit	Product code 07002220190 and 09051554190
Normal Human Plasma Negative Control (NHP-NC)	1 mL x 16 vials

Item	Description
cobas omni reagents for sample preparation	
cobas omni MGP Reagent (MGP)	P/N 06997546190, 480 tests
cobas omni Specimen Diluent (SPEC DIL)	P/N 06997511190, 4 x 875 mL
cobas omni Lysis Reagent (LYS)	P/N 06997538190, 4 x 875 mL
cobas omni Wash Reagent (WASH)	P/N 06997503190, 4.2 L
cobas Specimen Pre-Extraction Reagent	
cobas Specimen Pre-Extraction Reagent (SPER)	P/N 08064695190, 15 x 40mL
Additional Materials	
cobas omni Processing Plate	P/N 05534917001
cobas omni Amplification Plate	P/N 05534941001
cobas omni Pipette Tips	P/N 05534925001
cobas omni Liquid Waste Container	P/N 07094388001
cobas omni Lysis Reagent	P/N 06997538190
cobas omni MGP Reagent	P/N 06997546190
cobas omni Specimen Diluent	P/N 06997511190
cobas omni Wash Reagent	P/N 06997503190
Solid Waste Bag	P/N 07435967001
Solid Waste Container	P/N 07094361001
Materials and consumables required for PSC dried plasma spot application only.	

cobas Plasma Separation Card*	
Sterile or disposable forceps or tweezers**	
140 µL capillary (e.g., Vitrex plastic tube) with compatible dispenser (e.g., Vitrex pipette holder)*	
Single Use lancing device (e.g. Greiner Bio-one: MiniCollect Safety Lancet penetration depth 2.00 mm)*	
Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams) (for PSC storage and delivery, see PSC Method Sheet ms_07963084190 for more information)	
Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)	
Pipette (e.g., Multistepper pipette)	
Eppendorf Thermomixer (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes	
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Greiner Bio-one Cryo.s) with caps	

*See PSC Method Sheet ms_07963084190 for more information about the PSC sample collection.

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

Instrumentation and software required

The cobas 5800 System software and cobas HIV-1 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 Systems software and cobas HIV-1 analysis package for the cobas 6800/8800 Systems shall be installed on the instrument(s). For dried PSC plasma spot application on the cobas 6800/8800 Systems, the cobas HIVPSC ASAP (Assay Specific Analysis Package) shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the systems.

Equipment	Product Number (P/N)
cobas 5800 System	08707464001
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 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.

Storage:

- **cobas** HIV-1 kit, Positive and Negative Control Kit, **cobas omni** reagents for sample preparation and **cobas** Specimen Pre-Extraction Reagent are stored at 2-8 °C.
- **cobas omni** Wash Reagent is stored at 15-30°C.

Shelf-life upon manufacture:

- **cobas** HIV-1 and **cobas** HBV/HCV/HIV-1 Control Kit: 24 months.
- HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L (+) C) and HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H (+) C): 24 months.

Warnings/limitations:

Refer to the instructions for use.

Prioritization for prequalification

Based on the established eligibility criteria, the **cobas** HIV-1 Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems was given priority for WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abridged prequalification assessment, Roche Diagnostics GmbH was not required to submit a product dossier for **cobas** HIV-1 Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems as per the *“Instructions for compilation of a product dossier”* (PQDx_018 version 3). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Manufacturing site inspection

An onsite inspection of Roche Diagnostics GmbH located at Nonnenwald 2, Penzberg, 82377 Germany, was conducted from 11 July 2022. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and gives a summary of the assessment findings.

Information on the most current desk assessment can be found at:

<https://extranet.who.int/pgweb/inspection-services/prequalification-reports/whopirs-vitro-diagnostics>

All published WHOPIRs are with the agreement of the manufacturer.

The onsite inspection was accepted on 21 February 2023.

Product performance evaluation

cobas HIV-1 Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems was evaluated by the International Laboratory Branch (ILB), Division of Global HIV and TB, CDC Atlanta and the NHLS HIV PCR Lab, Charlotte Maxeke Johannesburg Academic Hospital, South Africa on behalf of WHO, according to protocol PQDx_215, version 6. The evaluation took place from July 9, 2018, to September 17, 2018, at the CDC ILB lab and from June 24, 2019, to January 17, 2020, at NHLS HIV PCR Lab.

Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 405 plasma specimens and 360 specimens on Plasma Separation Cards (PSC) from HIV-positive individuals and 91 plasma and 92 PSC from HIV-negative individuals was used. The specimens were characterized using COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0.

Clinical performance characteristics in comparison with an agreed reference standard		
	Plasma	Plasma Separation Card (PSC)
Sensitivity* % (95% CI) (N=)	98.0 (95.3-99.1) (N=245)	94.6 (91.2-96.8) (N=260)
Specificity* % (95% CI) (N=)	94.9 (89.9-97.5) (N=138)	94.1 (87.8-97.3) (N=102)
Specificity among specimens from HIV-negative individuals (N=)	100 (96.0-100) (N=91)	100 (96.1-100) (N=92)
Invalid rate % (N=)	4.4 (N=497)	1.7 (N=463)
Bias Limits of agreement	0.117 log ₁₀ cp/mL -0.346 to 0.580 log ₁₀ cp/mL	0.108 log ₁₀ cp/mL -0.386 to 0.601 log ₁₀ cp/mL

* Sensitivity and specificity for detection of virological failure at 1000 cp/mL

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD)	The LoD was estimated at 12.5 cp/mL (95% CI: 8.8-43.5) for plasma (500µL) and 489 cp/mL (95% CI: 330-1256) for PSC. The LoD claimed by the manufacturer (13.2 cp/mL for plasma (500µL) and 790.2 cp/mL for PSC) were verified.
Within-run precision (repeatability)	For plasma, all CV% were < 2% For PSC, all CV% were < 3.3%
Within-laboratory precision (reproducibility)	For plasma, all CV% were < 2% For PSC, all CV% were < 3.3%
Linearity	Linearity of the assay was verified for HIV-1 subtypes A, B, C, D, and A/G over a range of viral loads from 10 ² to 10 ⁶ cp/mL for plasma and over a range of viral loads from 10 ³ to 10 ⁶ cp/mL for PSC.
Cross-contamination/carry-over	No cross-contamination was observed when high positive and negative specimens were tested together.

Operational characteristics and ease of use

This assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. The instrument requires a stable source of electricity, air conditioning and significant physical space. Furthermore, training and implementation of good laboratory practice are essential to obtaining accurate results. Adequate technical support from the manufacturer or representative is critical.

The assay was found easy to use by the operators performing the evaluation, who received a 5-day training on the use of the assay.

Key operational characteristics	
Number of steps for one specimen*	4 steps in total (+ 4 additional steps for PSC preparation) No step with precision pipetting
Number of steps for instrument management**	5 steps per run/weekly maintenance
Time to result for one run (96 tests including 3 controls)	3 hours 30 minutes

Operator hands-on time for one run	1 hour 5 minutes
Level of automation	Fully automated
Quality controls	QCs are provided by the manufacturer and should be purchased separately. Three controls (high positive, low positive, negative) should be tested with each run.
Operating temperature	15-32°C
Result display and connectivity	Results are displayed on the instrument. They may be printed using a printer provided with the instrument. The results can be exported to the laboratory information system and other health information systems.
Power sources	Main power The use of a UPS is recommended, as stable electricity is required.
Biosafety (<i>outside of infectious specimen handling</i>)	Operators reported biosafety concerns for the user. Samples, reagents and waste need to be treated as biologically hazardous materials and should be handled according to the manufacturer's instructions. If not, they may cause infections, corrosions or may be carcinogenic. Remote user interface software is available to access the instrument software remotely and is necessary for the user to extract data electronically. Archive software is necessary to review the extracted data.
Waste	The volume of liquid was approx. 2.2 L per run. Waste disposal does not require specific measures besides usual laboratory biohazard waste disposal procedures.
Calibration	No calibration is required.
Maintenance	Weekly maintenance is required.
Other specific requirements	Floor stability must be formally assessed prior to installation.

* *Steps for one specimen: each action required to obtain a result for one specimen (excluding specimen collection, instrument management, maintenance/calibration), e.g. add specimen to the cartridge, close the cartridge, scan/type specimen ID, load the cartridge on the instrument, press start (5 steps) OR scan/type specimen ID, load the specimen collection tube into the instrument, press start (3 steps)*

** *Steps for instrument management: each action required daily or per run to set up and shut down the instrument, e. g. switch on the instrument, login, maintain supplies, maintain reagents, discard*

liquid waste, discard solid waste, archive results, switch off instrument (8 steps)

Based on these results, the performance evaluation for the **cobas** HIV-1 Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems meets the WHO prequalification requirements.

Labelling

1. Labels
2. Instructions for use

1. Labels

cobas

IVD



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

P/N: 07000995190

REF

cobas® **HIV-1**

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

Quantitativer Nukleinsäuretest zur Verwendung
auf den **cobas**® 6800/8800 Systems

Test quantitatif d'acide nucléique destiné à une
utilisation sur les **cobas**® 6800/8800 Systems

Test quantitativo dell'acido nucleico per l'uso sui
cobas® 6800/8800 Systems

Prueba cuantitativa de ácidos nucleicos para el uso en
los **cobas**® 6800/8800 Systems

Teste de ácido nucleico quantitativo para utilização
com os **cobas**® 6800/8800 Systems

Kvantitatív nukleinsyretest til brug på
cobas® 6800/8800 Systems

Kvantitatív nukleinsyretest för användning med
cobas® 6800/8800 Systems

Isotzowy test kwasu nukleinowego przeznaczony do
stosowania w **cobas**® 6800/8800 Systems

Kvantitatívny test nukleových kyselín na použítie v
cobas® 6800/8800 Systems

Cont.

HIV-1 Cassette
MMX-R1
HIV-1 MMX-R2
EB
RNA-QS
PASE

x 1
5.5 mL
6 mL
13 mL
13 mL
13 mL

EUH210
EUH208

Contains: Subtilisin, 9014-01-1
Enthält: Subtilisin, 9014-01-1
Contient : subtilisine, 9014-01-1
Contiene: Subtilisina, 9014-01-1
Contiene: subtilisina, 9014-01-1

Contém: Subtilisina, 9014-01-1
Ineholder: Subtilisin, 9014-01-1
Innehåller: Subtilisin, 9014-01-1
Zawiera: subtylizynę, 9014-01-1
Obsahuje: subtilizin, 9014-01-1

HIV-1

Manufactured in the United States



Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany



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 Valles
Barcelona, Spain

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

Roche Diagnostics
2, Avenue du Vercoors
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

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

COBAS and AMPERASE are trademarks of Roche. © 2019 Roche Molecular Systems, Inc. Made in USA



cobas® **HIV-1**

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

cobas

CE
0123

HIV-1

UDI label here

07174365001-03

cobas



IVD

Rx Only
192

REF 09040803190

cobas[®] HIV-1

CONTENT

HIV-1 Cassette	x 1
PASE	22.3 mL
RNA-QS	21.2 mL
EB	21.2 mL
MMX-R1	7.5 mL
HIV-1 MMX-R2	9.7 mL

For USA*
HIV-1 Cassette x 1
PASE 22.3 mL
 (Proteinase Solution)
 Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% (w/v) Proteinase

RNA-QS 21.2 mL
 (RNA Quantitation Standard)
 Tris buffer, < 0.05% EDTA, < 0.001% Non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% Sodium azide

EB 21.2 mL
 (Elution Buffer)
 Tris buffer, 0.2% Methyl-4 hydroxybenzoate

MMX-R1 7.5 mL
 (Master Mix Reagent 1)
 Manganese acetate, Potassium hydroxide, < 0.1% Sodium azide

HIV-1 MMX-R2 9.7 mL
 (HIV-1 Master Mix Reagent 2)
 Tricine buffer, potassium acetate, 18% Dimethyl sulfoxide, Glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% Upstream and downstream HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% Fluorescent-labeled oligonucleotide probes specific for HIV and the HIV Quantitation Standard, < 0.01% Oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% Sodium azide

*Composition information required for US only.

For USA:
cobas[®] HIV-1 for use on the **cobas[®] 6800/8800** Systems is an *in vitro* nucleic acid amplification test for the quantitation of HIV-1 in EDTA plasma of HIV-1-infected individuals. **cobas[®] HIV-1** is not intended for use as a screening test for the presence of HIV-1 in donated blood or plasma or as a diagnostic test to confirm the presence of HIV-1 infection.
 Refer to the product specific Instructions For Use for detailed information regarding hazards.

HIV-1

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

Roche Diagnostics GmbH
 Sandhofer Strasse 116
 68305 Mannheim, Germany

EC Importer¹

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)²

¹ Symbol text required by USA only.
² For USA only.

COBAS and AMPERASE are trademarks of Roche. ©2021 Roche Molecular Systems, Inc.

Made in USA



cobas[®] HIV-1

cobas

HIV-1

EC REP

Roche Diagnostics GmbH
 Sandhofer Str. 116
 68305 Mannheim
 Germany

CE
2797

UDI label here

09112286001-03

EU/210
 Safety data sheet available on request.
 Sicherheitsdatenblatt auf Anfrage erhältlich.
 Fiche de données de sécurité disponible sur demande.
 Scheda dati di sicurezza disponibile su richiesta.
 Puede solicitarse la ficha de datos de seguridad.
 Ficha de seguridad fornecida a pedido.
 Sakerhetsdatablad kan på ammanding rekterers.
 Sakerhetsdatablad finns att rekterers.
 Karta charakterystiky dostupna na zpdanie.
 Na pozadanie možno poskytnúť kartu bezpečnostných údajov.

EU/208
 Contenu Subtilien. May produce an allergic reaction.
 Contenu Subtilien. Kann allergische Reaktionen hervorrufen.
 Contenu de la subtiline. Peut produire une réaction allergique.
 Contiene Subtilina. Può provocare una reazione allergica.
 Contém subtilina. Pode provocar uma reação alérgica.
 Indeholder subtilin. Kan udløse allergisk reaktion.
 Innehåller subtilin. Kan orsaka en allergisk reaktion.
 Zawiera subtilyzynę. Może powodować wystąpienie reakcji alergicznej.
 Obsahuje subtilizín. Může vyvolat alergickou reakci.

2. Instructions for use²

² English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.



Rx Only

cobas[®] HIV-1

Quantitative nucleic acid test for use on the cobas[®] 5800/6800/8800 Systems

For in vitro diagnostic use

cobas[®] HIV-1	P/N: 09040803190
cobas[®] HBV/HCV/HIV-1 Control Kit	P/N: 09040773190
cobas[®] NHP Negative Control Kit	P/N: 09051554190

Table of contents

Intended use	5
Summary and explanation of the test.....	5
Reagents and materials.....	8
cobas® HIV-1 reagents and controls	8
cobas omni reagents for sample preparation.....	11
cobas® Specimen Pre-Extraction Reagent.....	12
Reagent storage requirements.....	13
Additional materials required for cobas® 5800 System.....	15
Additional materials required for cobas® 6800/8800 Systems.....	15
Instrumentation and software required.....	16
Precautions and handling requirements	17
Warnings and precautions	17
Reagent handling.....	17
Good laboratory practice.....	18
Plasma sample collection, transport, and storage	18
EDTA plasma samples.....	18
PSC dried plasma spot samples	19
Instructions for use.....	21
Procedural notes	21
PSC dried plasma spot sample preparation and pre-analytic procedure.....	21
Running cobas® HIV-1 on cobas® 5800 System.....	26
Running cobas® HIV-1 on cobas® 6800/8800 Systems	27
Results.....	29
Quality control and validity of results on the cobas® 5800 System.....	29
Control results on cobas® 5800 System.....	29
Quality control and validity of results on the cobas® 6800/8800 Systems	29
Control flags on cobas® 6800/8800 Systems	30

Interpretation of results	30
Interpretation of results on the cobas ® 5800 System	31
Interpretation of results on the cobas ® 6800/8800 Systems.....	31
Procedural limitations.....	31
Non-clinical performance evaluation	32
Key performance characteristics for EDTA plasma samples performed on the cobas ® 6800/8800 Systems	32
Limit of Detection (LoD)	32
Linear range.....	33
Precision - within laboratory.....	35
Subtype verification.....	36
Specificity.....	37
Analytical specificity.....	38
Analytical specificity – interfering substances.....	38
Method correlation - Performance compared to COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.....	39
Whole system failure.....	40
Cross contamination	40
Key performance characteristics for PSC dried plasma spot samples.....	40
Limit of Detection (LoD) using the PSC.....	40
Linear range using the PSC	42
Subtype verification using the PSC	43
Specificity using the PSC	44
Whole system failure using the PSC.....	44
Performance of PSC dried plasma spot samples compared to plasma samples	44
Clinical performance evaluation	46
Reproducibility	46
Validation of viral load quantitation	47
Clinical evaluation.....	50
Conclusion	53
System equivalency / system comparison	53

Additional information	54
Key test features for EDTA plasma samples	54
Key test features for PSC dried plasma spot samples	54
Symbols.....	55
Technical Support	56
Manufacturer and importer	56
Trademarks and patents	56
Copyright.....	56
References.....	57
Document revision.....	59

Intended use

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

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

Summary and explanation of the test

Background

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

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

Rationale for HIV-1 testing

At the moment, a great number of antiretroviral drugs are available, targeting the viral protease, integrase, envelope and reverse transcriptase. Genotypic analyses of viruses of different clades have shown naturally-occurring and drug-induced nucleotide changes, polymorphisms and secondary mutations within reverse transcriptase, integrase and protease regions of the HIV-1 pol gene. Resistance testing has become an important diagnostic tool in the management of HIV-1 infections and is initiated once a patient's viral load has risen to a level detectable by sequencing assays. Viral load monitoring has been shown to reduce the risk of drug resistance, and viral load is clinically considered to be a sentinel indicator of active viral replication heralding viral evolution in patients on therapy.^{5,6} Multiple national and international guidelines therefore recommend that HIV-1 viral load should be measured.^{3,7-9}

For a number of years the guidelines indicated that a key goal of treatment is suppression of the HIV-1 viral load below the limit of detection of a test (e.g., 50 copies/mL). In 2011, the United States guidelines began to indicate that viral load results of up to 200 copies/mL in patients on ART may not be indicative of treatment failure as some patients may experience low level viremia without virological breakthrough.³ In Europe, the guidelines continue to state that viral load results should be suppressed below 50 copies/mL.⁷ Low-end differences between viral load tests may lead to important differences in the clinical interpretation of viral load results when monitoring treatment response,^{10,11} as the goal of treatment is suppression

of virus to a level below which drug resistance is least likely to emerge, a level that has not been fully defined.¹² In July 2013, the WHO also began recommending the use of viral load testing in resource-limited settings, defining 1000 copies/mL as the threshold for defining virological failure requiring treatment management decisions. WHO also now recommends the use of dried spot specimens to expand the reach of viral load testing in resource limited settings without ready access to phlebotomy services or robust EDTA plasma sample transportation capabilities.⁸ A PSC dried plasma spot, which also stabilizes the HIV RNA in dried plasma can improve viral load testing coverage in these settings by enabling sample transportation over longer distances and harsher environmental conditions than EDTA plasma.

In addition to monitoring response to therapy, guidelines recommend the use of viral load assessment for determining whether a patient whose CD4+ cell count is > 500 cells/mm³ (viral load $> 100,000$ copies/mL) should initiate ART and for ensuring that drug resistance sequencing will be successful in appropriate patients (patients with a viral load $> 1,000$ copies/mL or suboptimal viral load response to ART). The use of viral load assessment should be performed in the prenatal setting to determine whether Caesarean section delivery is needed to prevent mother-to-child transmission of HIV infection (for pregnant women with a viral load $> 1,000$ copies/mL).

Explanation of the test

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

Principles of the procedure

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

Nucleic acid from patient samples, external controls and added armored RNA (RNA-QS) molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

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

use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).¹³⁻¹⁵ Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

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

Reagents and materials





cobas® HIV-1 reagents and controls

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

Table 1 cobas® HIV-1

cobas® HIV-1 Store at 2-8°C 192 test cassette (P/N 09040803190)		
Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	22.3 mL
RNA Quantitation Standard (RNA-QS)	Tris buffer, < 0.05% EDTA, < 0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HIV-1 Master Mix Reagent 2 (HIV-1 MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the HIV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL



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

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

cobas® NHP Negative Control Kit Store at 2-8°C (P/N 09051554190)			
Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  <p>WARNING</p> <p>H317: May cause an allergic skin reaction.</p> <p>P261: Avoid breathing dust/fume/gas/mist/vapours/spray.</p> <p>P272: Contaminated work clothing should not be allowed out of the workplace.</p> <p>P280: Wear protective gloves.</p> <p>P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.</p> <p>P362 + P364: Take off contaminated clothing and wash it before reuse.</p> <p>P501: Dispose of contents/container to an approved waste disposal plant.</p> <p>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)</p>

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	<p>DANGER</p> <p>H302 + H332: Harmful if swallowed or if inhaled. H314: Causes serious skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol</p>
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the cobas® HIV-1 test kit. See listing of additional materials required (Table 11).


** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

cobas® Specimen Pre-Extraction Reagent

Note: This reagent is optional and should only be used in conjunction with the PSC to generate dried plasma spot samples. See PSC Method Sheet ms_07963084190.

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)	 <p>DANGER</p> <p>H302: Harmful if swallowed.</p> <p>H314 Causes severe skin burns and eye damage.</p> <p>H412: Harmful to aquatic life with long lasting effects.</p> <p>EUH032: Contact with acids liberates very toxic gas.</p> <p>P273: Avoid release to the environment.</p> <p>P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.</p> <p>P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.</p> <p>P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.</p> <p>P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.</p> <p>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.</p> <p>593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol</p>

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 6, Table 7 and Table 8. **cobas®** Specimen Pre-Extraction Reagent, used in the PSC dried plasma spot workflow, shall be stored and handled as specified in Table 9. PSC storage and handling requirements are specified in the **PSC Method Sheet ms_07963084190**.

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

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

Reagent	Storage temperature
cobas® HIV-1	2–8°C
cobas® HBV/HCV/HIV-1 Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent handling requirements for cobas® 5800 System

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 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®** 5800 System.

Table 7 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	Date not passed	90 days from first usage	Max 40 runs	Max 36 days*
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable ^a	Not applicable	Max 36 days*
cobas® NHP Negative Control Kit	Date not passed	Not applicable ^a	Not applicable	Max 36 days*
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

^a Single use reagent

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

Reagent handling requirements for 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 8 are met. The system automatically prevents use of expired reagents. Table 8 allows the user to understand the reagent handling conditions enforced by the cobas® 6800/8800 Systems.

Table 8 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	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable ^a	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable ^a	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

^a Single use reagent

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

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 for cobas® 5800 System

Table 10 Material and consumables for use on cobas® 5800 System

Material	P/N
cobas omni Processing Plate 24	08413975001
cobas omni Amplification Plate 24	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 cobas® 6800/8800 Systems

Table 11 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 and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001

Table 12 Other materials and consumables required for PSC dried plasma spot application only

Materials
cobas® Plasma Separation Card*
Sterile or disposable forceps or tweezers**
140 µL capillary (e.g., Vitrex plastic tube) with compatible dispenser (e.g., Vitrex pipette holder)*
Single Use lancing device (e.g. Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)*
Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams) (for PSC storage and delivery, see PSC Method Sheet ms_07963084190 for more information)
Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)
Pipette (e.g., Multistep® pipette)
Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Greiner Bio-one Cryo.s™) with caps

* See **PSC Method Sheet ms_07963084190** for more information about the PSC sample collection.

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

Instrumentation and software required

The cobas® 5800 System software and cobas® HIV-1 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 Systems software and cobas® HIV-1 analysis package for the cobas® 6800/8800 Systems shall be installed on the instrument(s). For dried PSC plasma spot application on the cobas® 6800/8800 Systems, the cobas® HIV-PSC ASAP (Assay Specific Analysis Package) shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the systems.

Table 13 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
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® 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.
- **cobas® HIV-1** has not been evaluated for use as a screening test for the presence of HIV-1 in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.^{18,19} Only personnel proficient in handling infectious materials and the use of **cobas® HIV-1** and **cobas® 5800/6800/8800 Systems** should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
 - If spillage of PSC dried plasma spot samples in **cobas® Specimen Pre-Extraction Reagent** (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- **cobas® HBV/HCV/HIV-1 Control Kit** and **cobas® NHP Negative Control Kit** contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Refer to **PSC Method Sheet ms_07963084190** for additional warnings and precautions.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- **cobas® Specimen Pre-Extraction Reagent** is light sensitive and shipped in light protective bottles.
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Inform your local competent authority about any serious incidents which may occur when using this assay.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.

- **cobas**® HIV-1 kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent or **cobas**® Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® HIV-1 kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 instrument, follow the instructions in the **cobas**® 5800 Systems User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).
- 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 instrument(s).

Plasma sample collection, transport, and storage

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

Store all samples at specified temperatures.

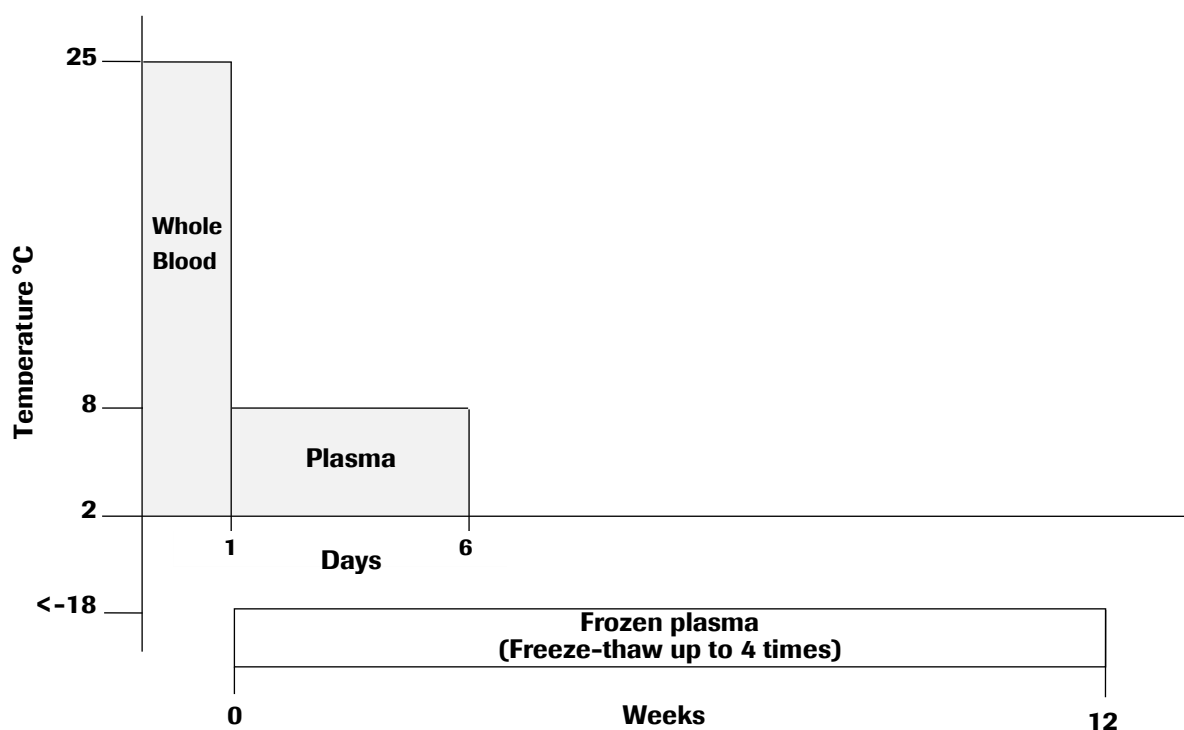
Sample stability is affected by elevated temperatures.

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

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

EDTA plasma samples

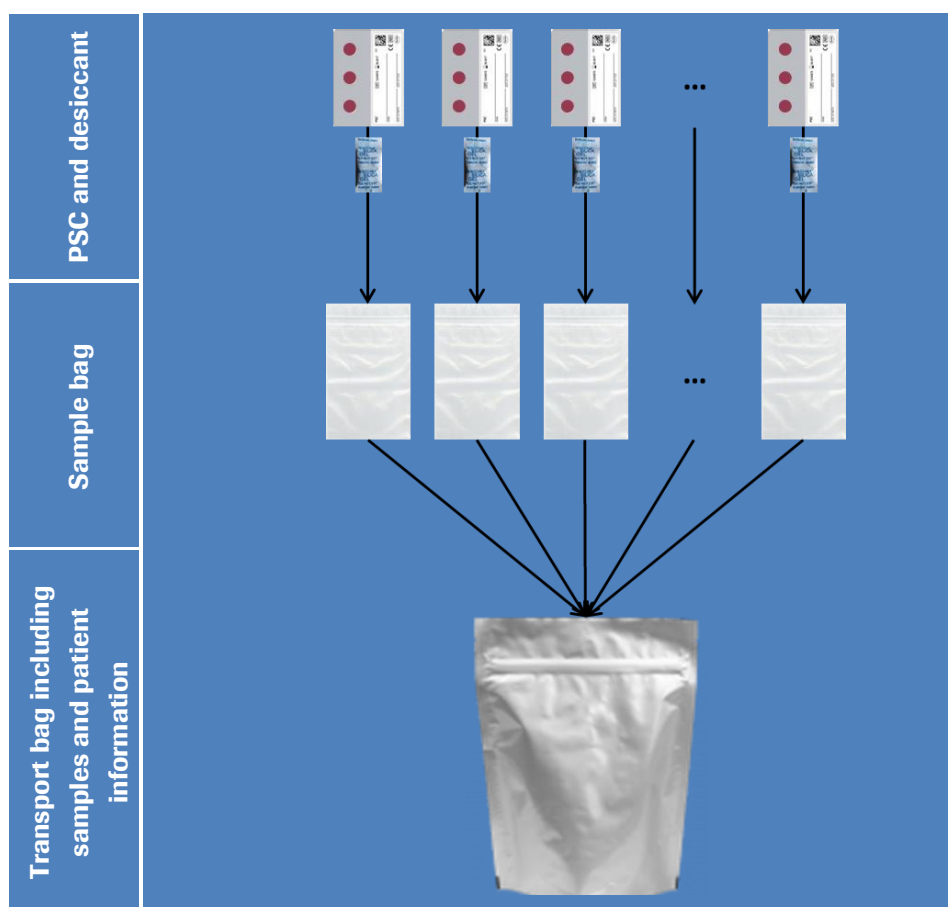
- Whole blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in EDTA tubes may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation EDTA plasma samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at $\leq -18^{\circ}\text{C}$. For long-term storage, temperatures at $\leq -60^{\circ}\text{C}$ are recommended.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at $\leq -18^{\circ}\text{C}$.

Figure 1 EDTA plasma sample storage conditions

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

PSC dried plasma spot samples

- Collect PSC dried plasma spot samples using appropriate clinical procedures (refer to **PSC Method Sheet ms_07963084190**).
- Check the expiry date of the PSC and proceed only if the PSC has not expired yet.
- Make sure that the bag in which the PSC is sealed is completely closed and intact.
- Label the PSC with patient's name, date of birth, date and time of sample collection.
- Apply 140 µL of whole blood on each circle of the PSC membrane delineated by the spotting layer using an appropriate capillary and a dispenser. It is recommended to fill all three spots on the PSC, in order to allow retesting.
- Do not apply samples from more than one patient on the same PSC.
- Do not allow the membranes to get in contact with gloves, tools or any potentially contaminated surfaces during this process.
- Ensure that BOTH sides of the PSC spots (front: membrane with blood; back: spot with plasma) are saturated after 5 minutes. Check the back side through the transparent back layer.
- Allow the PSC to dry at room temperature for at least 4 hours (to maximum overnight), protecting it from direct sunlight.
- Do not remove the spotting layer. This will be done at the laboratory, prior to sample extraction.
- After drying, store the PSC in an individual sample bag with 4 grams of desiccant and seal the bag. The collected sample bags must be packed in a transport bag together with their relative Patient Information Sheet. It is recommended to pack a maximum number of 25 PSCs per transport bag (see Figure 2 for an overview).

Figure 2 Overview of the packaging concept for PSC transport

- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents. PSCs may be transported for a period of 28 days before being analyzed at 18-45°C and up to 85% humidity. PSCs in individual sample bags with 4 grams of desiccant, within a transport bag may be stored after transportation at room temperature (18-30°C), at 2- 8°C or at $\leq -10^{\circ}\text{C}$ for up to 56 days (with and without layer separation).

Instructions for use

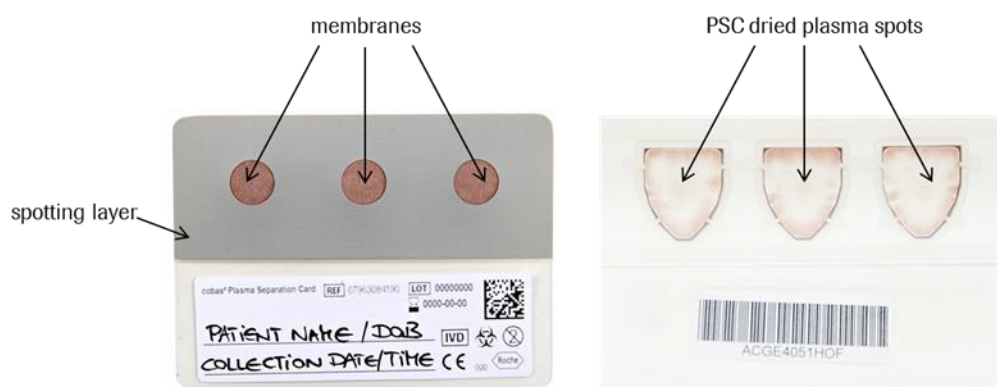
Procedural notes

- Do not use cobas® HIV-1 reagents, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, or cobas omni reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the cobas® 5800 System or cobas® 6800/8800 Systems User Assistance and/or User Guides for proper maintenance of instruments.

PSC dried plasma spot sample preparation and pre-analytic procedure

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

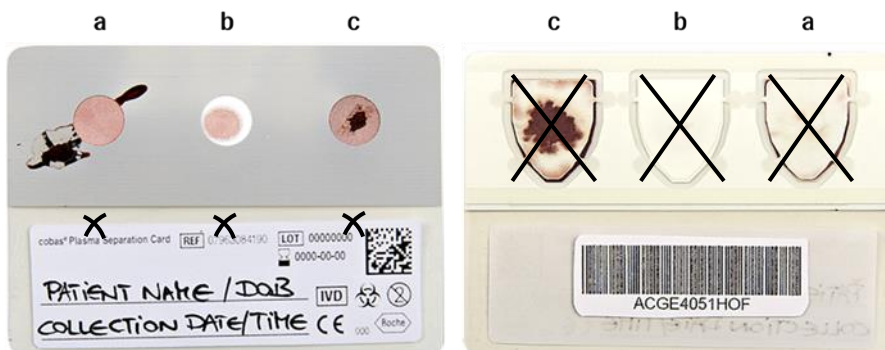
Figure 3 PSC dried plasma spots to be processed (left: front side. right: back side)



- PSC dried plasma spots should be marked and rejected if:
 - blood spills are visible (Figure 4a) or the membrane is not completely covered with blood (Figure 4b) and therefore the PSC spot contains an inhomogeneous front area and/or a back side not completely covered with plasma (visible through the carrier).
 - the membrane is damaged (Figure 4c) and therefore the PSC spot contains an inhomogeneous front area and a dark red-brownish back side (visible through the carrier).

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

Figure 4 Rejection criteria for PSC spots (left: front side; right: back side). Spots with spills (a), not covered membrane (b) or visibly damaged membranes (c) should not be processed. Spots should be clearly marked when rejected.

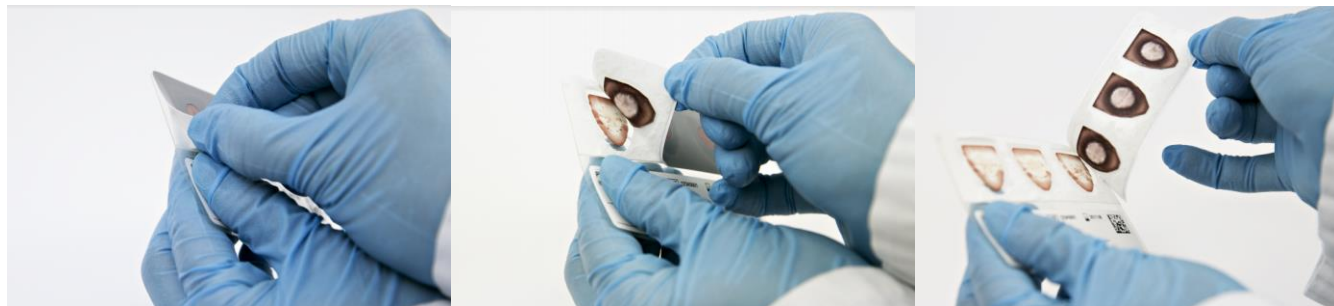


- Label a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Greiner Bio-one Cryo.s™]) for each PSC with its corresponding barcode of the laboratory request form (Figure 5) and place them into a rack. Transfer tubes into a laminar flow hood together with the sample bags containing the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

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



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

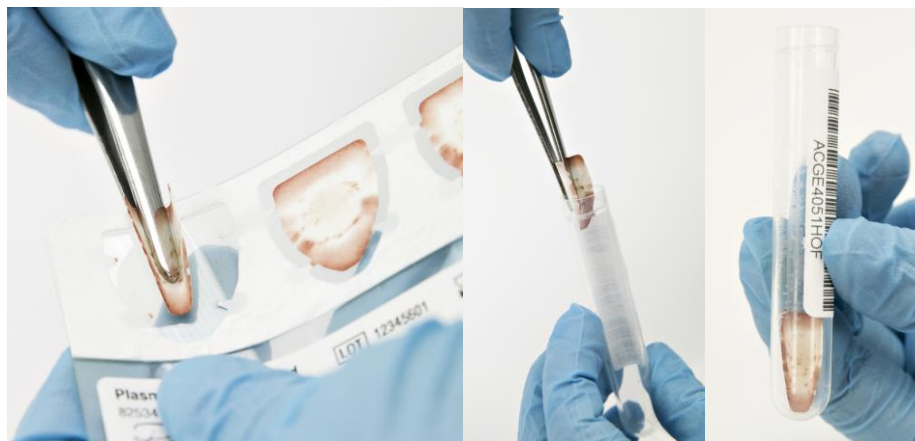
Figure 6 Removal of spotting layer

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

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

Figure 7 Removal of the PSC dried plasma spot and bending

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

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

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

Figure 9 PSC in sample bag for potential retest

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

Figure 10 1300 μL SPER addition

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

Figure 11 Incubation

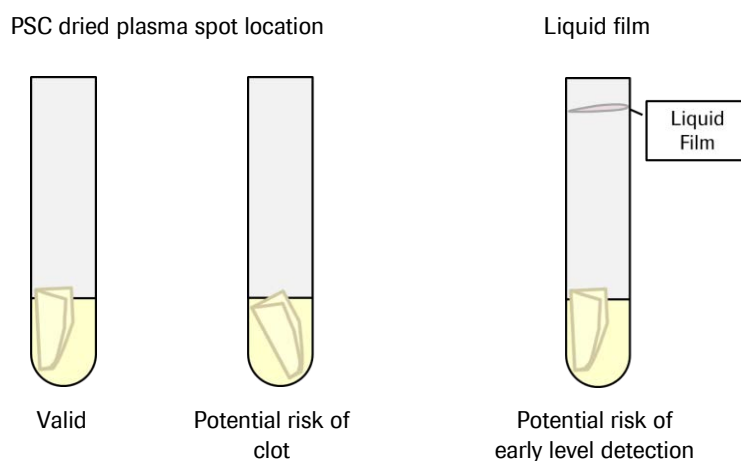


- Transfer the tubes onto a sample rack and uncapping the tubes one by one to minimize cross-contamination (Figure 12). Change gloves after removing the caps.

Figure 12 Uncapping of the tubes



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

Figure 13 PSC dried plasma spot preparation before the analytic workflow

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

Running cobas® HIV-1 on cobas® 5800 System

cobas® HIV-1 can be run with two minimum required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the cobas® 5800 System User Assistance and/or User Guide. Figure 14 below summarizes the procedure.

cobas® HIV-1 can be run with 1300 µL cobas® Specimen Pre-Extraction Reagent (for the 850 µL PSC sample workflow). Please note, cobas® HIV-1 PSC workflow can be run in mixed batch mode with plasma or serum samples, and cobas® HIV-1/HIV-2 Qualitative Dried Blood Spot Procedure. The test procedure is described in detail in the cobas® 5800 Systems User Assistance and/or User Guide. Figure 14 summarizes the procedure also for PSC dried plasma spot samples.

Verify that the correct sample type (**PSC**) and the correct ASAP is used (**cobas® HIV-PSC ASAP**) before starting the test procedure.

Figure 14 cobas® HIV-1 test procedure on cobas® 5800 System

1	Log onto the system
2	Loading samples onto the system <ul style="list-style-type: none"> • Load sample racks onto the system • The system prepares automatically • Order tests
3	Refill reagents and consumables as prompted by the system <ul style="list-style-type: none"> • 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 <ul style="list-style-type: none"> • 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 on cobas® 6800/8800 Systems

cobas® HIV-1 can be run with two minimum required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the **cobas® 6800/8800 Systems User Assistance and/or User Guide**. Figure 15 below summarizes the procedure.

cobas® HIV-1 can be run with 1300 µL **cobas® Specimen Pre-Extraction Reagent** (for the 850 µL PSC sample workflow). Please note, **cobas® HIV-1 PSC workflow** has been evaluated in mixed batch functionality only with the **cobas® HIV-1/ HIV-2 Qualitative Dried Blood Spot workflow**. The test procedure is described in detail in the **cobas® 6800/8800 Systems User Assistance and/or User Guide**. Figure 15 summarizes the procedure also for PSC dried plasma spot samples.

Verify that the correct sample type (**PSC**) and the correct ASAP is used (**cobas® HIV-PSC ASAP**) before starting the test procedure.

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

1	Log onto the system Press Start to prepare the system Order tests
2	Refill reagents and consumables as prompted by the system <ul style="list-style-type: none">• Load test specific reagent cassette• Load control cassettes• Load pipette tips• Load processing plates• Load MGP reagent• Load amplification plates• Refill specimen diluent• Refill lysis reagent• Refill wash reagent
3	Loading samples onto the system <ul style="list-style-type: none">• Load sample racks and clotted tip racks onto the sample supply module• Confirm samples have been accepted into the transfer module
4	Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full
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 <ul style="list-style-type: none">• Unload empty control cassettes• Empty amplification plate drawer• Empty liquid waste• Empty solid waste

Results

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

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

- One negative control [(-) C] and two positive controls, a low positive control [HIV-1 L (+) C] and a high positive control [HIV-1 H (+) C] are processed 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 batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HIV-1 L (+) C, HIV-1 H (+) C. The negative control result is displayed as (-) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

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 **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 negative control (-) C and two positive controls, a low positive control HIV-1 L(+)C and a high positive control HIV-1 H(+)C, are processed with each batch
- 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, which includes one negative control and two positive controls: HIV-1 L(+)C, HIV-1 H(+)C. The negative control result is displayed as (-) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

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

Control flags on cobas® 6800/8800 Systems

Table 14 Control flags for negative and positive controls

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

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

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

Interpretation of results

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

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

Table 15 Target results for individual target result interpretation

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

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

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the cobas® 5800 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:

- 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 15 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.

Interpretation of results on the cobas® 6800/8800 Systems

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

- Samples are marked with “Yes” in the column ‘Valid’ if all requested Target Results reported valid results. Samples marked with “No” in the column ‘Valid’ may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 15 above.

Procedural limitations

- cobas® HIV-1 plasma workflow has been evaluated only for use in combination with the cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 5800/6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome covered by cobas® HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- cobas® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in blood or blood products.

Non-clinical performance evaluation

Key performance characteristics for EDTA plasma samples performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

WHO International Standard

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

The results for EDTA plasma from both sample processing volumes are shown in Table 16 and Table 17. The study demonstrates that cobas® HIV-1 detected HIV-1 RNA at a concentration of 13.2 cp/mL (22.0 IU/mL) as determined by Probit with a hit rate of 95% for the 500 µL sample processing volume and at a concentration of 35.5 cp/mL (59.2 IU/mL) or greater as determined by Probit with a hit rate of 95% for the 200 µL sample processing volume.

Table 16 Limit of detection in EDTA plasma (500 µL)

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

Table 17 Limit of detection in EDTA plasma (200 µL)

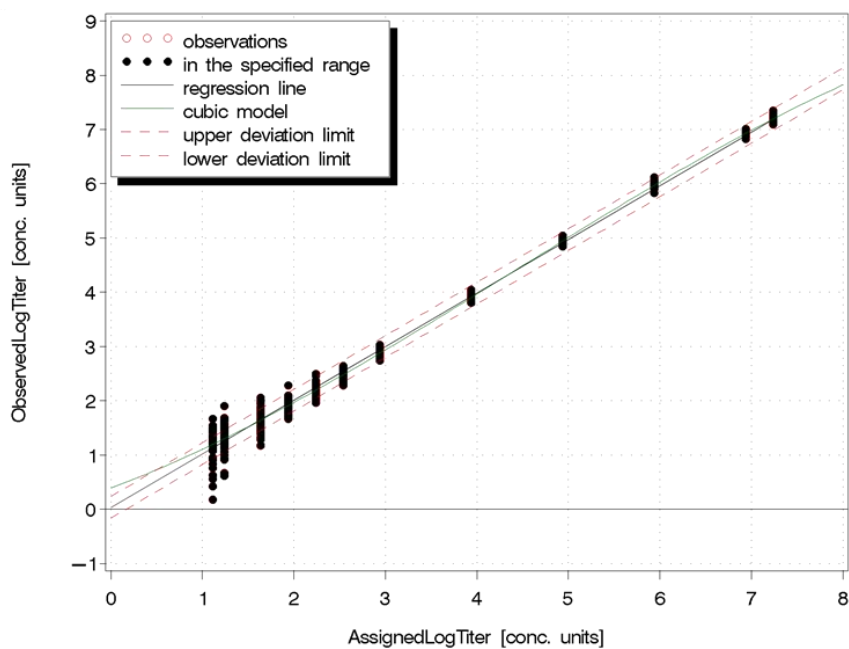
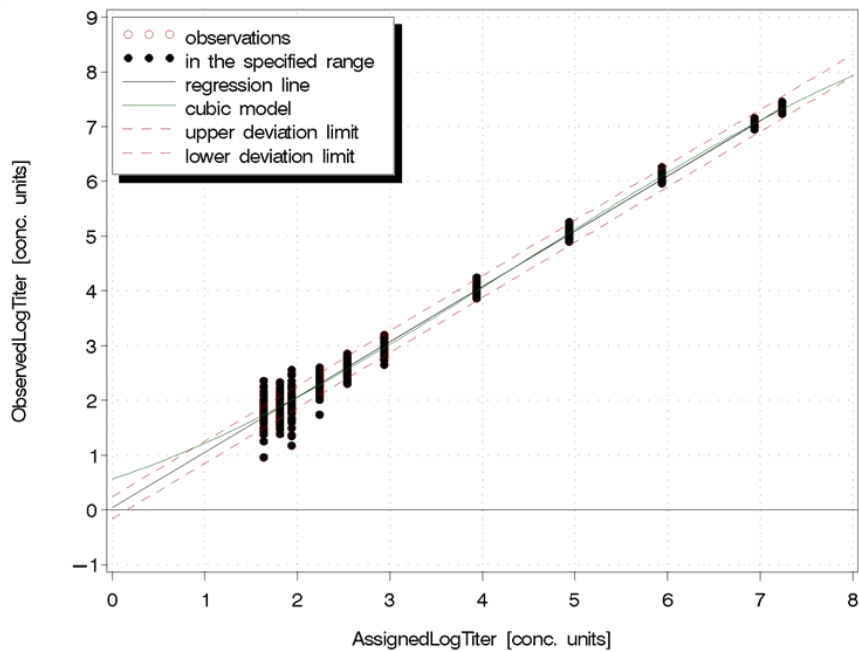
Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
200.0	333.3	189	189	100.0%
100.0	166.7	188	188	100.0%
50.0	83.3	189	186	98.4%
25.0	41.7	189	164	86.8%
12.5	20.8	189	112	59.3%
0.0	0.0	188	0	0.0%
LoD by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

Linear range

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

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

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

Figure 16 Linear range determination in EDTA plasma (500 µL)**Figure 17** Linear range determination in EDTA plasma (200 µL)

Precision - within laboratory

Precision of cobas® HIV-1 was determined by analysis of serial dilutions of an HIV-1 high positive sample (Group M Subtype B; cultured virus) in HIV negative EDTA plasma. Eight dilution levels (500 µL sample processing volume) and seven dilution levels (200 µL sample processing volume) were tested in 48 replicates for each level and processing volume across three lots of cobas® HIV-1 test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire cobas® HIV-1 test procedure on the fully automated cobas® 6800/8800 Systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 18 and Table 19.

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

Table 18 Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – processing volume of 500 µL)*

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

*Titer data are considered to be log-normally distributed and are analyzed following log₁₀ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 19 Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – processing volume of 200 µL)*

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

*Titer data are considered to be log-normally distributed and are analyzed following log₁₀ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Subtype verification

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

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

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

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

Table 20 LoD verification of HIV-1 group M subtypes, group O, and group N in 500 µL EDTA plasma

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

* Upper one-sided 95% confidence interval

Similarly, the limit of detection was verified for the 10 subtypes tested with 200 µL input volume. The data are summarized in Table 21. These results verify that cobas® HIV-1 detected HIV RNA for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), and HIV-1N at the claimed concentration of 50 cp/mL or below with an upper one-sided 95% confidence interval being equal to or greater to the expected hit rate of 95%. HIV-1O was verified at 100 cp/mL.

Table 21 LoD verification of HIV-1 group M subtypes, group O and group N in 200 µL EDTA plasma

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

* Upper one-sided 95% confidence interval

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

The dilution series used in the verification of subtypes linearity study of cobas® HIV-1 consists of seven panel members (500 µL sample processing volume) and six panel members (200 µL sample processing volume) spanning the linear range. Panel members were prepared from high titer HIV-1 RNA positive cell culture supernatant specimens of the respective subtype. Testing was conducted with two lots of cobas® HIV-1 reagent; 14 replicates per level were tested in EDTA plasma. The linear range of cobas® HIV-1 was verified for group M subtypes, group O and group N. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.2 log₁₀.

Specificity

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

09198911001-02EN

Analytical specificity

The analytical specificity of cobas® HIV-1 was evaluated by diluting a panel of microorganisms with HIV RNA positive and HIV RNA negative EDTA plasma. The microorganisms were added to negative human EDTA plasma and tested with and without HIV RNA. None of the non-HIV pathogens interfered with test performance. Negative results were obtained with cobas® HIV-1 for all microorganism samples without HIV-1 target and positive results were obtained on all of the microorganism samples with HIV-1 target. The mean log₁₀ titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.3 log₁₀ of the mean log₁₀ titer of the respective positive spike control.

Table 22 Microorganisms tested for cross-reactivity

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

Analytical specificity – interfering substances

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

In addition, drug compounds listed in Table 23 were tested at three times the C_{max} in presence and absence of HIV RNA.

All potentially interfering substances show no interference with the test performance. Negative results were obtained with cobas® HIV-1 for all samples without HIV target and positive results were obtained on all of the samples with HIV-1 target. The mean log₁₀ titer of each of the positive HIV-1 samples containing potentially interfering substances was within ± 0.3 log₁₀ of the mean log₁₀ titer of the respective positive spike control.

Table 23 Drug compounds tested for interference with the quantitation of HIV RNA by cobas® HIV-1

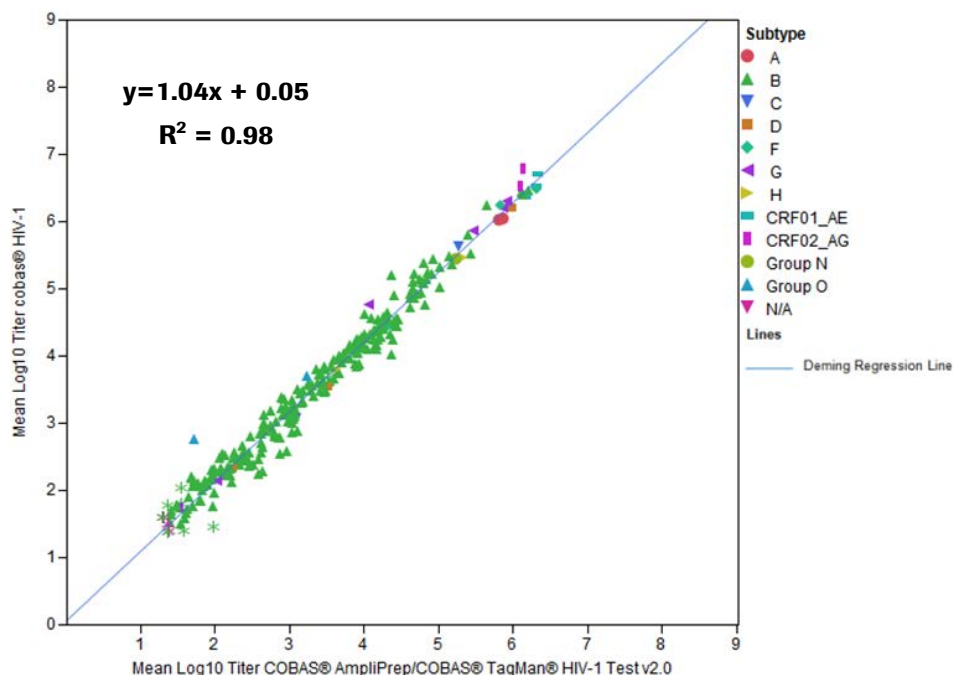
Class of drug	Generic drug name	
Immune Modulators	Peginterferon α-2a	Peginterferon α-2b
	Ribavirin	
HIV Entry Inhibitor	Maraviroc	
HIV Integrase Inhibitors	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitors	Efavirenz	Nevirapine
	Etravirine	Rilpivirine
HIV Protease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

Method correlation - Performance compared to COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

The performance of cobas® HIV-1 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 (TaqMan® HIV-1 Test, v2.0) were compared by analysis of 251 EDTA plasma specimens from patients infected with HIV-1 and by analysis of 16 diluted cell culture supernatants. The specimens comprised of HIV-1 M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1 O and HIV-1N and were tested in duplicate at an external site. Titers below the quantitation range (< 20 cp/mL) were generated for one of the two replicates for five samples on both the COBAS® AmpliPrep/COBAS® TaqMan® System and the cobas® 6800/8800 Systems, and for five samples on the COBAS® AmpliPrep/COBAS® TaqMan® System only. One sample on COBAS® AmpliPrep/COBAS® TaqMan® generated one valid aliquot only. Titers below the quantitation range were excluded from analysis. The Deming regression was performed considering log-transformed titers. For samples where both replicates were within the quantitation range, the mean log₁₀ titer was used for analysis.

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

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



Whole system failure

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

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

Cross contamination

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

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

Key performance characteristics for PSC dried plasma spot samples

Limit of Detection (LoD) using the PSC

The plasma limit of detection of cobas® HIV-1 in combination with the PSC was determined by analysis of plasma titers assigned to serial dilutions of HIV-1 group M cell culture supernatant, in HIV-negative human whole blood. Panels of five concentration levels plus a negative were tested over three lots of PSCs and three lots of cobas® HIV-1 test reagents,

multiple runs, days, operators, and instruments. Samples of the highest panel member were centrifuged and the plasma was titer assigned by Calibrator Bracketing Method (CBM) using cobas® HIV-1 with the 3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B for preparation of the high and low calibrators.

The combined results from three PSC lots, negative donors and dilution series with individual plasma titer assignments (CBM) are shown in Table 24. The study demonstrates that cobas® HIV-1 in combination with the PSC detected HIV-1 RNA at a concentration of 790.2 cp/mL with a hit rate of 95% as determined by Probit analysis.

Table 24 Limit of detection in combination with the PSC

Assigned plasma titer concentration (HIV-1 RNA cp/mL)	Number of valid replicates	Number of positives	Hit rate in %
1971.1	62	62	100.00
1925.3	63	63	100.00
1358.1	62	62	100.00
985.5	63	62	98.41
962.6	63	62	98.41
679	63	59	93.65
657	63	59	93.65
641.8	63	57	90.48
452.7	63	52	82.54
328.5	63	47	74.60
320.9	63	46	73.02
226.3	62	49	79.03
164.3	63	36	57.14
160.4	63	35	55.56
113.2	63	38	60.32
0	189	0	0.00
LoD by PROBIT at 95% hit rate	790.2 cp/mL; 95% confidence range: 658.9 – 1003.6 cp/mL 1317 IU//mL; 95% confidence range: (1098.2 – 1672.7 IU/mL)		

Estimation of LoD in whole blood

The corresponding titers in whole blood of the same sample cannot be exactly determined, since whole blood is not a sample type for viral load testing. Whole blood titers based on the amount of HIV-1 RNA spiked into the whole blood samples were not used for LoD estimation because the amount of spiked RNA does not necessarily correspond to the amount of RNA in plasma. Even after centrifugation, RNA can remain in the buffy coat or associated with the cellular fraction of whole blood. However, since other technologies like dried blood spots have used spiked whole blood titers to estimate their limit of detection in whole blood, an estimate for the PSC whole blood LoD can be provided based on an empirical factor which is assumed to be related to the average hematocrit content (45%) of the samples (Table 25).

Whole blood LoD values are estimates based on the assumption:

Whole blood LoD estimate = PSC plasma LoD / 1.8

Table 25 Whole blood LoD estimate

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

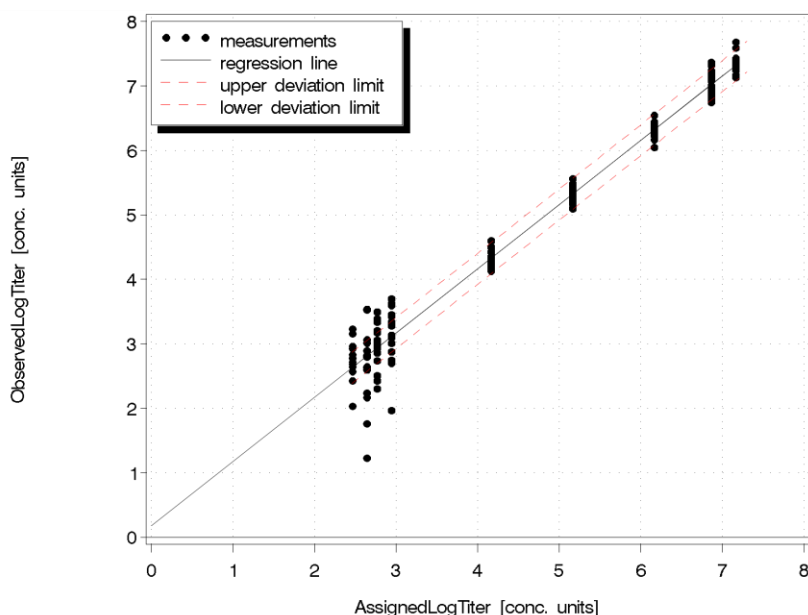
Linear range using the PSC

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

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

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

Figure 19 Linear range determination using the PSC



Precision - within laboratory using the PSC

Precision of cobas® HIV-1 in combination with the PSC was determined by analysis of serial dilutions of an HIV-1 high positive sample (high titer HIV-1 RNA positive cell culture supernatant specimen) in HIV negative EDTA whole blood. Five dilution levels were tested in 48 replicates for each level and processing volume across two lots of PSC and two lots of cobas® HIV-1 test reagents using two instruments and two operators over 12 days. Each sample was carried through the entire PSC workflow and cobas® HIV-1 procedure on the fully automated cobas® 6800/8800 Systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 26.

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

Table 26 Within laboratory precision of cobas® HIV-1 in combination with the PSC

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

Subtype verification using the PSC

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

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

The results are shown in Table 27. These results confirm that cobas® HIV-1 in combination with the PSC detected and correctly quantified HIV-1M subtypes A, C and D.

Table 27 Verification of HIV-1 group M subtypes A, C and D

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

Specificity using the PSC

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

Whole system failure using the PSC

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

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

Performance of PSC dried plasma spot samples compared to plasma samples

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

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

Figure 20 Deming regression

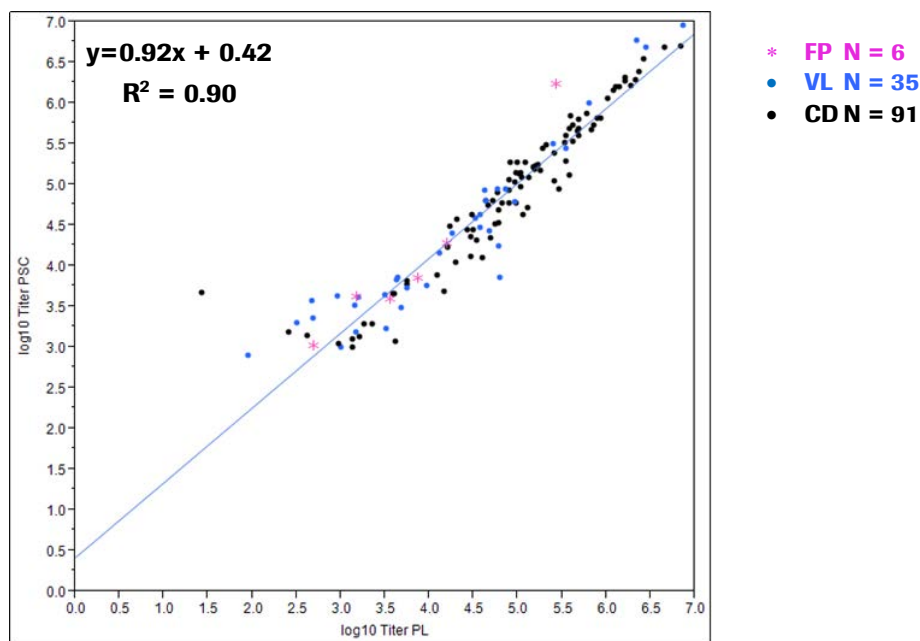


Table 28 Summary of statistical data

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

Clinical performance evaluation

Reproducibility

Reproducibility of cobas® HIV-1 was evaluated in EDTA plasma using the 500 µL sample processing volume on the cobas® 6800 System. The study was performed using panels constructed from well characterized HIV-1 group M, subtype B cultured virus stock and from EDTA plasma that was negative for HIV-1 RNA and HIV-1/2 antibodies. The 8-member panel included one negative panel member and 7 positive panel members covering the linear range of cobas® HIV-1 as well as key medical decision points for the intended use, supported by the 2015 Department of Health and Human Services Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents.³ Testing was done with three reagent lots, three sites, two operators per site, two runs per day, 6 days of testing per reagent lot, and three replicate per run. Reproducibility was evaluated using a random effects model including lot, site, operator, day, run, and within-run. Table 29 shows the total variance, total precision SDs, and lognormal CVs for cobas® HIV-1 as determined by analysis of variance. The within-run component contributed the most variability for the majority of the panel members.

Table 29 Attributable percentage of total variance, total precision standard deviation, and lognormal CV of HIV RNA quantitation by positive panel member on the cobas® 6800 System (reproducibility)

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

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

^a Calculated using SAS MIXED procedure based on log₁₀ transformed measurements.

^b Calculated using the total variability from the SAS MIXED procedure based on log₁₀ transformed measurements.

^c Number of valid tests with detectable viral load.

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

CV(%) = percent coefficient of variation; HIV = human immunodeficiency virus; No. = number; RNA = ribonucleic acid;

SD = standard deviation; sqrt = square root.

In Table 30 below, the negative percent agreement (NPA) for the cobas® 6800 System using all valid negative panel member tests was 100%.

Table 30 Negative percent agreement using the negative panel member

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

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

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

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

RNA = ribonucleic acid.

Validation of viral load quantitation

The performance of cobas® HIV-1 on the cobas® 6800 System was compared to that of the FDA-approved COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 (TaqMan® HIV-1 Test, v2.0) by analysis of paired EDTA plasma specimens from 410 subjects with HIV-1 viral loads spanning the linear range of both tests. Demographic characteristics of the subjects are shown in Table 31.

Table 31 Summary of demographic characteristics

Demographic Characteristics	Statistics
	(N=410)
Age (years)	
Mean (SD)	41.8 (11)
Median	43
Range	19 – 72
Sex, n (%)	
Male	321 (78.3%)
Female	89 (21.7%)
Race, n (%)	
Asian	5 (1.2%)
Black	163 (39.8%)
Latino	17 (4.1%)
White	94 (22.9%)
Other	91 (22.2%)
Unknown	40 (9.8%)
Ethnicity, n (%)	
Hispanic	101 (24.6%)
Non-Hispanic	231 (56.3%)
Unknown	78 (19.0%)
Antiviral Medication, n (%)	
Yes	208 (50.7%)
No	137 (33.4%)
Unknown	65 (15.9%)
CD4 Cell Count (cells/μL), n (%)	
N	391
Mean (SD)	438.1 (267.7)
Median	401
Range	0 – 1548

SD = standard deviation.

Of 410 paired samples tested, 305 paired samples had viral load measurements within the linear range of both assays. Table 32 shows the mean paired viral load difference between cobas® HIV-1 and the TaqMan® HIV-1 Test, v2.0.

Table 32 Mean of paired viral load difference between cobas® HIV-1 and the TaqMan® HIV-1 Test, v2.0

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

CI = confidence interval.

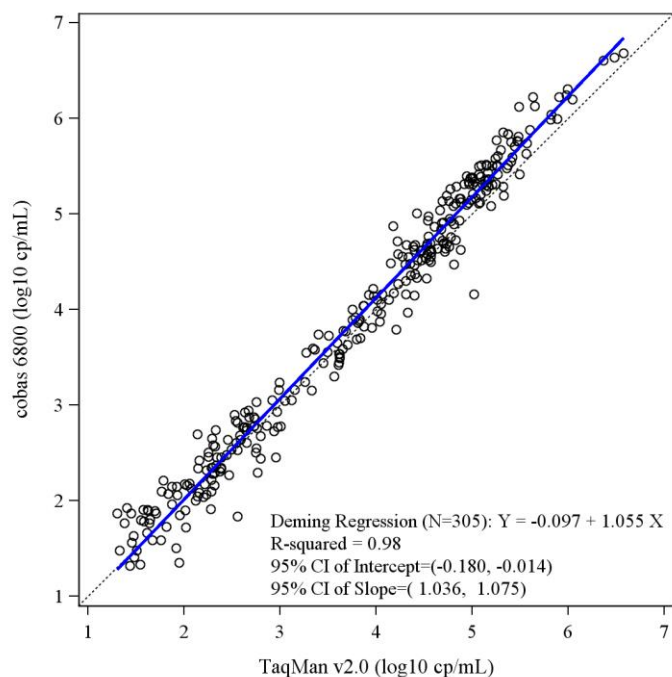
The results of Deming regression analysis between cobas® HIV-1 and the TaqMan® HIV-1 Test, v2.0 are tabulated in Table 33 and shown graphically in Figure 21. The dashed line indicates perfect agreement between the two test methods.

Table 33 Parameter estimates of Deming regression analysis between cobas® HIV-1 on the cobas® 6800 System and the TaqMan® HIV-1 Test, v2.0

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

CI = confidence interval.

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



Subsets of paired samples were also tested to compare cobas® HIV-1 results using the 200 µL and 500 µL sample processing volumes on both the cobas® 6800 and cobas® 8800 Systems. All comparisons showed a mean of paired difference of less than 0.095 log₁₀ copies/mL.

For the comparison across sample volumes, Table 34 shows the mean paired difference between cobas® HIV-1 results using the 200 µL and 500 µL sample processing volumes on the cobas® 6800 System.

Table 34 Mean of paired viral load difference between the 200 µL and 500 µL sample processing volumes on the cobas® 6800 System

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

CI = confidence interval.

Table 35 shows the mean paired difference between cobas® HIV-1 results using the 200 µL and 500 µL sample processing volumes on the cobas® 8800 System.

Table 35 Mean of paired viral load difference between the 200 µL and 500 µL sample processing volumes on the cobas® 8800 System

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

CI = confidence interval.

For the comparison across systems, Table 36 shows the mean paired difference between cobas® HIV-1 results on the cobas® 6800 System and the cobas® 8800 System using the 200 µL sample processing volume.

Table 36 Mean of paired viral load difference between the cobas® 6800 System and cobas® 8800 System using the 200 µL sample processing volume

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

CI = confidence interval.

Table 37 shows the mean paired difference between cobas® HIV-1 results on the cobas® 6800 System and the cobas® 8800 System using the 500 µL sample processing volume.

Table 37 Mean of paired viral load difference between the cobas® 6800 System and cobas® 8800 System using the 500 µL sample processing volume

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

CI = confidence interval.

A subset of paired samples spanning the linear range of the assay was also tested to compare cobas® HIV-1 results from BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods and EDTA plasma tubes without a gel separator. This comparison was done using the cobas® 6800 System. The results are shown in Table 38.

Table 38 Mean of paired viral load difference between BD Vacutainer® PPT™ Plasma Preparation Tubes and EDTA plasma tubes

Number of Paired Samples	Mean of Paired Difference (log₁₀ copies/mL)	Standard Error for Mean of Paired Difference	95% CI for Mean of Paired Difference
42	0.026	0.027	(-0.029, 0.081)

CI = confidence interval.

Clinical evaluation

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

Table 39 shows the demographic characteristics of the 355 subjects included in the study.

Table 39 Demographics characteristics

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

SD = standard deviation.

The results of comparisons using the 50 copies/mL and 200 copies/mL thresholds at 24 and 48 weeks of treatment with virological failure are shown in Table 40 and Table 41.

The analyses of the 50 copies/mL virological threshold with virological failure (at Week 24 and Week 48) are shown in Table 40. At Week 24, the PPV was 15.6% (10/64, 95% CI: 7.8%, 26.9%), and, at Week 48, the PPV was 25.7% (9/35, 95% CI: 12.5%, 43.3%). At Week 24, the NPV was 90.9% (251/276, 95% CI: 86.9%, 94.1%), and, at Week 48, the NPV was 91.1% (285/313, 95% CI: 87.3%, 94%). At Week 24, the OR was 1.86 (95% CI: 0.75, 4.29), which was not statistically significant ($p = 0.191$). At Week 48, the OR was 3.51 (95% CI: 1.31, 8.71) which was statistically significant ($p = 0.012$).

Table 40 Comparison of a 50 copies/mL virological threshold with virological failure

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

⁺ Valid results obtained by cobas® HIV-1.

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

When 200 cp/mL thresholds were used to define virological failure as shown in Table 41, at Week 24, the PPV was 22.2% (2/9, 95% CI: 2.8%, 60%), and, at Week 48, the PPV increased to 100% (2/2, 95% CI: 15.8%, 100%). At Week 24, the NPV was 90.0% (298/331, 95% CI: 86.3%, 93%), and, at Week 48, the NPV was 89.9% (311/346, 95% CI: 86.2%, 92.9%). At Week 24, the OR was 2.57 (95% CI: 0.25, 14.27), which was not statistically significant (p = 0.469). At Week 48, the OR was 20.76 (95% CI: 2.46, Not Calculable) which was statistically significant (p = 0.022).

Table 41 Comparison of a 200 copies/mL virological threshold with virological failure

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

⁺ Valid results obtained by cobas® HIV-1.

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

All odds ratios were above 1 and increased between 24 to 48 weeks of treatment. Statistically significant odds ratios were seen for both thresholds at Week 48. At both thresholds, the high NPV demonstrates the ability of the test to predict which patients are not failing treatment at each timepoint.

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

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

Conclusion

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

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 for EDTA plasma samples

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



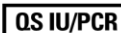



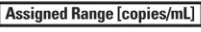




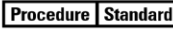

















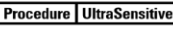






















Key test features for PSC dried plasma spot samples

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

Symbols

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

Table 40 Symbols used in labeling for Roche PCR diagnostics products

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

Technical Support

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

Manufacturer and importer

Table 41 Manufacturer and importer



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

Made in USA



Roche Diagnostics GmbH
 Sandhofer Strasse 116
 68305 Mannheim, Germany

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, 8129118, and 6727067, and foreign equivalent patents of each.

COBAS, COBAS OMNI, AMPERASE, AMPLIPREP, and TAQMAN are trademarks of Roche.

The trademark “Armored RNA®” is owned by Asuragen, Inc. and Cenetron Diagnostics, Ltd.

ProClin® is a registered trademark of Rohm and Haas Company.

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase® enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

Certain components of this product are covered by one or more US Patents and their foreign equivalents issued to Novartis Vaccines and Diagnostics, Inc. and licensed to Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd.

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

Copyright

©2021 Roche Molecular Systems, Inc.



Roche Diagnostics GmbH
 Sandhofer Str. 116
 68305 Mannheim
 Germany



References

1. Mellors JW, Muñoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* 1997;126:946-54. PMID: 9182471.
2. Mellors JW, Margolick JB, Phair JP, et al. Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 Cell count slope for progression to AIDS and death in untreated HIV-1 infection. *JAMA.* 2007;297:2349-50. PMID: 17551128.
3. Department of Health and Human Services. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV. <https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/AdultandAdolescentGL.pdf>. Accessed December 3, 2020.
4. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med.* 2011;365:493-505. PMID: 21767103.
5. Phillips AN, Pillay D, Garnett G, et al. Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings. *AIDS.* 2011;25:843-50. PMID: 21192233.
6. Sigaloff KC, Hamers RL, Wallis CL, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr.* 2011;58:23-31. PMID: 21694603.
7. European AIDS Clinical Society. European AIDS Clinical Society 2017 Guidelines. https://www.eacsociety.org/files/guidelines_9.0-english.pdf. Accessed December 3, 2020.
8. World Health Organization. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection – Recommendations for a Public Health Approach. <https://www.who.int/hiv/pub/arv/arv-2016/en/>. Accessed December 3, 2020.
9. Günthard HF, Aberg JA, Eron JJ, et al. Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA.* 2014;312:410-25. PMID: 25038359.
10. Do T, Duncan J, Butcher A, Liegler T. Comparative frequencies of HIV low-level viremia between real-time viral load assays at clinically relevant thresholds. *J Clin Virol.* 2011;52 Suppl 1:S83-9. PMID: 21995930.
11. Paba P, Fabeni L, Ciccozzi M, Perno CF, Ciotti M. Performance evaluation of the COBAS/TaqMan HIV-1 v2.0 in HIV-1 positive patients with low viral load: a comparative study. *J Virol Methods.* 2011;173:399-402. PMID: 21419171.
12. Deeks SG. Durable HIV treatment benefit despite low-level viremia: reassessing definitions of success or failure. *JAMA.* 2001;286:224-6. PMID: 11448286.

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. PMID: 2227421.
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. PMID: 7845459.
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. PMID: 7697717.
16. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology (N Y)*. 1992;10:413-7. PMID: 1368485.
17. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res*. 1996;6:986-94. PMID: 8908518.
18. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. <https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF>. Accessed December 2, 2020.
19. Clinical and Laboratory Standards Institute. Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. https://clsi.org/media/1459/m29a4_sample.pdf. Accessed December 2, 2020.
20. Clinical and Laboratory Standards Institute. EP06-A. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. https://clsi.org/media/1437/ep06a_sample.pdf. Accessed October 3, 2020.
21. Vubil A, Zicai AF, Siteo N, et al. Accurate HIV viral load measurement in primary health care settings using the cobas® plasma separation card. *PLoS One*. 2020;15:e0232122. PMID: 32374748.

Document revision

Document Revision Information	
Doc Rev. 1.0 12/2020	First Publishing.
Doc Rev. 2.0 07/2021	Added cobas ® 5800 specific information. Added importer address. Removed distributors addresses. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.

cobas[®] **HIV-1**

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

For in vitro diagnostic use

cobas[®] **HIV-1**

P/N: 07000995190

cobas[®] **HBV/HCV/HIV-1 Control Kit**

P/N: 06997767190

cobas[®] **NHP Negative Control Kit**

P/N: 07002220190

Table of contents

Intended use	4
Summary and explanation of the test.....	4
Reagents and materials.....	7
cobas® HIV-1 reagents and controls.....	7
cobas omni reagents for sample preparation.....	10
cobas® Specimen Pre-Extraction Reagent.....	11
Reagent storage and handling requirements.....	12
Additional materials required	13
Instrumentation and software required	14
Precautions and handling requirements	15
Warnings and precautions	15
Reagent handling.....	15
Good laboratory practice.....	16
Plasma sample collection, transport, and storage	16
EDTA plasma samples.....	16
PSC dried plasma spot samples	17
Instructions for use.....	19
Procedural notes	19
PSC dried plasma spot sample preparation and pre-analytic procedure.....	19
Running cobas® HIV-1.....	24
Results.....	26
Quality control and validity of results.....	26
Interpretation of results	27
Procedural limitations.....	27

Non-clinical performance evaluation	28
Key performance characteristics for EDTA plasma samples	28
Limit of Detection (LoD)	28
Linear range	29
Precision - within laboratory.....	31
Subtype verification.....	32
Specificity.....	33
Analytical specificity.....	34
Analytical specificity – interfering substances.....	34
Performance compared to COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0	35
Whole system failure.....	36
Cross contamination	36
Key performance characteristics for PSC dried plasma spot samples.....	36
Limit of Detection (LoD)	36
Linear range using the PSC	38
Precision - within laboratory using the PSC	39
Subtype verification using the PSC	39
Specificity using the PSC	40
Whole system failure using the PSC.....	40
Performance of PSC dried plasma spot samples compared to plasma samples	40
Additional information	42
Key test features for EDTA plasma samples.....	42
Key test features for PSC dried plasma spot samples	42
Symbols.....	43
Manufacturer and distributors	44
Trademarks and patents.....	44
Copyright.....	44
References.....	45
Document revision.....	47

Intended use

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

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

Summary and explanation of the test

Background

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

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

Rationale for HIV-1 testing

At the moment, a great number of antiretroviral drugs are available, targeting the viral protease, integrase, envelope and reverse transcriptase. Genotypic analyses of viruses of different clades have shown naturally-occurring and drug-induced nucleotide changes, polymorphisms and secondary mutations within reverse transcriptase, integrase and protease regions of the HIV-1 pol gene. Resistance testing has become an important diagnostic tool in the management of HIV-1 infections and is initiated once a patient's viral load has risen to a level detectable by sequencing assays. Viral load monitoring has been shown to reduce the risk of drug resistance, and viral load is clinically considered to be a sentinel indicator of active viral replication heralding viral evolution in patients on therapy.^{5,6} Multiple national and international guidelines therefore recommend that HIV-1 viral load should be measured.^{3,7-9}

For a number of years the guidelines indicated that a key goal of treatment is suppression of the HIV-1 viral load below the limit of detection of a test (e.g., 50 copies/mL). In 2011, the United States guidelines began to indicate that viral load results of up to 200 copies/mL in patients on ART may not be indicative of treatment failure as some patients may experience low level viremia without virological breakthrough.³ In Europe, the guidelines continue to state that viral load results should be suppressed below 50 copies/mL.⁷ Low-end differences between viral load tests may lead to important differences in the

clinical interpretation of viral load results when monitoring treatment response,^{10,11} as the goal of treatment is suppression of virus to a level below which drug resistance is least likely to emerge, a level that has not been fully defined.¹² In July 2013, the WHO also began recommending the use of viral load testing in resource-limited settings, defining 1000 copies/mL as the threshold for defining virological failure requiring treatment management decisions. WHO also now recommends the use of dried spot specimens to expand the reach of viral load testing in resource limited settings without ready access to phlebotomy services or robust EDTA plasma sample transportation capabilities.⁸ A PSC dried plasma spot, which also stabilizes the HIV RNA in dried plasma can improve viral load testing coverage in these settings by enabling sample transportation over longer distances and harsher environmental conditions than EDTA plasma.

In addition to monitoring response to therapy, guidelines recommend the use of viral load assessment for determining whether a patient whose CD4+ cell count is > 500 cells/mm³ (viral load > 100,000 copies/mL) should initiate ART and for ensuring that drug resistance sequencing will be successful in appropriate patients (patients with a viral load > 1,000 copies/mL or suboptimal viral load response to ART). The use of viral load assessment should be performed in the prenatal setting to determine whether Caesarean section delivery is needed to prevent mother-to-child transmission of HIV infection (for pregnant women with a viral load > 1,000 copies/mL).

Explanation of the test

cobas® HIV-1 is a quantitative test performed on the **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® HIV-1 enables the detection and quantitation of HIV-1 RNA in EDTA plasma or from a PSC dried plasma spot of infected patients. Two probes are used to detect and quantify, but not discriminate group M subtypes of HIV-1 and of HIV-1 group O and group N. The viral load is quantified against a non-HIV-1 armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample processing. The RNA-QS functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

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

Nucleic acid from patient samples, external controls and added armored RNA (RNA-QS) molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of the HIV-1 genome. The HIV-1 gag gene and the HIV-1 LTR region (dual target) are amplified by **cobas**® HIV-1. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HIV-1 genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and

RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).¹³⁻¹⁵ Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

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

Reagents and materials

cobas® HIV-1 reagents and controls

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





Table 1 cobas® HIV-1

cobas® HIV-1 Store at 2-8°C 96 test cassette (P/N 07000995190)		
Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	13 mL
RNA Quantitation Standard (RNA-QS)	Tris buffer, < 0.05% EDTA, < 0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
HIV-1 Master Mix Reagent 2 (HIV-1 MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the HIV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL

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

Store at 2–8°C



(P/N 06997767190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+))C)	<p>< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods.</p> <p>0.1% ProClin® 300 preservative**</p>	5.2 mL (8 x 0.65 mL)	  <p>WARNING</p> <p>H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247- 500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1)</p>
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+))C)	<p>< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods.</p> <p>0.1% ProClin® 300 preservative**</p>	5.2 mL (8 x 0.65 mL)	  <p>WARNING</p> <p>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)</p>

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Table 3 cobas® NHP Negative Control Kit

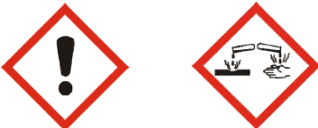
cobas® NHP Negative Control Kit			
Store at 2-8°C (P/N 07002220190)			
Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  <p>WARNING H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant. 55965-84-9 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)</p>

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polidocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 <p>DANGER</p> <p>H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol</p>
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

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


** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

cobas® Specimen Pre-Extraction Reagent

Note: This reagent is optional and should only to be used in conjunction with the PSC to generate dried plasma spot samples. See PSC Method Sheet ms_07963084190.

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)	 <p>DANGER</p> <p>H302: Harmful if swallowed.</p> <p>H314: Causes severe skin burns and eye damage.</p> <p>H412: Harmful to aquatic life with long lasting effects.</p> <p>EUH032: Contact with acids liberates very toxic gas.</p> <p>P273: Avoid release to the environment.</p> <p>P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.</p> <p>P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.</p> <p>P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.</p> <p>P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.</p> <p>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 if you feel unwell.</p> <p>593-84-0 guanidinium thiocyanate 9002-92-0 Polidocanol</p>

* 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 6 and Table 7. **cobas**® Specimen Pre-Extraction Reagent, used in the PSC dried plasma spot workflow, shall be stored and handled as specified in Table 8. PSC storage and handling requirements are specified in the **PSC Method Sheet ms_07963084190**.

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	2–8°C
cobas ® HBV/HCV/HIV-1 Control Kit	2–8°C
cobas ® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 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	Date not passed	30 days from first usage	Max 10 runs	Max 8 hours
cobas ® HBV/HCV/HIV-1 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.

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 8.

Table 8 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 9 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 10 Other materials and consumables required for PSC dried plasma spot application only**Materials**

cobas® Plasma Separation Card*

Sterile or disposable forceps or tweezers**

140 µL capillary (e.g., Vitrex plastic tube) with compatible dispenser (e.g., Vitrex pipette holder)*

Single Use lancing device (e.g. Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)*

Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams)
(for PSC storage and delivery, see **PSC Method Sheet ms_07963084190** for more information)

Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)

Pipette (e.g., Multistep® pipette)

Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes

Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Greiner Bio-one Cryo.s™) with caps

* See **PSC Method Sheet ms_07963084190** for more information about the PSC sample collection.

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

Instrumentation and software required

The cobas® 6800/8800 software and cobas® HIV-1 analysis package shall be installed on the instrument(s). For dried PSC plasma spot application, the cobas® HIV-1 PSC ASAP shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 11 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 has not been evaluated for use as a screening test for the presence of HIV-1 in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.^{18,19} Only personnel proficient in handling infectious materials and the use of **cobas**® HIV-1 and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
 - If spillage of PSC dried plasma spot samples in **cobas**® Specimen Pre-Extraction Reagent (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- **cobas**® HBV/HCV/HIV-1 Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Refer to **PSC Method Sheet ms_07963084190** for additional warnings and precautions.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- **cobas**® Specimen Pre-Extraction Reagent is light sensitive and shipped in light protective bottles.
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® HIV-1 kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a

preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.

- Do not allow **cobas omni** Lysis Reagent or **cobas**® Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® HIV-1 kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Plasma sample collection, transport, and storage

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

Store all samples at specified temperatures.

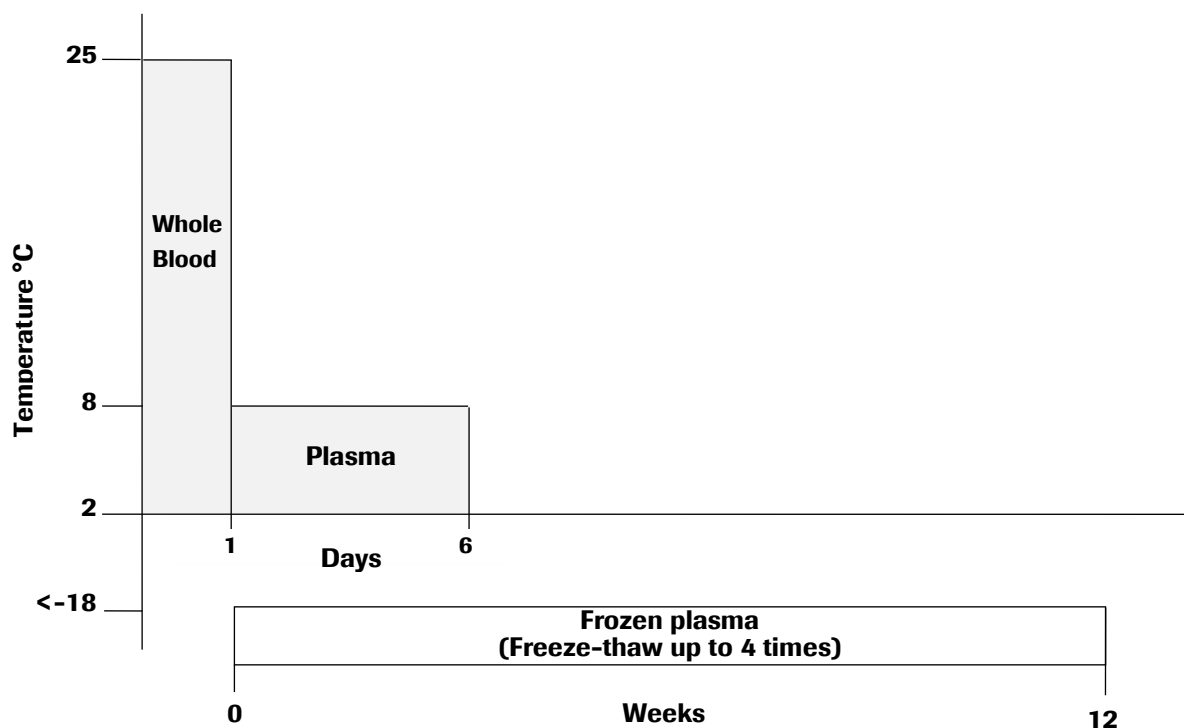
Sample stability is affected by elevated temperatures.

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

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

EDTA plasma samples

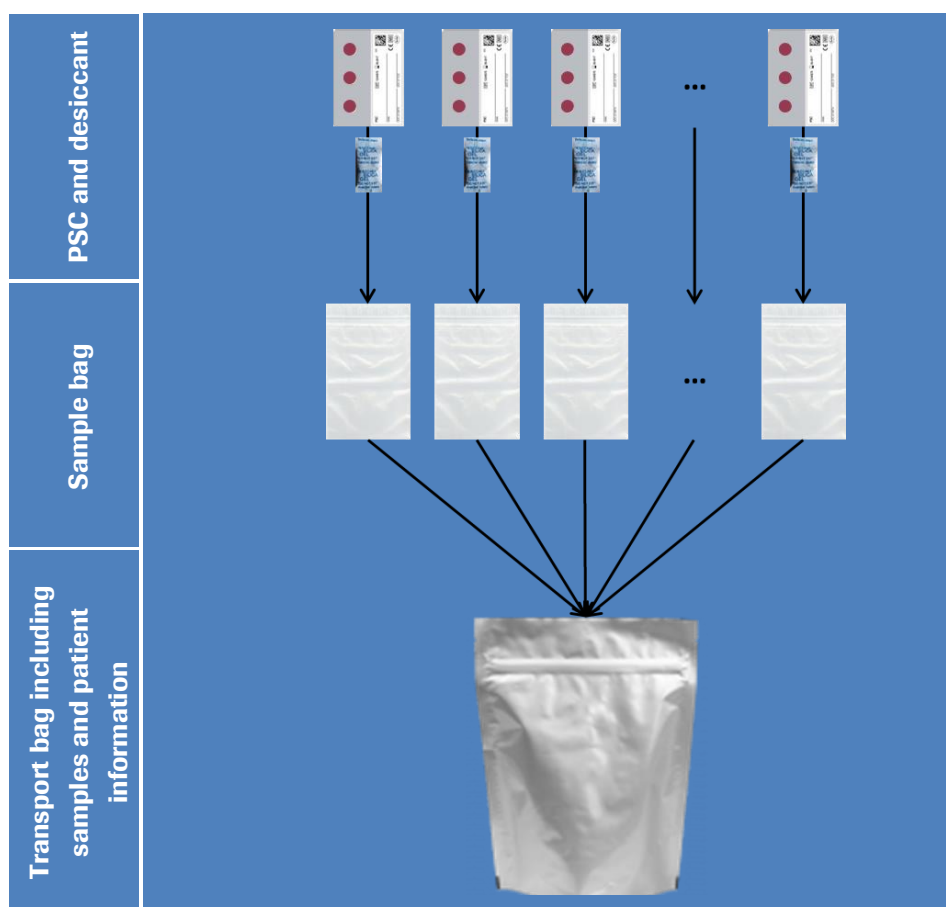
- Blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in EDTA tubes may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation EDTA plasma samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. For long-term storage, temperatures at ≤ -60°C are recommended.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 EDTA plasma sample storage conditions

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

PSC dried plasma spot samples

- Collect PSC dried plasma spot samples using appropriate clinical procedures (refer to **PSC Method Sheet ms_07963084190**).
- Check the expiry date of the PSC and proceed only if the PSC has not expired yet.
- Make sure that the bag in which the PSC is sealed is completely closed and intact.
- Label the PSC with patient's name, date of birth, date and time of sample collection.
- Apply 140 μ L of whole blood on each circle of the PSC membrane delineated by the spotting layer using an appropriate capillary and a dispenser. It is recommended to fill all three spots on the PSC, in order to allow retesting.
- Do not apply samples from more than one patient on the same PSC.
- Do not allow the membranes to get in contact with gloves, tools or any potentially contaminated surfaces during this process.
- Ensure that BOTH sides of the PSC spots (front: membrane with blood; back: spot with plasma) are saturated after 5 minutes. Check the back side through the transparent back layer.
- Allow the PSC to dry at room temperature for at least 4 hours (to maximum overnight), protecting it from direct sunlight.
- Do not remove the spotting layer. This will be done at the laboratory, prior to sample extraction.
- After drying, store the PSC in an individual sample bag with 4 grams of desiccant and seal the bag. The collected sample bags must be packed in a transport bag together with their relative Patient Information Sheet. It is recommended to pack a maximum number of 25 PSCs per transport bag (see Figure 2 for an overview).

Figure 2 Overview of the packaging concept for PSC transport

- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents. PSCs may be transported for a period of 28 days before being analyzed at 18-45°C and up to 85% humidity.
- PSCs in individual sample bags with 4 grams of desiccant, within a transport bag may be stored after transportation at room temperature (18-30°C), at 2-8°C or at $\leq -10^{\circ}\text{C}$ for up to 56 days (with and without layer separation).

Instructions for use

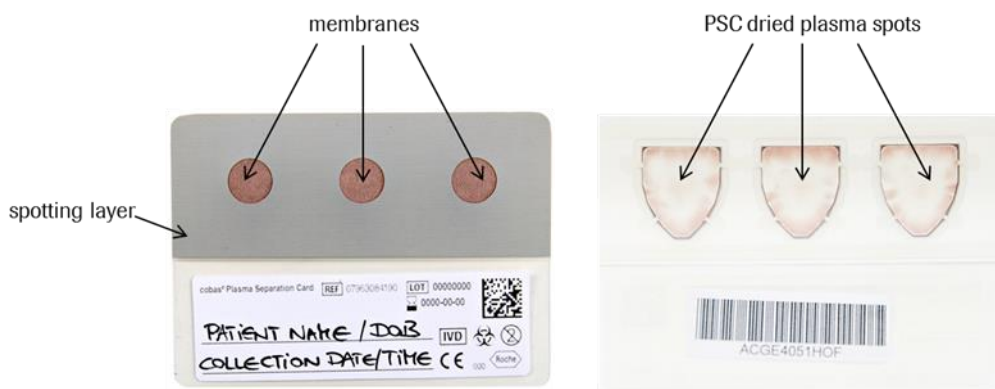
Procedural notes

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

PSC dried plasma spot sample preparation and pre-analytic procedure

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

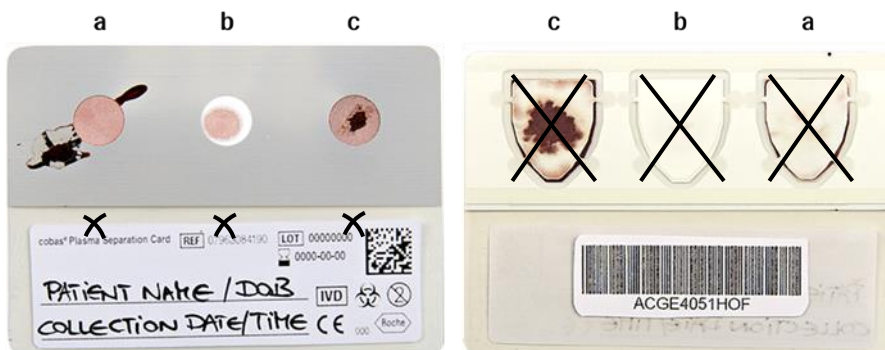
Figure 3 PSC dried plasma spots to be processed (left: front side. right: back side)



- PSC dried plasma spots should be marked and rejected if:
 - blood spills are visible (Figure 4a) or the membrane is not completely covered with blood (Figure 4b) and therefore the PSC spot contains an inhomogeneous front area and/or a back side not completely covered with plasma (visible through the carrier).
 - the membrane is damaged (Figure 4c) and therefore the PSC spot contains an inhomogeneous front area and a dark red-brownish back side (visible through the carrier).

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

Figure 4 Rejection criteria for PSC spots (left: front side; right: back side). Spots with spills (a), not covered membrane (b) or visibly damaged membranes (c) should not be processed. Spots should be clearly marked when rejected.

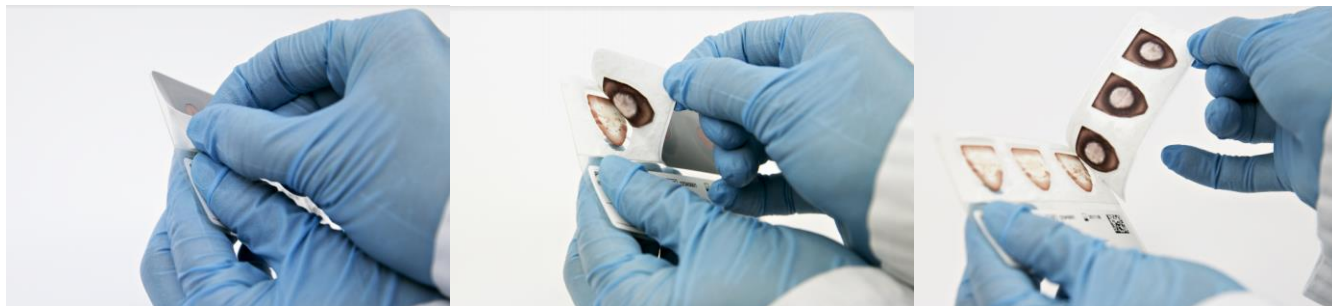


- Label a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Greiner Bio-one Cryo.s™]) for each PSC with its corresponding barcode of the laboratory request form (Figure 5) and place them into a rack. Transfer tubes into a laminar flow hood together with the sample bags containing the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

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



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

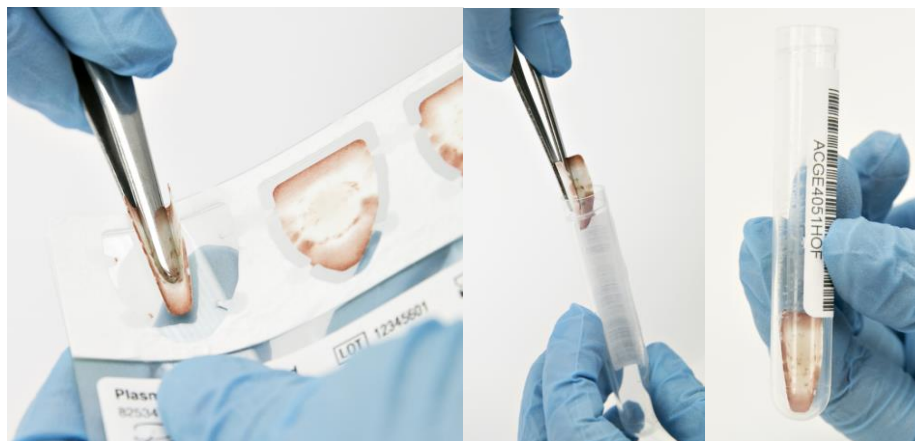
Figure 6 Removal of spotting layer

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

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

Figure 7 Removal of the PSC dried plasma spot and bending

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

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

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

Figure 9 PSC in sample bag for potential retest

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

Figure 10 1300 μL SPER addition

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

Figure 11 Incubation

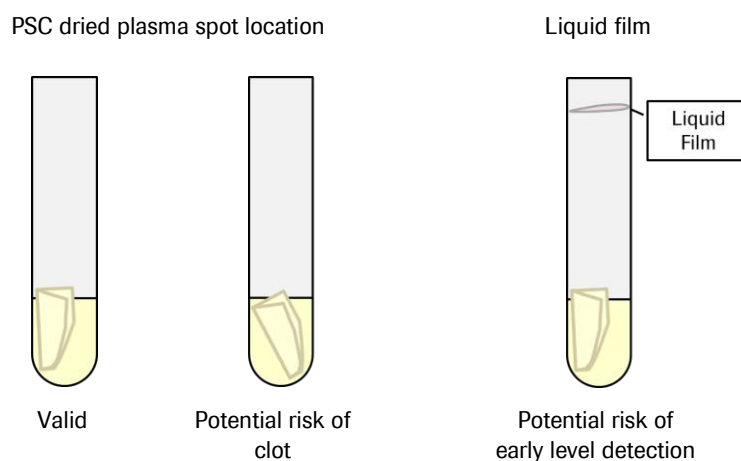


- Transfer the tubes onto a sample rack and uncap the tubes one by one to minimize cross-contamination (Figure 12). Change gloves after removing the caps.

Figure 12 Uncapping of the tubes



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

Figure 13 PSC dried plasma spot preparation before the analytic workflow

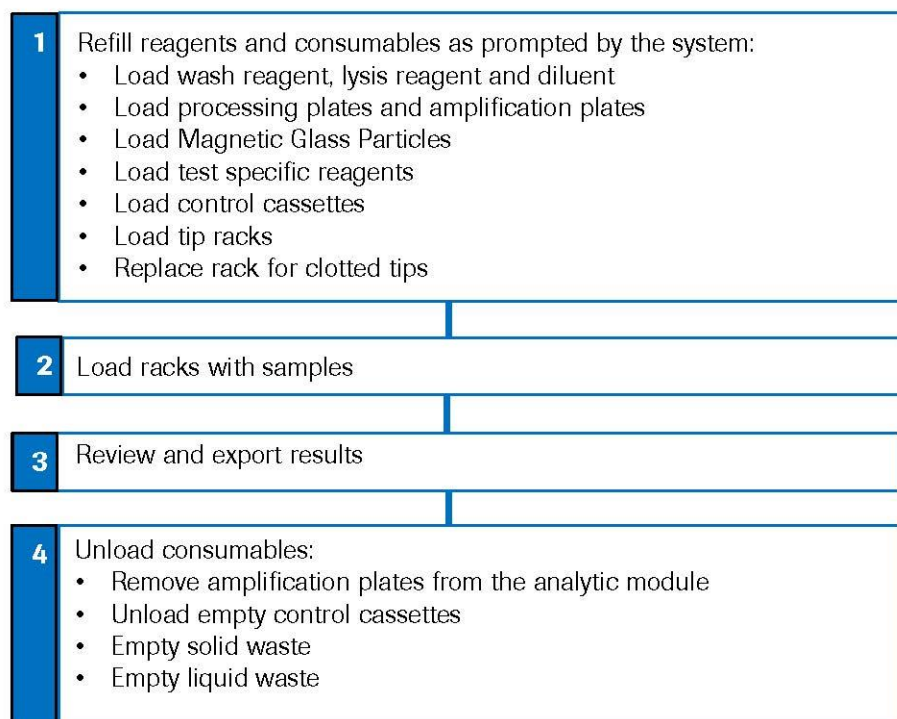
Note: Please be sure to remove any liquid film created during the process.

Running cobas® HIV-1

cobas® HIV-1 can be run with two minimum required sample volumes of 350 μL (for the 200 μL sample workflow) and 650 μL (for the 500 μL sample workflow). The test procedure is described in detail in the cobas® 6800/8800 Systems Operator's Manual. Figure 14 below summarizes the procedure.

cobas® HIV-1 can be run with 1300 μL cobas® Specimen Pre-Extraction Reagent (for the 850 μL PSC sample workflow). The test procedure is described in detail in the cobas® 6800/8800 Systems Operator's Manual. Figure 14 summarizes the procedure also for PSC dried plasma spot samples.

Verify that the correct sample type (**PSC**) and the correct ASAP is used (**cobas® HIV-PSC ASAP**) before starting the test procedure.

Figure 14 cobas® HIV-1 test procedure

Results

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

Quality control and validity of results

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

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

Control flags

Table 12 Control flags for negative and positive controls

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

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

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

Interpretation of results

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

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

Table 13 Target results for individual target result interpretation

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

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

Procedural limitations

- **cobas**® HIV-1 plasma workflow has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- **cobas**® HIV-1 PSC workflow has been evaluated in mixed batch functionality only with **cobas**® HIV-1/ HIV-2 Qualitative Dried Blood Spot Procedure.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HIV-1 may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- **cobas**® HIV-1 is not intended for use as a screening test for the presence of HIV-1 in blood or blood products.

Non-clinical performance evaluation

Key performance characteristics for EDTA plasma samples

Limit of Detection (LoD)

WHO International Standard

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

The results for EDTA plasma from both sample processing volumes are shown in Table 14 and Table 15. The study demonstrates that cobas® HIV-1 detected HIV-1 RNA at a concentration of 13.2 cp/mL (22.0 IU/mL) as determined by Probit with a hit rate of 95% for the 500 µL sample processing volume and at a concentration of 35.5 cp/mL (59.2 IU/mL) or greater as determined by Probit with a hit rate of 95% for the 200 µL sample processing volume.

Table 14 Limit of detection in EDTA plasma (500 µL)

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

Table 15 Limit of detection in EDTA plasma (200 µL)

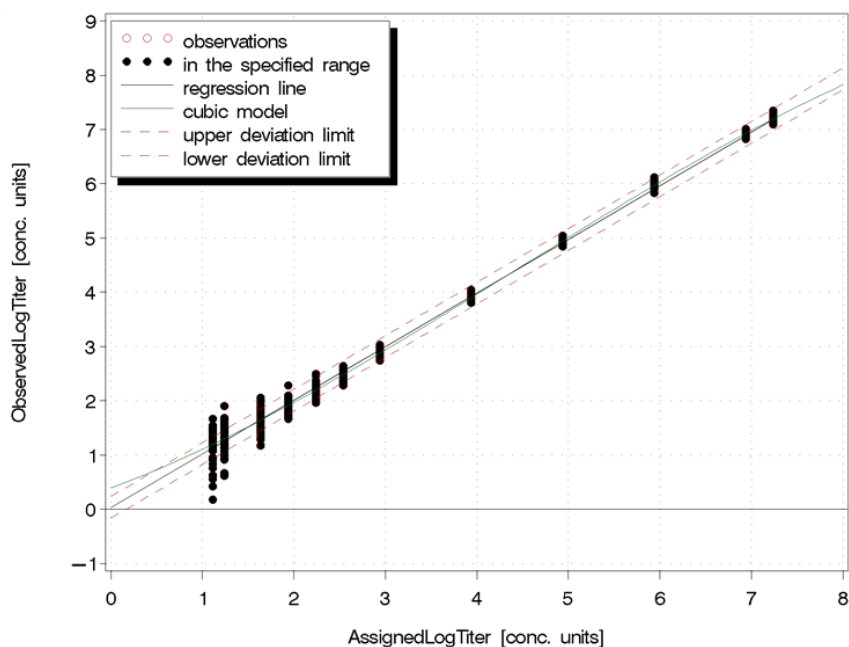
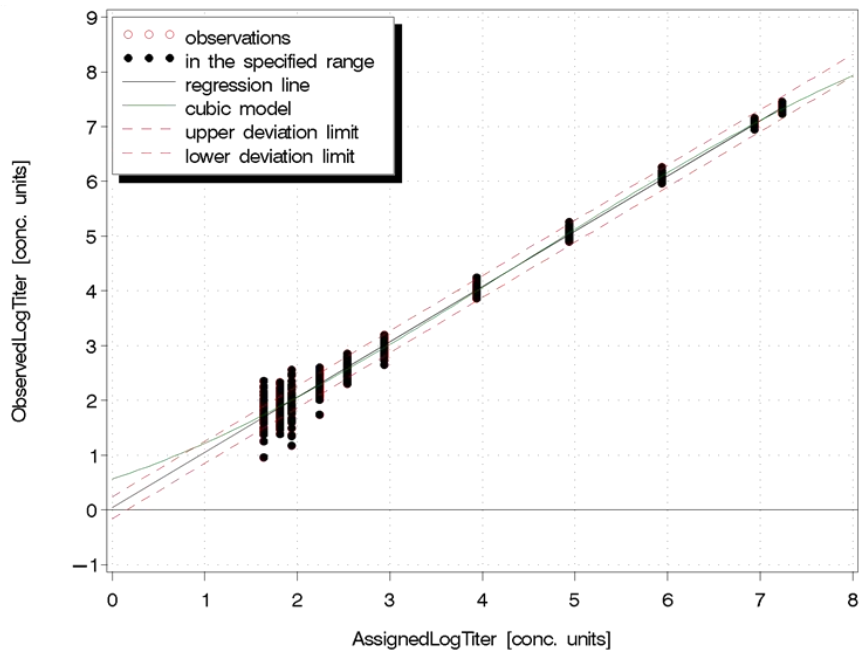
Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
200.0	333.3	189	189	100.0%
100.0	166.7	188	188	100.0%
50.0	83.3	189	186	98.4%
25.0	41.7	189	164	86.8%
12.5	20.8	189	112	59.3%
0.0	0.0	188	0	0.0%
LoD by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

Linear range

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

With 500 µL processing volume, **cobas**® HIV-1 is linear from 20 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than $\pm 0.2 \log_{10}$ in human EDTA plasma (see Figure 15). Across the linear range, the accuracy of the test was within $\pm 0.2 \log_{10}$.

With 200 µL processing volume, **cobas**® HIV-1 is linear from 50 cp/mL to 1.00E+07 cp/mL (83.3 IU/mL to 1.67E+07 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than $\pm 0.2 \log_{10}$ in human EDTA plasma (see Figure 16). Across the linear range, the accuracy of the test was within $\pm 0.2 \log_{10}$.

Figure 15 Linear range determination in EDTA plasma (500 µL)**Figure 16** Linear range determination in EDTA plasma (200 µL)

Precision - within laboratory

Precision of cobas® HIV-1 was determined by analysis of serial dilutions of an HIV-1 high positive sample (Group M Subtype B; cultured virus) in HIV negative EDTA plasma. Eight dilution levels (500 µL sample processing volume) and seven dilution levels (200 µL sample processing volume) were tested in 48 replicates for each level and processing volume across three lots of cobas® HIV-1 test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire cobas® HIV-1 test procedure on the fully automated cobas® 6800/8800 Systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 16 and Table 17.

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

Table 16 Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – processing volume of 500 µL)*

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

*Titer data are considered to be log-normally distributed and are analyzed following log₁₀ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 17 Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – processing volume of 200 µL)*

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

*Titer data are considered to be log-normally distributed and are analyzed following log₁₀ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Subtype verification

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

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

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

Cultured HIV-1 samples for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1O, HIV-1N were diluted to three different concentration levels in EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with 1 lot of cobas® HIV-1 reagents. The results from EDTA plasma using 500 µL are shown in Table 18. These results verify that cobas® HIV-1 detected HIV for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), HIV-1O, and HIV-1N at the claimed concentration of 20 cp/mL or below with an upper one-sided 95% confidence interval being equal to or greater to the expected hit rate of 95%.

Table 18 LoD verification of HIV-1 group M subtypes, group O, and group N in 500 µL EDTA plasma

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

* Upper one-sided 95% confidence interval

Similarly, the limit of detection was verified for the 10 subtypes tested with 200 µL input volume. The data are summarized

in Table 19. These results verify that **cobas**® HIV-1 detected HIV RNA for HIV-1M (A, C, D, F, G, H, CRF01_AE, CRF02_AG), and HIV-1N at the claimed concentration of 50 cp/mL or below with an upper one-sided 95% confidence interval being equal to or greater to the expected hit rate of 95%. HIV-1O was verified at 100 cp/mL.

Table 19 LoD verification of HIV-1 group M subtypes, group O and group N in 200 µL EDTA plasma

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

* Upper one-sided 95% confidence interval

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

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

The linear range of **cobas**® HIV-1 was verified for group M subtypes, group O and group N. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.2 log₁₀.

Specificity

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

Analytical specificity

The analytical specificity of cobas® HIV-1 was evaluated by diluting a panel of microorganisms with HIV RNA positive and HIV RNA negative EDTA plasma. The microorganisms were added to negative human EDTA plasma and tested with and without HIV RNA. None of the non-HIV pathogens interfered with test performance. Negative results were obtained with cobas® HIV-1 for all microorganism samples without HIV-1 target and positive results were obtained on all of the microorganism samples with HIV-1 target. The mean log₁₀ titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within $\pm 0.3 \log_{10}$ of the mean log₁₀ titer of the respective positive spike control.

Table 20 Microorganisms tested for cross-reactivity

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

Analytical specificity – interfering substances

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

In addition, drug compounds listed in Table 21 were tested at three times the C_{max} in presence and absence of HIV RNA.

All potentially interfering substances show no interference with the test performance. Negative results were obtained with cobas® HIV-1 for all samples without HIV target and positive results were obtained on all of the samples with HIV-1 target. The mean log₁₀ titer of each of the positive HIV-1 samples containing potentially interfering substances was within $\pm 0.3 \log_{10}$ of the mean log₁₀ titer of the respective positive spike control.

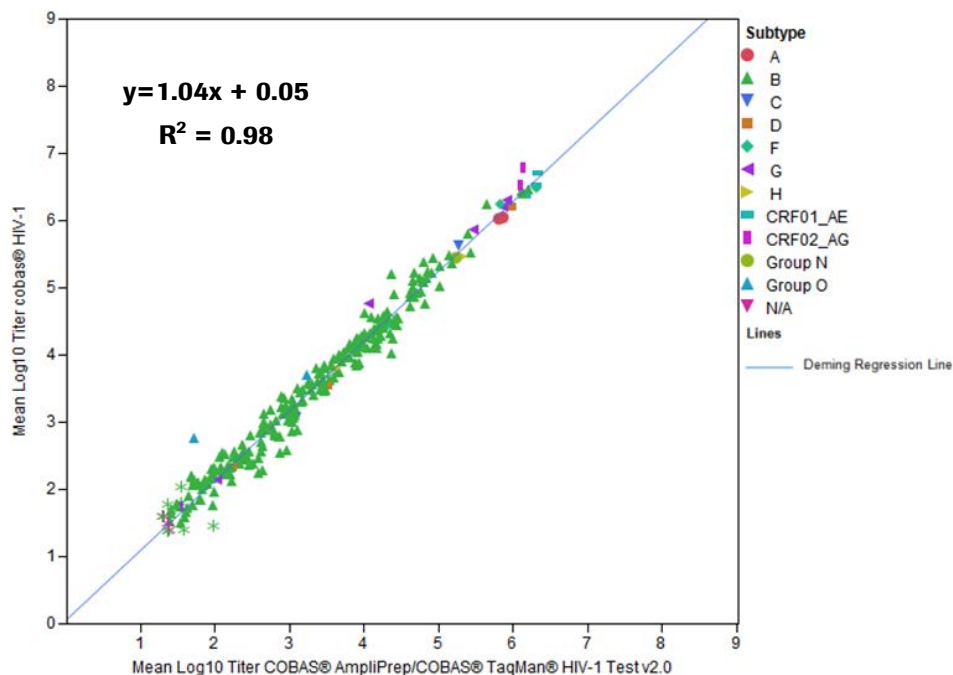
Table 21 Drug compounds tested for interference with the quantitation of HIV RNA by cobas® HIV-1

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

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

The performance of cobas® HIV-1 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 (TaqMan® HIV-1 Test, v2.0) were compared by analysis of 251 EDTA plasma specimens from patients infected with HIV-1 and by analysis of 16 diluted cell culture supernatants. The specimens comprised of HIV-1 M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1 O and HIV-1N and were tested in duplicate at an external site. Titers below the quantitation range (< 20 cp/mL) were generated for one of the two replicates for five samples on both the COBAS® AmpliPrep/COBAS® TaqMan® System and the cobas® 6800/8800 Systems, and for five samples on the COBAS® AmpliPrep/COBAS® TaqMan® System only. One sample on COBAS® AmpliPrep/COBAS® TaqMan® generated one valid aliquot only. Titers below the quantitation range were excluded from analysis. The Deming regression was performed considering log-transformed titers. For samples where both replicates were within the quantitation range, the mean log₁₀ titer was used for analysis.

The Deming regression results are shown in Figure 17. The symbol * in Figure 17 shows single determination.

Figure 17 Regression analysis of cobas® HIV-1 vs TaqMan® HIV-1 Test, v2.0, EDTA plasma samples

Whole system failure

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

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

Cross contamination

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

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

Key performance characteristics for PSC dried plasma spot samples

Limit of Detection (LoD)

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

Standard, HIV-1 group M, subtype B for preparation of the high and low calibrators.

The combined results from three PSC lots, negative donors and dilution series with individual plasma titer assignments (CBM) are shown in Table 22. The study demonstrates that cobas® HIV-1 in combination with the PSC detected HIV-1 RNA at a concentration of 790.2 cp/mL with a hit rate of 95% as determined by Probit analysis.

Table 22 Limit of detection in combination with the PSC

Assigned plasma titer concentration (HIV-1 RNA cp/mL)	Number of valid replicates	Number of positives	Hit rate in %
1971.1	62	62	100.00
1925.3	63	63	100.00
1358.1	62	62	100.00
985.5	63	62	98.41
962.6	63	62	98.41
679	63	59	93.65
657	63	59	93.65
641.8	63	57	90.48
452.7	63	52	82.54
328.5	63	47	74.60
320.9	63	46	73.02
226.3	62	49	79.03
164.3	63	36	57.14
160.4	63	35	55.56
113.2	63	38	60.32
0	189	0	0.00
LoD by PROBIT at 95% hit rate	790.2 cp/mL; 95% confidence range: 658.9 – 1003.6 cp/mL 1317 IU//mL; 95% confidence range: (1098.2 – 1672.7 IU/mL)		

Estimation of LoD in whole blood

The corresponding titers in whole blood of the same sample cannot be exactly determined, since whole blood is not a sample type for viral load testing. Whole blood titers based on the amount of HIV-1 RNA spiked into the whole blood samples were not used for LoD estimation because the amount of spiked RNA does not necessarily correspond to the amount of RNA in plasma. Even after centrifugation, RNA can remain in the buffy coat or associated with the cellular fraction of whole blood. However, since other technologies like dried blood spots have used spiked whole blood titers to estimate their limit of detection in whole blood, an estimate for the PSC whole blood LoD can be provided based on an empirical factor which is assumed to be related to the average hematocrit content (45%) of the samples (Table 23).

Whole blood LoD values are estimates based on the assumption:

Whole blood LoD estimate = PSC plasma LoD / 1.8

Table 23 Whole blood LoD estimate

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

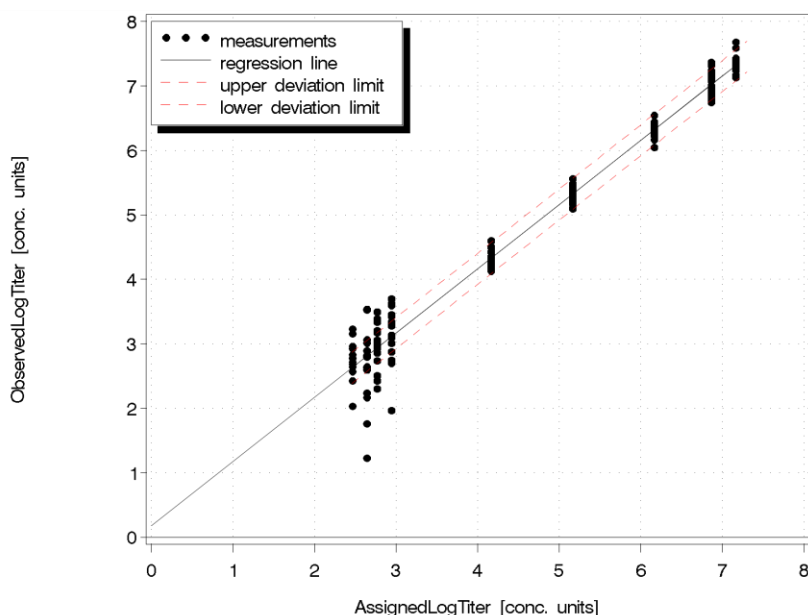
Linear range using the PSC

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

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

In combination with the PSC, cobas® HIV-1 is linear from 790 cp/mL to 1.00E+07 cp/mL and shows an absolute deviation from the better fitting non-linear regression of less than $\pm 0.24 \log_{10}$ with the PSC (see Figure 18). Across the linear range, the accuracy of the test was within $\pm 0.3 \log_{10}$ deviation from the linear regression fit.

Figure 18 Linear range determination using the PSC



Precision - within laboratory using the PSC

Precision of cobas® HIV-1 in combination with the PSC was determined by analysis of serial dilutions of an HIV-1 high positive sample (high titer HIV-1 RNA positive cell culture supernatant specimen) in HIV negative EDTA whole blood. Five dilution levels were tested in 48 replicates for each level and processing volume across two lots of PSC and two lots of cobas® HIV-1 test reagents using two instruments and two operators over 12 days. Each sample was carried through the entire PSC workflow and cobas® HIV-1 procedure on the fully automated cobas® 6800/8800 Systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 24.

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

Table 24 Within laboratory precision of cobas® HIV-1 in combination with the PSC

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

Subtype verification using the PSC

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

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

The results are shown in Table 25. These results confirm that cobas® HIV-1 in combination with the PSC detected and correctly quantified HIV-1M subtypes A, C and D.

Table 25 Verification of HIV-1 group M subtypes A, C and D

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

Specificity using the PSC

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

Whole system failure using the PSC

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

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

Performance of PSC dried plasma spot samples compared to plasma samples

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

The Deming regression results are shown in Figure 19. The symbols * and ● in Figure 19 show single determinations.

Figure 19 Deming regression

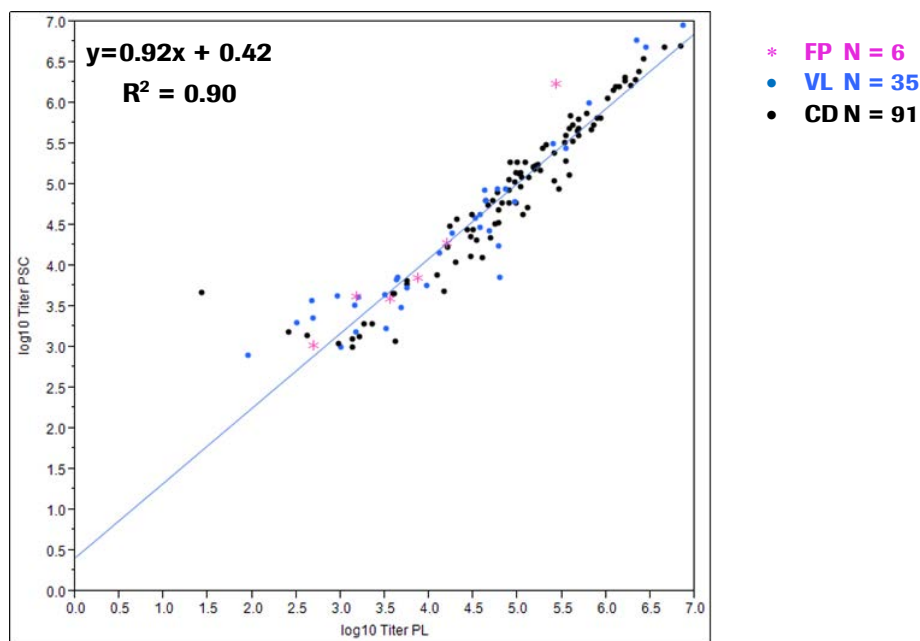


Table 26 Summary of statistical data

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

Additional information

Key test features for EDTA plasma samples

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




















































Key test features for PSC dried plasma spot samples

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

Symbols

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

Table 27 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.
	Ancillary Software		Device not for self-testing		
 Assigned Range [copies/mL]	Assigned Range (copies/mL)		Distributed by	 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	 IVD	In vitro diagnostic medical device		Temperature limit
 REF	Catalogue number	 LLR	Lower Limit of Assigned Range		Test Definition File
	CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device		Male		This way up
			Manufacturer	 Procedure UltraSensitive	Ultrasensitive Procedure
 Collect Date	Collect date	 CONTROL -	Negative control	 UDI	Unique Device Identification
	Consult instructions for use		Non sterile	 ULR	Upper Limit of Assigned Range
	Contains sufficient for <n> tests		Patient Name	 Urine Fill Line	Urine Fill Line
 CONTENT	Contents of kit		Patient number	 Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.
 CONTROL	Control		Peel here		Use-by date
	Date of manufacture	 CONTROL +	Positive control		
	Device for near-patient testing	 QS copies / PCR	QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.		
	Device for self-testing				

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 28 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)

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, 8129118, and 6727067, and foreign equivalent patents of each.

COBAS, COBAS OMNI, AMPERASE, AMPLIPREP, and TAQMAN are trademarks of Roche.

The trademark “Armored RNA®” is owned by Asuragen, Inc. and Cenetron Diagnostics, Ltd.

ProClin® is a registered trademark of Rohm and Haas Company.

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase® enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

Certain components of this product are covered by one or more US Patents and their foreign equivalents issued to Novartis Vaccines and Diagnostics, Inc. and licensed to Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd.

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

Copyright

©2021 Roche Molecular Systems, Inc.



Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany



References

1. Mellors JW, Muñoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* 1997;126:946-954.
2. Mellors JW, Margolick JB, Phair JP, et al. Prognostic value of HIV-1 RNA, CD4 cell count, and CD4 cell count slope for progression to AIDS and death in untreated HIV-1 infection. *JAMA.* 2007;297:2349-2350.
3. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at: <http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. Updated 2016.
4. Cohen MS, Chen YQ, McCauley M, et al. HPTN Study Team. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med.* 2011;365:493-505.
5. Phillips AN, Pillay D, Garnett G, et al. Effect on transmission of HIV-1 resistance of timing of implementation of viral load monitoring to determine switches from first to second-line antiretroviral regimens in resource-limited settings. *AIDS.* 2011;25:843-850.
6. Sigaloff KC, Hamers RL, Wallis CL, et al.; PharmAccess African Studies to Evaluate Resistance (PASER). Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr.* 2011;58:23-31.
7. European AIDS Clinical Society 2017 Guidelines. <http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html>
8. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection – Recommendations for a public health approach. World Health Organization HIV/AIDS Programme, Geneva. 2016.
9. Günthard HF, Aberg JA, Eron JJ, et al.; Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society – USA panel. *JAMA.* 2014;312:410-25.
10. Do T, Duncan J, Butcher A, Liegler T. Comparative frequencies of HIV low-level viremia between real-time viral load assays at clinically relevant thresholds. *J Clin Virol.* 2011;52 Suppl 1:S83-S89.
11. Paba P, Fabeni L, Ciccozzi M, Perno CF, Ciotti M. Performance evaluation of the COBAS/TaqMan HIV-1 v2.0 in HIV-1 positive patients with low viral load: a comparative study. *J Virol Methods.* 2011;173:399-402.
12. Deeks SG. Durable HIV treatment benefit despite low-level viremia: reassessing definitions of success or failure. *JAMA.* 2011;286:224-226.
13. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene.* 1990;93:125-128.
14. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature.* 1995;373:487-493.
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;80:869-878.
16. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology (NY).* 1992;10:413-417.

17. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. *Genome Res.* 1996;6:986-994.
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.
20. Clinical and Laboratory Standards Institute (CLSI) EP06-A. Evaluation of the linearity of quantitative measurement procedures: A statistical approach. CLSI Document EP06-A:Wayne, PA; CLSI, 2003.

Document revision

Document Revision Information	
Doc. Rev. 8.0 05/2021	Inserted Rx Only symbol on first page. Updated hazard warnings. Updated the harmonized symbol page. Added Technical support section. Added Made in statement. Updated distributors addresses. Updated Trademarks and patents section. Please contact your local Roche Representative if you have any questions.