

## WHO Prequalification of Diagnostics Programme PUBLIC REPORT

**Product: COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test,  
version 2.0 (TaqMan 96)  
Number: PQDx 0200-046-00**

### Abstract

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) with product codes 06693083190, 03587797190, 06989861190, 03051315001, 03121453001, 28127387001, 05807875001 and 03516440001, manufactured by Roche Diagnostics GmbH, CE-marked regulatory version, was accepted for the WHO list of prequalified diagnostics and was listed on 15 December 2014.

This public report was amended on 26 January 2015 to reflect a change to the legal manufacturer.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 is an in vitro diagnostic, total nucleic acid amplification test for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) DNA and RNA (or total nucleic acid, TNA) in human plasma or dried blood spots using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 96 Analyzer for automated amplification and detection.

The test is a diagnostic test, indicated for individuals who are suspected to be actively infected with HIV-1. Detection of HIV-1 TNA is indicative of active HIV infection. Infants born to mothers infected with HIV-1 may have maternal antibodies to HIV-1, and the presence of HIV-1 nucleic acid in the infant indicates active HIV-1 infection. In adults, the test may be used as an aid in the diagnosis of HIV-1 infection.

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is an in vitro diagnostic, total nucleic acid amplification test for the qualitative detection of HIV-1 in human EDTA plasma or dried blood spots (DBS) in combination with the COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent (SPEX). The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is based on three major processes: (1) specimen preparation to isolate HIV-1 nucleic acids; (2) reverse transcription of target RNA to generate complementary DNA (cDNA), and (3) PCR amplification of target DNA and cDNA, and simultaneous detection of cleaved dual-labeled oligonucleotide detection probes specific to the target and Internal Control (IC). The workflow is automated using the

COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 96 Analyzer. For DBS specimens, a manual pre-extraction step is required.

In order to perform the assay, the following components are required:

**Reagents:**

- COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0, 06693083190
- COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent 03587797190
- COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent 06989861190

**Instrumentation:**

- COBAS® TaqMan® 96 Analyzer, 03121453001
- COBAS® AmpliPrep Instrument, 03051315001

**Software and Hardware:**

- AMPLILINK Software, Version 3.3 Series or higher, 05807875001
- XP Data Station for AMPLILINK S/W with Printer, 03516440001

**Optional:**

- cobas p 630 instrument, 05527503001

**Disposables (Not provided):**

- Sample processing units (SPUs), 03755525001
- Sample input tubes (S-tubes) with barcode clips, 03137040001
- Racks of K-tips 03287343001
- K-tube (Box of 12 x 96), 03137082001
- Sample rack (SK 24 rack), 028122172001
- Reagent rack, 028122199001
- SPU rack, 05471664001
- K-tube capper, motorized, 03516539001
- K-tube capper, 03339874001
- K-carrier, 03341488001
- K-carrier transporter, 03517519001
- K-carrier rack, 03286436001

Other materials required but not provided:

- XP Data Station for the AMPLILINK S/W with printer, 03516440001
- Docking Station Short, 28127387001
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1000 µL)
- Disposable gloves, powderless

- Vortex mixer

**Storage:**

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0, 06693083190, should be stored at 2°C to 8°C.

COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent, 03587797190, should be stored at 2°C to 30°C.

COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent, 06989861190, should be stored at 2°C to 8°C.

**Shelf-life:**

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0, 06693083190: 22 months

COBAS AmpliPrep/COBAS TaqMan Wash Reagent, 03587797190: 24 months

COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent 06989861190: 12 months

**Summary of prequalification status for COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96)**

	Initial acceptance	
	Date	Outcome
<b>PQ amended</b>	26 January 2016	amended
<b>Status on PQ list</b>	15 December 2014	listed
<b>Dossier assessment</b>	28 November 2014	MR
<b>Inspection status</b>	04 September 2014	MR
<b>Laboratory evaluation</b>	FT	MR

MR: Meets Requirements

NA: Not Applicable

FT: Fast tracked

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

## **Background information**

The Manufacturer submitted an application for prequalification of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96). Based on the established prioritization criteria, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) was given priority for prequalification.

### **Product dossier assessment**

The Manufacturer, submitted a product dossier for COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) as per the Instructions for compilation of a product dossier (PQDx\_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx\_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) for prequalification.

### **Manufacturing site inspection**

A comprehensive inspection was performed at the site of manufacture (Branchburg, New Jersey, USA) of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Quantitative Test, version 2.0 (TaqMan 96), in September 2011 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx\_014 v1). The inspection found that the Manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

A quality management documentation review was performed in September 2014, and it was established that the Manufacturer continuously implemented a quality management system in compliance with ISO 13485:2003 and that no significant changes were implemented since the first inspection.

### **Laboratory evaluation**

Given the regulatory version of the product submitted for prequalification and the quality of the data submitted as part of the product dossier to support the claims for its intended use, the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 (TaqMan 96) assay has been found eligible to undergo the WHO fast track procedure. Subsequently, the product will not be required to undergo a laboratory evaluation.

## **Labelling**

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

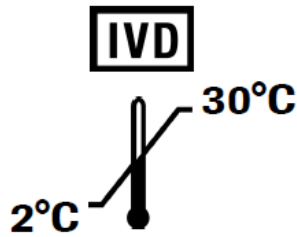
1. Labels

4 Pcs.

06693083190

# HIV-1 (-) C, v2.0 Clip

## HIV-1 v2.0 – COBAS® AmpliPrep/COBAS® TaqMan® Tests



KIT **LOT**

CTL **LOT**

Manufactured in the United States



Roche Diagnostics GmbH  
 Sandhofer Straße 116  
 68305 Mannheim, Germany

**Print Form**

07114460001-03


**cobas**<sup>®</sup> 07113412001

**CAP/CTM**  
**(-)C, v2.0**

1.0 mL


**2-8°C**

**IVD**



H317

Manufactured in the United States  
 Roche Diagnostics GmbH  
 68305 Mannheim, Germany

**LOT** 

07113382001-03

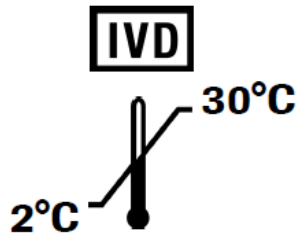
← 5/16" x 35/64"  
unvarnished  
area

4 Pcs.

06693083190

# HIV-1 (+) C, v2.0 Clip

## HIV-1 – COBAS® AmpliPrep/COBAS® TaqMan® Tests



KIT **LOT**

CTL **LOT**

Manufactured in the United States



Roche Diagnostics GmbH  
 Sandhofer Straße 116  
 68305 Mannheim, Germany

Print Form

07114478001-03

**cobas**<sup>®</sup> 07002408001


**HIV-1 (+)C, v2.0**

1.0 mL


**2-8°C**

**IVD**

Manufactured in the United States  
 Roche Diagnostics GmbH  
 68305 Mannheim, Germany

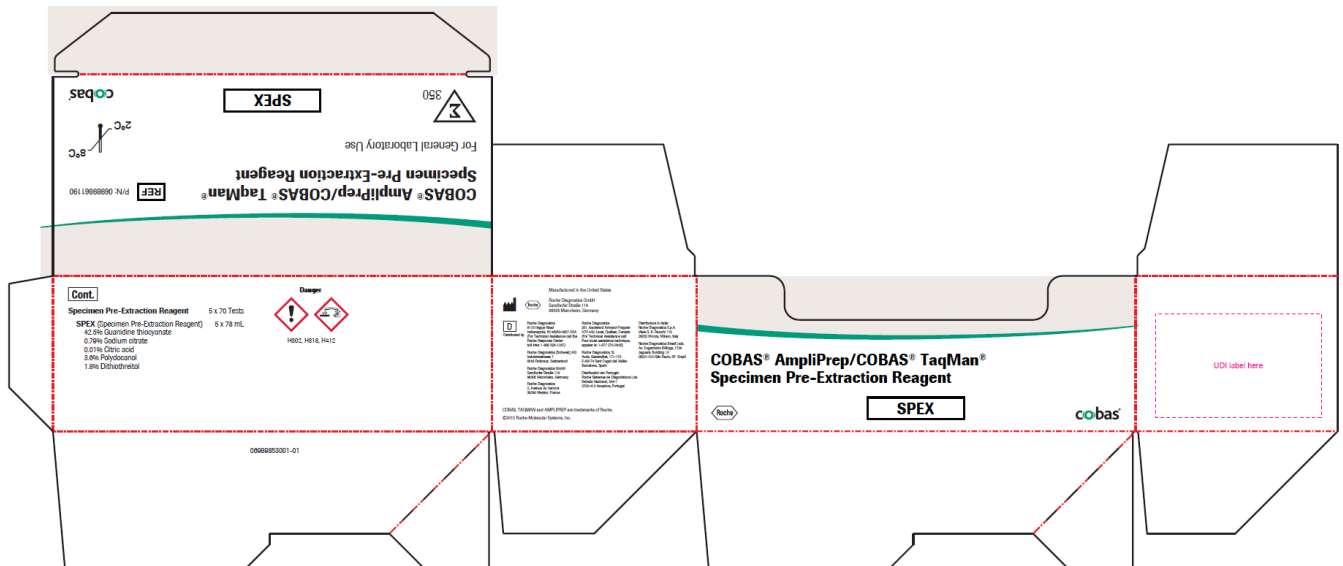
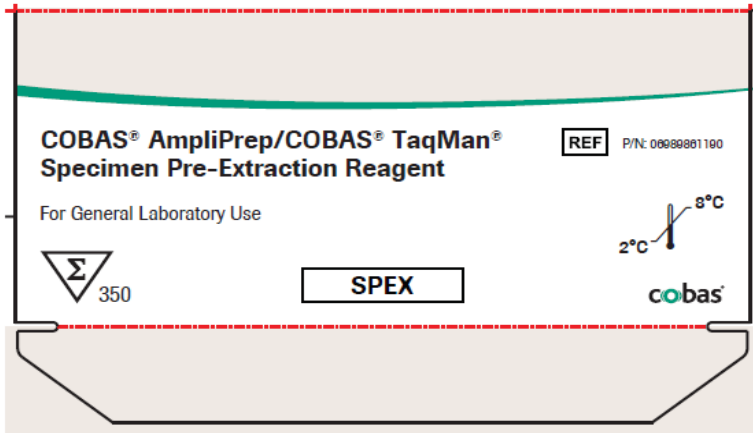
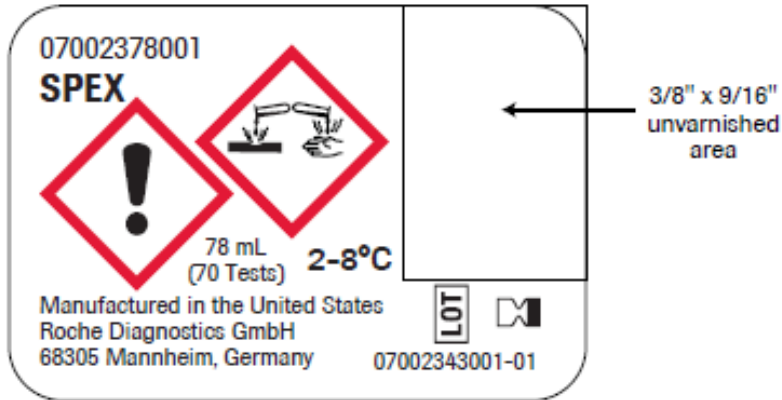


H317

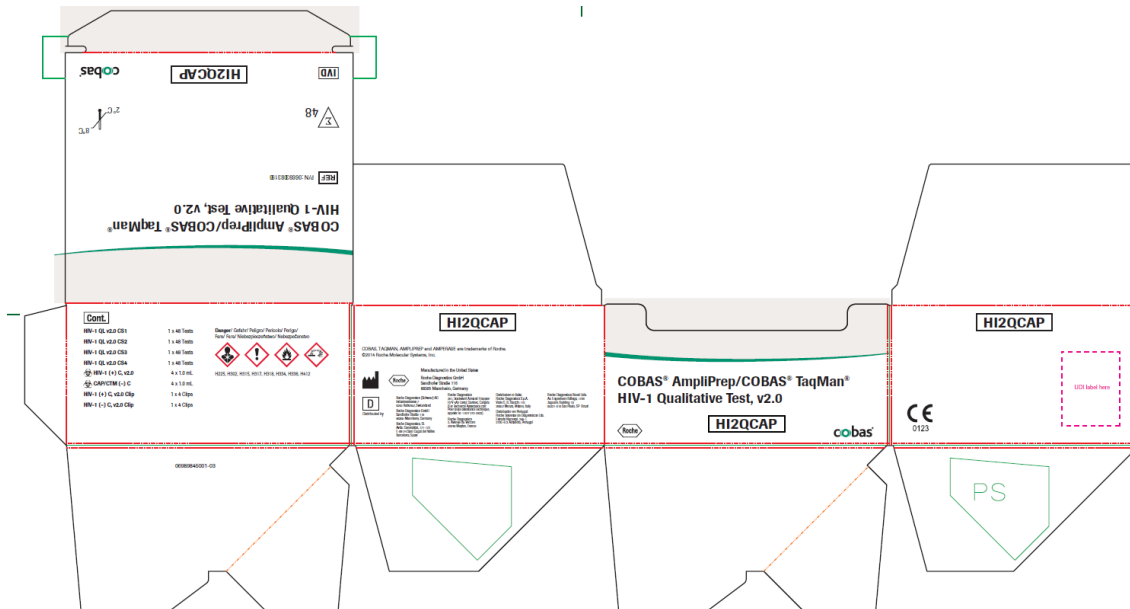
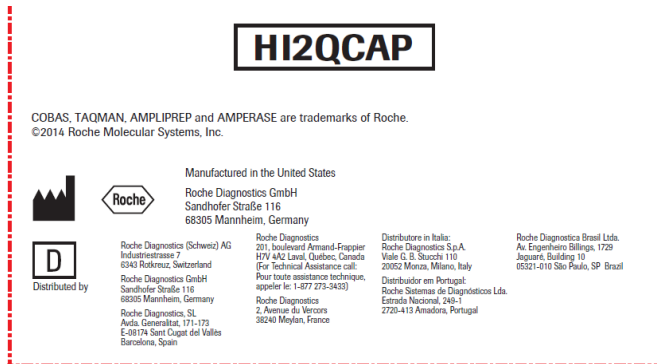
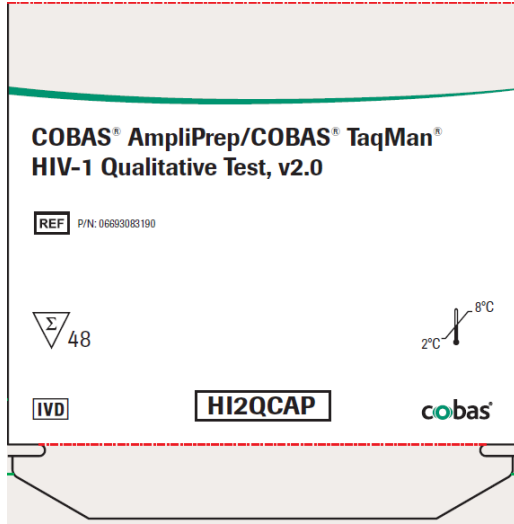
**LOT** 

06989900001-03


← 5/16" x 35/64" unvarnished area







HIV-1 QL v2.0 CS2

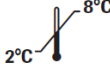


**HIV-1 QL v2.0 CS2** **48 Tests**

CAP/CTM HIV-1 Qual Test, v2.0


06693083190

IVD



**Cont.**  
LYS 78 mL


**Danger/ Gefahr/ Peligro/ Pericolo/ Perigo/**  
**Fare/ Fara/ Niebezpieczeństwo/ Nebezpečnostvo**



42.5% (w/w, Gew.-%, m/m, p/p, v/v, V/V)  
Guanidine thiocyanate/ Guanidinthiocyanat/  
Thiocyanate de guanidine/ Tiocianato di guanidina/  
Tiocianato de guanidina/ Guanidintiocyanat/  
Tiocyanian guanidyny/ Guanidin tiocyanat


H302, H315, H318, H412

Manufactured in the United States

 Roche Diagnostics GmbH  
Sandhofer Straße 116  
68305 Mannheim, Germany

07003340001-03

HIV-1 QL v2.0 CS2

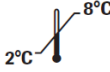


**HIV-1 QL v2.0 CS2** **48 Tests**

CAP/CTM HIV-1 Qual Test, v2.0


06693083190

IVD



**Cont.**  
LYS 78 mL


**Danger/ Gefahr/ Peligro/ Pericolo/ Perigo/**  
**Fare/ Fara/ Niebezpieczeństwo/ Nebezpečnostvo**



42.5% (w/w, Gew.-%, m/m, p/p, v/v, V/V)  
Guanidine thiocyanate/ Guanidinthiocyanat/  
Thiocyanate de guanidine/ Tiocianato di guanidina/  
Tiocianato de guanidina/ Guanidintiocyanat/  
Tiocyanian guanidyny/ Guanidin tiocyanat


H302, H315, H318, H412

Manufactured in the United States

 Roche Diagnostics GmbH  
Sandhofer Straße 116  
68305 Mannheim, Germany

07003340001-03

HIV-1 QL v2.0 CS3

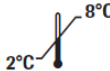


**HIV-1 QL v2.0 CS3** **48 Tests**

CAP/CTM HIV-1 Qual Test, v2.0


06693083190

IVD



**Cont.**  
B: Pase 3.8 mL  
A: \_\_\_\_\_  
C: EB 7.0 mL


**Danger/ Gefahr/ Peligro/ Pericolo/ Perigo/**  
**Fare/ Fara/ Niebezpieczeństwo/ Nebezpečnostvo**



≤ 7.8% (w/w, Gew.-%, m/m, p/p, v/v, V/V)  
Proteinase/ Protéinase/ Proteinasi/ Proteinasa/  
Proteinasa/ Proteinaza/ Proteináza


H318, H334

Manufactured in the United States

 Roche Diagnostics GmbH  
Sandhofer Straße 116  
68305 Mannheim, Germany

07003358001-03

HIV-1 QL v2.0 CS4

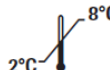


**HIV-1 QL v2.0 CS4** **48 Tests**

CAP/CTM HIV-1 Qual Test, v2.0


06693083190

IVD



**Cont.**  
B: HIV-1 QS 3.6 mL  
A: CAP/CTM Mn<sup>2+</sup> 19.8 mL  
C: HIV-1 MMX 2.5 mL

Manufactured in the United States

 Roche Diagnostics GmbH  
Sandhofer Straße 116  
68305 Mannheim, Germany

07003374001-03

**COBAS® AmpliPrep/COBAS® TaqMan®  
HIV-1 Qualitative Test, v2.0**

**HI2QCAP**

KIT **LOT**

**IVD**

**LOT**

**LOT**

HIV-1 QL v2.0 CS1

CAP/CTM (-) C

HIV-1 QL v2.0 CS2

HIV-1 (+) C, v2.0

HIV-1 QL v2.0 CS3

HIV-1 (-) C, v2.0 Clip

HIV-1 QL v2.0 CS4

HIV-1 (+) C, v2.0 Clip



**COBAS® TaqMan® 48 Analyzer**

HI2QLP48 TDF v1.0  
(AMPLILINK Software v3.3, v3.4)

**REF** 06989977190

HI2QLD48 TDF v1.0  
(AMPLILINK Software v3.3, v3.4)

**REF** 06989934190

**COBAS® TaqMan® Analyzer**

HI2QLP96 TDF v1.0  
(AMPLILINK Software v3.3, v3.4)

**REF** 07003030190

HI2QLD96 TDF v1.0  
(AMPLILINK Software v3.3, v3.4)

**REF** 06989969190



website: <http://e-labdoc.roche.com>

Method Sheet Catalog No.: 06693083190  
Doc Rev. 4.0

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Manufactured in the United States



Roche Diagnostics GmbH  
Sandhofer Strasse 116  
68305 Mannheim, Germany

**Print Form**

07114451001-04

## 2. Instructions for use

# COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0	<b>HI2QCAP</b>	48 Tests	P/N: 06693083 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	<b>PG WR</b>	5.1 Liters	P/N: 03587797 190
COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent	<b>SPEX</b>	5 x 78 mL	P/N: 06989861 190

### INTENDED USE

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, version 2.0 is an *in vitro* diagnostic, total nucleic acid amplification test for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) DNA and RNA (or total nucleic acid, TNA) in human plasma or dried blood spots using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

The test is a diagnostic test, indicated for individuals who are suspected to be actively infected with HIV-1. Detection of HIV-1 TNA is indicative of active HIV infection. Infants born to mothers infected with HIV-1 may have maternal antibodies to HIV-1, and the presence of HIV-1 nucleic acid in the infant indicates active HIV-1 infection. In adults, the test may be used as an aid in the diagnosis of HIV-1 infection.

### SUMMARY AND EXPLANATION OF THE TEST

Human Immunodeficiency Virus (HIV) is the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS)<sup>1-3</sup>. HIV infection can be transmitted by sexual contact, exposure to infected blood or blood products, or by an infected mother to the fetus<sup>4</sup>. Within three to six weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms and associated with high levels of viremia<sup>5-8</sup>. In most infected individuals this is followed by an HIV-specific immune response and a decline of plasma viremia, usually within four to six weeks of the onset of symptoms<sup>9,10</sup>. After seroconversion, infected individuals typically enter a clinically stable, asymptomatic phase that can last for years<sup>11-13</sup>. The asymptomatic period is characterized by persistent, low level plasma viremia<sup>14</sup> and a gradual depletion of CD4<sup>+</sup> T lymphocytes, leading to severe immunodeficiency, multiple opportunistic infections, malignancies and death<sup>15</sup>. 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<sup>+</sup> cells are balanced by equally high rates of virus clearance, death of infected cells and replenishment of CD4<sup>+</sup> cells, resulting in relatively stable levels of both plasma viremia and CD4<sup>+</sup> cells<sup>16-18</sup>.

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<sup>42</sup>.

Detection of HIV-1 in plasma and dried blood spots may provide evidence for current infection, using nucleic acid amplification technologies, such as the Polymerase Chain Reaction (PCR)<sup>27,28</sup>. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (CAP/CTM HIV-1 Qual Test, v2) uses PCR technology to achieve maximum sensitivity for the qualitative detection of HIV-1 in EDTA anti-coagulated plasma and dried blood spots from whole blood.

### PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is an *in vitro* diagnostic, total nucleic acid amplification test for the qualitative detection of HIV-1 in human EDTA plasma or dried blood spots (DBS) in combination with the COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent (SPEX).

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is based on three major processes: (1) specimen preparation to isolate HIV-1 nucleic acids; (2) reverse transcription of target RNA to generate complementary DNA (cDNA), and (3) PCR amplification of target DNA and cDNA, and simultaneous detection of cleaved dual-labeled oligonucleotide detection probes specific to the target and Internal Control (IC). The workflow is automated using the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer. For DBS specimens, a manual pre-extraction step is required.

### Target Selection

Selection of the target sequence for HIV-1 depends on identification of regions within the HIV-1 genome that show maximum sequence conservation among the various HIV-1 group M subtypes and HIV-1 group N and O specimens. In order to address the high genetic variability of the virus, two regions of HIV genome are simultaneously targeted for amplification and detection by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0.

*The Document Revision Information section is located at the end of this document.*

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Doc Rev. 4.0



Two target-specific and one IC-specific dual-labeled oligonucleotide probes permit independent identification of the HIV-1 amplicon and of the HIV-1 IC amplicon. Accordingly, the appropriate selection of the primers and the dual-labeled oligonucleotide probes is critical to the ability of the test to amplify and detect the HIV-1 group M subtypes and HIV-1 group O. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 uses reverse transcription and PCR amplification primers that define sequences within the highly conserved regions of the HIV-1 *gag* gene<sup>32</sup> and of the HIV-1 LTR region.

### Specimen Preparation

#### Pre-Analytical Procedure for Dried Blood Spots

Dried blood spots (DBS) are prepared by spotting 70 µL of human whole blood onto a paper filter card, and allowing it to dry. Prior to preparing the nucleic acid material for amplification and detection, the samples are extracted from the paper filter. This extraction is executed manually, by punching out a circle of the filter paper card with the dried blood spot into a COBAS® AmpliPrep S-tube, adding COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent (SPEX), and incubating at an elevated temperature with mixing. The samples are then ready for automated specimen preparation on the COBAS® AmpliPrep Instrument. See *INSTRUCTIONS FOR USE* for the detailed workflow.

#### Automated Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. Cells and virus particles are lysed by incubation at elevated temperature with a chaotropic lysis/binding buffer that releases nucleic acids and protects the released RNA and DNA from nucleases in the specimen. Magnetic glass particles along with the lysis reagent and a known quantity of HIV-1 Internal Control (IC, Armored RNA molecules) are introduced into each specimen tube. Subsequently, the mixture is incubated and the nucleic acids from the specimen and HIV-1 IC RNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the magnetic glass particles as well as released HIV-1 nucleic acids and HIV-1 IC RNA, is added to the amplification mixture by the COBAS® AmpliPrep Instrument, and transferred to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

### Reverse Transcription and PCR Amplification

The reverse transcription and PCR amplification reaction is performed with the thermostable recombinant enzyme *Thermus specie* Z05 DNA Polymerase (Z05). In the presence of manganese ( $Mn^{2+}$ ) and under the appropriate buffer conditions, Z05 has both reverse transcriptase and DNA polymerase activity<sup>30,31</sup>. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow the downstream primers to anneal specifically to the HIV-1 target RNA and to the HIV-1 IC RNA. In the presence of  $Mn^{2+}$  and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, Z05 polymerase extends the annealed primers forming DNA strands complementary to the RNA target.

#### Target Amplification

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. Following reverse transcription of the HIV-1 target RNA and the HIV-1 IC RNA, the Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the RNA:cDNA hybrids and the HIV-1 DNA and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05 in the presence of  $Mn^{2+}$  and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, extends the annealed primers along the target template to produce double-stranded DNA molecules termed amplicon. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the two regions of the HIV-1 genome between the primers; the entire HIV-1 genome is not amplified.

#### Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine<sup>33</sup>, but not DNA containing deoxythymidine.

Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is

included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55°C, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

#### Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 utilizes real-time<sup>34,35</sup> PCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HIV-1 and HIV-1 IC-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 the HIV-1 and HIV-1 IC probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HIV-1 target and HIV-1 IC RNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HIV-1 target and HIV-1 IC RNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

#### REAGENTS

**Note: Product safety labeling primarily follows EU GHS guidance**

**COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0**  
(P/N: 06693083 190)

**HI2QCAP**

**48 Tests**

##### **HIV-1 QL v2.0 CS1**

(HIV-1 Magnetic Glass Particles Reagent Cassette)  
Magnetic glass particles  
93% Isopropanol

**1 x 48 Tests**



93% (w/w) Isopropanol

H225, H319, H336

##### **HIV-1 QL v2.0 CS2**

(HIV-1 Lysis Reagent Cassette)  
Sodium citrate dihydrate  
42.5% Guanidine thiocyanate  
< 14% Polydocanol  
0.9% Dithiothreitol

**1 x 48 Tests**



42.5% (w/w) Guanidine thiocyanate

H302, H315, H318, H412

##### **HIV-1 QL v2.0 CS3**

HIV-1 Multi-Reagent Cassette containing:

**1 x 48 Tests**

##### **Pase**



(Proteinase Solution)  
Tris buffer  
< 0.05% EDTA  
Calcium chloride  
Calcium acetate  
≤ 7.8% Proteinase  
Glycerol

**1 x 3.8 mL**



≤ 7.8% (w/w) Proteinase

H318, H334

<b>EB</b> (Elution Buffer) Tris-base buffer 0.2% Methylparaben	1 x 7.0 mL
<b>HIV-1 QL v2.0 CS4</b> HIV-1 Test-Specific Reagent Cassette containing:	<b>1 x 48 Tests</b>
<b>HIV-1 IC</b> (HIV-1 Internal Control) Tris-HCl buffer EDTA < 0.005% Poly rA RNA (synthetic) < 0.001% Armored HIV-1 RNA construct containing HIV-1 primer binding sequences and a unique probe binding region (non-infectious RNA in MS2 bacteriophage) 0.05% Sodium azide	1 x 3.6 mL
<b>HIV-1 MMX</b> (HIV-1 Master Mix) Tricine buffer Potassium acetate Potassium hydroxide 20% Dimethylsulfoxide Glycerol < 0.04% dATP, dCTP, dGTP, dUTP, dTTP < 0.003% Upstream and downstream primers to the <i>gag</i> and the LTR region of HIV-1 < 0.003% Oligonucleotide aptamer < 0.003% Fluorescent-labeled oligonucleotide probes specific for HIV-1 and the HIV-1 Internal Control < 0.05% Z05 DNA Polymerase (microbial) < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide	1 x 2.5 mL
<b>CAP/CTM Mn<sup>2+</sup></b> (CAP/CTM Manganese Solution) < 0.5% Manganese acetate Glacial acetic acid 0.09% Sodium azide	1 x 19.8 mL
<b>HIV-1 (+)C, v2.0</b> (HIV-1 Positive Control, v2.0) < 0.001% Armored HIV-1 RNA construct containing HIV-1 sequences (non-infectious RNA in MS2 bacteriophage) Negative Human Plasma, non-reactive by tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin <sup>®</sup> 300 preservative	4 x 1.0 mL
 (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one	
<b>CTM (-) C</b> [COBAS <sup>®</sup> TaqMan <sup>®</sup> Negative Control (Human Plasma)] Negative Human Plasma, non-reactive by tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin <sup>®</sup> 300 preservative	4 x 1.0 mL
 (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one	
<b>HIV-1 (+)C, v2.0 Clip</b> (HIV-1 Positive Control, v2.0 Barcode Clip)	1 x 4 Clips
<b>HIV-1 (-) C Clip</b> (HIV-1 Negative Control, v2.0 Barcode Clip)	1 x 4 Clips
07002475001-04EN	4
	Doc Rev. 4.0

**COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent**  
(P/N: 03587797 190)

PG WR
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1 x 5.1 L

**PG WR**

(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)  
Sodium citrate dihydrate  
< 0.1% N-Methylisothiazolone-HCl

**COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent**  
(P/N: 06989861 190)

SPEX
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5 x 78 mL

**SPEX**

(COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent)

42.5% Guanidine thiocyanate  
0.79% Sodium citrate  
0.01% Citric acid  
3.6% Polydocanol  
1.8% Dithiothreitol



42.5% (w/w) Guanidine thiocyanate

H302, H318, H412

**WARNINGS AND PRECAUTIONS****A. FOR IN VITRO DIAGNOSTIC USE.**

- B. This test is not intended for use in screening blood or plasma donors.
- C. This test is for use with human plasma and dried blood spots prepared from fresh whole blood collected in the anticoagulant EDTA.
- D. Do not pipette by mouth.
- E. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- F. **Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control vials.**
- G. **The use of sterile disposable pipettes and RNase-free pipette tips is recommended.**
- H. Do not pool controls from different lots or from different bottles of the same lot.
- I. Do not mix reagent cassettes or controls from different kits.
- J. Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles.
- K. Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.
- L. Do not use a kit after its expiration date.
- M. Safety Data Sheets (SDS) are available on request from your local Roche office.
- N. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*<sup>37</sup> and in the CLSI Document M29-A3<sup>38</sup>. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

**Note: Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.**

- O. **CAUTION: CTM (-) C and HIV-1 (+)C, v2.0** contain Human Plasma derived from human blood. The source material has been tested and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No



known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all human sourced material should be considered potentially infectious. **CTM (-) C** and **HIV-1 (+)C, v2.0** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*<sup>37</sup> and in the CLSI Document M29-A3<sup>38</sup>. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

- P. **HIV-1 IC, CAP/CTM Mn<sup>2+</sup>** and **HIV-1 MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.
- Q. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. 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 of these reagents occur, dilute with water before wiping dry.
- R. Do not allow **HIV-1 QL v2.0 CS2, SPEX** (used in dried blood spot workflow) and liquid waste from the COBAS<sup>®</sup> AmpliPrep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.
- S. When disposing of used COBAS<sup>®</sup> AmpliPrep Sample Processing Units (SPUs), which contain guanidine thiocyanate, avoid any contact with sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

#### STORAGE AND HANDLING REQUIREMENTS

##### A. Do not freeze reagents or controls.

- B. Store **HIV-1 QL v2.0 CS1, HIV-1 QL v2.0 CS2, HIV-1 QL v2.0 CS3** and **HIV-1 QL v2.0 CS4** at 2-8°C. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 28 days at 2-8°C or until the expiration date, whichever comes first. **HIV-1 QL v2.0 CS1, HIV-1 QL v2.0 CS2, HIV-1 QL v2.0 CS3** and **HIV-1 QL v2.0 CS4** can be used for a maximum of 4 instrument cycles, up to a maximum of 64 hours cumulative on board the COBAS<sup>®</sup> AmpliPrep Instrument. Reagents must be stored at 2-8°C between instrument cycles.
- C. Store **HIV-1 (+)C, v2.0** and **CTM (-) C** at 2-8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.
- D. Store Barcode clips [**HIV-1 (+)C, v2.0 Clip** and **HIV-1 (-) C Clip**] at 2-30°C.
- E. Store **PG WR** at 2-30°C. **PG WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.
- F. Store **SPEX** (used in dried blood spot workflow) at 2-8°C. **SPEX** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.

#### MATERIALS PROVIDED

COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 Qualitative Test, v2.0  
(P/N: 06693083 190)

HI2QCAP
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**HIV-1 QL v2.0 CS1**  
(HIV-1 Magnetic Glass Particles Reagent Cassette)

**HIV-1 QL v2.0 CS2**  
(HIV-1 Lysis Reagent Cassette)

**HIV-1 QL v2.0 CS3**  
(HIV-1 Multi-Reagent Cassette)

**HIV-1 QL v2.0 CS4**  
(HIV-1 Test-Specific Reagent Cassette)

**HIV-1 (+)C, v2.0**  
(HIV-1 Positive Control, v2.0)

**CTM (-) C**  
[COBAS<sup>®</sup> TaqMan<sup>®</sup> Negative Control (Human Plasma)]

**HIV-1 (+)C, v2.0 Clip**  
(HIV-1 Positive Control, v2.0 Barcode Clip)

**HIV-1 (-) C Clip**  
(HIV-1 Negative Control, v2.0 Barcode Clip)

**COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent**  
(P/N: 03587797 190)

<b>PG WR</b>
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**PG WR**  
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)

**COBAS® AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent**  
(P/N: 06989861 190)

<b>SPEX</b>
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#### **MATERIALS REQUIRED BUT NOT PROVIDED**

##### **Instrumentation and Software**

- COBAS® AmpliPrep Instrument
  - COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
  - Optional: Docking Station
  - AMPLILINK Software Version 3.3 or Version 3.4 Series
  - Control Unit for the AMPLILINK Software with printer
  - Instrumentation and Software Manuals:
    - COBAS® AmpliPrep Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
    - COBAS® TaqMan® Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
    - COBAS® TaqMan® 48 Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
    - AMPLILINK Software Version 3.3 Series Application Manual for use with COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® Taqman® 48 Analyzer, COBAS® AMPLICOR® Analyzer and **cobas p 630** Instrument
- or
- AMPLILINK Software Version 3.4 Series Application Manual
  - Test Definition File (TDF). See Product Information Card, provided with the kit, for the name and current version of the TDF.

##### **Disposables**

- Sample processing units: SPUs
- Sample input tubes (S-tubes) with barcode clips
- Racks of K-tips
- K-tube Box of 12 x 96

**OTHER MATERIALS REQUIRED BUT NOT PROVIDED**

- Sample Rack (SK 24 rack)
- Reagent Rack
- SPU rack
- K-tube capper, motorized
- K-tube capper
- K-carrier
- K-carrier Transporter
- K-carrier rack
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1000 µL)\*
- Disposable gloves, powderless
- Vortex mixer
- Eppendorf Thermomixer C with SmartBlock 1.5 mL including Eppendorf IsoRack (for DBS application only) (or use equivalent equipment: Eppendorf Thermomixer Comfort or Thermomixer R including Eppendorf IsoRack)
- Dessiccant bags (for DBS storage)
- Whatman 903<sup>®</sup> filter cards or Munktell Specimen Collection card TFN Brand 5 or equivalent (for DBS application only)

\* Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement RNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.

**SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

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

**Note:** This test has been validated for use with only human plasma collected in EDTA anticoagulant and whole blood collected on dried blood spots. Testing of other specimen types may result in inaccurate results.

**A. Specimen Collection**

**Note:** Sample labeling shall reflect sample type to allow correct workflow application.

EDTA Plasma

The COBAS<sup>®</sup> AmpliPrep/COBAS<sup>®</sup> TaqMan<sup>®</sup> HIV-1 Qualitative Test, v2.0 is for use with plasma specimens. Blood should be collected in sterile tubes using EDTA (lavender top) as the anticoagulant and mixed adequately according to the tube manufacturer's instructions. Store whole blood at 2-25°C for no longer than 24 hours. Separate plasma from whole blood within 24 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer plasma to a sterile polypropylene tube.

Dried Blood Spots (DBS)

Collect dried blood spot samples (DBS) using appropriate clinical procedures. It is recommended to apply a minimum of 70 µL of capillary blood inside each delineated circle on the Whatman 903<sup>®</sup> filter card or the Munktell Specimen Collection card TFN Brand 5 or equivalent. Use filter paper ensuring that BOTH sides of the paper are saturated beyond the delineated circle. Allow DBS to dry at room temperature (18-25°C) for at least 3 hours, protecting the DBS card from direct sunlight. For further details consult package insert of filter cards used. It is recommended to prepare 3 paper disks per patient sample.

**B. Specimen Transport**

Transportation of whole blood, plasma or DBS must comply with country, federal, state and local regulations for the transport of etiologic agents<sup>30</sup>. Whole blood must be transported at 2-25°C and centrifuged within 24 hours of collection. Plasma may be transported at 2-8°C or frozen at -20°C to -80°C. DBS may be transported at 15-30°C within 3 months.

**C. Specimen Storage**EDTA Plasma

Plasma specimens may be stored at room temperature (25-30°C) for up to 1 day or at 2-8°C for up to 5 days. Plasma specimens were shown to be stable for six weeks if frozen at -20°C to -80°C. It is recommended that specimens be stored in 1100-1200 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt 72.694.006).

Plasma specimens may be frozen and thawed up to three times without a significant loss of HIV-1 target.

#### Dried Blood Spots

Store dried blood spots in individual re-sealable bags with a desiccant sachet each at 15–30°C for up to three months.

### **INSTRUCTIONS FOR USE**

For detailed operating instructions, a description of the possible configurations, printing results and interpreting flags, comments and error messages, refer to AMPLILINK Software – Version 3.3 or Version 3.4 Series manuals, as listed in section Instrumentation and Software.

#### **Batch Size and Workflow**

Each kit contains reagents sufficient for 48 tests, which may be performed in batches of 12 to 24 tests. At least one of each control [CTM (-) C and HIV-1 (+)C, v2.0] must be included in each batch (see "Quality Control" section). The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

#### **Specimen and Control Preparation**

*Note: If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3-5 seconds before use. Controls should be removed from 2-8°C storage and equilibrated to ambient temperature before use.*

#### **COBAS® AmpliPrep Instrument Set-up**

##### **Part A. Maintenance and Priming**

- A1. The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.
- A2. Turn the Control Unit for the AMPLILINK software **ON**. Prepare the Control Unit as follows:
  1. Log onto the Microsoft Windows Operating System.
  2. Double click the AMPLILINK software icon.
  3. Log onto AMPLILINK software by entering the assigned User ID and password.

A3. Check the supply of **PG WR** using the **Status** Screen and replace if necessary.

A4. Perform all Maintenance that is listed in the Due Tab. The COBAS® AmpliPrep Instrument will automatically prime the system.

##### **Part B. Loading of Reagent Cassettes**

*Note: All reagent cassettes should be removed from 2-8°C storage, immediately loaded onto the COBAS® AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is to be processed. Do not let reagent cassettes come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels.*

- B1. Place **HIV-1 QL v2.0 CS1** onto a reagent rack. Place **HIV-1 QL v2.0 CS2**, **HIV-1 QL v2.0 CS3** and **HIV-1 QL v2.0 CS4** onto a separate reagent rack.
- B2. Load the reagent rack containing **HIV-1 QL v2.0 CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument.
- B3. Load the reagent rack containing **HIV-1 QL v2.0 CS2**, **HIV-1 QL v2.0 CS3** and **HIV-1 QL v2.0 CS4** onto rack position **B, C, D** or **E** of the COBAS® AmpliPrep Instrument. (See Table 1 for additional information).

##### **Part C. Loading of Disposables**

*Note: Determine the number of COBAS® AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (S-tubes), K-tips and K-tubes needed. One SPU, one Input S-tube, one K-tip and one K-tube are needed for each specimen or control.*

Multiple workflows for use of the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer are possible. For reference, see Table 1 below. Depending on the workflow used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks onto the respective rack positions of the COBAS® AmpliPrep Instrument (See Table 1 for additional information).



- C1. Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position **J, K or L** of the COBAS® AmpliPrep Instrument.
- C2. Depending on the workflow used, load full K-tube rack(s) onto rack position **M, N, O or P** of the COBAS® AmpliPrep Instrument.
- C3. Load full K-tip rack(s) onto rack position **M, N, O or P** of the COBAS® AmpliPrep Instrument.
- C4. For workflow 3 using the COBAS® TaqMan® 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position **M & N, or O & P** of the COBAS® AmpliPrep Instrument.

**Table 1**  
Possible Workflows for using COBAS® AmpliPrep Instrument with  
COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer

Workflow		Transfer Mode to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer	Racks, Carriers and Disposables	Position on COBAS® AmpliPrep Instrument
1	COBAS® AmpliPrep Instrument plus Docking Station plus COBAS® TaqMan® Analyzer	Automated transfer of K-carrier	K-tubes in full K-tube racks	M - P
			K-tips in full K-tip racks	M - P
			Input S-tubes containing specimens and controls on sample racks	F - H
			SPUs in SPU racks	J - L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B - E
2	COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer	Manual transfer of K-tubes via sample rack(s) onto COBAS® TaqMan® Analyzer	K-tubes in full K-tube racks	M - P
			K-tips in full K-tip racks	M - P
			Input S-tubes containing specimens and controls on sample racks	F - H
			SPUs in SPU racks	J - L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B - E
		<u>After specimen processing is finished:</u> K-tubes on sample racks (ready for manual transfer)	Same as above (F - H)	
3	COBAS® AmpliPrep Instrument plus COBAS® TaqMan® 48 Analyzer(s)	Manual transfer of K-carrier via K-carrier rack(s) onto COBAS® TaqMan® 48 Analyzer	K-tubes on sample racks	F - H
			K-tips in full K-tip racks	M - P
			Input S-tubes containing specimens and controls on sample racks	F - H
			SPUs in SPU racks	J - L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B - E
			Empty barcoded K-carrier on K-carrier rack	M - P
	<u>After specimen processing is finished:</u> K-tubes in K-carrier on K-carrier rack	Same as above (M - P)		

#### Part D. Ordering and Loading of Specimens

- D1. Prepare sample racks as follows: Attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [CTM (-) C and HIV-1 (+)C, v2.0] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip.
- D2. Using the AMPLILINK software, create specimen orders for each specimen and control in the **Orders** window **Sample** folder. **Select the appropriate test file** (EDTA or DBS Test Definition File) and complete by saving. (See product information card (PIC) provided with the kit for appropriate TDF name and version.)

D3. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be for the rack prepared in Step D1.

D4. Print the **Sample Rack Order** report to use as a worksheet.

D5. Prepare specimen and control racks in the designated area for specimen and control addition as follows: Vortex each specimen and control [CTM (-) C and HIV-1 (+)C, v2.0] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.

**Note:** For EDTA plasma samples proceed with D6.

**Note:** For Dried Blood Spot samples proceed with D7.

**Note:** Do not mix EDTA plasma and Dried Blood Spot samples on the same sample rack.

**Note:** Allow SPEX to equilibrate to ambient temperature before use.

D6. Place one Input S-tube into each position containing a barcode label clip. Transfer 1000 to 1050 µL of each specimen to the appropriate barcode labeled Input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. **Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube.** Specimens are transferred to tube positions as assigned and recorded on the worksheet in Step D4. **Avoid contaminating the upper part of the S-tubes with specimens. Skip D7 and proceed with D8.**

D7. Place input S-tubes in each of the positions 1 - 24 on a Thermomixer IsoRack. Excise Dried Blood Spots from Whatman 903<sup>®</sup> filter card or the Munktell Specimen Collection card TFN Brand 5 or equivalent (according to filter card user instruction) and transfer into the Input S-tube. **For all DBS card types used, it is crucial to ensure the dried blood spot is attached to the S-input tube wall (to avoid sample clots) and completely covered with SPEX reagent.** 1000 µl of SPEX reagent is pipetted into an S-input tube containing the dried blood spot. For elution of the dried whole blood, the S-input tube is placed on an Eppendorf Thermomixer for 10 minutes, at 56°C and 1000 rpm. Transfer S-input tubes to tube position on sample rack as assigned on the worksheet in Step D4.

D8. Transfer 1000 to 1050 µL of each control [CTM (-) C and HIV-1 (+)C, v2.0] to the appropriate barcode labeled Input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. Controls should be transferred to tube positions as assigned and recorded on the worksheet in Step D4. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Assign the right control to the position with the appropriate control barcode clip. **Avoid contaminating the upper part of the S-tubes with controls.**

D9. For workflows 1 and 2, load the sample rack(s) filled with Input S-tubes onto rack positions **F, G** or **H** of the COBAS<sup>®</sup> AmpliPrep Instrument.

D10. For workflow 3 using the COBAS<sup>®</sup> TaqMan<sup>®</sup> 48 Analyzer, load sample rack(s) with Input S-tubes and K-tubes (one for each Input S-tube, loaded in the right position adjacent to Input S-tubes) onto rack position **F, G** or **H** of the COBAS<sup>®</sup> AmpliPrep Instrument.

#### **Part E. Start of COBAS<sup>®</sup> AmpliPrep Instrument Run**

E1. Start the COBAS<sup>®</sup> AmpliPrep Instrument using the AMPLILINK software.

#### **Part F. End of COBAS<sup>®</sup> AmpliPrep Instrument Run and Transfer to COBAS<sup>®</sup> TaqMan<sup>®</sup> Analyzer or COBAS<sup>®</sup> TaqMan<sup>®</sup> 48 Analyzer (for workflow 2 and 3 only)**

F1. Check for flags or error messages.

F2. Remove processed specimens and controls from the COBAS<sup>®</sup> AmpliPrep Instrument on either sample racks (for COBAS<sup>®</sup> TaqMan<sup>®</sup> Analyzer without Docking Station) or K-carrier racks (for COBAS<sup>®</sup> TaqMan<sup>®</sup> 48 Analyzer), depending on the workflow (for further details see Part G).

F3. Remove waste from the COBAS<sup>®</sup> AmpliPrep Instrument.

**Note:** All processed specimens and controls should not be exposed to light after completion of specimen and control preparation.

**Note:** Do not freeze or store processed specimens and controls at 2-8°C.

**Amplification and Detection****COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Set-up**

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

**Part G. Loading Processed Specimens**

G1. Depending on the workflow, perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer:

*Workflow 1:* Automated transfer of K-carrier via docking station to COBAS® TaqMan® Analyzer. Manual intervention is unnecessary.

*Workflow 2:* Manual transfer of K-tubes in sample rack(s) to COBAS® TaqMan® Analyzer

*Workflow 3:* Manual transfer of K-carrier on K-carrier rack(s) to the COBAS® TaqMan® 48 Analyzer. Manual transfer of K-carriers into COBAS® TaqMan® 48 Analyzer using the K-carrier Transporter.

**Part H. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run**

H1. Start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer by one of the options below depending on the workflow used:

*Workflow 1:* No intervention necessary.

*Workflow 2:* Automatic start of the COBAS® TaqMan® Analyzer after insertion of sample rack(s).

*Workflow 3:* Fill K-carrier with empty K-tubes if there are fewer than 6 K-tubes on the K-carrier. Filling is guided by the AMPLILINK software. Open thermal cycler cover, load K-carrier into thermal cycler and close lid. Start the COBAS® TaqMan® 48 Analyzer run.

**Part I. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run**

I1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print Results Report. Check for flags or error messages in the Result report. Specimens with flags and comments are interpreted as described in the Results section. After acceptance, store data in archive.

I2. Remove used K-tubes from the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

**RESULTS**

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the presence of the HIV-1 for the specimens and controls.

**AMPLILINK Software:**

- Determines the Cycle Threshold value (Ct) for the HIV-1 and the HIV-1 IC RNA.
- Determines the presence of HIV-1 and HIV-1 IC RNA based upon the Ct values for the HIV-1 and HIV-1 IC RNA.

**Batch Validation**

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid.

For control orders, a check is made to determine if the Ct value for the control is within its specified range. If the Ct value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [HIV-1 (+)C, v2.0 and CTM (-) C].

The batch is not valid if any of the following flags appear for the HIV-1 Controls:

**Negative Control**

Flag	Result	Interpretation
NC_INVALID	Invalid	An invalid result or the result for the negative control is not negative.

**HIV-1 Positive Control, v2.0**

Flag	Result	Interpretation
PC_INVALID	Invalid	An invalid result or the result for the positive control is not positive.

If the batch is invalid, repeat the entire batch including specimen and control preparation, amplification and detection.

**Interpretation of Results**

For a valid batch, check each individual specimen for flags or comments on the result printout.

⇒ A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

**Specimen results are interpreted as follows:**

Sample Type	Detection Result	Interpretation
EDTA Plasma	Not Detected	Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as "HIV-1 not detected".
	Detected	Report results as "HIV-1 detected".
DBS	Not Detected DBS	Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as "HIV-1 not detected".
	Detected DBS	Report results as "HIV-1 detected".

If specimen result display element is "Failed", "Invalid" or "Aborted" please refer to the AMPLILINK Software Version 3.3 and 3.4 Series Application Manual as listed in section "Materials required but not provided".



**PROCEDURAL LIMITATIONS**

1. This test has been validated for use with human plasma collected in EDTA anticoagulant and dried blood spots from whole blood collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.
2. The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 has not been evaluated with specimens containing HIV-2.
3. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
4. The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 Master Mix reduces the risk of amplicon contamination. However, contamination from HIV-1 positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.
5. Use of this product should be limited to personnel trained in the techniques of PCR.
6. This product can only be used with the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.
7. Though rare, mutations within the highly conserved regions of the viral genome covered by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 primers and/or probes may result in the failure to detect the virus.
8. Detection of HIV-1 RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors, (i.e., age, presence of symptoms, stage of the infection, viral load setpoint and antiretroviral medications).
9. Due to inherent differences between technologies, it is recommended that, before switching from one technology to the next, users perform method correlation studies in their laboratory to quantify technology differences.

**INTERFERING SUBSTANCES**

Elevated levels of triglycerides (up to 30 g/L), conjugated and unconjugated bilirubin (up to 0.2 g/L), human serum albumin (up to 60 g/L), hemoglobin (up to 2 g/L) and human DNA (up to 2 mg/L) in Dried Blood Spots and plasma specimens were shown not to interfere with the detection of HIV-1 and did not impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0. Autoantibodies from patients with a reactive Antinuclear Antibody were shown not to interfere with the test in Dried Blood Spot and plasma specimen. Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) plasma specimen were shown to interfere with the detection of HIV-1 which may lead to false negative results in rare cases. The before mentioned autoimmune disease markers have also been tested in Dried Blood Spots and did not show interference. The evaluation was performed according to CLSI Guideline EP7-A2 using more than one lot of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 reagents.

Foscarnet sodium (CMV drug, viral polymerase inhibitor) at concentrations greater than 2x the Cmax reported by the manufacturer, was observed to interfere with the assay's Internal Control, leading to invalidation of some tests. No false positive or false negative result calls were observed. The following drug compounds tested at 3 times the Peak Plasma Level (Cmax) have been shown not to interfere with the detection of HIV-1 or impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0:

<b>HIV drugs:</b>	
<b>Protease Inhibitors</b> Atazanavir Saquinavir Ritonavir Lopinavir/Ritonavir Nelfinavir mesylate Darunavir Tipranavir Fosamprenavir	<b>Nucleoside/Nucleotide Analogue Inhibitors of Reverse Transcriptase</b> Zidovudine, AZT Abacavir sulfate, ABC Lamivudine, 3TC Tenofovir DF, TDF Emtricitabine, FTC Stavudine, d4T
<b>Integrase Inhibitor</b> Raltegravir Elvitegravir	<b>Non-nucleoside Inhibitors of Reverse Transcriptase</b> Efavirenz Rilpivirine Etravirine Nevirapine
<b>Entry Inhibitor</b> Maraviroc	<b>Pharmacokinetic boosting agent</b> Cobicistat
<b>HBV and / or HCV drugs:</b>	
<b>Nucleotide analogue</b> Adefovir dipivoxil	<b>Nucleoside analogue</b> Entecavir Telbivudine
<b>Immune Modulator</b> Peginterferon alfa-2a Peginterferon alfa-2b Ribavirin	<b>Protease inhibitor</b> Boceprevir Telaprevir
<b>Compounds for Treatment of Herpes Viruses:</b>	
<b>Nucleoside analogues</b> Acyclovir Ganciclovir Valganciclovir HCl Cidofovir	
<b>Compounds for Treatment or Prevention of Opportunistic Infections in HIV Disease:</b>	
<b>Anti Infective</b> Sulfamethoxazole Trimethoprim	<b>Anti fungal</b> Fluconazole
<b>Anti mycobacterial</b> Azithromycin Isoniazid Rifampin Rifabutin Ethambutol Clarithromycin Pyrazinamide	

**NON-CLINICAL PERFORMANCE EVALUATION****A. Limit of Detection**

The limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was determined by testing the 2<sup>nd</sup> International HIV-1 RNA WHO Standard, NIBSC Code 97/650<sup>41</sup>, HIV-1 subtype B, diluted in HIV-1-negative human EDTA plasma or whole blood for dried blood spots. One copy of HIV-1 RNA is equivalent to 1.7 ± 0.1 International Unit (IU). The limit of detection was determined for three reagent lots. Three dilution series were analyzed for each reagent lot. A total of approximately 126 replicates per concentration level were tested for EDTA plasma and a total of approximately 189 replicates per concentration level were tested for dried blood spots. The evaluation was performed according to CLSI Guideline EP17-A2.

The combined results for all three lots tested for EDTA plasma and dried blood spots are shown in Table 2 and Table 3 and demonstrate that the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 detected HIV-1 RNA at concentrations of 20 cp/mL in EDTA Plasma and 300 cp/mL in dried blood spots respectively, or greater with a hit rate of ≥ 95% as determined by PROBIT Analysis.

**Table 2**  
*Limit of Detection in EDTA plasma of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 using the WHO International Standard and PROBIT analysis*

Nominal Input (HIV-1 RNA cp/mL)	No. Replicates	No. Positives	Positivity Rate
60	126	126	100
40	186	185	99
30	126	125	99
<b>20</b>	<b>126</b>	<b>124</b>	<b>98</b>
15	59	53	90
10	126	108	86
5	125	66	53
0	126	0	0
<b>PROBIT 95% Hit Rate</b>	<b>16.5 cp/mL</b> <b>95% confidence range: 14.3 – 19.8 cp/mL</b>		

**Table 3**  
*Limit of Detection in dried blood spots of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 using the WHO International Standard and PROBIT analysis*

Nominal Input (HIV-1 RNA cp/mL)	No. Replicates	No. Positives	Positivity Rate
500	189	189	100
400	187	185	99
<b>300</b>	<b>188</b>	<b>183</b>	<b>97</b>
150	188	166	88
75	188	108	57
0	188	0	0
<b>PROBIT 95% Hit Rate</b>	<b>221.8 cp/mL</b> <b>95% confidence range: 195.6 – 260.5 cp/mL</b>		

**B. Precision**

The precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was determined by analysis of serial dilutions of a HIV-1 cell culture supernatant specimen (HIV-1 subtype B) in HIV-1-negative human EDTA plasma and Dried blood spot specimens. The titer assignment of the cell culture supernatant (stock concentration) was performed by a method that ensures traceability to the 1<sup>st</sup> International HIV-1 RNA WHO Standard, NIBSC Code 97/656<sup>42</sup>. One copy of HIV-1 RNA is equivalent to 1.7 ± 0.1 International Unit (IU). For EDTA Plasma three reagent lots were analyzed, which are split between 11 runs. Each run consisted of 7 dilution levels and 14 replicates at each level. For dried blood spots three reagent lots were analyzed, which are split between 15 runs. Each run consisted of 6 dilution levels and 21 replicates at each level. Each specimen was taken through the entire COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 procedure, including specimen preparation, amplification and detection. The study was performed

with three lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 reagents. All valid precision data were evaluated by calculating the Hit Rate in % for each panel member by reagent lot for plasma and dried blood spots. The COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 demonstrates consistent performance at concentrations levels 60, 40, 30, 20, 10, 5 and 0 cp/mL for EDTA plasma and at levels 500, 400, 300, 150, 75 and 0 cp/mL for dried blood spots across all three reagent lots tested (Table 4 and Table 5).

**Table 4**  
*Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (EDTA Plasma)*

Nominal Concentration (cp/mL)	Hit rate %		
	Lot 1	Lot 2	Lot 3
60	100	100	100
40	100	99	100
30	98	100	100
20	95	100	100
10	88	88	81
5	52	49	57
0	0	0	0

**Table 5**  
*Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (Dried Blood Spots)*

Nominal Concentration (cp/mL)	Hit rate %		
	Lot 1	Lot 2	Lot 3
500	100	100	100
400	100	98	98
300	100	95	97
150	95	84	86
75	60	56	57
0	0	0	0

### C. Inclusivity of HIV-1 Group M, N and O

Eight subtype categories have been proposed for HIV-1 group M based on nucleotide divergence. These subtypes are designated with capital alphabetical letters from A through H<sup>40</sup>. The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 on each HIV-1 group M subtype A through H, CRF01\_AE, group O, and group N was evaluated by verifying the Limit of Detection.

The evaluation of the 8 HIV-1 group M subtype isolates, CRF01\_AE, one group N and one group O isolate were performed at up to four different concentration levels in EDTA plasma or dried blood spots and a Hit Rate determination was performed for each level with up to 49 replicates. The study was conducted with up to two lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 reagents. The results for EDTA plasma and dried blood spots are shown in Table 6 and Table 7 and verify that COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 detected HIV-1 for ten different genotypes/subtypes at concentrations of 7.5E+01 cp/mL (or lower) for plasma and 1.0E+3 cp/mL (or lower) for dried blood spots with a hit rate of  $\geq 95\%$ .

**Table 6**  
**Genotype Verification of Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (EDTA Plasma)**

Subtype	Isolate Designation	Lowest Concentration Level $\geq$ 95% Hit Rate (cp/mL)
group M subtype A	92UG029	1.0E+01
group M subtype A	4237A/98	2.9E+01
group M subtype B	92TH026	3.2E+01
group M subtype B	8E5/LAV	7.5E+01
group M subtype C	92BR025	3.0E+01
group M subtype C	3777A/97	1.1E+01
group M subtype D	92UG021	3.0E+01
group M subtype D	92UG035	1.1E+01
group M subtype E	92TH022	1.2E+01
group M subtype E	92TH009	1.4E+01
group M subtype F	93BR020	3.2E+01
group M subtype G	ARP173/RU570	1.3E+01
group M subtype H	HIV V1557	1.6E+01
group M subtype CRF01_AE	1019-HIV-0027	6.0E+01
group O	MVP5180	5.0E+01
group N	YBF30	6.0E+01

**Table 7**  
**Genotype Verification of Limit of Detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 (Dried Blood Spots)**

Subtype	Isolate Designation	Lowest Concentration Level $\geq$ 95% Hit Rate (cp/mL)
group M subtype A	92UG029	2.0E+02
group M subtype C	92BR025	3.0E+02
group M subtype D	92UG021	2.0E+02
group M subtype E	92TH022	2.0E+02
group M subtype F	93BR020	3.0E+02
group M subtype G	ARP173/RU570	2.0E+02
group M subtype H	HIV V1557	4.0E+02
group M subtype CRF01_AE	1019-HIV-0027	1.0E+03
group O	MVP5180	4.0E+02
group N	YBF30	1.0E+03

#### D. Diagnostic Sensitivity

The diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was determined by analyzing individual HIV-1 positive EDTA plasma samples (169 total results) and dried blood spot samples from adults (172 total results) with three lots of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 reagents. All EDTA plasma and adult DBS specimens tested positive for HIV-1. In this panel, the diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is 100% (one sided lower 95% confidence limit:  $\geq$  98.2%) in EDTA plasma and adult DBS (one sided lower 95% confidence limit:  $\geq$  98.3%).

In addition, the diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was evaluated during seroconversion. The members of 10 commercially available HIV-1 seroconversion panels, each collected from an individual plasma donor during a period of HIV-1 antibody seroconversion, were tested with one lot of COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 reagents and Abbott RealTime HIV-1 Qualitative assay. COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 detected HIV-1 earlier in 5 out of 10 seroconversion panels (one panel was not detected at all by the Abbott RealTime HIV-1



Qualitative assay), and 5 out of 10 seroconversion panels were detected at the same time, compared to the Abbott RealTime HIV-1 Qualitative assay. In addition, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was compared to a serology reference method. Nine out of ten seroconversion panels were detected earlier and one out of ten was detected at the same time.

**E. Specificity**

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was determined with two reagent lots by analysis of HIV-1-negative EDTA plasma and Dried Blood Spot (DBS) specimens from blood donors. A total of 1298 individual EDTA plasma specimens and 998 DBS showed valid results. With the exception of two EDTA plasma specimens and one DBS specimen all results were negative for HIV-1 in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0. Based on these results, the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 is 99.8% (one-sided lower 95% confidence limit: ≥ 99.5%) in EDTA plasma and 99.9% (one-sided lower 95% confidence limit: ≥ 99.5%) in DBS specimens.

**F. Analytical Specificity**

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was evaluated by adding cultured organisms (viruses, bacteria, yeast) at 1.0E+06 particles/mL and Hepatitis C virus at 1.0E+07 particles/mL input concentration into HIV-1-negative human EDTA plasma and into HIV-1-positive EDTA plasma at 6.0E+01 cp/mL HIV-1 and into HIV-1-positive EDTA plasma at 1.0E+03 for dried blood spots (see Table 8).

None of the organisms tested showed cross reaction with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0.

**Table 8**  
**Analytical Specificity Specimens**

<p><b>Virus</b></p> <ul style="list-style-type: none"> <li><i>Adenovirus type 5</i></li> <li><i>Cytomegalovirus</i></li> <li><i>Epstein-Barr virus</i></li> <li><i>Human Herpes virus type 6</i></li> <li><i>Herpes simplex virus type 1</i></li> <li><i>Herpes simplex virus type 2</i></li> <li><i>Human T-Cell Lymphotropic virus type 1</i></li> <li><i>Human T-Cell Lymphotropic virus type 2</i></li> <li><i>Influenza A</i></li> <li><i>Hepatitis A virus</i></li> <li><i>Hepatitis B virus</i></li> <li><i>Hepatitis C virus</i></li> <li><i>Hepatitis G virus</i></li> <li><i>Hepatitis E virus</i></li> <li><i>Human Papiloma virus</i></li> <li><i>Varicella Zoster virus</i></li> <li><i>West Nile virus</i></li> <li><i>St. Louis Encephalitis virus</i></li> <li><i>Murray Valley Encephalitis virus</i></li> <li><i>Dengue virus type 1, 2, 3 and 4</i></li> <li><i>Yellow Fever virus</i></li> <li><i>Zika virus</i></li> <li><i>Tick-borne encephalitis virus – strain HYPR</i></li> <li><i>Parvovirus B19</i></li> <li><i>Chikungunya-virus</i></li> </ul>	<p><b>Bacteria</b></p> <ul style="list-style-type: none"> <li><i>Staphylococcus aureus</i></li> <li><i>Staphylococcus epidermidis</i></li> <li><i>Staphylococcus haemolyticus</i></li> <li><i>Streptococcus sanguinis</i></li> <li><i>Propionibacterium acnes</i></li> <li><i>Escherichia coli</i></li> </ul> <hr/> <p><b>Yeast</b></p> <ul style="list-style-type: none"> <li><i>Candida albicans</i></li> </ul>
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**G. Method Correlation**

EDTA plasma

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was compared to the Abbott RealTime HIV-1 Qualitative assay by analysis of 169 undiluted confirmed HIV-1 positive clinical EDTA plasma specimens. The specimens comprised HIV-1 group M subtypes A to H and were analyzed at one external site. Results of 169 samples with titers between 1.0E+02 and 5.0E+05, including titers close to the limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 were evaluated, leading to a 100% positive agreement between the two tests. All negative EDTA plasma specimens tested

(100 total results) were valid and returned a negative result, leading to a 100% negative agreement between COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 and the Abbott RealTime HIV-1 Qualitative assay.

#### Adult DBS

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was compared to the Abbott RealTime HIV-1 Qualitative assay by analysis of a total of 272 clinical specimens: 172 undiluted confirmed HIV-1 positive clinical DBS specimens from adult donors and 100 DBS specimen from healthy HIV-1 negative adult donors. The HIV-1 positive specimens comprised HIV-1 group M subtypes A to H, Group O and N and were analyzed at one external site. Results of 172 samples were evaluated, 167 were found positive in both tests, leading to a 100% positive agreement. However, five confirmed HIV-1 positive specimens could not be detected in Abbott RealTime HIV-1 Qualitative assay. All negative adult DBS specimens tested (100 total results) were valid and returned a negative result, leading to a 95.2% negative agreement between COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 and the Abbott RealTime HIV-1 Qualitative assay (including the five positive specimens tested negative in Abbott RealTime HIV-1 Qualitative assay).

#### Early infant DBS

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 was compared to the Abbott RealTime HIV-1 Qualitative assay by analysis of 99 prospectively collected, undiluted HIV-1 positive clinical early infant DBS specimens. The specimens comprised HIV-1 group M subtypes A to H and were analyzed at one external site. Results of 99 samples were evaluated, leading to a 100% agreement between the two tests. All negative early infant DBS specimens tested (201 total results) were valid and returned a negative result, leading to a 100% negative agreement between COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 and the Abbott RealTime HIV-1 Qualitative assay.

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Document Revision Information	
Doc Rev. 2.0 08/2014	Update Legal Manufacturer to Roche Diagnostics GmbH. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 03/2015	<p>In sections: <b>SUMMARY AND EXPLANATION OF THE TEST, PRINCIPLES OF THE PROCEDURE, SPECIMEN COLLECTION, TRANSPORT AND STORAGE, RESULTS, INTERFERING SUBSTANCES, NON-CLINICAL PERFORMANCE EVALUATION</b> (Subsections: "Inclusivity of HIV-1 Group M, N and O" and "Specificity") deletion of the reference to "RNA" (were appropriate).</p> <p>In the <b>Target Selection</b> section, add "group N".</p> <p>Under the <b>Warnings and Precautions</b> section, letter (r), add "SPEX (used in dried blood spot workflow)."</p> <p>In the <b>Storage and Handling</b> section, add storage information for the <b>SPEX</b> reagent. In the <b>OTHER MATERIALS REQUIRED BUT NOT PROVIDED</b> section, add ordering information for the Eppendorf Thermomixer C.</p> <p>In the <b>SPECIMEN COLLECTION, TRANSPORT AND STORAGE</b> section, include instructions for labeling all specimen types; include instructions for transport of DBS; and provide a temperature range for DBS.</p> <p>In the <b>Specimen and Control Preparation</b> section, clarify instructions for handling frozen specimens, storage of processed specimens and controls.</p> <p>In the <b>INSTRUCTIONS FOR USE</b> section include instruction on handling of SPEX before use.</p> <p>In <b>INTERFERING SUBSTANCES</b> section, correct spelling of Peginterferon alfa-2a</p> <p>In the <b>Procedural Limitations</b> section, remove "HIV-1 group N" from no. 2 limitation.</p> <p>In the <b>Diagnostic Sensitivity</b> section, provide clarification for the Abbott and Serology study results when compared to the CAP/CTM HIV-1 Qual v2.</p> <p>Added GTIN symbol and description to the harmonized symbol page.</p> <p>Added statement to clarify that product safety labeling primarily follows EU GHS guidance.</p> <p>Please contact your local Roche Representative if you have any questions.</p>
Doc Rev. 4.0 07/2015	Updated AMPLILINK versions, manuals, terminology and Operating System. Please contact your local Roche Representative if you have any questions.



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07/2015  
Doc Rev. 4.0

07002475001-04

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