WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT
Product: cobas HIV-1 Nucleic acid test for use on the cobas 4800 System[1]
WHO reference number: PQDx 0710-118-00

cobas HIV-1 Nucleic acid test for use on the cobas 4800 System with product code 08792992190 and 06979572190 manufactured by Roche Diagnostics GmbH, CE-Mark, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 14 September 2022.

Summary of WHO prequalification assessment for cobas HIV-1 Nucleic acid test for use on the cobas 4800 System

	Date	Outcome
Prequalification listing	14 September 2022	listed
Dossier review	N/A	MR
Desk Assessment) of the quality management system	4 December 2019	MR
Product performance evaluation	10 August 2021 and 7 November 2023	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 WHO has been notified and has undertaken a review. Amendments to the report are summarized in the table below.

Version	Summary of amendment	Date of report amendment
2.0	Removal of product codes for controls and other materials required but not provided with the prequalified product.	23 November 2022

[1] Dossier assessment and product performance evaluation for the cobas HIV-1 Nucleic acid test for use on the cobas 4800 System were considered from the previous assessment of the prequalified product, cobas HIV-1 Quantitative nucleic acid test for use on the cobas 4800 System (PQDx 0373-118-00) , which was prequalified in 2021. Based on the product dossier assessment and product performance evaluation, cobas HIV-1 Nucleic acid test for use on the cobas 4800 System meets WHO prequalification requirements. The summaries of the dossier, manufacturing site inspection and performance evaluation from the initial assessment were included in this public report.

	Added a footnote on the intended use section of the public report to clarify the WHO's position on the confirmatory claim on the product's intended use.	
3.0	Merging of the public report for the cobas HIV-1 Quantitative nucleic acid test for use on the cobas 4800 System and cobas HIV-1 Nucleic acid test for use on the cobas 4800 System and the inclusion of the performance evaluation results for the cobas HIV-1 Nucleic acid test for use on the cobas 4800 System.	16 November 2023.

Intended use[2]:

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 and for the qualitative detection of HIV-1 in dried blood spots of HIV-1-infected individuals including infants born to mothers infected with HIV-1. The test is to be used with cobas 4800 System. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progression for the clinical management of HIV-1-infected patients.*

When used as a quantitative test, this test can be used as an aid in diagnosis for confirmation of HIV-1 infection in antibody reactive individuals and to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

When used as a qualitative test with dried blood spots, detection of HIV-1 nucleic acids is indicative of active HIV-1 infection in infants born to HIV-infected mothers who have maternal antibodies to HIV-1. This test can also be used for confirmation of HIV-1 infection in individuals reactive for HIV-1 antibodies or antigens."

Description of the quantitative test:

According to the claim of assay description from Roche Diagnostics GmbH, *"cobas HIV-1 can be used as a quantitative nucleic acid test performed on the cobas 4800 System. The cobas HIV-1 quantitative test enables the detection and quantitation of HIV 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, N and O subtypes. The viral load is quantified against a non-HIV armored RNA quantitation standard (RNA QS), which is introduced into each*

[2] The intended use claim that cobas HIV-1 Nucleic acid test for use on the cobas 4800 System to confirm HIV-1 infection in an individual with specimens reactive for HIV-1 antibodies or antigens has not been verified in the prequalification assessment. The WHO Global HIV, Hepatitis, and STI Programme does not recommend using NAT assays to confirm HIV-1 or HIV-2 infection in individuals older than 18 months of age with specimens reactive for HIV-1 or HIV-2 antibodies or antigens (outside of infant diagnosis, for which NAT assays are recommended as an aid to diagnose HIV infection).

sample during sample processing. The RNA QS functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control."

Description of the qualitative test:

According to the claim of assay description from Roche Diagnostics GmbH, *"cobas HIV-1 can be used as a qualitative nucleic acid test performed on the cobas 4800. The cobas HIV-1 qualitative test enables the detection of HIV-1 nucleic acid in DBS of infected patients. Two probes are used to detect, but not discriminate group M, N and O subtypes.*
The RNA QS provided with this kit functions as an internal control to monitor the entire sample preparation and PCR amplification process in the context of the qualitative DBS application. In addition, the test utilizes two external controls: the low titer positive control and a negative control (the high positive control is not needed for cobas HIV-1 qualitative test)."

Test kit contents:

Component	**tests (product code)**
cobas HIV-1	120 (08792992190)
cobas HBV/HCV/HIV-1 Control Kit	10 Sets (06979572190)

Items required but not provided:

Item	**Description (P/N)**
cobas 4800 System Sample Preparation Kit 2	240 (06979513190) 960 (06979521190)
cobas 4800 System Wash Buffer Kit	240 (05235863190) 960 (05235871190)
cobas 4800 System Specimen Diluent 2	240 (06979556190)
cobas 4800 System Lysis Kit 2	240 (06979530190) 960 (06979548190)
cobas 4800 System Extraction (deep well) Plate 2.0 mL	06884008001
cobas 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001

Item	Description (P/N)
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Lab gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

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

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

Storage:

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

Note: Do not freeze reagents.

Shelf-life upon manufacture:
24 months.

Warnings/limitations:

Please refer to the instructions for use attached to this report.

Prioritization for Prequalification

In 2021, Roche Diagnostics GmbH submitted a change notification for their prequalified product cobas HIV-1 Quantitative nucleic acid test for use on the cobas 4800 System (with application number PQDx 0373-118-00) to introduce a new configuration with a qualitative claim resulting in the new product name cobas HIV-1 Nucleic acid test for use on the cobas 4800 System, with corresponding product code 08792992190.

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 Nucleic acid test for use on the cobas 4800 System 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

A manufacturing site inspection of Roche Diagnostics GmbH, located at Sandhofer Strasse 116, Mannheim, 68305, Germany, was conducted from 14 to 15 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 manufacturing site inspection performed at a manufacturing site for in vitro diagnostic products and summarises the desk assessment findings.

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

https://extranet.who.int/prequal/inspection-services/prequalification-reports/whopirs-vitro-diagnostics

All published WHOPIRs are with the agreement of the manufacturer.

The manufacturer's responses to the non-conformities found during the manufacturing site inspection were accepted on 13 April 2023.

Product performance evaluation

The cobas HIV-1 Quantitative nucleic acid test for use on the cobas 4800 System 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, as part of the initial assessment of this product (PQDx 0373-118-00, refer to footnote 1). The evaluation took place from July 9, 2018, to September 17, 2018, at the CDC ILB lab and from 25 Oct 2019 to 26 June 2020, at the NHLS HIV PCR Lab[3]. This evaluation focused on the claim for quantitative detection of HIV-1 using plasma and plasma separation cards (PSC).

As part of the assessment of the change request, including the addition of a claim for qualitative detection of HIV-1 using DBS, the manufacturer was required to coordinate a prequalification performance evaluation using the standardized protocol for evaluating HIV qualitative nucleic acid tests by 31 March 2023. The prequalification listing was contingent upon a positive outcome of the prequalification performance evaluation. The commitment was fulfilled. The issue is closed.
The cobas HIV-1 Nucleic acid test for use on the cobas 4800 System was evaluated on behalf of WHO by the Central Public Health Laboratories, Kampala, Uganda, for the clinical evaluation in the 1st quarter of 2023 according to protocol PQDx_199 version 3.3 and at the CDC, United States, for the analytical evaluation in the 3rd quarter of 2021 according to protocol PQDx_199 version 3.0. This evaluation focused on the claim for qualitative detection of HIV-1 using dried blood spots (DBS).

Clinical performance evaluation -quantitative detection of HIV-1 using plasma and PSC

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 549 plasma specimens and 462 PSC specimens was used. A total of 87 plasma specimens could not be repeated after a run error and were excluded from this analysis. Another 40 plasma specimens and 23 PSC specimens with individual errors that could not be repeated are included in the calculation of the invalid rate but not in the performance analysis. The resulting panel included 332 plasma specimens, 348 PSC specimens from HIV-positive

[3] The performance evaluation also included an evaluation on dried plasma spots prepared using the cobas Plasma Separation Card (PSC), which was not in the scope of this initial submission. The addition of this specimen type will be reviewed as a change request, and corresponding results of the performance evaluation reported accordingly.

individuals, 90 plasma specimens and 91 PSC specimens from HIV-negative individuals. The specimens were characterized using the following reference assay on plasma: COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 (Roche Diagnostics GmbH).

Clinical performance characteristics in comparison with an agreed reference standard		
	Plasma	**PSC**
Sensitivity for detection of virological failure among HIV-positive individuals (% (95% CI)	96.8 % (95% CI 93.8 to 98.4) (N=248)	96.0% (95% CI 92.8 to 97.8) (N=251)
Specificity for detection of virological failure among HIV-positive individuals % (95% CI)	98.8% (95% CI 93.6 to 99.8) (N=84)	96.9% (95% CI 91.3 to 98.9) (N=97)
Specificity among HIV-negative individuals	100% (95% CI 96.0 to 100) (N=90)	100% (95% CI 96.0 to 100) (N=91)
Bias Limits of agreement	0.032 $\log_{10}$ cp/mL -0.436 to 0.499 $\log_{10}$ cp/mL	0.049 $\log_{10}$ cp/mL -0.410; 0.508 cp/mL
Invalid rate %	There were three run failures during the evaluation. In addition, 55/521 individual errors were reported for plasma specimens and 62/508 for PSC specimens (including repeats). Most of these were considered user errors (clots or insufficient volume due to the need to perform the index and reference assays). After excluding user errors, the invalid rate was 2.3% for plasma specimens and 1.0% for PSC.	

Clinical performance evaluation – qualitative detection of HIV-1 using DBS

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 430 DBS specimens were used, of which 5 were excluded due to discrepant reference results on repeat testing. The specimens were characterized using the following reference assay on DBS: COBAS AmpliPrep/COBAS TaqMan HIV-1 Qualitative Test, version 2.0 (Roche Diagnostics).

Clinical performance characteristics		
	Infants < 18 months	**Adults**
Sensitivity (N=121 infants / 50 adults)	100 % (95% CI: 97.0% - 100%)	98.0% (95% CI: 89.4% - 99.9%)
Specificity % (N= 199 infants / 55 adults)	100% (95% CI: 98.2% - 100%)	98.2% (95% CI: 90.3% - 100 %)
Invalid/error rate % (N= 425)	0%	

Analytical performance evaluation -quantitative detection of HIV-1 using plasma and PSC

Analytical performance characteristics		
	Plasma	**PSC**
Limit of detection (LoD)	The LoD was estimated at 20.6 cp/mL (95% CI: 14.9-40.5). The LoD claimed by the manufacturer (14.2 cp/mL; 95% confidence range: 12.5-16.6) was verified.	The LoD was estimated at 745.2 copies/mL (95% CI: 470-2129). The LoD claimed by the manufacturer (598.6 cp/mL; 95% confidence range: 526.4-707.0) was verified.
Within-run precision (repeatability)	At 10^3 cp /mL, CV% < 4 % At 10^4 cp /mL, CV% < 2 %	At $2.5x10^3$ cp /mL, CV% < 4 % At 10^4 cp /mL, CV% < 3 %
Within-laboratory precision (reproducibility)	At 10^3 cp /mL, CV% < 4 % At 10^4 cp /mL, CV% < 3 %	At $2.5x10^3$ cp /mL, CV% < 4 % At 10^4 cp /mL, CV% < 3 %
Linearity	Linearity of the assay was verified for subtypes A, B, C, D and CRF02_AG over a range of viral loads from 10^2 to 10^6 cp /mL for plasma and from 10^3 to 10^6 cp /mL for PSC.	
Cross-contamination/carry-over	No cross-contamination was observed when high positive and negative plasma or PSC specimens were tested alternatively.	

Analytical performance evaluation– qualitative detection of HIV-1 using DBS

Analytical performance characteristics (DBS specimens)	
Limit of detection (LoD) using the WHO 4th International Standard for HIV-1 RNA – DBS specimens	The LoD for HIV-1 was estimated at 1034 IU/mL (95% CI: 747 – 1665 IU/mL), or 620 cp/mL (95% CI: 448 – 999 cp/mL) using the conversion factor provided by the manufacturer. The LoD claimed by the manufacturer was 325.1 cp/mL (95% confidence range: 284.1-385.8 cp/mL).
Within-laboratory precision (reproducibility)	The hit rate for HIV-1 subtype B, at 975 cp/mL, was 100% (40/40). The hit rate for HIV-1 subtype C, at 975 cp/mL, was 100% (40/40).
Subtype detection	HIV-1 subtypes D, A and AG were detected at 5000 cp/mL.
Cross-contamination/carry-over	No cross-contamination/carry-over was observed when high positive and negative specimens were tested alternatively.

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 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 3-day training on the use of the assay.

Key operational characteristics	
Number of steps for one specimen*	4 steps in total for plasma specimens; no step with precision pipetting Additional 8 steps for DBS or PSC processing, including one with precision pipetting and one-timed step using thermomixer.
Number of steps for instrument management**	5 steps per day.
Time to result for one run.	4 hrs and 5 minutes (2h40 for extraction and 1hr 25 for amplification and detection) for a 96 test run.
Operator hands-on time for one run.	90 minutes (preparing specimens, loading reagents, consumables and specimens for a full run)
Level of automation	Semi-automated: Plates must be removed from cobas x 480 after extraction, manually sealed, and then placed on cobas z 480 within 40 minutes after extraction.
Quality controls	The manufacturer provides QC, which should be purchased separately. Three controls (high positive, low positive, negative) should be tested with each run.
Operating temperature	15-30 °C.
Result display and connectivity	Results are displayed on the connected computer. They may be printed using a standard printer. According to the manufacturer, the results can be exported to the laboratory and other health information systems.
Power sources	Main power. The use of a UPS is recommended, as stable electricity is required.
Biosafety *(outside of infectious specimen handling)*	Operators reported biosafety concerns for the user. Specimens, reagents and waste must be handled according to the manufacturer's instructions. If not,

	they may cause infections, irritations, corrosions, explosions, or carcinogens. The cobas 4800 lysis buffer 2 and Specimen Pre-Extraction reagent contain guanidine thiocyanate, a potentially hazardous chemical.
Waste	Very low liquid volume waster per run (approx.. 400 mL).
Calibration	No calibration is required.
Maintenance	Daily and weekly maintenance is required. Two preventive maintenance per year.

** 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 Nucleic acid test for use on the cobas 4800 System meets the WHO prequalification requirements.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

1.1 cobas HIV-1 Quantitative nucleic acid test (product code 08792992190)

Cont.

MMX R1	10 x 1.75 mL
HIV-1 MMX R2	10 x 0.5 mL
RNA QS	10 x 1.75 mL

*Nucleic acid test for use on the **cobas**® 4800 System*
*Nukleinsäuretest zur Verwendung auf dem **cobas**® 4800 System*
*Test d'acide nucléique à utiliser avec le système **cobas**® 4800*
*Test dell'acido nucleico per l'uso con il sistema **cobas**® 4800*
*Prueba de ácidos nucleicos para uso en el sistema **cobas**® 4800*
*Teste de ácido nucleico para utilização no sistema **cobas**® 4800*
*Nukleinsyretest til brug på **cobas**® 4800-systemet*
*Nukleinsyretest för användning på **cobas**® 4800-systemet*
*Test kwasu nukleinowego przeznaczony do użytku z systemem **cobas**® 4800*
*Test nukleových kyselín na použite v systéme **cobas**® 4800*

c4800 HIV-1 AMP/DET

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

D

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

08793000001-01

PS

cobas® HIV-1
Nucleic acid test
*for use on the **cobas**® 4800 System*

c4800 HIV-1 AMP/DET

Roche

cobas®

CE
0123

UDI label here

cobas® HIV-1
Nucleic acid test
*for use on the **cobas**® 4800 System*

REF P/N: 08792992190

IVD

2°C 8°C

Σ 120

c4800 HIV-1 AMP/DET

cobas®

Drawing: 9698

cobas® HIV-1

IVD

Nucleic acid test
for use on the cobas® 4800 System

c4800 HIV-1 AMP/DET

KIT LOT

	LOT
MMX R1	
HIV-1 MMX R2	
RNA QS	

SW

cobas® 4800 system
cobas® HIV-1 AP v1.2 or higher
cobas® HIV-1-qual-DBS AP v1.0 or higher
cobas® 4800 system
Application Software Version 2.2 or higher

Non-USA

website: http://e-labdoc.roche.com
Method Sheet Catalog No.: 08792992190
Doc Rev. 4.0

CANADA

website: http://e-labdoc.roche.com
Method Sheet Catalog No.: 08792992190
Doc Rev. 4.0

Please contact your local Roche representative if you require a printed copy free of charge or need technical support to access the package insert./
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Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

08793018001-06

1.3 Component Labels

cobas® 4800

MMX R1

1.75 mL IVD 2-8°C

06978304001

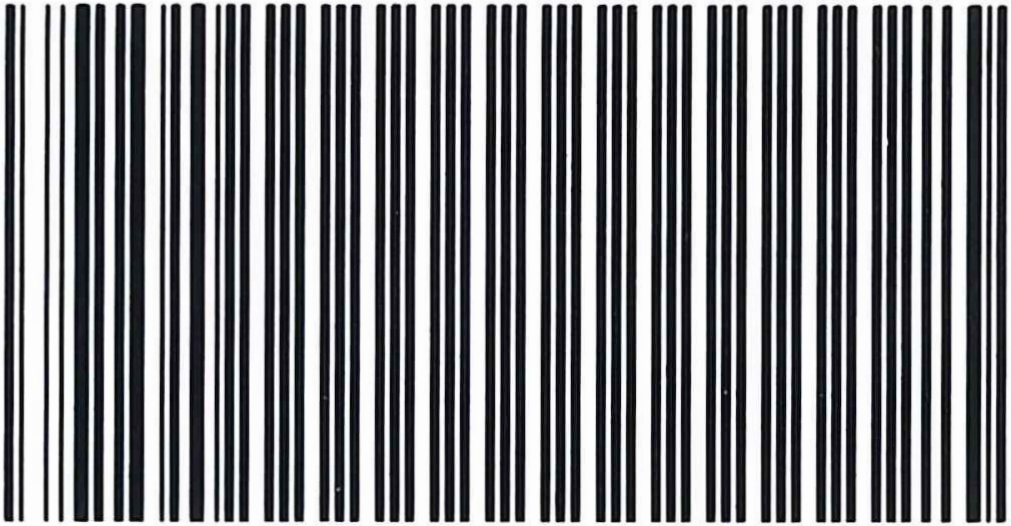
07275412001-02

LOT ????????

??????????

Manufactured in the United States

Roche Diagnostics GmbH, 68305 Mannheim, Germany



0T2????????????

cobas® 4800

RNA QS

1.75 mL IVD 2-8°C

06978339001

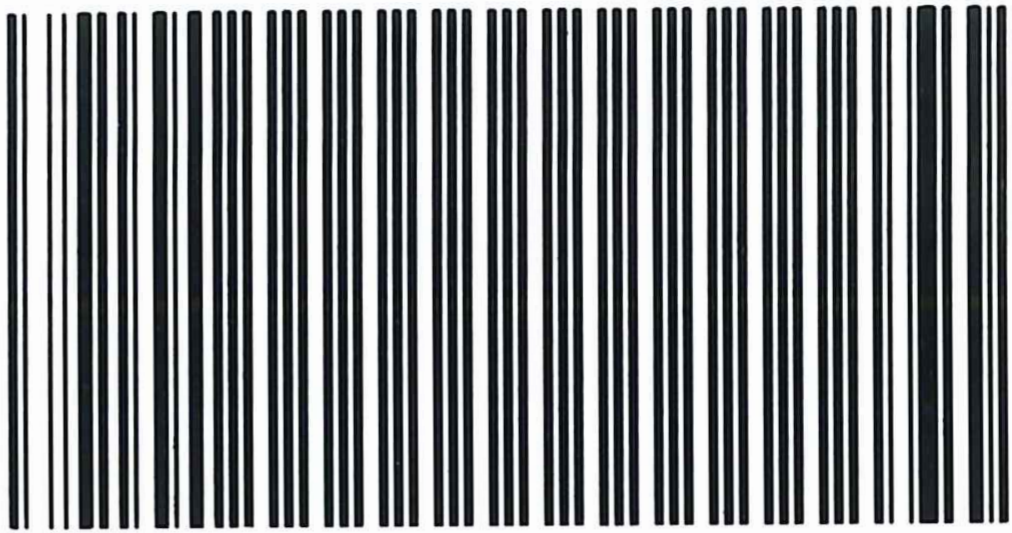
07275447001-02

LOT ????????

??????????

Manufactured in the United States

Roche Diagnostics GmbH, 68305 Mannheim, Germany



0Q1????????????

cobas® 4800

HIV-1 MMX R2

0.5 mL IVD 2-8°C

06653154001

07275455001-01

LOT ????????

??????????

Manufactured in the United States

Roche Diagnostics GmbH, 68305 Mannheim, Germany

BM0???????????

1.2 cobas HBV/HCV/HIV-1 Control Kit (product code 06979572190)

cobas® HBV/HCV/HIV-1 Control Kit

REF P/N: 06979572190

10 E

IVD

8°C 2°C

c4800 HBV/HCV/HIV CTLS

cobas®

NOTICE
The instructions for use for this product are contained in the target-specific **cobas®** 4800 test package insert.

HINWEIS
Die Gebrauchsanleitung für dieses Produkt finden Sie in der testspezifischen Packungsbeilage des jeweiligen **cobas®** 4800-Tests.

INDICATION
Les instructions d'utilisation de ce produit sont contenues dans la notice relative au test **cobas®** 4800 correspondant.

AVVISO
Le istruzioni per l'uso di questo prodotto sono contenute nel foglio illustrativo del test **cobas®** 4800 specifico per il target.

AVISO
Las instrucciones de uso de este producto figuran en el boletín técnico de la prueba **cobas®** 4800 específica para el objetivo.

AVISO
As instruções de utilização deste produto encontram-se no folheto informativo do teste **cobas®** 4800 específico do alvo.

BEMÆRKNING
Brugsvejledningen til dette produkt findes på det targetspecifikke pakningsindlæg til **cobas®** 4800-testen.

MEDDELANDE
Bruksanvisningen för den här produkten finns på den target-specifika bipacksedeln för **cobas®** 4800-testet.

UWAGA
Instrukcje dotyczące stosowania tego produktu umieszczono na wkładce do opakowania testu **cobas®** 4800.

OZNÁMENÍ
Návod na použitie pre tento produkt nájdete v cieľovo špecifickom príbalovom letáku testov **cobas®** 4800.

Cont.

(–) C	10 x 0.75 mL
HBV/HCV/HIV-1 L(+)C	10 x 0.75 mL
HBV/HCV/HIV-1 H(+)C	10 x 0.75 mL

H317, P261, P272, P280, P333 + P313, P362 + P364, P501

*For use on the **cobas®** 4800 System*
*Zur Verwendung auf dem **cobas®** 4800 System*
*À utiliser avec le système **cobas®** 4800*
*Per l'uso con il sistema **cobas®** 4800*
*Para uso en el sistema **cobas®** 4800*
*Para utilização no sistema **cobas®** 4800*
*Til brug på **cobas®** 4800-systemet*
*För användning på **cobas®** 4800-systemet*
*Do użytku z systemem **cobas®** 4800*
*Určené na použitie v systéme **cobas®** 4800*

c4800 HBV/HCV/HIV CTLS

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Distributed by D

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

Roche Diagnostics GmbH
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68305 Mannheim, Germany

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Barcelona, Spain

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

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38240 Meylan, France

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20052 Monza, Milano, Italy

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Made in USA

Component Lot Label Here

cobas® HBV/HCV/HIV-1 Control Kit

c4800 HBV/HCV/HIV CTLS

UDI label here

Roche cobas®

CE 0123

07499256001-02

PS

Drawing: 9698

REF (240)08792992190

GTIN (01)00875197006209

UDI 

LOT (10)Z1234567

2038-01-31

2030-01-31 02

2. Instructions for use[4]

[4] 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

Nucleic acid test

for use on the cobas® 4800 System

For in vitro diagnostic use

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

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Intended use

cobas® HIV-1 is an in vitro nucleic acid amplification test for the quantitation of human immunodeficiency virus type 1 (HIV-1) in EDTA plasma or from a **cobas**® Plasma Separation Card (**PSC**) dried plasma spot and for the qualitative detection of HIV-1 in dried blood spots of HIV-1-infected individuals including infants born to mothers infected with HIV-1. The test is to be used with **cobas**® 4800 System. This test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progression for the clinical management of HIV-1-infected patients.

When used as a quantitative test, this test can be used as an aid in diagnosis for confirmation of HIV-1 infection in antibody reactive individuals and to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiretroviral therapy by measuring changes in HIV-1 RNA levels during the course of antiretroviral treatment.

When used as a qualitative test with dried blood spots, detection of HIV-1 nucleic acids is indicative of active HIV-1 infection in infants born to HIV-infected mothers who have maternal antibodies to HIV-1. This test can also be used for confirmation of HIV-1 infection in individuals reactive for HIV-1 antibodies or antigens.

Information about the contents

cobas® HIV-1 is comprised of a quantitative and a qualitative workflow, and both the quantitative and qualitative workflows share the same amplification/detection kit and control kit. The quantitative and qualitative test, however, have fundamental differences, and therefore, the descriptions of the two tests are provided in independent sections.

In the earlier sections of this document, information regarding the summary and explanation of the **cobas**® HIV-1 quantitative test, the required materials and reagents, precautions and handling requirements, instructions for use, results and non-clinical performance evaluation are provided. This information is followed by equivalent sections covering the **cobas**® HIV-1 qualitative test.

Summary and explanation of the cobas® HIV-1 for quantification of HIV-1 RNA in plasma and dried plasma spots

Background

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

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

Rationale for HIV-1 quantitative testing

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

For a number of years, the guidelines indicated that a key goal of treatment is suppression of the HIV-1 viral load below the limit of detection of a test (e.g., 50 copies/mL). In 2011, the United States HIV treatment guidelines began to indicate that viral load results of up to 200 copies/mL in patients on ART may not be indicative of treatment failure.[3] European guidelines continue to recommend the use of 50 copies/mL as the threshold for determining treatment failure.[7] The correct threshold to use has not been determined in a rigorous clinical trial. Low-end differences between viral load tests may lead to important differences in the clinical interpretation of viral load results when monitoring treatment response,[10,11] as the goal of treatment is suppression of virus to a level below which drug resistance is least likely to emerge, 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.[13] 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/mm^3 (viral load > 100,000 copies/mL) should initiate ART and for ensuring that drug resistance sequencing will be successful in appropriate patients (patients with a viral load > 1,000 copies/mL or suboptimal viral load response to ART). The use of viral load assessment should be

performed in the prenatal setting to determine whether Caesarean section delivery is needed to prevent mother-to-child transmission of HIV infection (for pregnant women with a viral load > 1,000 copies/mL).

Explanation of the quantitative test

cobas® HIV-1 can be used as a quantitative nucleic acid test performed on the **cobas**® 4800 System. The **cobas**® HIV-1 quantitative test enables the detection and quantitation of HIV 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, N and O subtypes. The viral load is quantified against a non-HIV armored RNA quantitation standard (RNA QS), which is introduced into each sample during sample processing. The RNA QS functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

The **cobas**® HIV-1 quantitative test is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 4800 System consists of the **cobas x** 480 sample preparation instrument and the **cobas z** 480 real-time PCR analyzer. Automated data management is performed by the **cobas**® 4800 software which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HIV RNA detected, a value in the linear range LLoQ ≤ x ≤ ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

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

Selective amplification of target nucleic acids from the sample is achieved by the use of target virus-specific forward and reverse primers, which are selected from highly conserved regions of HIV. The HIV-1 gag gene and the HIV-1 LTR region (dual target) are amplified by **cobas**® HIV-1. Selective amplification of RNA QS is achieved by the use of sequence-specific forward and reverse primers, which are selected to have no homology with the HIV genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[14-16] Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, any newly formed amplicon is not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

cobas® HIV-1 master mix contains two detection probes specific for the HIV-1 target sequences and one for RNA QS. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target and RNA QS in two different detection channels.[17,18] When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA QS, respectively.

Materials and reagents for the cobas® HIV-1 quantitative test

Reagents

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

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HIV-1 120 Tests (P/N: 08792992190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 x 1.75 mL	N/A
	HIV-1 MMX R2 (**cobas**® **HIV-1** Master Mix Reagent 2) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A
	RNA QS (**cobas**® RNA Quantitation Standard) Tris buffer, < 0.05% EDTA, < 0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	10 x 1.75 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HBV/HCV/HIV-1 Control Kit 10 Sets (P/N: 06979572190)	**HBV/HCV/HIV-1 L(+)C** (**cobas**® HBV/HCV/HIV-1 Low Positive Control) < 0.001% synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	  **WARNING** H317 May cause an allergic skin reaction. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 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 H(+)C (**cobas**® HBV/HCV/HIV-1 High Positive Control) < 0.001% synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	
	(–) C (**cobas**® Negative Control) Normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	

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

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	 **DANGER** H302+H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. 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. 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. P342 + P311 If experiencing respiratory symptoms: 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 39450-01-6 Proteinase, Tritirachium album serine
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 27 mL	

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	 **DANGER** H302+H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. 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. 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. P342 + P311 If experiencing respiratory symptoms: 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 39450-01-6 Proteinase, Tritirachium album serine
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 84 mL	

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

cobas® Specimen Pre-Extraction Reagent

Note: This reagent is optional and should only be used in conjunction with the cobas® Plasma Separation Card (PSC) to generate dried plasma spot samples. See PSC Method Sheet ms_09411763190.

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® **Specimen Pre-Extraction Reagent** (P/N 08064695190)	**SPER** (**cobas**® Specimen Pre-Extraction Reagent) 28% (w/w) guanidine thiocyanate[b], 6% (w/v) polydocanol[b], 1% (w/v) dithiothreitol, dihydro sodium citrate	15 x 40 mL	 **DANGER** H302 Harmful if swallowed. 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. P273 Avoid release to the environment. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. 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

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

Reagent storage and handling requirements

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

Do not freeze reagents.

Additional materials required

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

Additional materials required for PSC dried plasma spot application only

Materials
cobas® Plasma Separation Card (P/N 07963084190 or P/N 09411763190)[a]
Sterile or disposable forceps or tweezers[b]
140 µL capillary (e.g., Vitrex plastic tube) with compatible dispenser (e.g., Vitrex pipette holder)[a]
Single Use lancing device (e.g. Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)[a]
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_09411763190** for more information)
Transport bag (e.g., Wicoseal 180 x 60 x 240 mm)
Pipette (e.g., Multistepper pipette)
Thermomixer (e.g., Eppendorf Thermomixer® model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (e.g., Greiner Bio-one Cryo.s™) with caps

[a] See **PSC Method Sheet ms_09411763190** for more information about the **PSC** sample collection.

[b] 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.

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

Glossary of simplified terms for the cobas® HIV-1 quantitative test

The following simplified terms are used, within the content of the instructions for use, to refer to some of the materials and reagents listed in this section.

Simplified term	**Material/Reagent**
H(+)C	**cobas**® HBV/HCV/HIV-1 High Positive Control
High positive control	**cobas**® HBV/HCV/HIV-1 High Positive Control
L(+)C	**cobas**® HBV/HCV/HIV-1 Low Positive Control
Low positive control	**cobas**® HBV/HCV/HIV-1 Low Positive Control
LYS 2	**cobas**® 4800 Lysis Buffer 2
Microwell plate	**cobas**® 4800 System AD (microwell) Plate 0.3 mL
MGP 2	**cobas**® 4800 MGP Reagent 2
MMX R1	**cobas**® Master Mix Reagent 1
MMX R2	**cobas**® HIV-1 Master Mix Reagent 2
Negative control	**cobas**® Negative Control
P 2	**cobas**® 4800 Protease 2
PSC	**cobas**® Plasma Separation Card
QS	**cobas**® RNA Quantitation Standard
SD 2	**cobas**® 4800 System Specimen Diluent 2
SPER	**cobas**® Specimen Pre-Extraction Reagent
WB	**cobas**® 4800 System Wash Buffer

Instrumentation and software required, but not provided, for the cobas® HIV-1 quantitative test

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.2.0 or higher
cobas® 4800 System **cobas**® HIV-1 AP v1.2.0 or higher

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

Supported sample tubes by the cobas® HIV-1 quantitative test

The **cobas**® HIV-1 quantitative test accepts commonly used primary and secondary tubes.

The following sample tubes are supported:

Primary tubes for plasma sample type

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

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

Secondary tubes for plasma sample type

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

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

Secondary tubes for PSC sample type

Nominal Diameter (mm)	SPER input volume (400 µL processing volume)
12.5	800 µL

For specific sample tube order information, contact your local Roche representative.

Sample tubes for **PSC** sample type can be loaded only with the 32-position sample carrier.

Precautions and handling requirements for the cobas® HIV-1 quantitative test

Warnings and precautions

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

- For in vitro diagnostic use only.
- **cobas**® HIV-1 quantitative test 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.[19,20] Only personnel proficient in handling biohazardous materials and the use of **cobas**® HIV-1 and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
- **cobas**® HBV/HCV/HIV-1 Control Kit contains plasma derived from human blood. The source material has been tested by a licensed antibody test and found to be non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg and antibody to HBc. Testing by PCR methods showed no detectable HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Refer to **PSC Method Sheet ms_09411763190** for additional warnings and precautions.
- Prevent exposure of MGP to sources of magnetic field.
- **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.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the appropriate **cobas**® 4800 System - User Assistance.

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

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the lab gloves.

- Wear eye protection, lab coats and disposable lab gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- Maintain a consistent temperature in the laboratory that conforms to the environmental specifications of the system, as provided in the **cobas**® 4800 System - User Assistance.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 and **cobas**® Specimen Pre-Extraction Reagent contain guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® HIV-1, **cobas**® 4800 Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® 4800 Lysis Buffer 2 or **cobas**® Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

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

Integrity

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

Disposal

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

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

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 and **cobas**® Specimen Pre-Extraction Reagent contain guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. Do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- If spillage of human-sourced material 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. FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas x** 480 instrument, follow the instructions in the appropriate **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or the **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or the **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - User Assistance.

Sample collection, transport, and storage

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

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

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

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

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.

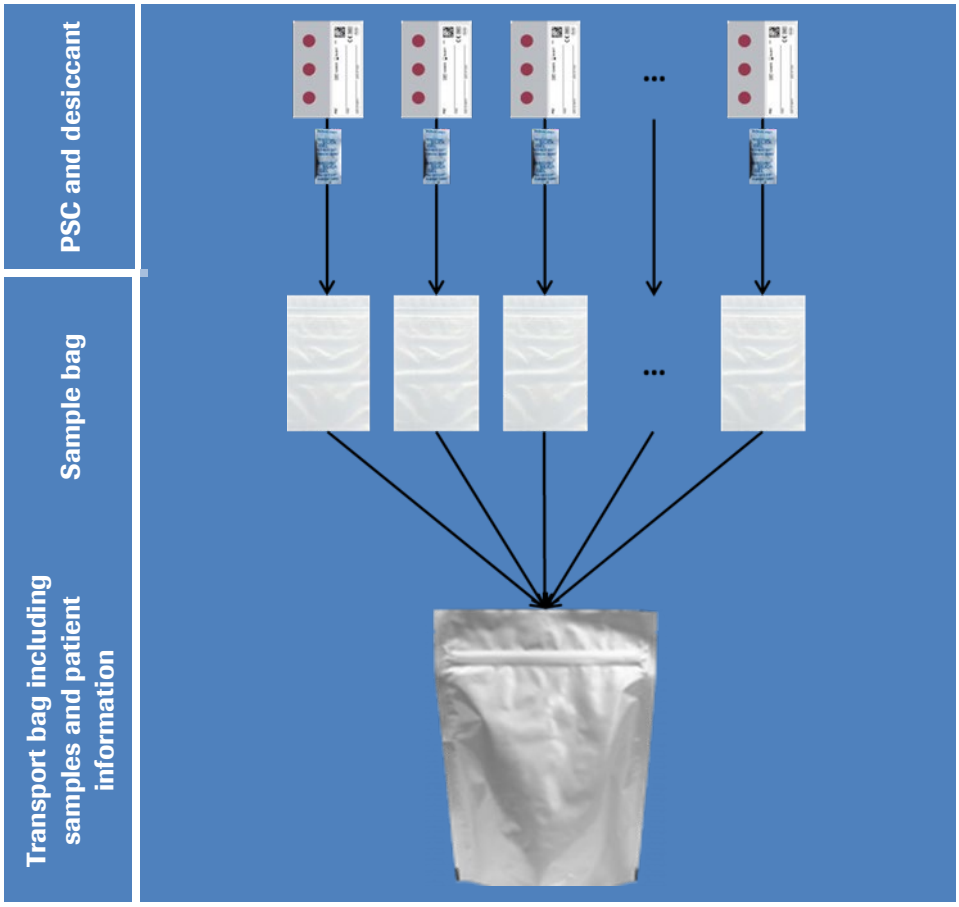
Note: The user must follow the guidance provided by the tube manufacturer for plasma preparation.

- Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation and subsequent testing.
- Plasma samples may be stored in secondary tubes for up to 24 hours at 2°C to 30°C, up to 72 hours at 2°C to 8°C or up to 6 weeks at ≤ -18°C. Separated plasma samples in secondary tubes are stable for up to three freeze/thaw cycles when stored frozen at ≤ -18°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

PSC dried plasma spot samples

- Collect **PSC** dried plasma spot samples using appropriate clinical procedures (refer to **PSC Method Sheet ms_09411763190**).
- 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 and date of birth, as well as with the date and time of sample collection.
- Perform a finger prick and 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 come 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 **PSC**s per transport bag (see Figure 1 for an overview).

Figure 1: Overview of the packaging concept for PSC transport



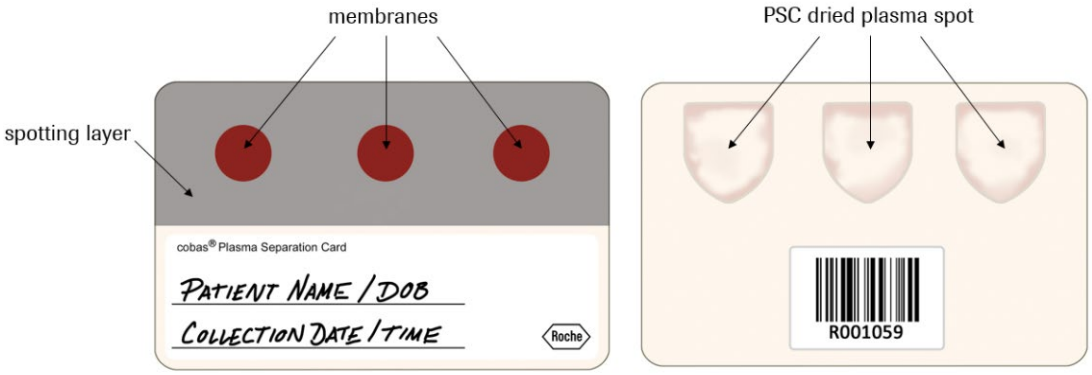
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents. **PSC**s may be transported for a period of 28 days before being analyzed at 18-45°C and up to 85% humidity.
- **PSC**s 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 ≤ -10°C for up to 56 days (with and without layer separation).

Instructions for use for the cobas® HIV-1 quantitative test

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 and transportation occurred in the past 28 days, and before the expiration date of the **PSC**.
  - 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 2).

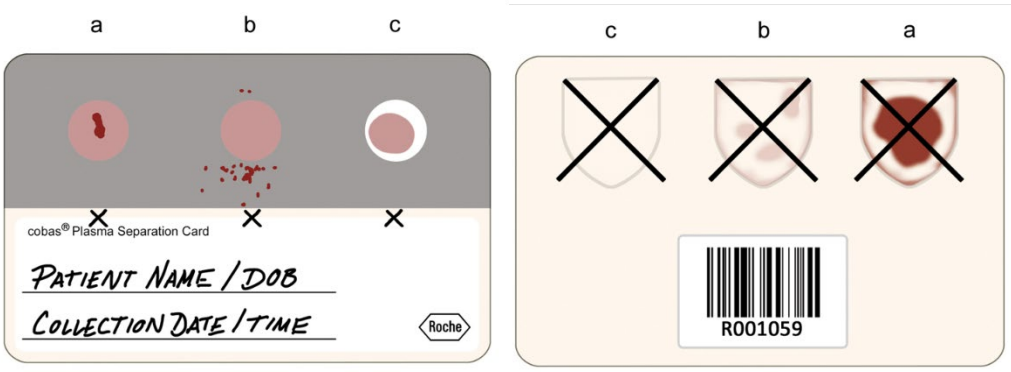
Figure 2: PSC dried plasma spots to be processed (left: front side, right: back side)



  - **PSC** dried plasma spots should be marked and rejected if:
    - § blood spills are visible (Figure 3b) or the membrane is not completely covered with blood (Figure 3c) 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 3a) 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 testing and 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 3: Rejection criteria for PSC spots (left: front side; right: back side). Spots with spills (b), not covered membrane (c) or visibly damaged membranes (a) should not be processed. Spots should be clearly marked when rejected.

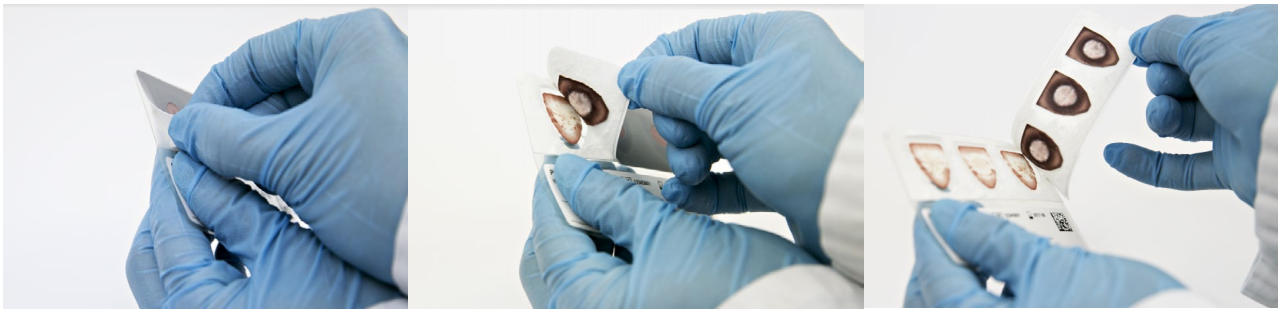


- Label a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Greiner Bio-one Cryo.s™]) for each **PSC** with its corresponding barcode of the laboratory request form (Figure 4) and place them into a rack. Transfer tubes into a laminar flow hood together with the sample bags containing the **PSC**s. Refer to the **cobas**® 4800 System - User Assistance for proper barcode labeling.

Figure 4: 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 5).

Figure 5: Removal of spotting layer



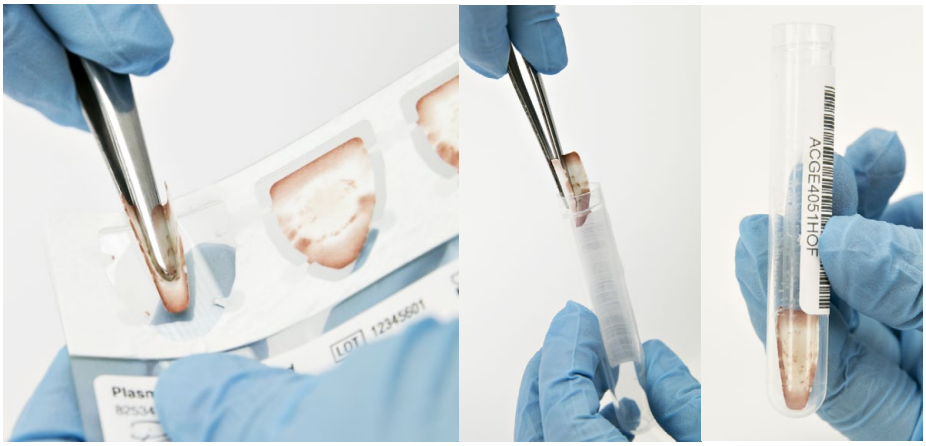
- Slightly bend the **PSC** and remove one dried plasma spot with sterile forceps or tweezers by pulling it up. Bend the removed dried plasma spot on the **PSC** to facilitate tube insertion (Figure 6). Use one pair of forceps or tweezers per patient.

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

Figure 6: 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 **PSC** dried plasma spot reaches the bottom of the tube and is attached to the tube wall to prevent pipetting errors (Figure 7). 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.

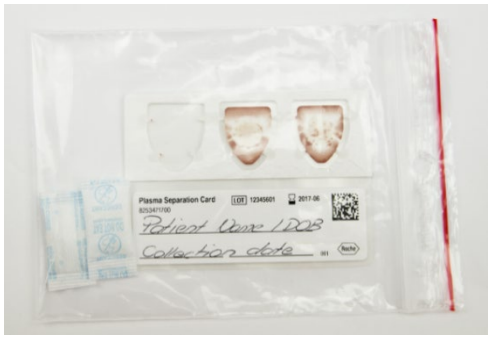
Figure 7: Transfer of the PSC dried plasma spot into the tube



- Place **PSC** with remaining dried plasma spots back to its original sample bag containing 4 grams of fresh desiccant for retesting, if required (Figure 8). **PSC**s 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 ≤ -10°C).

Note: Make sure not to re-use the desiccant from the original sample bag, but to use a fresh desiccant.

Figure 8: PSC in sample bag for potential retest



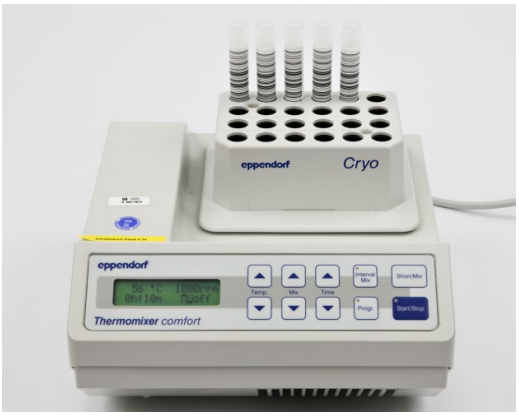
- Allow **cobas**® Specimen Pre-Extraction Reagent (SPER) to equilibrate to ambient temperature before use. Pipette 800 µL of SPER into the tubes containing the **PSC** dried plasma spots (Figure 9) and cap the tubes. Make sure the tubes are properly capped to prevent evaporation.

Note: Make sure to only use SPER and no different type of pre-extraction reagent (e.g., SPEX). It is important to pipette 800 µL of SPER (i.e., not more and not less than 800 µL) for correct assay performance.

Figure 9: 800 µL SPER addition

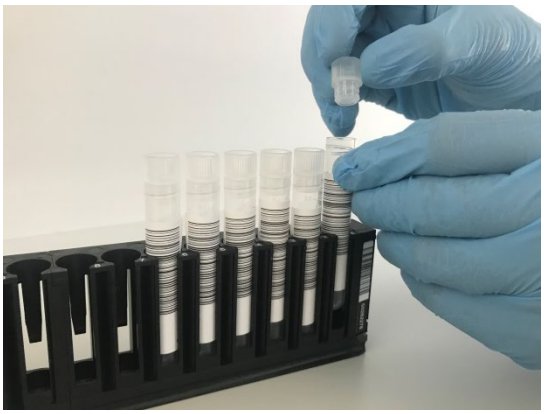
- Place capped tubes on a preheated Thermomixer (e.g., Eppendorf Thermomixer® model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes and incubate for 10 minutes, at 56°C and 1000 rpm to extract the virus from the dried plasma (Figure 10). Start the incubation right after the addition of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

Figure 10: Incubation



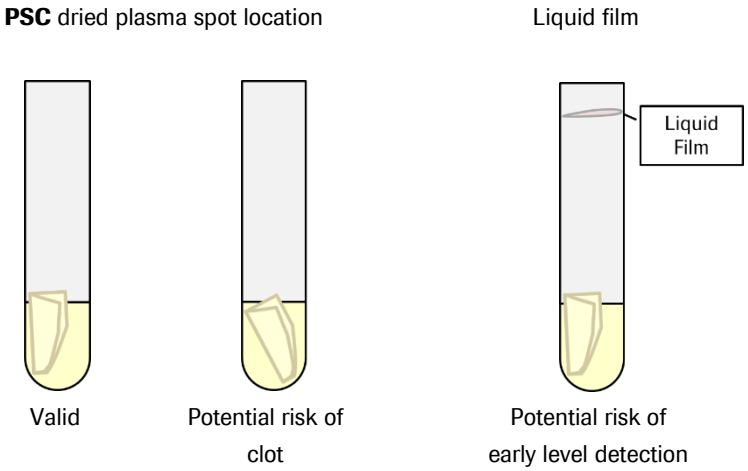
- Transfer the tubes onto a sample rack and uncap the tubes one by one to minimize cross-contamination (Figure 11). Change gloves after removing the caps.

Figure 11: **Uncapping of the tubes**



- Ensure the **PSC** dried plasma spot is correctly placed along the tube walls (Figure 12) 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**® 4800 System using the 32-position sample carrier only.

Figure 12: PSC dried plasma spot preparation before the analytic workflow



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

Running the cobas® HIV-1 quantitative test

Sample types and processing volume for the cobas® HIV-1 quantitative test

The **cobas**® HIV-1 quantitative test supports three sample types: plasma, diluted plasma and **PSC**. The processing volume for each of these sample types is as follows:

Supported sample types and processing volume

Sample type	Processing volume [µL]
Plasma	400
Diluted plasma[a]	200
PSC	400

[a] When the "diluted plasma" sample (specimen) type is chosen during the work order creation, the software will prompt the user to load the **cobas**® 4800 System Specimen Diluent 2 as an additional reagent during the reagent loading phase.

Run size for the cobas® HIV-1 quantitative test

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

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

Workflows for the cobas® HIV-1 quantitative test – single and mixed batch

The **cobas**® HIV-1 quantitative test is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer.

To start a **cobas**® HIV-1 quantitative test, the HIV-1 test type must be selected.

The HIV-1 quantitative test may be performed alone as a single batch or in mixed-batch mode with tests that share the same automated sample preparation process and PCR profile for amplification and detection. At the test selection step the software will display tests that are compatible with the HIV-1 quantitative test for mixed-batch mode.

Overviews of the HIV-1 quantitative workflows can be found as follows:

- HIV-1 quantitative test single batch is shown in Figure 13.
- HIV-1 quantitative test mixed batch with HIV-1 qualitative test (HIV-1-qual-DBS) is shown in Figure 14.
- Generic overview of mixed batch runs, including HIV-1 quantitative test with any applicable test, is available in the **cobas**® 4800 System - User Assistance.

Figure 13: Overview of HIV-1 quantitative test single batch – reagents and workflow

Reagents	
Generic sample preparation · **cobas**® 4800 System Sample Preparation Kit 2 o **MGP 2** o **EB 2** · **cobas**® 4800 System Lysis Kit 2 o **P 2** o **LYS 2** · **cobas**® 4800 System Wash Buffer Kit o **WB** For diluted plasma: · **cobas**® 4800 System Specimen Diluent 2 o **SD 2** For Plasma Separation Card (**PSC**): · **cobas**® Specimen Pre-Extraction Reagent o **SPER** Amplification / detection · **cobas**® HIV-1 o **MMx R1** o **HIV-1 MMx R2** o **RNA QS** Controls · **cobas**® HBV/HCV/HIV-1 Control Kit o **H(+)C** o **L(+)C** o **(–)C**	
Workflow	
1	Start the system
2	Perform instrument maintenance
3	Collect samples and reagents from storage
4	Start run and select HIV-1
5	Scan parameter cards
6	Load samples
7	Create work order: · With LIS: confirm work order · Without LIS: create work order
8	Load consumables
9	Load sample preparation reagents
10	Load AMP/DET reagents and controls
11	Start sample preparation run
12	Unload microwell plate
13	Seal microwell plate
14	Load sealed microwell plate into analyzer
15	Remove samples, used reagents, and deepwell plate
16	Review results · Optional: If LIS connected, send results to LIS
17	Unload analyzer

Figure 14: Overview of HIV-1 quantitative test mixed batch with HIV-1 qualitative test (HIV-1-qual-DBS) – reagents and workflow

Reagents	
Generic sample preparation · **cobas**® 4800 System Sample Preparation Kit 2 o **MGP 2** o **EB 2** · **cobas**® 4800 System Lysis Kit 2 o **P 2** o **LYS 2** · **cobas**® 4800 System Wash Buffer Kit o **WB** For diluted plasma: · **cobas**® 4800 System Specimen Diluent 2 o **SD 2** For Plasma Separation Card (**PSC**) and Dried Blood Spot (DBS): · **cobas**® Specimen Pre-Extraction Reagent o **SPER** Amplification / detection · **cobas**® HIV-1 o **MMx R1** o **HIV-1 MMx R2** o **RNA QS** Controls · **cobas**® HBV/HCV/HIV-1 Control Kit o **H(+)C** *Required for the HIV-1 quantitative test only* o **L(+)C** o **(–)C**	
Workflow	
1	Start the system
2	Perform instrument maintenance
3	Collect samples and reagents from storage
4	Start run and select HIV-1 and HIV-1-qual-DBS
5	Scan parameter cards for the HIV-1 *The same kits can be used for both the HIV-1 and HIV-1-qual-DBS.*
6	Load samples
7	Create work order: · With LIS: confirm work order · Without LIS: create work order
8	Load consumables
9	Load sample preparation reagents
10	Load AMP/DET reagents and controls *For the HIV-1:* · *The QS and the L(+)C, H(+)C and (-)C from the amplification/detection kit and the control kit, respectively, are required.* *For the HIV-1-qual-DBS:* · *The software wizard prompts the loading of QS, which is used as the Internal Control (IC).* · *The software wizard also prompts the loading of the L(+)C and (–)C from the control kit. The H(+)C is not required for the HIV-1-qual-DBS.*

11	Start sample preparation run
12	Unload microwell plate
13	Seal microwell plate
14	Load sealed microwell plate into analyzer
15	Remove samples, used reagents, and deepwell plate
16	Review results · Optional: If LIS connected, send results to LIS *In the results for the HIV-1-qual-DBS, the QS is reported as the IC and the L(+)C is reported as the positive control.*
17	Unload analyzer

The HIV-1 quantitative test single batch and the HIV-1 quantitative test mixed batch with the HIV-1 qualitative test (HIV-1-qual-DBS) workflows are described, respectively, in the following two sections.

For a detailed description of the HIV-1 qualitative test single batch workflow, refer to section Workflow single batch – HIV-1 qualitative test (HIV-1-qual-DBS).

Workflow single batch – HIV-1 quantitative test

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

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

4. Start a new run and select the HIV-1 test type.
5. Follow the software wizard guide and scan the barcode on the control ranges and calibration coefficients parameter cards.

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

6. Load the samples. Primary or secondary sample tubes can be loaded and minimum sample volume depends on the tube type and size. Refer to the supported sample tubes section for more details.

   Plasma and diluted plasma samples can be loaded with either the 24-position or 32-position sample carrier. **PSC** samples can be loaded only with the 32-position sample carrier.
7. Create the work order. There are three ways to create a work order:

   · By using the sample editor before sample rack is loaded into **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary. The specimen type must be selected as plasma, diluted plasma or **PSC**. After the specimen type has been selected, the requested result is automatically set as HIV-1.

- By following the software wizard for the new run, and loading samples into **cobas x** 480 instrument when prompted. The sample barcodes will be automatically scanned, and the specimen type and requested results for each specimen must be defined. When selecting the specimen type, select plasma, diluted plasma or **PSC** as applicable. After the specimen type has been selected, the requested result is automatically set as HIV-1.
- By using your institution's LIS system.

Note: Select correct specimen type. The software does not check that specimen type selection corresponds to the loaded sample. For example, if a PSC sample is loaded, make sure to select PSC specimen type (and not plasma or diluted plasma).

Refer to the **cobas**® 4800 System - User Assistance for more details. Load samples and define/select work order or use LIS as appropriate.

8. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
9. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir (MGP vial must be vortexed prior to dispensing)
   - Place the filled reagent reservoir into the designated position on the reagent carrier

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

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

10. Load amplification/detection reagent vials [MMX R2, MMX R1 and RNA QS], control vials [L(+)C, H(+)C and (–) C] and generic reagent vials [P 2 and SD 2 as required] directly onto the reagent carrier.

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

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

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

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

Workflow mixed batch – HIV-1 quantitative and qualitative (HIV-1-qual-DBS) tests

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

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

4. Start a new run and select the HIV-1 and HIV-1-qual-DBS test types.

   The HIV-1 test type represents the HIV-1 quantitative test and the HIV-1-qual-DBS represents the HIV-1 qualitative test.

   HIV-1, HIV-1-qual-DBS, CMV, HCV and HCV GT test types can be mixed batch. Up to four tests can be mixed batch in a single run.

   In this section, a mixed batch run including only HIV-1 and HIV-1-qual-DBS is described. Refer to the **cobas**® 4800 System - User Assistance for generic mixed batch workflows.

5. The HIV-1 and HIV-1-qual-DBS both use the amplification/detection and the HBV/HCV/HIV-1 control kits. In a mixed-batch run involving HIV-1 and HIV-1-qual-DBS, a single amplification/detection kit and a single control kit can be used. Control ranges and calibration coefficient parameters are required for the HIV-1. Follow the software wizard guide and scan the barcode on the control ranges and calibration coefficients parameter cards.

   If multiple kits of either the amplification/detection or the control kit are to be used for a mixed-batch HIV-1 and HIV-1-qual-DBS, ensure that the control range and calibration coefficient parameter cards are scanned for the reagents that will be used for the HIV-1. The cards from kit(s) used for the HIV-1-qual-DBS do not need to be scanned.

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

6. Load the samples. Primary or secondary sample tubes can be loaded and minimum sample volume depends on the tube type and size. Refer to the supported sample tubes section for more details.

   Plasma and diluted plasma samples can be loaded with either the 24-position or 32-position sample carrier. **PSC** and DBS samples can be loaded only with the 32-position sample carrier.

7. Create the work order. There are three ways to create a work order:

   - By using the sample editor before sample rack is loaded into **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary. The specimen type must be selected as plasma, diluted plasma, **PSC** or DBS as applicable. The requested result is then automatically set as HIV-1 for plasma, diluted plasma or **PSC**, and as HIV-1-qual-DBS for DBS.
   - By following the software wizard for the new run and loading samples into **cobas x** 480 instrument when prompted. The sample barcodes will be automatically scanned, and the specimen type and requested results for each specimen must be defined. When selecting the specimen type, select plasma, diluted plasma, **PSC** or DBS as applicable. After the specimen type is selected, the requested result is then automatically set as HIV-1 for plasma, diluted plasma or **PSC**, and as HIV-1-qual-DBS for DBS.
   - By using your institution's LIS system.

Note: Select correct specimen type. The software does not check that specimen type selection corresponds to the loaded sample. For example, if a PSC sample is loaded, make sure to select PSC specimen type (and not plasma, diluted plasma or DBS).

Refer to the **cobas**® 4800 System - User Assistance for more details. Load samples and define/select work order or use LIS as appropriate.

8. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
9. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir (MGP vial must be vortexed prior to dispensing)
   - Place the filled reagent reservoir into the designated position on the reagent carrier

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

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

10. Load amplification/detection reagent vials [MMX R2, MMX R1 and RNA QS], control vials [L(+)C, H(+)C and (–) C for the HIV-1, and L(+)C and (–) C for the HIV-1-qual-DBS] and generic reagent vials [P 2 and SD 2 as required] directly onto the reagent carrier.

    The HIV-1 and the HIV-1-qual-DBS each requires its own set of amplification/detection reagent vials and control vials, and each set can originate from the same kit or from different ones.

    For the HIV-1-qual-DBS, the software prompts the user to load QS. This QS is used as Internal Control (IC) and it is reported as IC in the results.

    Also for the HIV-1-qual-DBS, the software prompts the user to load L(+)C. This L(+)C is used as positive control and it is reported as positive control in the results. The H(+)C is not required for the HIV-1-qual-DBS.

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

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

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

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

Results from the cobas® HIV-1 quantitative test

The **cobas**® 4800 System automatically determines the HIV-1 RNA concentration for the samples and controls. The HIV-1 RNA concentration is expressed in copies per milliliter (cp/mL) or International Units per milliliter (IU/mL). The conversion factor for the **cobas**® HIV-1 Test is 0.6 cp/IU.

Quality control and validity of results from the cobas® HIV-1 quantitative test

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

Control result interpretation for the cobas® HIV-1 quantitative test

Table 1: Control result interpretation for negative and positive controls

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

Interpretation of results from the cobas® HIV-1 quantitative test

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

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

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

Table 2: Target results for individual target result interpretation

HIV-1	Result Report and Interpretation
Target Not Detected	HIV RNA not detected. Report results as "HIV not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HIV detected, less than (Titer Min)". Titer min = 2.00E+01 cp/mL and 3.33E+01 IU/mL (400 µL plasma) Titer min = 6.00E+01 cp/mL and 1.00E+02 IU/mL (200 µL diluted plasma) Titer min = 5.99E+02 cp/mL and 9.98E+02 IU/mL (**PSC** samples)
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as "(Titer) of HIV-1 detected".
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "HIV detected, greater than (Titer Max)". Titer max = 1.00E+07 cp/mL and 1.67E+07 IU/mL (400 µL plasma, 200 µL diluted plasma and **PSC** samples)
Invalid	Target is invalid, Internal control is invalid or negative; or An external control is invalid

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

List of result flags for the cobas® HIV-1 quantitative test

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

Table 3: List of flags

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

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

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

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

Procedural limitations

1. The **cobas**® HIV-1 quantitative test has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2, **cobas**® 4800 System Wash Buffer Kit and **cobas**® 4800 System Specimen Diluent 2.
2. Reliable results are dependent on adequate sample collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and **cobas**® 4800 System - User Assistance.
3. The **cobas**® HIV-1 quantitative test has been validated only for use with EDTA plasma or **cobas**® Plasma Separation Card (**PSC**) dried plasma spot. Testing of other sample types may result in inaccurate results.
4. Quantitation of HIV-1 RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
5. Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HIV-1, may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
6. The predictive value of an assay depends on the prevalence of the disease in any particular population.
7. The addition of AmpErase enzyme into the **cobas**® HIV-1 Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
8. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
9. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
10. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
11. Cross-contamination can cause false positive results. The sample-to-sample cross-contamination rate of **cobas**® HIV-1 has been determined in a non-clinical study to be 0.0%. Run to run cross-contamination has not been observed.
12. The **cobas**® HIV-1 quantitative test is not intended for use as a screening test for the presence of HIV-1 in blood or blood products.

Non-clinical performance evaluation for the cobas® HIV-1 quantitative test

Key performance characteristics for EDTA plasma samples

Limit of Detection (LoD) for EDTA plasma samples

The limit of detection of **cobas**® HIV-1 was determined by analysis of serial dilutions of the WHO International Standard for HIV-1 RNA for Nucleic Acid Amplification Technology Assays (2nd WHO International Standard) group M subtype B obtained from NIBSC, in HIV negative EDTA plasma using sample processing volumes of 400 µL and 200 µL. Panels of six concentration levels plus a negative were tested over three lots of **cobas**® HIV-1 reagents, multiple runs, days, operators, and instruments.

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

Table 4: Limit of detection in EDTA plasma (400 µL)

Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
60.0	100.0	252	252	100.0%
30.0	50.0	251	251	100.0%
20.0	33.3	252	247	98.0%
10.0	16.7	252	227	90.1%
5.0	8.3	252	160	63.5%
2.0	3.3	252	86	34.1%
0.0	0.0	71	0	0.0%
LoD by PROBIT at 95% hit rate		14.2 cp/mL; 95% confidence range: 12.5–16.6 cp/mL 23.7 IU/mL; 95% confidence range: 20.8–27.7 IU/mL		

Table 5: Limit of detection in EDTA plasma (200 µL)

Input titer concentration (HIV-1 RNA cp/mL)	Input titer concentration (HIV-1 RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
100.0	166.7	251	251	100.0%
60.0	100.0	251	249	99.2%
30.0	50.0	251	227	90.4%
15.0	25.0	250	172	68.8%
7.0	11.7	250	110	44.0%
3.5	5.8	250	83	33.2%
0.0	0.0	68	0	0.0%
LoD by PROBIT at 95% hit rate		43.9 cp/mL; 95% confidence range: 37.7–52.7 cp/mL 73.4 IU/mL; 95% confidence range: 62.9–88.1 IU/mL		

Linear range for EDTA plasma samples

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

With the 400 µL sample processing volume, **cobas**® HIV-1 was linear from 20.0 cp/mL to 1.00E+07 cp/mL (33.3 IU/mL to 1.67E+07 IU/mL) and showed a maximal deviation from the better fitting non-linear regression of less than ±0.07 $\log_{10}$ (see Figure 15). Across the linear range, the accuracy of the test was within ±0.18 $\log_{10}$.

With the 200 µL sample processing volume, **cobas**® HIV-1 was linear from 60.0 cp/mL to 1.00E+07 cp/mL (100.0 IU/mL to 1.67E+07 IU/mL) and showed a maximal deviation from the better fitting non-linear regression of less than ±0.08 $\log_{10}$ (see Figure 16). Across the linear range, the accuracy of the test was within ±0.19 $\log_{10}$.

Figure 15: Linear range determination in EDTA plasma (400 µL)

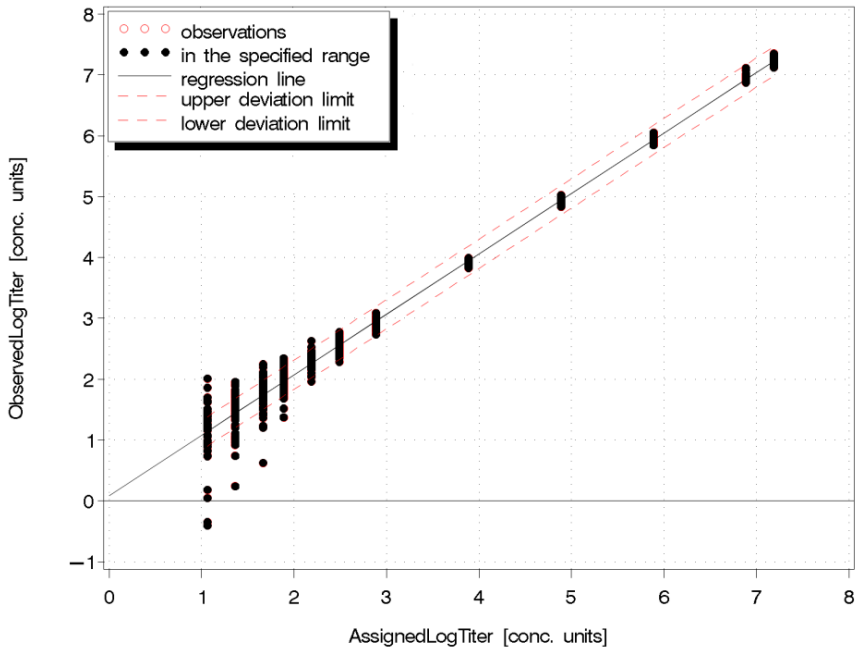
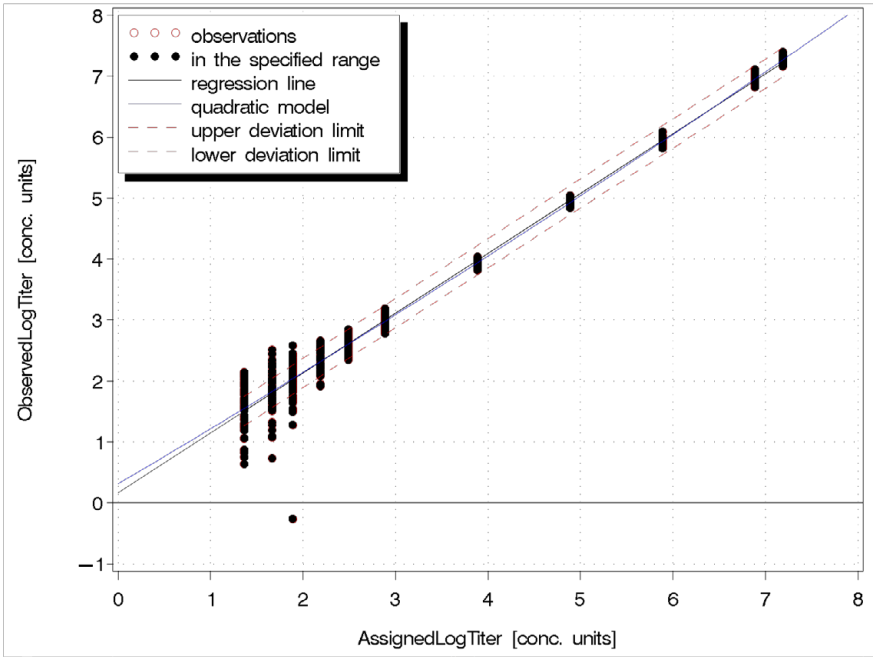


Figure 16: Linear range determination in EDTA plasma (200 µL)



Precision – within laboratory for EDTA plasma samples

Precision of **cobas**® HIV-1 was determined by analysis of serial dilutions of an HIV-1 high positive sample (Group M Subtype B; cultured virus) in HIV negative EDTA plasma. Seven dilution levels (400 µL sample processing volume) and five dilution levels (200 µL sample processing volume) were tested in 16 replicates for each level and sample processing volume across three lots of **cobas**® HIV-1 reagents using three instruments and four operators over 15 days. The results are shown in Table 6 and Table 7.

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

Table 6: Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – sample processing volume of 400 µL)[a]

Nominal concentration (cp/mL)	Assigned concentration (cp/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
2.0E+07	1.54E+07	Cell Culture	0.05	0.05	0.04	0.05
1.0E+06	7.70E+05	Cell Culture	0.05	0.05	0.05	0.05
1.0E+05	7.70E+04	Cell Culture	0.05	0.05	0.05	0.05
1.0E+04	7.70E+03	Cell Culture	0.04	0.06	0.07	0.06
1.0E+03	7.70E+02	Cell Culture	0.09	0.10	0.07	0.09
4.0E+02	3.08E+02	Cell Culture	0.08	0.10	0.09	0.09
1.0E+02	7.70E+01	Cell Culture	0.18	0.24	0.15	0.19

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

Table 7: Within laboratory precision of cobas® HIV-1 (EDTA plasma samples – sample processing volume of 200 µL)[a]

Nominal concentration (cp/mL)	Assigned concentration (cp/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
2.0E+07	1.54E+07	Cultured Virus	0.04	0.04	0.04	0.04
1.0E+07	7.70E+06	Cultured Virus	0.04	0.04	0.04	0.04
1.0E+06	7.70E+05	Cultured Virus	0.03	0.04	0.05	0.04
1.0E+05	7.70E+04	Cultured Virus	0.04	0.05	0.03	0.04
1.0E+04	7.70E+03	Cultured Virus	0.05	0.04	0.04	0.04

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

Group/subtype verification for EDTA plasma samples

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

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

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

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

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

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

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

The dilution series used in the verification of subtypes linearity study of **cobas**® HIV-1 consists of seven panel members spanning the linear range. Panel members were prepared from high titer HIV-1 RNA positive cell culture supernatant specimens of the respective group/subtype. The tested linear range of **cobas**® HIV-1 spanned from the LLoQ (20.0 cp/mL for a sample processing volume of 400 µL) to the ULoQ (1.0E+07 cp/mL) and included at least two medical decision points. Twelve replicates per level were tested in EDTA plasma.

The linear range of **cobas**® HIV-1 was verified for group M subtypes, group O and group N. The maximal deviation between the linear regression and the better fitting non-linear regression was less than 0.12 $\log_{10}$.

Specificity for EDTA plasma samples

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

Analytical specificity

The analytical specificity of **cobas**® HIV-1 was evaluated by diluting a panel of pathogens with HIV RNA positive and HIV RNA negative EDTA plasma (Table 9). The pathogens were added to negative EDTA plasma and tested with and without HIV RNA. None of the non-HIV pathogens interfered with test performance. Negative results were obtained with **cobas**® HIV-1 for all pathogen samples without HIV-1 target and positive results were obtained on all of the pathogen

samples with HIV-1 target. Furthermore, the mean log_{10} titer of each of the positive HIV-1 samples containing potentially cross-reacting organisms was within ± 0.32 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 9: Pathogens tested for cross-reactivity

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

Analytical specificity – interfering substances

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

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

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® HIV-1 for all samples without HIV target and positive results were obtained on all of the samples with HIV-1 target. Furthermore, the mean log_{10} titer of each of the positive HIV-1 samples containing potentially interfering substances was within ± 0.20 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 10: Drug compounds tested for interference with the quantitation of HIV RNA by cobas® HIV-1

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

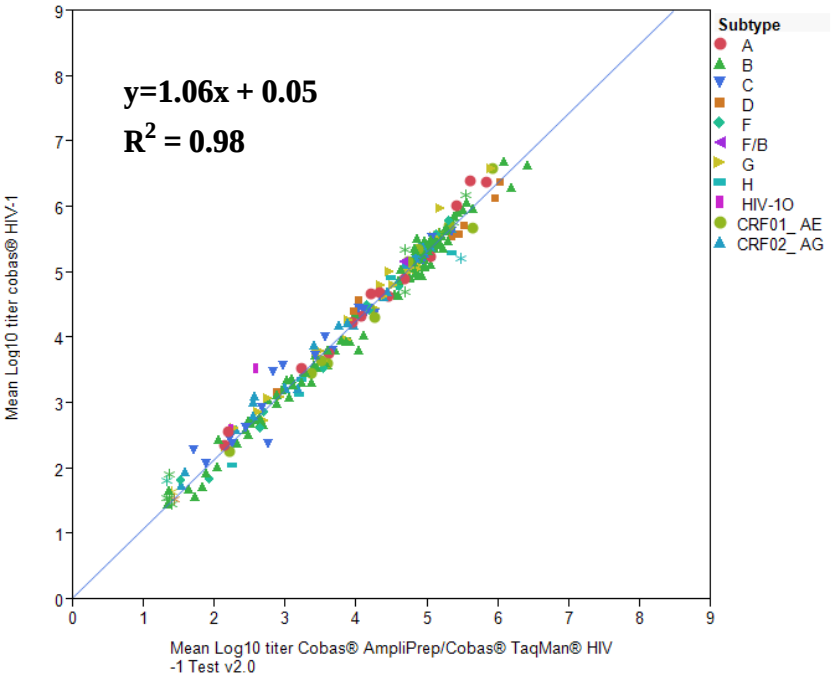
Method correlation for EDTA plasma samples

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

The performance of **cobas**® HIV-1 and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 (TaqMan® HIV-1 Test, v2.0) were compared by analysis of 243 EDTA plasma samples from patients infected with HIV-1. The samples comprised of HIV-1 M (A–D, F–H, F/B, CRF01_AE, CRF02_AG) and HIV-1 O were tested in duplicate at an external site. The Deming regression was performed considering log-transformed titers.

The Deming regression results are shown in Figure 17. The symbol * in the figures shows single determination. The color represents the subtype.

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



Whole system failure for EDTA plasma samples

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

The results of this study determined that all replicates were valid and positive for the HIV-1 target, resulting in a whole system failure rate of 0.0%. The two-sided 95% exact confidence interval was 0.0% for the lower bound and 3.6% for the upper bound [0%: 3.6%].

Cross contamination for EDTA plasma samples

The cross-contamination rate for **cobas**® HIV-1 was determined by testing 230 replicates of HIV negative EDTA-plasma samples and 235 replicates of a high titer HIV-1 sample at 1.9E+07 cp/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

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

Key performance characteristics for PSC dried plasma spot samples

Limit of Detection (LoD)

The 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 **PSC**s 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 11. The study demonstrates that **cobas**® HIV-1 in combination with the **PSC** detected HIV-1 RNA at a concentration of 598.6 cp/mL (997.7 IU/mL) with a hit rate of 95% as determined by Probit analysis.

Table 11: 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
2356.6	63	63	100.0%
1970.4	63	63	100.0%
2262.4	63	63	100.0%
1177.4	63	63	100.0%
984.5	63	62	98.4%
1130.3	63	63	100.0%
783.8	63	60	95.2%
655.3	63	63	100.0%
752.4	62	61	98.4%
391.9	63	58	92.1%
327.7	63	54	85.7%
376.2	62	41	66.1%
195.9	63	33	52.4%
163.8	63	32	50.8%
188.1	63	25	39.7%
0	144	1	0.7%
LoD by PROBIT at 95% hit rate	598.6 cp/mL; 95% confidence range: 526.4 –707.0 cp/mL 997.7 IU/mL; 95% confidence range: 877.3 – 1178.3 IU/mL		

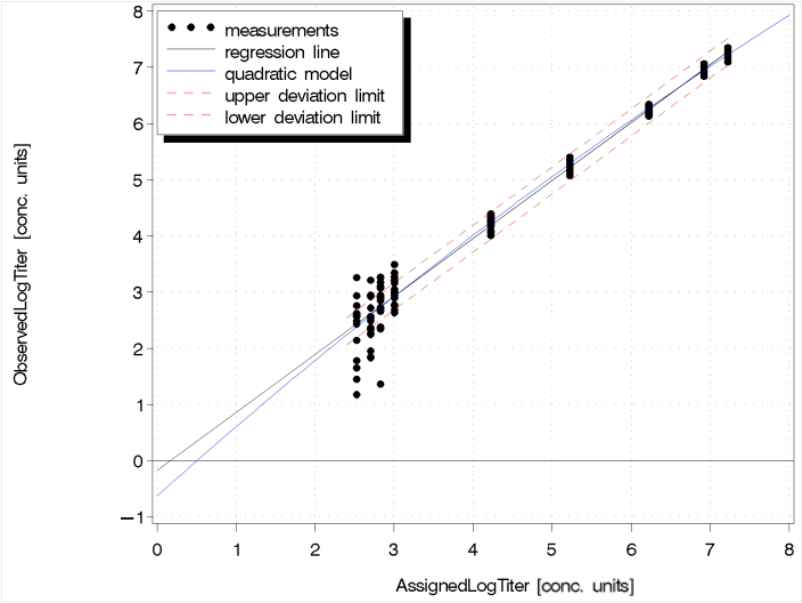
Linear range using the PSC

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.[21] Two **PSC** lots and two reagent lots were analyzed on two **cobas**® 4800 Systems, by one operator 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 599 cp/mL to 1.00E+07 cp/mL (998 IU/mL to 1.67E+07 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.07 $\log_{10}$ with the **PSC** (see Figure 18). Across the linear range, the accuracy of the test was within ± 0.04 $\log_{10}$ 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 whole blood. Five dilution levels were tested in 48 replicates for each level 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 **cobas**® 4800 Systems. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 12.

cobas® HIV-1 in combination with the **PSC** showed high precision for two lots of **PSC** and reagents tested across a concentration range of 1.50E+03 cp/mL to 1.00E+07 cp/mL.

Table 12: Within laboratory precision of cobas® HIV-1 in combination with the PSC

Nominal concentration (cp/mL)	Mean Observed Log_{10} Titer	Source material	PSC		
			Lot 1	Lot 2	All Lots
			SD	SD	Pooled SD
1.0E+07	6.98	Cell Culture	0.08	0.07	0.08
1.0E+06	5.92	Cell Culture	0.09	0.09	0.09
1.0E+05	4.88	Cell Culture	0.09	0.11	0.10
1.0E+04	3.97	Cell Culture	0.17	0.14	0.16
1.5E+03	3.19	Cell Culture	0.26	0.19	0.23

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 whole blood spiked with HIV-1 group M subtype B. These samples were tested at a target concentration of approximately 3 x LoD.

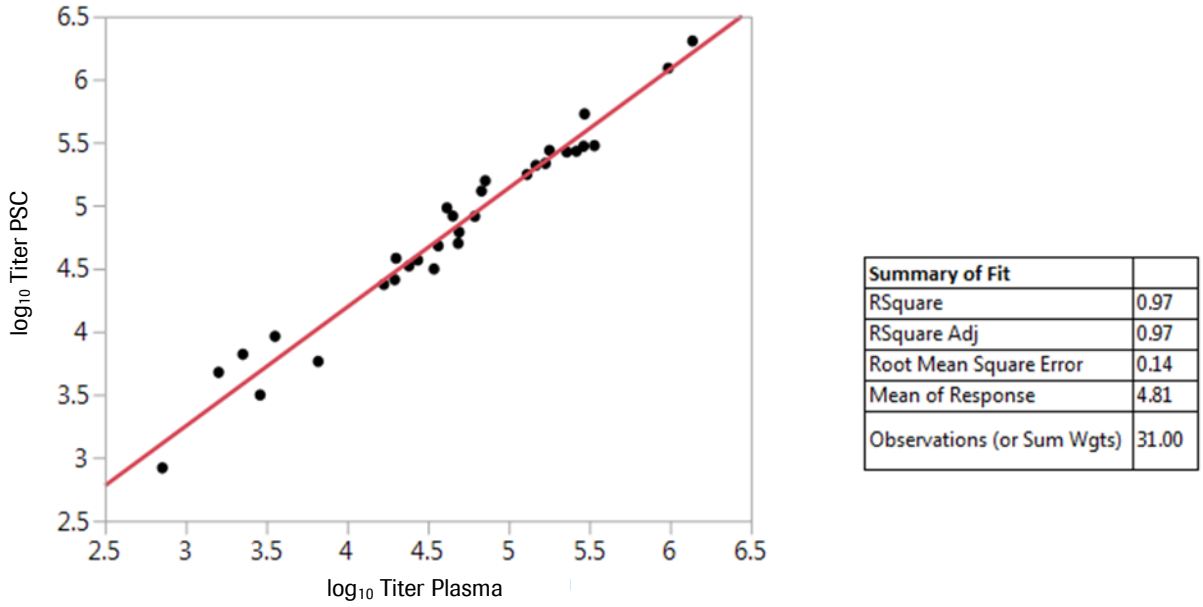
The results of this study determined that all replicates were valid and positive for the HIV-1 target, resulting in a whole system failure rate of 0.0%. The two-sided 95% exact confidence interval was 0.0% for the lower bound and 3.6% for the upper bound [0%: 3.6%].

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 31 specimens from patients infected with HIV-1. The specimens were tested one replicate each (**PSC** dried plasma spot and EDTA plasma). 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 and Table 13.

Figure 19: Deming regression



Summary of Fit	
RSquare	0.97
RSquare Adj	0.97
Root Mean Square Error	0.14
Mean of Response	4.81
Observations (or Sum Wgts)	31.00

Table 13: 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	31	0.15	[0.10; 0.21]	0.94	0.42	0.97

Summary and explanation of the cobas® HIV-1 for qualitative detection of HIV-1 in dried blood spots

Background

HIV is the etiologic agent of acquired immunodeficiency syndrome (AIDS) with over 35 million people infected[22] worldwide. After seroconversion, infected individuals typically enter a clinically stable, relatively asymptomatic phase that can last for years. Without antiretroviral treatment, individuals typically progress to AIDS, which is marked by immune system depletion of CD4+ cells, susceptibility to opportunity infections, and eventual death.[22]

Rationale for HIV-1 qualitative testing

Historically, HIV testing has been based on the antibody response that patients make to the virus. Although these antibodies are ineffective at combating the virus, they are found in almost all chronically infected patients.

Nucleic acid amplification tests can diagnose HIV infection during the first 18 months of life while the infant's blood still contains maternal antibodies that complicate the interpretation of serologic tests. Detection of HIV-1 in dried blood spots may provide evidence for current infection, using nucleic acid amplification technologies, such as the Polymerase Chain Reaction (PCR).

Explanation of the HIV-1 qualitative test

cobas® HIV-1 can be used as a qualitative nucleic acid test performed on the **cobas**® 4800. The **cobas**® HIV-1 qualitative test enables the detection of HIV-1 nucleic acid in DBS of infected patients. Two probes are used to detect, but not discriminate group M, N and O subtypes.

The RNA QS provided with this kit functions as an internal control to monitor the entire sample preparation and PCR amplification process in the context of the qualitative DBS application. In addition, the test utilizes two external controls: the low titer positive control and a negative control (the high positive control is not needed for **cobas**® HIV-1 qualitative test).

Principles of the procedure

The **cobas**® HIV-1 qualitative test is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 4800 System consists of the **cobas x** 480 sample preparation instrument and the **cobas z** 480 real-time PCR analyzer. Automated data management is performed by the **cobas**® 4800 software which assigns test results for all tests as detected, not detected, or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acids from patient samples, external controls and RNA QS molecules (which serve as the sample preparation and amplification/detection process control) are simultaneously extracted. In summary, viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature.

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

amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[14-16] Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step. However, any newly formed amplicon is not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

cobas® HIV-1 master mix contains two detection probes specific for the HIV-1 target sequences and one for RNA QS. The probes are labeled with target specific fluorescent reporter dyes allowing simultaneous detection of HIV-1 target and RNA QS in two different detection channels.[17,18]When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA QS, respectively.

Materials and reagents for the cobas® HIV-1 qualitative test

Reagents

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

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HIV-1 120 Tests (P/N: 08792992190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 x 1.75 mL	N/A
	HIV-1 MMX R2 (**cobas**® **HIV-1** Master Mix Reagent 2) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% HIV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HIV and the Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A
	RNA QS used as Internal Control IC (**cobas**® RNA Quantitation Standard) Tris buffer, < 0.05% EDTA, < 0.001% non-HIV related armored RNA construct containing primer and probe specific sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	10 x 1.75 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HBV/HCV/HIV-1 Control Kit 10 Sets (P/N: 06979572190)	**HBV/HCV/HIV-1 L(+)C used as positive control** (**cobas**® HBV/HCV/HIV-1 Low Positive Control) < 0.001% synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	  **WARNING** H317 May cause an allergic skin reaction. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 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 H(+)C not used for DBS (**cobas**® HBV/HCV/HIV-1 High Positive Control) < 0.001% synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	
	(–) C (**cobas**® Negative Control) Normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative[b]	10 x 0.75 mL	

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

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	   **DANGER** H302+H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. 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. 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 or doctor/ physician. P342 + P311 If experiencing respiratory symptoms: 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 39450-01-6 Proteinase, Tritirachium album serine
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 27 mL	

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	 **DANGER** H302+H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. 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. 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. P342 + P311 If experiencing respiratory symptoms: 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 39450-01-6 Proteinase, Tritirachium album serine
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 84 mL	

cobas® Specimen Pre-Extraction Reagent (P/N 08064695190)	**SPER** (**cobas**® Specimen Pre-Extraction Reagent) 28% (w/w) guanidine thiocyanate[b], 6% (w/v) polydocanol[b], 1% (w/v) dithiothreitol, dihydro sodium citrate	15 x 40 mL	 **DANGER** H302: Harmful if swallowed. 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. P273: Avoid release to the environment. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. P301 + P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. 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

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

Reagent storage and handling requirements

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

Do not freeze reagents.

Additional materials required

Materials	P/N
cobas® 4800 System Extraction (deepwell) Plate 2.0 mL	06884008001
cobas® 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Lab gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.
Sterile or disposable forceps or tweezers[a]	Any tweezers are acceptable.
Dried Blood Spot Card	Whatman 903® filter card, Munktell Specimen Collection card TFN or equivalent (12–13 mm spot diameter)
Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of ≥ 0.5 grams).	N/A
Pipette (e.g., Multistepper pipette)	N/A
Thermomixer	e.g., Eppendorf Thermomixer® model R 5355 or C or equivalent with Thermoblock for 24 cryo tubes
Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene with caps	e.g., Greiner Bio-one Cryo.s™

[a] 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 as well as single-use tweezers are recommended.

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

Glossary of simplified terms for the cobas® HIV-1 qualitative test

The following simplified terms are used, within the content of the instructions for use, to refer to some of the material and reagents listed in this section.

Simplified term	Material / Reagent
(-)C	**cobas**® Negative Control
Amplification/detection kit	**cobas**® HIV-1
Control kit	**cobas**® HBV/HCV/HIV-1 Control Kit
DBS	Dried blood spot
Deepwell plate	**cobas**® 4800 System Extraction (deepwell) Plate 2.0 mL
	cobas® 4800 Elution Buffer 2
HBV/HCV/HIV-1 control kit	**cobas**® HBV/HCV/HIV-1 Control Kit
H(+)C	**cobas**® HBV/HCV/HIV-1 High Positive Control
High positive control	**cobas**® HBV/HCV/HIV-1 High Positive Control
L(+)C	**cobas**® HBV/HCV/HIV-1 Low Positive Control
Low positive control	**cobas**® HBV/HCV/HIV-1 Low Positive Control
LYS 2	**cobas**® 4800 Lysis Buffer 2
Microwell plate	**cobas**® 4800 System AD (microwell) Plate 0.3 mL
MGP 2	**cobas**® 4800 MGP Reagent 2
MMX R1	**cobas**® Master Mix Reagent 1
MMX R2	**cobas**® **HIV-1** Master Mix Reagent 2
Negative control	**cobas**® Negative Control
P 2	**cobas**® 4800 Protease 2
PSC	**cobas**® Plasma Separation Card
QS (reported as IC)	**cobas**® RNA Quantitation Standard
SD 2	**cobas**® 4800 System Specimen Diluent 2
SPER	**cobas**® Specimen Pre-Extraction Reagent
WB	**cobas**® 4800 System Wash Buffer

Instrumentation and software required but not provided for the cobas® HIV-1 qualitative test

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.2.0 or higher
cobas® 4800 System **cobas**® HIV-1-qual-DBS AP v1.0.0 or higher

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

Supported sample tubes by the cobas® HIV-1 qualitative test

The following sample tubes are supported:

Secondary tubes for DBS sample type

Nominal Diameter (mm)	SPER input volume (400 µL processing volume)
12.5	700 µL

For specific sample tube order information, contact your local Roche representative.

Sample tubes for DBS sample type can be loaded only with the 32-position sample carrier.

Precautions and handling requirements for the cobas® HIV-1 qualitative test

Warnings and precautions

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

- For in vitro diagnostic use only.
- 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.[19,20] Only personnel proficient in handling biohazardous materials and the use of **cobas**® HIV-1-qual-DBS and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
- **cobas**® HBV/HCV/HIV-1 Control Kit contains plasma derived from human blood. The source material has been tested by a licensed antibody test and found to be non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg and antibody to HBc. Testing by PCR methods showed no detectable HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Prevent exposure of MGP to sources of magnetic field.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- **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.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the appropriate **cobas**® 4800 System - User Assistance.

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

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the lab gloves.
- Wear eye protection, lab coats and disposable lab gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- Maintain a consistent temperature in the laboratory that conforms to the environmental specifications of the system, as provided in the **cobas**® 4800 System - User Assistance.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 and **cobas**® Specimen Pre-Extraction Reagent contain guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® HIV-1, **cobas**® 4800 Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® 4800 Lysis Buffer 2 or **cobas**® Specimen Pre-Extraction Reagent, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

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

Integrity

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

Disposal

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

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

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 and **cobas**® Specimen Pre-Extraction Reagent contain guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. Do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- If spillage of human-sourced material 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 dried blood 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. FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas x** 480 instrument, follow the instructions in the appropriate **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or the **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or the **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - User Assistance.

Sample collection, transport, and storage

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

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

Dried blood spots

- Check the expiry date of the DBS card and only proceed if the card has not expired.
- Collect DBS samples using appropriate clinical procedures.
- It is recommended to apply a minimum of 70 µL of capillary blood inside each delineated circle on the DBS card.
- Ensure that BOTH sides of the paper are saturated and completely fill the delineated circle.
- Place DBS card on a drying rack (one card per level) or on a flat surface and allow the DBS card to dry at room temperature (18-25°C) for at least 4 hours (to maximum overnight), protecting the card from direct sunlight.
- For further details, consult package insert of filter cards used.
- It is recommended to prepare at least 3 paper disks per patient sample.
- Store DBS in individual resealable bags with a desiccant sachet in each bag and protected from sunlight.
- DBS may be transported or stored at 15-30°C for up to three months.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use for the cobas® HIV-1 qualitative test

DBS dried blood spot sample preparation and pre-analytic procedure

- Open the transport bag and, for each sample bag, proceed only if:
  - The laboratory request form is completely filled out.
  - The barcodes of the laboratory request form and the DBS card match.
  - The sample bag is completely closed and contains a DBS card with a desiccant sachet.
  - The sample collection date is available, and the sample collection occurred before the expiration date of the DBS card.
- Allow **cobas**® Specimen Pre-Extraction Reagent (SPER) to equilibrate to ambient temperature before use.

Note: Make sure to only use SPER and no different type of pre-extraction reagent (e.g., SPEX).

- Excise one DBS from the DBS card.
- Transfer the spot into a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Cryo.s™]) using a new set of sterile or disposable forceps or tweezers for each patient.
- Ensure the DBS is located at the bottom of the tube as shown in Figure 20.
- Pipette 700 µL of SPER into the tube containing the DBS and cap the tube.

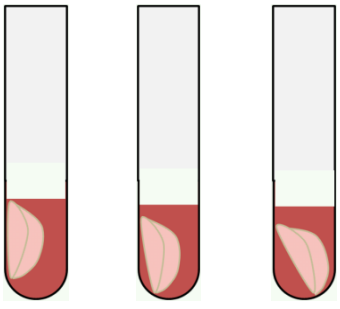
Note: It is important to pipette 700 µL of SPER (i.e., not more and not less than 700 µL) for correct assay performance.

- Ensure the DBS is completely covered with SPER.
- Place capped tubes on a preheated Thermomixer (e.g., Eppendorf Thermomixer® 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 whole blood.
- Uncap the tubes and ensure the DBS is attached to the tube wall (Figure 21) to avoid sample clots.
- Eliminate any potential liquid film located above the liquid level (Figure 22) using a sterile pipette tip (to avoid early level detection).
- Load the tubes onto the **cobas**® 4800 Systems.

Figure 20: DBS in tube

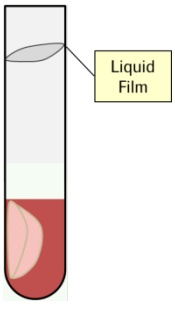
Valid

Figure 21: DBS location



Valid Valid Potential risk of clot

Figure 22: Liquid film



Potential risk of early level detection

Running the cobas® HIV-1 qualitative test

Sample type and processing volume for the cobas® HIV-1 qualitative test

The **cobas**® HIV-1 qualitative test supports one single sample type: DBS. The processing volume is as follows:

Supported sample type and processing volume

Sample type	Processing volume [µL]
DBS	400

Run size for the cobas® HIV-1 qualitative test

The generic sample preparation reagents (**cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit) are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and samples to be run. **cobas**® HIV-1 is available in a single kit size sufficient to test up to 120 (10×12) samples, including controls and samples. For each test batch, one HBV/HCV/HIV-1 Low Positive Control and one Negative Control must be used (HBV/HCV/HIV-1 High Positive Control is not needed for DBS). For a single test run, the maximum number of samples allowed is 94 specimens and 2 controls.

Note: For optimal use of reagents, the generic sample preparation reagents can be used for a run containing 1-22 total samples (10×24 test kit size) or 1-94 total samples (10×96 test kit size). However, different kit sizes of the cobas® 4800 System Wash Buffer Kit, cobas® 4800 System Sample Preparation Kit 2 and cobas® 4800 System Lysis Kit 2 cannot be combined. For example, if a 96-test Wash Buffer reagent bottle is scanned at the start of the run, 96-test size reagents from the other sample preparation reagent kits must also be used.

Workflows for the cobas® HIV-1 qualitative test

The **cobas**® HIV-1 qualitative test is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer.

To start a **cobas**® HIV-1 qualitative test, the HIV-1-qual-DBS test type must be selected.

The HIV-1 qualitative test may be performed alone as a single batch or in mixed-batch mode with tests that share the same automated sample preparation process and PCR profile for amplification and detection. At the test selection step the software will display tests that are compatible with the HIV-1 qualitative test (HIV-1-qual-DBS) for mixed-batch mode.

Overviews of the HIV-1 qualitative workflows can be found as follows:

- HIV-1 qualitative test single batch is shown in Figure 23.
- HIV-1 qualitative test mixed batch with HIV-1 quantitative test is shown in Figure 14.
- Generic overview of mixed batch runs, including HIV-1 qualitative test with any applicable test, is available in the **cobas**® 4800 System - User Assistance.

Figure 23: Overview of HIV-1 qualitative test (HIV-1-qual-DBS) single batch – reagents and workflow

Reagents	
Generic sample preparation · **cobas**® 4800 System Sample Preparation Kit 2 o **MGP 2** o **EB 2** · **cobas**® 4800 System Lysis Kit 2 o **P 2** o **LYS 2** · **cobas**® 4800 System Wash Buffer Kit o **WB** For Dried Blood Spot (DBS): · **cobas**® Specimen Pre-Extraction Reagent o **SPER** Amplification / detection · **cobas**® HIV-1 o **MMx R1** o **HIV-1 MMx R2** o **RNA QS** *Reported as IC* Controls · **cobas**® HBV/HCV/HIV-1 Control Kit o **L(+)C** *Reported as Positive Control* o **(–)C**	
Workflow	
1	Start the system
2	Perform instrument maintenance
3	Collect samples and reagents from storage
4	Start run and select HIV-1-qual-DBS
5	Load samples
6	Create work order: · With LIS: confirm work order · Without LIS: create work order
7	Load consumables
8	Load sample preparation reagents
9	Load AMP/DET reagents and controls · *The software wizard prompts the loading of QS, which is used as the Internal Control (IC).* · *The software wizard also prompts the loading of the L(+)C and (–)C from the control kit. The H(+)C is not required for the HIV-1-qual-DBS.*
10	Start sample preparation run
11	Unload microwell plate
12	Seal microwell plate
13	Load sealed microwell plate into analyzer
14	Remove samples, used reagents, and deepwell plate
15	Review results · Optional: If LIS is connected, send results to LIS *In the results, the QS is reported as the IC and the L(+)C is reported as the positive control.*
16	Unload analyzer

The HIV-1 qualitative test (HIV-1-qual-DBS) single batch workflow is described in detail in the following section.

The HIV-1 qualitative test (HIV-1-qual-DBS) mixed batch with HIV-1 quantitative test workflow is described in detail in the Workflow mixed batch – HIV-1 quantitative and qualitative (HIV-1-qual-DBS) tests section.

For a complete description of the HIV-1 quantitative test single batch workflow, refer to section Workflow single batch – HIV-1 quantitative test.

Workflow single batch – HIV-1 qualitative test (HIV-1-qual-DBS)

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

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

4. Start a new run and select the HIV-1-qual-DBS test type.
5. Load the DBS samples. Refer to the supported sample tubes section for sample tube details.

   DBS samples can be loaded only with the 32-position sample carrier.
6. Create the work order. There are three ways to create a work order:

- By using the sample editor before sample rack is loaded into **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary. Specimen type and requested result are automatically set as DBS and HIV-1-qual-DBS, respectively.
- By following the software wizard for the new run and loading samples into **cobas x** 480 instrument when prompted. The sample barcodes are automatically scanned, and the specimen type and requested result are automatically set as DBS and HIV-1-qual-DBS, respectively.
- By using your institution's LIS system.

  Refer to the **cobas**® 4800 System - User Assistance for more details. Load samples and define/select work order or use LIS as appropriate.

7. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
8. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir (MGP vial must be vortexed prior to dispensing)
   - Place the filled reagent reservoir into the designated position on the reagent carrier

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

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

9. Load amplification/detection reagent vials [MMX R2, MMX R1 and RNA QS], control vials [L(+)C and (–) C] and generic reagent vials [P2] directly onto the reagent carrier.

   The software prompts the user to load QS. This QS is used as Internal Control (IC) and it is reported as IC in the results.

   The software prompts the user to load L(+)C. This L(+)C is used as positive control and it is reported as positive control in the results. The H(+)C is not required for the HIV-1-qual-DBS.

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

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

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

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

Results from the cobas® HIV-1 qualitative test

The **cobas**® 4800 System detects HIV-1 in samples and controls and provides a qualitative result, showing a detected or not detected result for HIV-1 total nucleic acids.

The low positive control and the QS loaded during the HIV-1-qual-DBS workflow are used and reported as positive control and IC, respectively.

In case there is a need to identify the kit lot number of the positive control or IC, the corresponding kit lot number can be found under the labels Low positive control and RNA Quantitation Standard, respectively.

Quality control and validity of results from the cobas® HIV-1 qualitative test

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

Control result interpretation for the cobas® HIV-1 qualitative test

Table 14: Control result interpretation for negative and positive controls

Control	Result	Interpretation
(–) C	Valid	Control is valid. HIV-1 not detected.
	Invalid	The negative control is invalid.
HBV/HCV/HIV-1 L(+)C reported as Positive Control	Valid	Control is valid.
	Invalid	The positive control is invalid.

Interpretation of results from the cobas® HIV-1 qualitative test

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

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

For a valid batch, sample results are interpreted as shown in Table 15.

Table 15: Target results for individual target result interpretation

HIV-1-qual-DBS	Result Report and Interpretation
Detected	HIV-1 detected. Ct value
Not detected	HIV-1 not detected.
Invalid	Target is invalid, Target is invalid or negative and IC is invalid or negative; or An external control is invalid

Note: Specimen Ct values, if available, are reported in the result report and also exported to the LIS.

List of result flags for the cobas® HIV-1 qualitative test

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

Table 16: List of flags

Flag code	Description	Recommended action
R20	Positive control is invalid	Positive control values are invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R21	Negative control is invalid	Negative control values are invalid. To avoid carryover, use Good Laboratory Practice. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4807	Specimen target is invalid	The target is invalid due to calculation failure. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4808	Specimen internal control is invalid or negative and the specimen target is invalid or negative	Internal control values, of an invalid or negative specimen target, are invalid. 1. Rerun the sample with fresh reagents. 2. If the problem persists, contact Roche Service.
X3	Error: Clot was detected. Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

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

Procedural limitations

1. The **cobas**® HIV-1 qualitative test has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit.
2. Reliable results are dependent on adequate sample collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and **cobas**® 4800 System - User Assistance.
3. The **cobas**® HIV-1 qualitative test has been validated only for use with DBS. Testing of other sample types may result in inaccurate results.
4. 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 failure to detect the presence of virus.
5. The predictive value of an assay depends on the prevalence of the disease in any particular population.
6. The addition of AmpErase enzyme into the **cobas**® HIV-1 Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
7. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
8. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
9. 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.
10. Cross-contamination can cause false positive results. The sample-to-sample cross-contamination rate of **cobas**® HIV-1 has been determined in a non-clinical study to be 0.0% with an upper one-sided 95% confidence interval of 1.3% (Clopper Pearson). Run to run cross-contamination has not been observed.

Non-clinical performance evaluation for the cobas® HIV-1 qualitative test

Key performance characteristics for DBS samples

Limit of Detection (LoD) using DBS

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 (3rd WHO International Standard) group M subtype B obtained from NIBSC, in HIV negative whole blood applied on dried blood spot cards. Panels of five concentration levels plus a negative were tested over three lots of **cobas**® HIV-1 reagents, two different DBS card types, multiple runs, days, operators, and instruments.

The combined results for dried blood spots are shown in Table 17. The study demonstrates that **cobas**® HIV-1 detected HIV-1 RNA with a hit rate of ≥ 95%, as determined by PROBIT, at a concentration of 325.1 cp/mL (541.8 IU/mL) in dried blood spots.

Table 17: Limit of detection using dried blood spots

Input titer concentration (HIV-1 RNA cp/mL)	Number of valid replicates	Number of positives	Hit rate
969	63	63	100.0%
	63	63	100.0%
	62	62	100.0%
484.5	63	63	100.0%
	62	59	95.2%
	63	63	100.0%
323	63	61	96.8%
	63	61	96.8%
	63	59	93.7%
161.5	63	50	79.4%
	61	44	72.1%
	63	45	71.4%
80.75	63	28	44.4%
	63	32	50.8%
	63	26	41.3%
0	48	0	0.0%
	48	0	0.0%
	48	0	0.0%
LoD by PROBIT at 95% hit rate	325.1 cp/mL; 95% confidence range: 284.1 – 385.8 cp/mL 541.8 IU/mL; 95% confidence range: 473.5 – 643.0 IU/mL		

Repeatability using DBS

Repeatability of **cobas**® HIV-1 was determined by analysis of serial dilutions of Roche HIV-1 Secondary Standard in HIV negative whole blood applied on dried blood spot cards. Three dilution levels were tested in 2 within-run replicates for each level, across two types of dried blood spot cards and two lots of **cobas**® HIV-1 reagents using two instruments and two operators over 12 days. The results are shown in Table 18.

cobas® HIV-1 showed high repeatability for two types of dried blood spot cards and two lots of reagents tested at three concentration levels.

Table 18: Repeatability of cobas® HIV-1 using DBS

Concentration	Lot	Reactive / Valid Replicates	Hit Rate [%]	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
~3 x LoD	1	24/24	100.0%	85.8%	100.0%
	2	24/24	100.0%	85.8%	100.0%
~1 x LoD	1	21/24	87.5%	67.6%	97.3%
	2	21/24	87.5%	67.6%	97.3%
~0.6 x LoD	1	17/24	70.8%	48.9%	87.4%
	2	21/24	87.5%	67.6%	97.3%

Specificity using DBS

The specificity of **cobas**® HIV-1 was determined by analyzing HIV negative dried blood spot samples from individual donors. A total of 620 individual DBS samples were tested with two lots of the **cobas**® HIV-1 reagents and two types of DBS cards. All samples tested were found to be non-reactive for HIV-1 RNA. Based on these results, the specificity of **cobas**® HIV-1 using DBS was 100.0% [the lower bound of 95% one sided confidence interval (Clopper Pearson) was 99.5%].

Correlation using DBS

The performance of **cobas**® HIV-1 was compared to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 by analysis of clinical early infant DBS samples at one external site. For HIV-1 positive and HIV-1 negative DBS samples, a total percent agreement of 100% between the two tests was shown, demonstrating that the performance of **cobas**® HIV-1 and COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is equivalent (Table 19).

Table 19: Summary of results for correlation for HIV-1 DBS samples

Correlation **HIV-1 DBS samples**		**COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0**	
		Reactive	Non-reactive
cobas® HIV-1	Reactive	99	0
	Non-reactive	0	129
Total Percentage of Agreement		100% (95% CI: 98 – 100%)	

These results in a clinical sensitivity of 100% (with a two-sided 95% confidence interval of 96 – 100%) and clinical specificity of 100% (with a two-sided 95% confidence interval of 97 – 100%).

Whole system failure using DBS

The whole system failure rate for **cobas**® HIV-1 using DBS was determined by testing 100 replicates of 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 0.0% for DBS. The two-sided 95% exact confidence interval was 0.0% for the lower bound and 3.6% for the upper bound [0%: 3.6%].

Additional information

Key assay features for EDTA plasma samples

Sample type	EDTA plasma
Sample processing volume	400 µL or 200 µL
Analytical sensitivity	14.2 cp/mL (400µL)
	43.9 cp/mL (200 µL)
Linear range	400 µL: 20.0 cp/mL – 1.0E+07 cp/mL
	200 µL: 60.0 cp/mL – 1.0E+07 cp/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%)
Genotypes detected	HIV-1M (A–D, F–H, CRF01_AE, CRF02_AG), HIV-1O, HIV-1N

Key assay 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	400 µL
Analytical sensitivity	598.6 cp/mL
Linear range	599 cp/mL – 1.0E+07 cp/mL

Key assay features for DBS samples

Sample type	Dried Blood Spots
Minimum amount of sample required	70 µL whole blood
Sample processing volume	400 µL
Analytical sensitivity	325.1 cp/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%)

Workflow quick guide

	Single Batch		Mixed Batch
	HIV-1 quantitative – Plasma – Diluted plasma – PSC	**HIV-1 qualitative** – DBS	**HIV-1 quantitative** **HIV-1 qualitative**
1	Start the system		
2	Perform instrument maintenance		
3	Remove samples and reagents from storage		
4	Start run and select test type:		
	HIV-1	**HIV-1-qual-DBS**	**HIV-1** **HIV-1-qual-DBS**
5	Scan parameter cards (AVC) – MMx calibration coefficients – Control ranges	Parameter cards are not needed	Scan parameter cards (AVC) – MMx calibration coefficients – Control ranges
6	Load samples		
7	Create work order		
8	Load consumables		
9	Load sample preparation reagents		
10	Load AMP/DET reagents and controls:		
	– MMx1 & 2 + QS – L(+)C, H(+)C & (-)C – Generic reagents	– MMx1 & 2 + QS – L(+)C & (-)C – Generic reagents	– MMx1 & 2 + QS – L(+)C, H(+)C & (-)C – Generic reagents
11	Start sample preparation run		
12	Unload microwell plate		
13	Seal microwell plate		
14	Load sealed microwell plate into analyzer		
15	Remove samples, used reagents, and deepwell plate		
16	Review results		
17	Unload analyzer		

For the HIV-1 qualitative test (HIV-1-qual-DBS) the QS is reported as IC, the L(+)C as PC, and the H(+)C is not required.

- Details on single batch mode workflows
  - HIV-1 quantitative test workflow (See Workflow single batch – HIV-1 quantitative test section)
  - HIV-1 qualitative test workflow (HIV-1-qual-DBS) (See Workflow single batch – HIV-1 qualitative test (HIV-1-qual-DBS) section)
- Details on HIV-1 mixed batch workflow (See Workflow mixed batch – HIV-1 quantitative and qualitative (HIV-1-qual-DBS) tests section)

Symbols

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

Table 20: Symbols used in labeling for Roche PCR diagnostic products

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

Technical support

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

Manufacturer

Table 21: Manufacturer

Manufactured in the United States

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

Made in USA

Trademarks and patents

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

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

Vacutainer® is a registered trademark of Becton Dickinson & Company.

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

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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.

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Document revision

Document Revision Information	
Doc Rev. 4.0 01/2022	Updated reference to **PSC Method Sheet ms_09411763190** to reflect new M/N of PSC card. Updated Figure 2 and Figure 3 to be compliant to PSC ms_09411763190. Updated the harmonized symbol page. Added **Technical support** section. Added Made in statement. Removed distributors addresses. Updated **Trademarks and patents** section. Please contact your local Roche Representative if you have any questions.