Warsaw, January 25th, 2021

To Whom It May Concern

Roche Diagnostics Poland herby confirm that Instructions for Use (IFU) or Method Sheets of the following reagents:

- 04687787190 HBsAg G2 Elecsys cobas e 100
  (date of issue of current IFU version: 2020-12, V 18)
- 06923348190 Syphilis Elecsys cobas e 100
  (date of issue of current IFU version: 2021-01, V 6)
- 06368921190 Anti-HCV G2 Elecsys cobas e 100
  (date of issue of current IFU version: 2020-11, V 8)
- 05390095190 HIV combi PT Elecsys cobas e 100
  (date of issue of current IFU version: 2021-01, V 11.0)
- 05969484190 KIT s201 T-SCRN MPX v2.0 96T US-IVD
  (date of issue of current IFU version: 11-10-2019, Doc Rev. 4.0)

are an official documents, which are essential to perform conformity assessment in accordance to EU Directive 98/79/EC on In vitro Diagnostic Medical Device and for 05969484190 KIT s201 T-SCRN MPX v2.0 96T US-IVD to finalize FDA's approval. The content of IFU reflects product's performance characteristics supported by the manufacturer's technical documentation.

In addition, for high-risk products (04687787190 HBsAg G2 Elecsys cobas e 100, 06368921190 Anti-HCV G2 Elecsys cobas e 100, 05390095190 HIV combi PT Elecsys cobas e 100) conformity assessment was conducted by a Notified Body, which holds accreditation to check products' compliance to EU Directive 98/79/EC on In vitro Diagnostic Medical Devices in European Union. For those products, EC Certificate finalizes products' compliance.

Digitally signed by Lazari Cristina
Date: 2021.02.09 16:39:43 EET
Reason: MoldSign Signature
Location: Moldova

Roche Diagnostics Polska Sp. z o.o.
ul. Bobrowiecka 8, 00-728 Warszawa
Tel. +48 22 481 55 55
Faks +48 22 481 55 99

Sąd Rejonowy dla m. st. Warszawy
w Warszawie, XIII Wydział Gospodarczy
Krajowego Rejestru Sądowego,
KRS 0000132695

kapitał zakładowy 8 000 000 PLN
NIP 527-23-22-068

www.roche.pl

Attachments:

1. EC Certificates for products:

   Screening and Confirmatory Test for Hepatitis B marker
   Screening test for Hepatitis C marker
   Screening test for HIV-1/12 marker

2. Instructions for Use (Method Sheets) of reagents listed above.
3. FDA approval for KIT s201 T-SCRN MPX v2.0 96T US-IVD

Joanna Bućko

Product Manager
Roche Molecular & Blood Safety Solutions
Management Center Poland & East Europe
Roche Diagnostics Polska Sp. z o.o.

Mateusz Metlerski

Quality and Regulatory Specialist
Roche Diagnostics Polska Sp. z o.o.

Benannt durch/Designated by
Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten
www.zlg.de
ZLG-BS-245.10.07

Roche Diagnostics Polska Sp. z o.o.
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tel. 22 481 55 55, fax 22 481 55 99
NIP 527-23-22-068 REGON 016755430

TÜV SÜD Product Service

EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0610 Rev. 02

Model(s):

Elecsys HBsAg II
PreciControl HBsAg II
Elecsys HBsAg Confirmatory Test
Elecsys HBsAg II Auto Confirm
PreciControl HBsAg Auto Confirm
Elecsys and cobas e analyzers

Facility(ies):

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Nonnenwald 2, 82377 Penzberg, GERMANY

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Sandhofer Strasse 116, 68305 Mannheim, GERMANY

Roche Diagnostics International Ltd Centralised and Point of Care Solutions
Forrenstrasse 2, 6343 Rotkreuz, SWITZERLAND

Parameters:

Model Name:	Model No.:
–	
Elecsys HBsAg II	04687787190
	08814856190
Elecsys HBsAg II	07914482190
	08814864190
Elecsys HBsAg II	07251076190
	08814848190
PreciControl HBsAg II	04687876190
Elecsys HBsAg Confirmatory Test	11820648122
Elecsys HBsAg II Auto Confirm	08741034190
PreciControl HBsAg Auto Confirm	08741107190
Elecsys and cobas e analyzers	

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TÜV SÜD Product Service

EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0610 Rev. 02

Manufacturer: **Roche Diagnostics GmbH**
Sandhofer Strasse 116
68305 Mannheim
GERMANY

Product: **Screening and Confirmatory Test for Hepatitis B marker**

The Certification Body of TÜV SÜD Product Service GmbH declares that a design examination has been carried out on the respective devices in accordance with IVDD Annex IV (4). The design of the devices conforms to the requirements of this Directive. See also notes overleaf.

Report No.: 713179013

Valid from: 2020-05-08
Valid until: 2024-05-26

Date, 2020-05-08

Christoph Dicks
Head of Certification/Notified Body

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A4 / 07.17

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NIP 527-23-22-068 REGON 016755430

TÜV SÜD Product Service

EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0630 Rev. 00

Model(s):

Elecsys Anti-HCV II
PreciControl Anti-HCV
Elecsys and cobas e analyzers

Facility(ies):

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Nonnenwald 2, 82377 Penzberg, GERMANY

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Sandhofer Strasse 116, 68305 Mannheim, GERMANY

Roche Diagnostics International Ltd Centralised and Point of Care Solutions
Forrenstrasse 2, 6343 Rotkreuz, SWITZERLAND

Parameters:

Model Name:	Model No.:
--	
Elecsys Anti-HCV II	06368921190
Elecsys Anti-HCV II	06427405190
Elecsys Anti-HCV II	07026889190
PreciControl Anti-HCV	03290379190
Elecsys and cobas e analyzers	

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EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0630 Rev. 00

Manufacturer: **Roche Diagnostics GmbH**
Sandhofer Strasse 116
68305 Mannheim
GERMANY

Product: **Screening test for Hepatitis C marker**

The Certification Body of TÜV SÜD Product Service GmbH declares that a design examination has been carried out on the respective devices in accordance with IVDD Annex IV (4). The design of the devices conforms to the requirements of this Directive. All applicable requirements of the testing and certification regulation of TÜV SÜD Group have to be complied with. For details and certificate validity see: www.tuvsud.com/ps-cert?q=cert:V7 010283 0630 Rev. 00

Report No.: 713182822

Valid from: 2020-11-24
Valid until: 2024-05-26

Date, 2020-11-24

Christoph Dicks
Head of Certification/Notified Body

TÜV SÜD Product Service GmbH is Notified Body with identification no. 0123

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ZLG-BS-245.10.07

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tel. 22 481 55 55, fax 22 481 55 99
NIP 527-23-22-068 REGON 016755430

TÜV SÜD Product Service

EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0621 Rev. 01

Model(s): **Elecsys HIV combi PT**
Elecsys and cobas e analyzers

Facility(ies):

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Nonnenwald 2, 82377 Penzberg, GERMANY

Roche Diagnostics GmbH Centralised and Point of Care Solutions
Sandhofer Strasse 116, 68305 Mannheim, GERMANY

Roche Diagnostics International Ltd Centralised and Point of Care Solutions
Forrenstrasse 2, 6343 Rotkreuz, SWITZERLAND

Parameters:

Model Name:	Model No.:
–	
Elecsys HIV combi PT	05390095190 08924163190
Elecsys HIV combi PT	07914504190 08924180190
Elecsys and cobas e analyzers	

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EC Certificate

EC Design-Examination Certificate
Directive 98/79/EC on In Vitro Diagnostic Medical Devices (IVDD), Annex IV (4) (List A)

No. V7 010283 0621 Rev. 01

Manufacturer: **Roche Diagnostics GmbH**
Sandhofer Strasse 116
68305 Mannheim
GERMANY

Product: **Screening test for HIV-1/-2 marker**

The Certification Body of TÜV SÜD Product Service GmbH declares that a design examination has been carried out on the respective devices in accordance with IVDD Annex IV (4). The design of the devices conforms to the requirements of this Directive. All applicable requirements of the testing and certification regulation of TÜV SÜD Group have to be complied with. For details and certificate validity see: www.tuvsud.com/ps-cert?q=cert:V7 010283 0621 Rev. 01

Report No.: 713182792

Valid from: 2020-11-12
Valid until: 2024-05-26

Date, 2020-11-10

Christoph Dicks
Head of Certification/Notified Body

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

RECEIVED Jaq 01/06/2015

December 19, 2014

Our STN: BL125459/0

BLA APPROVAL

Roche Molecular Systems, Inc.
Attention: Ms. Angela Tucker
4300 Hacienda Drive
Pleasanton, CA 94588

Dear Ms. Tucker:

We have approved your biologics license application for HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT) under your existing Department of Health and Human Services U.S. License No. 1636. HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT) is indicated for use with the cobas s 201 system, is a qualitative in vitro test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA, and Hepatitis B Virus (HBV) DNA in human plasma.

This test is intended for use to screen for HIV RNA, HCV RNA, and HBV DNA in plasma specimens from human donors, including donors of Whole Blood, blood components, Source Plasma, and other living donors. This test is also intended for use in testing plasma to screen organ and tissue donors when specimens are obtained while the donor's heart is still beating. For donations of Whole Blood and blood components, plasma specimens may be tested individually or in pools comprised of equal aliquots of not more than 6 individual specimens. For donors of hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood, or cord blood, and for donors of donor lymphocytes for infusion (DLI), plasma may be tested in pools comprised of not more than 6 individual specimens. For donations of Source Plasma, the sample may be tested in pools comprised of equal aliquots of not more than 96 individual specimens. Whereas this test can detect HIV-1 Group O RNA and HIV-2 RNA, detection of HIV-1 Group O RNA or HIV-2 RNA in donor specimens negative for anti-HIV-1 Group O antibodies or anti-HIV-2 antibodies, respectively, has not been demonstrated in clinical studies. This test is intended to be used in conjunction with licensed serology tests for HIV, HCV, and HBV. For an individual specimen, results are simultaneously detected and discriminated for HIV, HCV, and HBV. This test is not intended for use as an aid in diagnosis of infection with HIV, HCV, or HBV.

Under this authorization, you are approved to manufacture HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT) at your facility in Branchburg Township, Somerville, New Jersey. You may label your product with the proprietary name cobas® TaqScreen MPX Test, version 2.0, and you may market it in the U.S.

Jenilee

We did not refer your application to the Blood Products Advisory Committee because our review of information submitted in your BLA, including the clinical study design and trial results, did not raise concerns or controversial issues which would have benefited from an advisory committee discussion.

The dating period for HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT) shall be 12 months when stored at the required temperature of the kit components. We have approved the stability protocol in your license application for the purpose of extending the expiration dating of your kit under 21 CFR 610.12.

Please submit final container samples of the product and each kit component together with protocols showing results of all applicable tests. You may not distribute any lots of product until you receive a notification of release from the Director, Center for Biologics Evaluation and Research (CBER).

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in, including but not limited to, the manufacturing, testing, packaging, or labeling of HIV-1 Group O and M, HIV-2, HCV and/or HBV (HIV-1/HIV-2/HCV/HBV/Multiplex Discriminatory NAT), or in the manufacturing facilities.

You must submit reports of biological product deviations under 21 CFR 600.14. You should identify and investigate all manufacturing deviations promptly, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, at the following address:

Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Ave.
WO71-G112
Silver Spring, MD 20993-0002

Please submit all final printed labeling at the time of use and include implementation information on Form FDA 356h and Form FDA 2567 as appropriate. Please provide a PDF electronic copy as well as three original paper copies for circulars and other labels.

Two draft copies of the proposed introductory promotional labeling may be voluntarily submitted for advisory comment with a Form FDA 2253 to the Advertising and Promotional Labeling Branch, at the following address:

Roche Diagnostics Polska Sp. z o.o.
ul. Bobrowiecka 8, 00-728 Warszawa
tel. 22 481 55 55, fax 22 481 55 99
NIP 527-23-22-068 REGON 016755430

Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Ave.
WO71-G112
Silver Spring, MD 20993-0002

You must submit adverse experience reports in accordance with the Medical Device Reporting requirements for medical devices (21 CFR 803) as required by (21 CFR 600.80(k)(2)). Since your product is characterized as a device as well as a biological, submit these reports to the MedWatch System using MedWatch Reporting Form 3500A. Required reports should be submitted to the Food and Drug Administration, Center for Devices and Radiological Health, Medical Device Reporting, P.O. Box 3002, Rockville, Maryland 20847-3002.

Statement on Pediatric Postmarket Surveillance of Devices

Pediatric postmarket surveillance of devices ordered under section 522 of the Federal Food, Drug and Cosmetic Act must be registered at ClinicalTrials.gov in accordance with the provisions of Section 801 of FDAAA.

Please contact register@clinicaltrials.gov to determine the best mechanism for using ClinicalTrials.gov to address this provision.

AGREED UPON POSTMARKETING COMMITMENTS

We acknowledge your written commitments as described in your letter of October 6, 2014 as outlined below:

Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.

1. RMS commits to monitor for instances of HCV true positive samples that are reported as invalid due to the presence of signal spiking effects in the MPX v2.0 through RMS' Complaint Handling Program. The unnormalized raw data associated with complaints of this nature will be analyzed to verify the presence of signal spiking effects and samples will be requested for retesting. The complaint investigations will be stored in the RMS case handling system, will be periodically reported to FDA as a PMC Submission-Status Update, and will be included in the Annual Reports to FDA through the life cycle of the product.

2. RMS commits to monitor for the occurrence of mutations that could result in failure to detect some HIV -1 positive specimens through its Complaint Handling Program. Customer complaints that meet the criteria for a potentially critical complaint, (e.g., failure to detect HIV-1 mutations) are escalated for handling and investigation to the Complaint Investigation Resolution (CIR) group.

Requests for samples that result in a failure to detect HIV -1 positive specimens are made for additional investigation activities, which may include sequence analysis or testing with an alternative platform that detects the LTR region of the HIV-1 genome.

Specific information pertaining to HIV -1 mutations complaint investigations will be stored in the RMS case-handling system, will be periodically reported to FDA as a PMC Submission-Status Update, and will be included in the Annual Reports to FDA through the life cycle of the product.

We request that you submit information concerning nonclinical and chemistry, manufacturing, and control postmarketing commitments and final reports to your BLA, STN BL 125459.

Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Correspondence**
- **Postmarketing Study Commitment – Final Study Report**
- **Supplement Contains Postmarketing Study Commitment – Final Study Report**

For each postmarketing commitment not subject to the reporting requirements of 21 CFR 601.70, you may report the status to FDA as a "PMC Submission – Status Update." The status report for each commitment should include:

- The original schedule for the commitment, and
- The status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted).

When you have fulfilled your commitment, submit your final report as PMC Submission – Final Study Report or Supplement Contains Postmarketing Study Commitment – Final Study Report.

Sincerely yours,

Ginette Y. Michaud MD.

Ginette Michaud, MD for
Jay S. Epstein, MD
Director
Office of Blood Research and Review
Center for Biologics
Evaluation and Research

Enclosure

Roche Diagnostics Polska Sp. z o.o.
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tel. 22 481 55 55, fax 22 481 55 99
NIP 527-23-22-068 REGON 016755430

ms_04687787190V18.0

Elecsys HBsAg II

cobas®

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170
04687787 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 900
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 250

Intended use

Immunoassay for the in vitro qualitative determination of hepatitis B surface antigen (HBsAg) in human serum and plasma.

The electrochemiluminescence Immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Regulatory approval

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for screening of blood donations and, according to Paul-Ehrlich-Institut (PEI) recommendation[1], for use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating).

Summary

The hepatitis B surface antigen (HBsAg), a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus (HBV) particle.[2,3] The blood of persons infected with HBV contains, in addition to intact infectious HBV particles, an excess of smaller non-infectious 'empty' envelope particles, or filaments, formed from HBsAg.[4]

The HBsAg determinant 'a', against which the immune response is mainly directed, is common to all HBsAg particles. Within this 'a' determinant several HBsAg subtype determinants could be defined as d, y, w1-w4, r and q.[5] Under selective pressure (caused by antiviral therapy or by the action of the immune system itself) the virus can express many different viable HBsAg mutants (so-called 'escape mutants'). Some mutants might lead to a loss of detection in commercially available HBsAg assays.[3,6]

The Elecsys HBsAg II assay was specifically developed to detect a multitude of these mutants. HBsAg is the first immunologic marker of HBV infection and is generally present some days or weeks before clinical symptoms begin to appear. Detection of HBsAg in human serum or plasma indicates the presence of acute or chronic HBV infection.[7]

HBsAg assays are used within the scope of diagnostic procedures to identify persons infected with HBV and prevent the transmission of the virus by blood and blood products.[4,8]

HBsAg assays can also be used to monitor the course of the disease and the efficacy of therapy in persons with acute or chronic HBV infections.[9]

In addition, HBsAg assays are recommended as part of prenatal care, in order to initiate suitable measures for preventing as far as possible the transmission of an HBV infection to the newborn child.[10]

The Elecsys HBsAg II assay uses monoclonal and polyclonal anti-HBs antibodies (mouse and sheep) to detect HBsAg.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex[a] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as HBSAG II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HBsAg-Ab~biotin (gray cap), 1 bottle, 8 mL:
Two biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.

R2 Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 7 mL:
Monoclonal anti-HBsAg antibody (mouse), polyclonal anti-HBsAg antibodies (sheep) labeled with ruthenium complex > 1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative.

HBSAG II Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each:
Human serum; preservative.

HBSAG II Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each:
HBsAg approximately 0.5 IU/mL in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

The calibrators have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAG II Cal1 only) and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing HBsAg (HBSAG II Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[11,12]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per calibrator bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Elecsys HBsAg II

cobas®

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	4 weeks
on **cobas e** 411	4 weeks
on **cobas e** 411	6 weeks if stored alternately in the refrigerator and on the analyzers (up to 42 hours at 20-25 °C)

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Specimen collected from living patients, blood donors, or individual organ, tissue or cell donors may be used, including donor samples obtained while the donor's heart is still beating.
Performance for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) was established according to Paul-Ehrlich-Institut recommendation[1] with samples obtained within 24 hours after death.[13] Qualitative differences of neat (non-reactive) or spiked (reactive) specimens from cadaveric compared to living donors were not observed.

Criterion: Mean value of cadaveric specimens compared to specimens from living donors within a recovery of 75-125 %.

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma as well as plasma tubes containing separating gel.

Criterion: Correct assignment of negative and positive samples.

Stability:
For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 6 times.
For cadaveric specimens: Stable for 3 days at 20-25 °C, 7 days at 2-8 °C. The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys HBsAg II assay has not been established with body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 04687876190, PreciControl HBsAg II, for 16 x 1.3 mL
- REF 11820648122, HBsAg Confirmatory Test, 2 x 1.0 mL each of confirmatory and control reagent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

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After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against the NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).

The following reference materials from the Paul-Ehrlich-Institute, Langen (Germany), were also measured (U/mL) and compared with the WHO standard:

PEI Standard AD (information sheet 1985, subtype AD; 1000 U/mL; inactivated)

PEI Standard AY (information sheet 1985, subtype AY; 1000 U/mL; inactivated)

(1 IU/mL WHO Standard corresponds to 0.34 U/mL PEI Standard AY and 1 IU/mL WHO Standard corresponds to 0.44 U/mL PEI Standard AD)

Calibration frequency:

Calibration must be performed once per reagent lot using HBSAG II Cal1, HBSAG II Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (HBSAG II Cal1): 600-1700
Positive calibrator (HBSAG II Cal2): 3000-11000

Quality control

For quality control, use PreciControl HBsAg II.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of HBSAG II Cal1 and HBSAG II Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Interpretation of the results

Samples with a cutoff index < 0.90 are non-reactive in the Elecsys HBsAg II assay. These samples are considered negative for HBsAg and do not need further testing.

Samples having a cutoff index in the range ≥ 0.90 to < 1.0 are considered borderline in the Elecsys HBsAg II assay.

Samples with a cutoff index ≥ 1.0 are considered reactive.

All initially reactive or borderline samples should be redetermined in duplicate using the Elecsys HBsAg II assay. If cutoff index values < 0.90 are found in both cases, the sample is considered negative for HBsAg. Initially reactive or borderline samples giving cutoff index values of ≥ 0.90 in either of the redeterminations are considered repeatedly reactive. Repeatedly reactive samples must be investigated using an independent neutralization test (Elecsys HBsAg Confirmatory Test).

Samples confirmed by neutralization with human anti-HBs are regarded as positive for HBsAg.

Limitations - Interference

The assay is unaffected by icterus (bilirubin < 684 µmol/L or < 40 mg/dL), hemolysis (Hb < 1.24 mmol/L or < 2 g/dL), lipemia (Intralipid < 22.8 mmol/L or < 2000 mg/dL) and biotin (< 164 nmol/L or < 40 ng/mL).

Criterion: Correct assignment of negative and positive samples.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 6210 IU/mL.

No false negative result due to high-dose hook effect was found with the Elecsys HBsAg II assay up to a concentration of 1.5 million IU/mL.

There is no indication for a significant loss in sensitivity or specificity with samples having elevated levels of IgM up to 3678 mg/dL, IgA up to 3250 mg/dL and IgG up to 3817 mg/dL.

No significant interfering effects of 21 commonly used therapeutic drugs could be detected.

According to the present state of knowledge, it can be assumed that available assays for the detection of HBsAg cannot identify all infected blood samples or persons. A negative test result does not exclude with certainty a possible exposure to or an infection with the hepatitis B virus. Negative test results obtained for persons with a past exposure may be caused by an antigen concentration below the detection limit of this assay or the lack of reactivity of the antigens to the antibodies used in this assay.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys HBsAg II assay is combined with all assays performed on the analyzer - including the Elecsys HBsAg II assay itself:

From test	Step	To test	Step 0	Step 1	Step 2
HBsAg II	1	HBsAg II	x	x	x
HBsAg II	1	each other assay	x	x	x

If new tests are installed make sure that the Special Wash List is updated accordingly.

For the Elecsys Anti-HBs assay make sure that "Step 1" and "Step 2" are activated:

From test	Step	To test	Step 0	Step 1	Step 2
Anti-HBs	1	HBsAg II	-	x	x

The described additions to the Special Wash List have to be entered manually. Please refer to the operator's manual.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Detection limit

In order to determine the sensitivity, the HBsAg concentration which corresponds to the measuring signal of the cutoff value was read off the standard curves of serial dilutions of HBsAg standards (ad and ay) in human HBV-negative serum.

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	Paul-Ehrlich-Institute standards				WHO standard 00/588	
	Subtype ad, 1985		Subtype ay, 1985		Subtype ad	
Sample	COI	U/mL	COI	U/mL	COI	IU/mL
1	88.4	1.999	566	10.0	39.4	2.00
2	44.7	1.005	289	5.04	19.9	0.998
3	3.09	0.047	12.7	0.200	1.64	0.052
4	0.396	0.000	0.421	0.000	0.409	0.000
Cutoff sensitivity (cutoff = 0.9)	≤ 0.04 U/mL		≤ 0.04 U/mL		≤ 0.1 IU/mL	

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human sera and controls.

cobas e 411 analyzer						
	Repeatability[b]			**Intermediate precision**[c]		
Sample	Mean COI[d]	SD COI	CV %	Mean COI	SD COI	CV %
HS[e], negative	0.333	0.026	7.7	0.429	0.043	10.1
HS, weakly positive	3.95	0.088	2.2	1.43	0.082	5.7
HS, positive	55.7	2.19	3.9	113	1.28	1.1
PreciControl HBSAG II 1	0.505	0.042	8.3	0.380	0.044	11.7
PreciControl HBSAG II 2	3.77	0.055	1.5	3.41	0.113	3.3

b) Repeatability = within-run precision (n = 21)

c) Intermediate precision = between-run precision(n = 10)

d) COI = cutoff index

e) HS = human serum

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability[b]			**Intermediate precision**[f]		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	0.412	0.036	8.7	0.346	0.082	23.8
HS, weakly positive	2.42	0.048	2.0	2.34	0.141	6.0
HS, positive	284	3.27	1.2	280	11.2	4.0
PreciControl HBSAG II 1	0.455	0.038	8.5	0.439	0.049	11.2
PreciControl HBSAG II 2	3.59	0.075	2.1	3.61	0.126	3.49

f) Intermediate precision = within-laboratory (modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60))

Analytical specificity

1596 samples containing potentially interfering substances were tested with the Elecsys HBsAg II assay comprising specimens:

- containing antibodies against HAV, HCV, HIV, HTLV, CMV, EBV, HSV, Rubella, Parvo virus, VZV, Toxoplasma gondii, Treponema pallidum, Borrelia, Listeriosis
- containing autoantibodies (ANA, SLE), elevated titers of rheumatoid factor or HAMA antibodies
- positive for Mumps, Measles, Malaria
- after vaccination against HBV and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma, patients undergoing dialysis or patients suffering from alcoholic liver disease
- from pregnant women

No false positive result was found. 14 samples were found to be positive for HBsAg (1 out of each group of the HAV, HIV, HTLV and EBV antibody positive patients; 1 from a patient undergoing dialysis and 9 from pregnant women). 2 samples (1 after HBV vaccination and 1 with elevated RF) were initially positive, but negative after performing a second measurement. The overall specificity was 100 % (lower confidence limit 95 %, one-sided: 99.81 %).

Clinical sensitivity

A total of 1025 selected HBsAg confirmed positive samples in various stages of the disease were tested with the Elecsys HBsAg II assay. 1024 samples were correctly identified (1 sample was repeatedly negative (COI 0.81-0.88), positively neutralized with the Elecsys HBsAg Confirmatory Test; negative in a 3rd HBsAg assay, anti-HBs negative, anti-HBe negative, HBeAg negative, anti-HBc positive). The sensitivity in that group of 1025 samples is 99.9 %.

A total of 156 genotyped samples (genotype A (30), B (8), C (11), C/E (1), D (68), E (17), F (17), G (3), not assigned (1)) and all known HBsAg subtypes (CNTS "Centre National de la Transfusion Sanguine", n = 9 subtype panels) were tested with the Elecsys HBsAg II assay. All of them were positive except for 6 samples (2 of genotype A, 1 of genotype D and 3 of genotype E) with negative or low HBV-DNA (also negative in other HBsAg tests). A total of 115 samples comprising different HBsAg mutations were tested with the Elecsys HBsAg II assay and compared to 3 registered HBsAg assays.

Mutant panel	Elecsys HBsAg II tested/positive
Native mutant panel 1 (strains displaying amino acid substitutions either linked to vaccine resistance, resistance to therapy with human HB immunoglobulin or impaired HBsAg reactivity)	41/40[g]
Native mutant panel 2 (strains displaying other amino acid changes)	24/24
Native mutant panel 3	19/17[h]
Recombinant mutant panel	31/31
Total	115/112

g) sample (mutation G145R) negative in all assays (COI 0.1-0.6); all measurements were performed in 1:40 dilution (FCS: fetal calf serum)

h) samples (mutation M133L/M143T/G145R and mutation T45S/I49R/I113T114/I186P, respectively) negative in all assays tested; 1st mutation tested in 3 assays (COI 0.03-0.76), 2nd mutation tested in 4 assays (COI 0.03-0.78)

For 8 performance panels (Boston Biomedica, Inc.) the Elecsys HBsAg II assay shows a very good concordance with the data given in the respective product information (140 positives out of 150 samples tested). All positive assigned samples were positive with the Elecsys HBsAg II assay, resulting in a 100 % sensitivity.

Clinical specificity

The specificity of the Elecsys HBsAg II assay in a group of 6360 blood donors was found to be as follows: initially reactive (IR) specificity 99.91 %; repeatedly reactive (RR) specificity 99.98 %.

In the group of the 3593 daily routine samples (hospitalized patients, outpatients, pre-surgery, health care workers and anonymous testing), the specificity (IR and RR) was 99.88 %.

Group	Number	Initially reactive	Repeatedly reactive	Confirmed positive
Blood donors	6360	8	3	2

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Group	Number	Initially reactive	Repeatedly reactive	Confirmed positive
Hospitalized patients	3593	181	176[i]	122[j]

i) 5 samples could not be repeated due to insufficient sample volume

j) 55 samples could not be neutralized due to insufficient sample volume; 1 sample was negative with the Elecsys HBsAg II assay

Seroconversion panels

Seroconversion sensitivity of the Elecsys HBsAg II assay has been shown by testing 56 commercial seroconversion panels in comparison to registered HBsAg assays. In all panels the Elecsys HBsAg II assay shows detection of seroconversion equal to or earlier than other HBsAg assays.

References

1 Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, anti-HCV-Assays, HBsAg and Anti-HBc assays for Use with Cadaveric Samples; PEI 08/05/2014.

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3 Lee JM, Ahn SH. Quantification of HBsAg: basic virology for clinical practice. World J Gastroenterol 2011;17:283-289.

4 Liaw YF. Clinical utility of hepatitis B surface antigen quantification in patients with chronic hepatitis B: a review. Hepatology 2011;54:W1-E9.

5 Norder H, Couroucé AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes and HBsAg subtypes. Intervirology 2004;47:289-309.

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10 US Preventative Services Task Force. Screening for hepatitis B virus infection in pregnancy: US Preventative Services Task Force Reaffirmation Recommendation Statement. Ann Int Med 2009;150:569-873.

11 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

12 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

13 Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

GTIN Global Trade Item Number

CE 0123

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Elecsys Anti-HCV II

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REF	Σ	SYSTEM
06368921 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1020
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 286

Intended use

The Elecsys Anti-HCV II assay is an in vitro diagnostic test for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Regulatory approval

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for screening of blood donations and, according to Paul-Ehrlich-Institut (PEI) recommendation[1], for use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating).

Summary

The hepatitis C virus (HCV), first identified in 1989, is a leading cause of liver disease and a major healthcare concern with over 170 million persons (roughly 3 % of the human population), infected worldwide.[2,3] The highest prevalence is found in Africa, the Eastern Mediterranean and Asian regions.[3,4] HCV is a member of the Flaviviridae family and has a single-stranded, positive-sense RNA genome.[5] Currently over 67 subtypes have been identified and these have been classified into 7 genotypes (1-7).[6]

Due to the high rate of asymptomatic infections, clinical diagnosis is difficult and screening assays are of major importance.[7] Infection with HCV can lead to acute and chronic hepatitis disease. Approximately 70-85 % of HCV infections progress to chronic disease, although this varies according to patient gender, age, race and immune status.[5,8] Chronic HCV infection may lead to cirrhosis and hepatocellular carcinoma,[9] therefore, early anti-HCV detection is the first step in the management of chronic hepatitis and in the selection of patients needing treatment.[7] HCV infection can be detected by measuring the amount of HCV RNA, alanine aminotransferase (ALT) and HCV-specific immunoglobulins (anti-HCV) in patient serum or plasma samples. This can also indicate if the infection is acute or chronic.[5,8]

Anti-HCV antibody tests are used alone or in combination with other tests (e.g. HCV RNA) to detect an infection with HCV and to identify blood and blood products of individuals infected with HCV. The Elecsys Anti-HCV II assay is a third-generation test.[10,11] The Elecsys Anti-HCV II assay uses peptides and recombinant proteins representing HCV core, NS3 and NS4 antigens for the determination of anti-HCV antibodies.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, 55 µL of a reagent containing biotinylated HCV-specific antigens and 55 µL of a reagent containing HCV-specific antigens labeled with a ruthenium complex[a] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as A-HCV II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HCV-specific antigens~biotin (gray cap), 1 bottle, 18 mL:
Biotinylated HCV-specific antigens, HEPES[b] buffer, pH 7.4; preservative.

R2 HCV-specific antigens~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 18 mL:
HCV-specific antigens labeled with ruthenium complex ≥ 0.3 mg/L, HEPES buffer, pH 7.4; preservative.

b) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

A-HCV II Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each:
Human serum, preservative.

A-HCV II Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each:
Human serum positive for anti-HCV Ab; preservative. Non-reactive for HBsAg, anti-HIV 1/2.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV (A-HCV II Cal1 only) and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HCV (A-HCV II Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[12,13]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

The Elecsys Anti-HCV II assay has a high dilution sensitivity. Avoid any sample cross-contamination during sample pre-analytics.

Reagent handling

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

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All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after first opening at 2-8 °C	8 weeks
on the analyzers	31 days if continuously stored onboard (20-25 °C) or 7 weeks and up to 80 hours in total onboard (20-25 °C) if stored alternately in the refrigerator and on the analyzer

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Specimen collected from living patients, blood donors, or individual organ, tissue or cell donors may be used, including donor samples obtained while the donor's heart is still beating.
Performance for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) was established according to Paul-Ehrlich-Institut recommendation[1] with samples obtained within 24 hours after death.[14] Qualitative differences of neat (non-reactive) or spiked (reactive) specimens from cadaveric compared to living donors were not observed.

Criterion: Mean value of cadaveric specimens compared to specimens from living donors within a recovery of 75-125 %.

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPDA and Na-citrate plasma as well as plasma tubes containing separating gel.
Criterion: Correct assignment of positive and negative samples within a recovery of 80-120 % of serum value.

CPD and CP2D plasma.
Criterion: Correct assignment of positive and negative samples within a recovery of 80-130 % of serum value.

Stability:
For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 6 times.
For cadaveric specimens: Stable for 3 days at 20-25 °C, 7 days at 2-8 °C. The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys Anti-HCV II assay has not been established with body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 03290379190, PreciControl Anti-HCV, for 16 x 1.3 mL
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

Elecsys Anti-HCV II

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After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

No internationally accepted standard for anti-HCV exists.

Every Elecsys Anti-HCV II reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the A-HCV II Cal1 and A-HCV II Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Range for electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (A-HCV II Cal1): 400-3000 (all analyzers)
Positive calibrator (A-HCV II Cal2): 25000-350000 (all analyzers)

Quality control

For quality control, use PreciControl Anti-HCV.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of A-HCV II Cal1 and A-HCV II Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff-index (COI; signal sample/cutoff).

Interpretation of the results

Samples with a cutoff-index < 0.9 are non reactive in the Elecsys Anti-HCV II assay.

Samples with a cutoff-index ≥ 0.9 and < 1.0 are considered borderline in the Elecsys Anti-HCV II assay.

Samples with a cutoff-index ≥ 1.0 are reactive in the Elecsys Anti-HCV II assay.

All initially reactive or borderline samples should be redetermined in duplicate using the Elecsys Anti-HCV II assay. If no reactivity is found in both cases, the sample is negative for anti-HCV. If the result from either of the two measurements is reactive or borderline then the sample is repeatedly reactive. Repeatedly reactive samples must be investigated by supplemental methods (e.g. immunoblot or detection of HCV RNA). If one or both measurements remain borderline the analysis of a follow-up sample is recommended.

Limitations - Interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.00 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 172 nmol/L or < 42 ng/mL).

Criterion: Recovery of positive samples within ± 20 % of initial value, cutoff-index for negative samples ± 0.2 of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals and 3 drugs used in HCV therapy. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Studies have been performed to assess the high-dose hook effect. Out of 765 positive samples no false negative result was found. Occurrence of high-dose hook effect cannot be completely excluded.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Due to a long time period from infection to seroconversion, negative anti-HCV test results may occur during early infection. If acute hepatitis C infection is suspected, measuring of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR e.g. by COBAS AMPLICOR) may give evidence of HCV infection.

The detection of anti-HCV antibodies indicates a present or past infection with hepatitis C virus, but does not differentiate between acute, chronic or resolved infection. It is recognized within the scientific community that presently available methods for anti-HCV detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HCV infection. The antibody concentration may be beneath the detection limit of this assay or the patient´s antibodies do not react with the antigens used in this test. In addition, non-specific results cannot be ruled out with the Elecsys Anti-HCV II assay.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer						
	Repeatability[c]			**Intermediate precision**[d]		
Sample	Mean COI[e]	SD COI	CV %	Mean COI	SD COI	CV %
HS[f], negative	0.071	0.001	1.6	0.071	0.003	4.1
HS, weakly positive	1.86	0.049	2.7	1.86	0.085	4.6
HS, positive	20.0	0.476	2.4	20.0	1.04	5.2
PreciControl A-HCV1	0.097	0.001	1.4	0.097	0.004	3.8
PreciControl A-HCV2	4.39	0.113	2.6	4.39	0.185	4.2

c) Repeatability = within-run precision
d) Intermediate precision = within-laboratory precision
e) COI = Cutoff index
f) HS = Human serum

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, negative	0.034	0.006	16.3	0.034	0.007	20.4
HS, weakly positive	1.89	0.017	0.9	1.89	0.033	1.8

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MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
HS, positive	20.9	0.138	0.7	20.9	0.339	1.6
PreciControl A-HCV1	0.055	0.001	1.1	0.055	0.001	2.3
PreciControl A-HCV2	4.00	0.028	0.7	4.00	0.160	4.0

Analytical specificity

1037 samples containing potentially interfering substances or derived from high-risk groups were tested with the Elecsys Anti-HCV II assay comprising specimens:

- containing antibodies against HBV, HAV, HEV, EBV, CMV, HSV, HIV, VZV, Parvovirus, Mumps, Dengue, tick-borne encephalitis virus (TBEV), Rubella, Toxoplasma gondii, Treponema pallidum
- containing autoantibodies and elevated titers of rheumatoid factor, IgG, IgM or IgA antibodies
- positive for HBsAg and E. coli
- after vaccination against HBV and Influenza
- non-viral liver diseases
- alcoholic liver disease
- high-risk groups: hemophiliacs, homosexuals and intravenous drug abusers

	N	Elecsys Anti-HCV II reactive	Positive by immunoblot or indeterminate	Negative by immunoblot
Specimens containing potentially interfering substances	1037	59	58 positive	1[g]

g) EBV IgM positive patients: 1 out of 69 samples

Clinical sensitivity

Of 765 samples from HCV infected patients with different stages of disease and infected with different HCV genotypes (type 1, 2, 3, 4, 5 and 6), all samples were found to be reactive with the Elecsys Anti-HCV II assay.

Group	N	Reactive
HCV infected persons with different stages of disease	224	224
HCV genotypes (type 1, 2, 3, 4, 5, 6)	541	541

In the above study the diagnostic sensitivity was 100 %. The 95 % lower confidence limit was 99.61 %.

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HCV II assay has been shown by testing 60 commercial seroconversion panels. The Elecsys Anti-HCV II assay detected more positive bleedings than all other registered anti-HCV assays tested and was more sensitive in the recognition of early HCV infection than the Elecsys Anti-HCV assay and the other registered anti-HCV screening assays.

Clinical specificity

In a group of randomly selected European blood donors the specificity of the Elecsys Anti-HCV II assay was found 99.85 % (RR). The 95 % confidence interval (2-sided) was 99.73-99.93 %.

The diagnostic specificity of the Elecsys Anti-HCV II assay in a group of hospitalized patients was found 99.66 %. The 95 % confidence interval (2-sided) was 99.41-99.82 %.

	N	Elecsys Anti-HCV II IR[h] COI ≥ 1	Elecsys Anti-HCV II RR[i] COI ≥ 1	Positive or indeterminate by immunoblot and/ or HCV RNA
European blood donors	6850	15	15	2 confirmed positive, 3 indeterminate
Hospitalized patients	3922	153[j]	152[k]	128 confirmed positive, 8 indeterminate
Dialysis patients	731	19	18	12 confirmed positive
Pregnant women	629	3	3	2 confirmed positive

h) IR = Initially Reactive

i) RR = Repeatedly Reactive

j) 4 (positive) samples had to be excluded from calculation due to "qns" for Immunoblot analysis, qns = quantity not sufficient

k) 4 (positive) samples had to be excluded from calculation due to "qns" for Immunoblot analysis

References

1 Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, anti-HCV-Assays, HBsAg and Anti-HBc assays for Use with Cadaveric Samples; PEI 08/05/2014.

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12 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

13 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

14 Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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Elecsys Anti-HCV II

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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

Roche

ms_05390095190V11.0

Elecsys HIV combi PT

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REF	Σ	SYSTEM
05390095 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 880
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 111

Intended use

Immunoassay for the in vitro qualitative determination of HIV-1 p24 antigen and antibodies to HIV-1, including group O, and HIV-2 in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Regulatory approval

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for screening of blood donations and, according to Paul-Ehrlich-Institut (PEI) recommendation,[1] for use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating).

Summary

The human immunodeficiency virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), belongs to the family of retroviruses. HIV can be transmitted through sexual contact, contaminated blood and blood products or from an HIV-infected mother to her child before, during and after birth.
Two types of HIV, called HIV-1 and HIV-2, have been identified to date.[2,3,4,5] HIV-1 can be divided into 4 distantly related groups: group M (for main), group N (for non-M, non-O), group O (for outlier) and group P.[6,7,8] Based on their genetic relationship, 9 different subtypes (A to D, F to H, J, K) as well as several circulating recombinant forms (CRFs) have been identified within HIV-1 group M.[9] The large majority of HIV-1 infections are caused by viruses belonging to group M, while geographical distribution of subtypes and CRFs within this group varies strongly.[10] Due to differences in the sequence of immunodominant epitopes, especially in the envelope proteins of HIV-1 group M, HIV-1 group O and HIV-2, specific antigens are necessary to avoid failure in the detection of an HIV infection by immunoassays.[11,12]

HIV p24 antigen in blood specimen of recently infected patients can be detected as early as 2-3 weeks after infection.[13,14] Anti-HIV antibodies are detectable in serum from around 4 weeks post infection.[13,15] The combined detection of HIV p24 antigen and anti-HIV antibodies in 4th generation HIV screening assays leads to improved sensitivity and therefore a shorter diagnostic window compared to traditional anti-HIV assays.[16,17]

With the Elecsys HIV combi PT assay the HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2 can be detected simultanously within one determination. The assay uses recombinant antigens derived from the *env*- and *pol*-region of HIV-1 (including group O) and HIV-2 to determine HIV-specific antibodies. For the detection of HIV-1 p24 antigen specific monoclonal antibodies are used. Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms. Confirmatory tests include Western Blot and HIV RNA tests.

Test principle

Sandwich principle. Total duration of assay: 27 minutes.

- 1st incubation: Pretreatment of 40 µL of sample with detergent agent.
- 2nd incubation: Biotinylated monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides, and monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 3rd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R0, R1, R2) is labeled as HIVCOMPT.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R0 MES buffer 50 mmol/L, pH 5.5; 1.5 % Nonidet P40; preservative (white cap), 1 bottle, 4 mL.

R1 Anti-p24~, HIV-1/-2-specific recombinant antigens (E. coli)~, HIV-1/-2-specific peptides~biotin (gray cap), 1 bottle, 7 mL:
Biotinylated monoclonal anti-p24 antibodies (mouse), biotinylated HIV-1/-2-specific recombinant antigens (E. coli), biotinylated HIV-1/-2 specific peptides > 1.3 mg/L; TRIS buffer 50 mmol/L, pH 7.5; preservative.

R2 Anti-p24~, HIV-1/-2-specific recombinant antigens (E. coli)~, HIV-1/-2-specific peptides~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 7 mL:
Monoclonal anti-p24 antibodies (mouse), HIV-1/-2 specific recombinant antigens, HIV-1/-2 specific peptides labeled with ruthenium complex > 1.5 mg/L; TRIS buffer 50 mmol/L, pH 7.5; preservative.

HIVCOMPT Cal1 Negative calibrator (white cap), 2 bottles (lyophilized) for 1.0 mL each:
Human serum, non reactive for anti-HIV-1 and anti-HIV-2.

HIVCOMPT Cal2 Positive calibrator (black cap), 2 bottles (lyophilized) for 1.0 mL each:
Anti-HIV-1 positive human serum (inactivated) in human serum negative for anti-HIV-1 and anti-HIV-2.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

Elecsys HIV combi PT

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H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious.

The negative calibrator (HIVCOMPT Cal1) has been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HIV-1 (HIVCOMPT Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[18,19]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit are ready-for-use (except for HIVCOMPT Cal1 and HIVCOMPT Cal2) and are supplied in bottles compatible with the system.

HIVCOMPT Cal1 and HIVCOMPT Cal2: Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

cobas e 411 analyzer: The reconstituted calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per calibrator bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the reconstituted calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on**cobas e** 411 at 20-25 °C	28 days
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	28 days

Stability of the calibrators	
lyophilized	up to the stated expiration date
reconstituted at 2-8 °C	12 weeks
on**cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Specimen collected from living patients, blood donors, or individual organ, tissue or cell donors may be used, including donor samples obtained while the donor's heart is still beating.
Performance for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) was established according to Paul-Ehrlich-Institut recommendation[1] with samples obtained within 24 hours after death.[20] Qualitative differences of neat (non-reactive) or spiked (reactive) specimens from cadaveric compared to living donors were not observed.

Criterion: Mean value of cadaveric specimens compared to specimens from living donors within a recovery of 75-125 %.

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma as well as Li-heparin plasma tubes containing separating gel.

Criterion: Correct assignment of negative and positive samples.

Sampling devices containing liquid anticoagulants have a dilution effect resulting in lower cutoff index (COI) values for individual patient specimens.

In order to minimize dilution effects it is essential that respective sampling devices are filled completely according to manufacturer's instructions.

Stability:
For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 4 weeks at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 5 times.
For cadaveric specimens: Stable for 3 days at 20-25 °C, 7 days at 2-8 °C. The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys HIV combi PT assay has not been established with body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

Elecsys HIV combi PT

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- 2 x 4 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 05162645190, PreciControl HIV, for 6 x 2.0 mL
- REF 06924107190, PreciControl HIV Gen II, for 6 x 2.0 mL
- REF 06924115190, PreciControl HIV; HIV-2+GrpO, for 4 x 2.0 mL (optional use)
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer
- Distilled or deionized water

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the reconstituted calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: No internationally accepted standard for anti-HIV-1 and anti-HIV-2 exists.

This method has been standardized against the Human Immunodeficiency Virus Type 1 (HIV-1 p24 Antigen) - 1st International Reference Reagent 1992, code 90/636 - available from NIBSC (National Institute for Biological Standards and Control).

Calibration frequency: Calibration must be performed once per reagent lot using HIVCOMPT Cal1, HIVCOMPT Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (HIVCOMPT Cal1):
1200-3500 (**cobas e** 411 analyzer)
550-2200 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers)

Positive calibrator (HIVCOMPT Cal2):
17000-75000 (**cobas e** 411 analyzer)
14000-70000 (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers)

Quality control

For quality control, use PreciControl HIV or PreciControl HIV Gen II. The use of PreciControl HIV; HIV-2+GrpO is optional. Note that all HIV results are sufficiently controlled if only PreciControl HIV Gen II is used.

All controls should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of HIVCOMPT Cal1 and HIVCOMPT Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Interpretation of the results

Samples with a cutoff index < 0.90 are non-reactive in the Elecsys HIV combi PT assay. These samples are considered negative for HIV-1 Ag and HIV-1/-2 specific antibodies and do not need further testing. Samples having a cutoff index in the range ≥ 0.90 to < 1.0 are considered borderline in the Elecsys HIV combi PT assay.

Samples with a cutoff index ≥ 1.0 are considered reactive in the Elecsys HIV combi PT assay.

All initially reactive or borderline samples should be redetermined in duplicate with the Elecsys HIV combi PT assay. If cutoff index values < 0.90 are found in both cases, the samples are considered negative for HIV-1 Ag and HIV-1/-2 specific antibodies.

Initially reactive or borderline samples giving cutoff index values of ≥ 0.90 in either of the redeterminations are considered repeatedly reactive. Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms. Confirmatory tests include Western Blot and HIV RNA tests.

Elecsys HIV combi PT

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Limitations - interference

The assay is unaffected by icterus (bilirubin < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 0.310 mmol/L or < 500 mg/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Correct assignment of negative and positive samples.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

No false negative result due to high-dose hook effect was found with the Elecsys HIV combi PT assay.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

A negative test result does not completely rule out the possibility of an infection with HIV. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of HIV infection can occasionally yield negative findings. Yet unknown HIV variants can also lead to a negative HIV finding. The presence of HIV antigen or antibodies to HIV is not a diagnosis of AIDS.

Limits and ranges

Antigen detection

Detection limit: ≤ 2 IU/mL

The stated sensitivity was determined by reading off the HIV Ag concentration corresponding to the signal of the cutoff value from standard curves obtained by serial dilutions of the Human Immunodeficiency Virus Type 1 (HIV-1 p24 Antigen) - 1st International Reference Reagent 1992, code 90/636 - in human HIV-negative serum.

Antibody detection

No international accepted standard for HIV-specific antibody detection exists.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability[b]		**Intermediate precision**[c]	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[d], negative	0.203	0.026	-	0.031	-
HS, positive for anti-HIV-1	126	1.09	0.9	1.77	1.4
HS, positive for anti-HIV-2	7.57	0.069	0.9	0.111	1.5
HS, positive for anti-HIV-1 group O	10.2	0.097	1.0	0.155	1.5
HS, positive for HIV Ag	3.52	0.031	0.9	0.062	1.8
PreciControl HIV 1	0.236	0.023	-	0.026	-
PreciControl HIV 2	18.3	0.283	1.5	0.380	2.1
PreciControl HIV 3	55.2	1.12	2.0	1.42	2.6

b) Repeatability = within-run precision

c) Intermediate precision = between-run

d) HS = human serum

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS, negative	0.141	0.016	-	0.025	-
HS, positive for anti-HIV-1	138	3.22	2.3	5.08	3.7
HS, positive for anti-HIV-2	8.47	0.214	2.5	0.326	3.9
HS, positive for anti-HIV-1 group O	10.8	0.255	2.3	0.432	4.0
HS, positive for HIV Ag	3.65	0.084	2.3	0.141	3.9
PreciControl HIV 1	0.194	0.017	-	0.022	-
PreciControl HIV 2	19.6	0.404	2.1	0.707	3.6
PreciControl HIV 3	62.2	1.50	2.4	2.38	3.8

Analytical specificity

1182 samples containing potentially interfering substances were tested with the Elecsys HIV combi PT assay comprising specimens:

- containing antibodies against HAV, HBV, HCV, HTLV, CMV, EBV, HSV, VZV, Toxoplasma gondii, Treponema pallidum, Borrelia, Parvovirus B19
- containing autoantibodies and elevated titers of rheumatoid factor
- positive for Candida, E. coli, Plasmodium falciparum/vivax, Mycobacterium tuberculosis
- after vaccination against HAV, HBV, and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma

	N	Elecsys HIV combi PT assay		Western Blot[e]	Analytical specificity
		IR[f] COI ≥ 1	RR[g] COI ≥ 1		
Specimens containing potentially interfering substances	1182	1[h]	1	0	99.92 % 95 % lower confidence limit: 99.53 %

e) Western Blot confirmed positive/indeterminate

f) IR = initially reactive

g) RR = repeatedly reactive

h) Patients with monoclonal gammopathy: 1 out of 21

Clinical sensitivity

Of 179 HIV samples from early seroconversion phase (according to CTS definition), 172 samples were found positive with the Elecsys HIV combi PT assay.

Of 1532 samples from HIV infected patients in different stages of the disease and infected with HIV-1 group M, O and HIV-2, 1532 were found to be reactive with the Elecsys HIV combi PT assay. The sensitivity of the Elecsys HIV combi PT assay in this study was 100 %.

The 95 % lower confidence limit was 99.76 %.

Group	N	Reactive
HIV-1 infected persons from various stages of disease	338	338
Infection with HIV-1 group M (subtypes A-J)	629	629
Infection with HIV-1 group O	8	8
Infection with HIV-2	472	472
HIV Ag positive specimens	85	85

Elecsys HIV combi PT

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53 lysates of cell culture supernatants including different HIV-1 group M subtypes (A-H), HIV-1 group O, and HIV-2 were tested and found reactive in the Elecsys HIV combi PT assay.

In 46 follow-ups of very early HIV infections, 100 out of 105 samples were detected positive with the Elecsys HIV combi PT assay.

Clinical specificity

In a group of 7343 randomly selected blood donors from Europe and Asia the specificity of the Elecsys HIV combi PT assay was found 99.88 % (RR). The 95 % lower confidence limit was 99.77 %.

In a group of 4103 samples from unselected daily routine, dialysis patients and pregnant women the specificity of the Elecsys HIV combi PT assay was found 99.81 % (RR). The 95 % lower confidence limit was 99.62 %.

	N	Elecsys HIV combi PT assay		Western Blot[i)]	Clinical specificity (95 % lower confidence limit)
		IR COI ≥ 1	RR COI ≥ 1		
Blood donors	7343	13	11	1/1	99.88 % (99.77 %)
Unselected samples from daily routine	2721	33	33	26	99.74 % (99.47 %)
Dialysis patients	251	1	1	0	99.60 % (97.80 %)
Pregnant women	1131	1	1	1	100 % (99.67 %)

i) Western Blot confirmed positive/indeterminate. Samples with indeterminate WB were excluded from calculation.

Seroconversion panels

Seroconversion sensitivity of the Elecsys HIV combi PT assay has been shown by testing 102 commercial seroconversion panels in comparison to registered HIV combi assays or anti-HIV immunoassays and/or HIV Ag assays.

References

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19 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

20 Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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ms_06923348190V6.0

Elecsys Syphilis

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REF	Σ	SYSTEM
06923348 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1360
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 160

Intended use

Immunoassay for the in vitro qualitative determination of total antibodies to Treponema pallidum in human serum and plasma. The test is intended as an aid in the diagnosis of syphilis infection.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Regulatory status

This assay has been CE marked according to Directive 98/79/EC. Test performance has been established for diagnostic use and for screening of blood donations and, according to Paul-Ehrlich-Institut (PEI) recommendation,[1] for use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating).

Summary

Syphilis is caused by the intracellular gram-negative spirochete bacterium Treponema pallidum (TP) subspecies pallidum.[2]

Syphilis is mainly transmitted sexually, but can also be transmitted from mother to fetus during pregnancy or birth. The global incidence of infection in 2008 was approximately 10.6 million and the total number of infections during that year was estimated to be 36.4 million.[3] In the USA the national infection rate rose to 6.3 cases per 100000 people, the highest rate since 1994.[4] Certain European countries have also seen increases in the rate of infection[5,6] and large localized outbreaks.[7] Each year, globally, an estimated 2 million pregnancies are affected.[8]

Congenital syphilis is still common in the developing world, as many women do not receive antenatal care or the scheme does not include syphilis screening.[9] Up to 80 % of syphilis infected pregnant women show adverse pregnancy outcomes.[8] The World Health Organization recommends all women to be tested at their first antenatal visit and again in the third trimester.[8] If they are positive, the recommendation also includes treatment of the partner.

Typically, symptoms of syphilis start with a painless ulcer at the site of entry to the body (primary syphilis) followed by a widespread rash as the bacteria disseminate (secondary syphilis). This is followed by a lengthy latent (asymptomatic) period. Eventually, tertiary syphilis ensues, characterized by the development of granulomatous dermal lesions, neurosyphilis, and/or cardiovascular syphilis (which can be fatal).[10]

The immune response to T. pallidum is the main driver of lesion development.[10] The antibody response is directed not only against antigens specific to T. pallidum (treponemal antibodies), but antibodies are also generated against antigens which are not specific (non-treponemal antibodies); for example, antigens released during the cellular damage caused by the organism. Therefore, treponemal and non-treponemal tests co-exist for the diagnosis of syphilis.[2]

Non-treponemal tests detect antibodies against lecithin, cholesterol and cardiolipin, which are present in many syphilis patients.[2] Treponemal tests detect antibodies directed against T. pallidum antigens such as TpN47, TpN17 and TpN15, for IgM and IgG detection.[2] A positive treponemal antibody test result indicates exposure to T. pallidum but cannot distinguish between treated and untreated syphilis. Non-treponemal assays are useful to help distinguish between treated and untreated syphilis and are also used for monitoring the progression of disease and treatment response.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, biotinylated TP-specific recombinant antigens and TP-specific recombinant antigens labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as Syphilis.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 TP-specific recombinant antigens (E. coli)-biotin (gray cap), 1 bottle, 9 mL:
Biotinylated TP-specific recombinant antigens (E. coli), 0.7 mg/L; MES[b)] buffer 50 mmol/L, pH 6.5; preservative.

R2 TP-specific recombinant antigens (E. coli)-$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
TP-specific recombinant antigens labeled with ruthenium complex 0.7 mg/L; MES buffer 50 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

Syphilis Cal1 Negative calibrator (white cap), 2 bottles (lyophilized) for 1.0 mL each:
Human serum, non reactive for anti-TP antibodies; preservative.

Syphilis Cal2 Positive calibrator (black cap), 2 bottles (lyophilized) for 1.0 mL each:
Human serum, reactive for anti-TP antibodies; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

N-Methylisothiazolone hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[11,12]

Elecsys Syphilis

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Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in bottles compatible with the system.

Calibrators

Syphilis Cal1 and Syphilis Cal2: Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

cobas e 411 analyzer: The reconstituted calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per calibrator bottle set should be performed.

If necessary, freeze in aliquots; see section on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the reconstituted calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at -20 °C (± 5 °C) for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	56 days
on the analyzers	28 days

The lyophilized calibrators are stable up to the stated expiration date.

Stability of the reconstituted calibrators	
either at -20 °C (± 5 °C)	6 months (3 freeze/thaw cycles possible)
or at 2-8 °C	28 days
on **cobas e** 411 at 20-25 °C	up to 6 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Specimen collected from living patients, blood donors, or individual organ, tissue or cell donors may be used, including donor samples obtained while the donor's heart is still beating.
Performance for the use of cadaveric blood specimens (specimens collected post-mortem, non-heart-beating) was established according to Paul-Ehrlich-Institut recommendation[1] with samples obtained within 24 hours after death.[13] Qualitative differences of neat (non-reactive) or spiked (reactive) specimens from cadaveric compared to living donors were not observed.

Criterion: Mean value of cadaveric specimens compared to specimens from living donors within a recovery of 75-125 %.

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA, K_3-EDTA, ACD, CPD, CP2D, CPDA and Na-citrate plasma as well as K_2-EDTA plasma tubes containing separating gel.

Criterion: Mean recovery of positive samples within ± 20 % of serum value. Absolute deviation of samples with COI (cutoff index) values from 0.00-1.0 within ± 0.2 COI.

Sampling devices containing liquid anticoagulants have a dilution effect resulting in lower COI values for individual patient specimens. In order to minimize dilution effects it is essential that respective sampling devices are filled completely according to manufacturer's instructions.

Stability:
For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 12 months at -20 °C (± 5 °C). The samples may be frozen 5 times.
For cadaveric specimens: Stable for 2 days at 20-25 °C, 7 days at 2-8 °C. The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes or systems that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys Syphilis assay has not been established with body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 06923364190, PreciControl Syphilis, for 4 x 2.0 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer
- Distilled or deionized water

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution

Elecsys Syphilis

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- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the reconstituted calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Calibration frequency: Calibration must be performed once per reagent lot using Syphilis Cal1, Syphilis Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (Syphilis Cal1): 450-4000
Positive calibrator (Syphilis Cal2): 22000-140000

Quality control

For quality control, use PreciControl Syphilis.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of Syphilis Cal1 and Syphilis Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff).

Interpretation of the results

Samples with a cutoff index < 1.00 are non-reactive in the Elecsys Syphilis assay. These samples are considered negative for syphilis-specific antibodies and do not need further testing.

Samples with a cutoff index ≥ 1.00 are considered reactive in the Elecsys Syphilis assay.

All initially reactive samples should be redetermined in duplicate with the Elecsys Syphilis assay. If cutoff index values < 1.00 are found in both cases, the samples are considered negative for syphilis-specific antibodies.

Initially reactive samples giving cutoff index values of ≥ 1.00 in either of the redeterminations are considered repeatedly reactive. Repeatedly reactive samples must be confirmed according to recommended confirmatory algorithms.

Limitations - Interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.310 mmol/L or ≤ 500 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 246 nmol/L or ≤ 60 ng/mL
Rheumatoid factors	≤ 1500 IU/mL
Human serum albumin	≤ 10 g/dL
IgG	≤ 3.2 g/dL
IgA	≤ 2.8 g/dL
IgM	≤ 1.0 g/dL

Criterion: Mean recovery of positive samples within ± 15 %. Absolute deviation of samples with COI values from 0.00-1.0 within ± 0.2 COI.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No false negative result due to high-dose hook effect was found with the Elecsys Syphilis assay.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

A negative test result does not completely rule out the possibility of an infection with Treponema pallidum. Serum or plasma samples from the very early (pre-seroconversion) phase or the late phase of a syphilis infection can occasionally yield negative findings.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

Elecsys Syphilis

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cobas e 411 analyzer					
		Repeatability		Intermediate precision	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[c], negative	0.103	0.002	1.6	0.003	3.2
HS, positive 1	1.01	0.028	2.8	0.033	3.2
HS, positive 2	1.12	0.018	1.6	0.022	1.9
HS, positive 3	9.99	0.171	1.7	0.262	2.6
HS, positive 4	50.2	0.986	2.0	1.24	2.5
PC[d] Syphilis1	0.106	0.003	2.4	0.004	4.1
PC Syphilis2	4.95	0.101	2.1	0.161	3.2

c) HS = human serum

d) PC = PreciControl

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS, negative	0.062	0.001	2.0	0.002	3.6
HS, positive 1	1.10	0.017	1.5	0.055	5.0
HS, positive 2	1.19	0.014	1.2	0.055	4.6
HS, positive 3	11.1	0.146	1.3	0.508	4.6
HS, positive 4	54.6	0.910	1.7	2.69	4.9
PC Syphilis1	0.064	0.001	1.8	0.002	2.5
PC Syphilis2	5.36	0.082	1.5	0.138	2.6

Analytical specificity

236 samples containing antibodies against Borrelia, EBV, Rubella, HAV, HBV, HCV, HIV, CMV, HSV, E. coli, Toxoplasma gondii, ANA and rheumatoid factor, respectively, were tested with the Elecsys Syphilis assay. 227 samples were tested negative, 9 samples were tested positive for anti-TP antibodies (confirmed by Western Blot and other anti-TP assays). No cross-reactivity was found.

Clinical sensitivity

A total of 924 samples from patients with suspected syphilis infection (diagnostic routine and blood screening) from Europe and Asia were tested with the Elecsys Syphilis assay. Four additional samples were excluded due to probable handling errors with banked samples. 922 samples were found to be positive for anti-TP antibodies (either clinically defined or confirmed by FTA-Abs[h] and other anti-TP assays). Two samples were found to be indeterminate. Overall, 922 samples were found to be repeatedly reactive (RR) with the Elecsys Syphilis assay. The two indeterminate samples were found to be non-reactive with the Elecsys Syphilis assay. The resulting sensitivity of confirmed positive samples is 100 %. The 95 % lower confidence limit was 99.60 %.

Cohort	N	Confirmed positive samples	Indeterminate samples	False negative samples[e]	Sensitivity[f] %
Primary syphilis	101	101	0	0	100
Secondary syphilis	124	124	0	0	100
Latent syphilis	470	470	0	0	100
Syphilis, stage of the disease unknown	229	227	2	0	100
Total[g]	**924**	**922**	**2**	**0**	**100**

e) Elecsys Syphilis assay (RR)

f) Sensitivity of confirmed positive samples

g) Four additional samples were excluded due to probable handling errors with banked samples.

h) FTA (Fluorescent Treponemal Antibody) - Abs (absorption)

Clinical specificity

A total of 8079 samples (diagnostic routine and blood screening) from Europe and Asia were tested with the Elecsys Syphilis assay. 14 samples were found to be positive for anti-TP antibodies (confirmed by FTA-Abs and other anti-TP assays), 8063 samples were found to be negative and 10 samples were found to be repeatedly false reactive with the Elecsys Syphilis assay (negative in FTA-Abs and other anti-TP assays). The resulting specificity in the study is 99.88 %. The 95 % lower confidence limit was 99.77 %.

Cohort	N	Confirmed positive samples	Confirmed negative samples	False positive samples[i]	Specificity %
Diagnostic routine samples	3500	14	3486	7	99.80
Blood donor samples	4579	0	4577*	3	99.93
Overall specificity for all samples (routine cohorts and blood donations)	**8079**	**14**	**8063***	**10**	**99.88**

i) Elecsys Syphilis assay (RR)

* Two samples were excluded due to indeterminate confirmation results.

References

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Elecsys Syphilis

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NIP 527-23-22-068 REGON 016755430

cobas®

12 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

13 Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://e-labdoc.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

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

Roche

cobas® TaqScreen MPX Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

cobas® TaqScreen MPX Test, v2.0	MPX v2.0	96 Tests	P/N: 05969484 190
cobas® TaqScreen MPX Control Kit, v2.0	MPX CTL v2.0	6 Sets	P/N: 05965390 190
cobas® TaqScreen Wash Reagent	TS WR	5.1 L	P/N: 04404220 190

For testing cadaveric specimens with the **cobas®** TaqScreen MPX Test, version 2.0, the following kit is required by the user, in addition to the kits above:

cobas® TaqScreen Cadaveric Specimen Diluent Kit	CADV SPEC DIL	96 Tests	P/N: 05002125 190

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

INTENDED USE

The **cobas®** TaqScreen MPX Test, version 2.0 (v2.0) for use with the **cobas s 201** system, is a qualitative *in vitro* test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) Group M RNA, HIV-1 Group O RNA, Human Immunodeficiency Virus Type 2 (HIV-2) RNA, Hepatitis C Virus (HCV) RNA, and Hepatitis B Virus (HBV) DNA in human plasma.

This test is intended for use to screen for HIV-1 Group M RNA, HCV RNA, and HBV DNA in plasma specimens from individual human donors, including donors of whole blood, blood components, source plasma and other living donors. The test is also intended for use in testing plasma specimens to screen organ and tissue donors for HIV-1 Group M RNA, HCV RNA, and HBV DNA when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart beating) donors. This test is not intended for use on samples of cord blood. Plasma from all donors may be screened as individual specimens. For donations of whole blood and blood components, plasma specimens may be tested individually or in pools comprised of not more than 6 individual specimens. For donors of hematopoietic stem/progenitor cells (HPCs) sourced from bone marrow, peripheral blood or cord blood, and for donors of donor lymphocytes for infusion (DLI), plasma may be tested in pools comprised of not more than 6 individual specimens. For donations of source plasma, sample may be tested in pools comprised of not more than 96 individual specimens.

Whereas this test can detect HIV-1 Group O RNA and HIV-2 RNA, detection of HIV-1 Group O RNA or HIV-2 RNA in donor specimens negative for anti-HIV-1 Group O antibodies or anti-HIV-2 antibodies, respectively, has not been demonstrated in clinical studies.

This test is intended to be used in conjunction with licensed serology tests for HIV, HCV, and HBV.

For an individual specimen, results are simultaneously detected and discriminated for HIV, HCV, and HBV.

This test is not intended for use as an aid in diagnosis of infection with HIV, HCV, or HBV.

The **cobas®** TaqScreen MPX Test, v2.0 can be considered a supplemental test that confirms HIV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HIV and reactive for HIV on the **cobas®** TaqScreen MPX Test, v2.0.

The **cobas®** TaqScreen MPX Test, v2.0 can be considered a supplemental test that confirms HCV infection for specimens that are repeatedly reactive on a licensed donor screening test for antibodies to HCV and reactive for HCV on the **cobas®** TaqScreen MPX Test, v2.0.

The **cobas®** TaqScreen MPX Test, v2.0 can be considered a supplemental test that confirms HBV infection for specimens that are repeatedly reactive on a licensed donor screening test for Hepatitis B surface antigen, and reactive for HBV on the **cobas®** TaqScreen MPX Test, v2.0.

SUMMARY AND EXPLANATION OF THE TEST

A major concern regarding the transfusion of blood and blood components is the potential for transmission of viral infections, particularly with Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV). These agents are primarily transmitted by exposure to contaminated blood or blood and plasma products, exposure to certain body tissues or fluids, by sexual contact or by an infected mother to the newborn child.

HIV-1 is prevalent globally, with an estimated overall prevalence of 1.1% (0.56% in North America and 0.25% in Western Europe). Persons infected with HIV-1 can experience a brief, initially acute, flu-like illness associated with high levels of viremia in peripheral blood within 3 - 6 weeks of initial infection.[1] There are currently three principal genetic groups for HIV-1: Group M (main), Group N (non-M, non-O), and Group O (outlier). Group M is highly prevalent and is divided into 9 subtypes, as well as several circulating recombinant forms (CRFs).[2]

HIV-2 was first isolated in 1986 from patients in West Africa. Both HIV-1 and HIV-2 have the same modes of transmission and are associated with similar opportunistic infections and Acquired Immunodeficiency Syndrome (AIDS).[3] The prevalence of HIV-2 in some African nations reaches more than 1%, and HIV-2 is a growing concern in certain parts of Europe and India.[4] The first case of HIV-2 infection in the United States was diagnosed in 1987. The Centers for Disease Control and Prevention (CDC) advise that continued surveillance is needed to monitor HIV-2 in the US population.[3]

HCV is considered to be the principal etiologic agent responsible for 90%–95% of the post transfusion non-A and non-B hepatitis cases.[5,6] HCV occurs globally but the incidence is not well known because the infection is generally asymptomatic. However, the reported prevalence varies from 0.5 ~ 2.0% in Western Europe to as high as 20% in Egypt.

More than 2 billion people alive today have been infected with HBV at some time in their lives. Of these, about 350 million remain infected chronically and become carriers of the virus.[7-11] Three quarters of the world's population live in areas where there are high levels of infection. Every year there are over 4 million acute clinical cases of HBV.

Serological screening assays have greatly reduced, but not eliminated, the risk of transmission of viral infections by transfusion of blood and blood products. Testing of whole blood and source plasma donations for HBV was initiated with HBsAg assays in the early 1970s and anti-HBc in the 1980s. In addition to HBV screening, blood and plasma donations are routinely tested for HIV-1 and HIV-2 by screening with enzyme immunoassays (EIAs) and for anti-HCV by EIAs. Public demand for higher standards of screening for infectious agents in transfusion products has fueled the advancement of nucleic acid amplification technology (NAT). Studies have shown that testing for viral nucleic acids (HIV RNA,[12-14] HCV RNA,[8,13-16] and HBV DNA[17-20]) can further reduce the transmission risk of these agents in blood donations made during the seroconversion window period. This window period has been estimated as 22 days on average, but may be as long as 6 months, for HIV-1[21] With the implementation of HIV-1 mini-pool NAT testing, the infectious window period has been significantly shortened and the current risk of HIV-1 transmission is estimated to be approximately 1 in 2 million donations.[13-15] Similarly, the introduction of HCV RNA NAT reduced the antibody negative window period by approximately 60 days,[13-16] with a current estimated risk of approximately 1 – 2 in 1 million donations. HBV DNA NAT screening is being increasingly adopted. Studies from countries with low, moderate and high HBV prevalence have demonstrated NAT yield from window period and late stage HBV-infected donors, thus demonstrating reduced incidence of transfusion transmitted HBV.[15,19,20,22,23]

To improve the efficiency of testing for multiple targets, a multiplex (MPX) polymerase chain reaction (PCR) for simultaneous detection of multiple viruses has been developed. In MPX PCR, more than one target sequence is amplified and detected by using multiple pairs of primers and probes in one reaction tube.

The **cobas**® TaqScreen MPX Test, v2.0 is a qualitative multiplex test that enables the simultaneous detection and discrimination of HIV RNA, HCV RNA, and HBV DNA in a single test. The **cobas**® TaqScreen MPX Test, v2.0 uses a generic nucleic acid preparation technique on the COBAS® AmpliPrep Instrument. HIV RNA (HIV-1 Groups M and O RNA, HIV-2 RNA), HCV RNA and HBV DNA are amplified, detected and discriminated using automated, real time PCR on the COBAS® TaqMan® Analyzer. The test does not discriminate between HIV-1 Group M, HIV-1 Group O and HIV-2. The test also incorporates an Internal Control (IC) for monitoring test performance in each individual test as well as the AmpErase enzyme to reduce potential contamination by previously amplified material (amplicon).

PRINCIPLES OF THE PROCEDURE

The **cobas**® TaqScreen MPX Test, v2.0 used on the **cobas s** 201 system is based on 4 major processes:

1. Automated Specimen Pooling/Pipetting and Control Pipetting using the Hamilton MICROLAB® STAR/STARlet IVD Pipettor (optional)
2. Automated Specimen Preparation using the COBAS® AmpliPrep Instrument
3. Automated Amplification of Nucleic Acid and Real Time Automated Detection and discrimination of PCR products using the COBAS® TaqMan® Analyzer
4. Automated Data Management using the Pooling and Data Management (PDM) Software

Automated Specimen Pooling and Pipetting using the Hamilton MICROLAB STAR/STARlet IVD Pipettor

The Hamilton MICROLAB STAR/STARlet IVD Pipettor automates pipetting of pools and individual donor specimens, transfer of aliquots to Deep Well Plates (optional) and pipetting of Test Controls as part of the **cobas s** 201 system. The **cobas s** 201 system is used for testing donor pools and resolution testing of reactive pools to identify the reactive individual donor specimens. The **cobas s** 201 system is designed to process specimens in batches. A batch is defined as a collection of specimens and controls that are pipetted, extracted, amplified and detected together. When the pipetting of a batch is completed on the Hamilton MICROLAB STAR/STARlet IVD Pipettor, the batch is transferred into the COBAS® AmpliPrep Instrument for the next phase of the process. Manually pipetted specimens may be loaded directly onto the COBAS® AmpliPrep Instrument without prior use of the Hamilton MICROLAB STAR/STARlet IVD Pipettor.

Note: For testing of cadaveric specimens, the specimen should first be manually diluted 1:5 in cobas® TaqScreen Cadaveric Specimen Diluent (CADV SPEC DIL) prior to pipetting using the Hamilton MICROLAB STAR/STARlet IVD Pipettor.

Automated Specimen Preparation using the COBAS® AmpliPrep Instrument

Nucleic acids from the targets and added Armored RNA Internal Control (IC) molecules (which serve as the specimen preparation and amplification/detection process control) are simultaneously processed. The **cobas**® TaqScreen MPX Test, v2.0 contains reagents that accomplish five sequential steps on the COBAS® AmpliPrep Instrument. The Proteinase Solution digests proteins to promote lysis, inactivate nucleases and facilitate the release of RNA and DNA from viral particles. Addition of Lysis Reagent to the specimen results in viral lysis and nuclease inactivation by denaturation of proteins. RNA and DNA are released and simultaneously protected from nucleases. The released nucleic acids bind to the silica surface of the added Magnetic Glass Particles. This is mainly due to the net positive charge on the glass particle surface and net negative charge of the nucleic acids under the chaotropic salt concentration and ionic strength of the Lysis reaction. Wash Reagent removes unbound substances and impurities such as denatured proteins, cellular debris and potential PCR inhibitors (such as hemoglobin, etc.), and reduces the salt concentration. Purified nucleic acids are released from the Magnetic Glass Particles at an elevated temperature with Elution Buffer.

Automated Amplification of Nucleic Acid using the COBAS® TaqMan® Analyzer

After isolation of the purified nucleic acids from human plasma during automated specimen preparation, the **cobas**® TaqScreen MPX Test, v2.0 Master Mix (MPX2 MMX) is used for the amplification, detection and discrimination of HIV RNA, HCV RNA, HBV DNA, and IC RNA. Once activated by the addition of manganese acetate, the **cobas**® TaqScreen MPX Test, v2.0 Master Mix permits reverse transcription (for RNA targets), followed by PCR amplification of highly conserved regions of HIV-1 Group M, HIV-1 Group O, HIV-2 and HCV RNAs, HBV DNA, and IC RNA using specific primers. Concurrent detection of the amplified nucleic acid is accomplished by the generation of fluorescent signals from 5'-nucleolytic degradation of the HIV-1 (Groups M and O), HIV-2, HCV, HBV, and IC probes, also present in the Master Mix. Four unique fluorescent dyes are used: one dye labels the IC probe, and other three dyes label the HIV, HCV, and HBV probes, permitting independent identification of the HIV, HCV and HBV targets, and the IC. All three HIV targets are identified using the same dye and thus are not discriminated from each other.

Reverse Transcription and PCR Amplification

Reverse transcription and amplification reactions are performed with a thermostable recombinant enzyme, Z05D DNA Polymerase. In the presence of manganese (Mn^{2+}), Z05D DNA Polymerase has reverse transcriptase and DNA polymerase activities. This allows both reverse transcription and PCR amplification to occur in the same reaction mixture.

PCR amplification is accomplished using the Z05D DNA Polymerase, which extends the annealed primers along the target templates to produce a double-stranded DNA (amplicon). This process is repeated for multiple cycles, with each cycle doubling the amount of amplicon DNA. Amplification occurs only in the region of the target genomes between the primers; the entire genomes are not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® TaqScreen MPX 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[24], but not DNA containing deoxythymidine, or RNA containing ribouridine.[25,26] Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate as one of the dNTPs in the MPX2 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 **cobas**® TaqScreen MPX Test, v2.0 Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the MPX2 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 and therefore does not destroy target amplicon formed after PCR.

Real time Automated Detection of PCR Products using the COBAS® TaqMan® Analyzer

During PCR amplification, the intermittent high temperature during the cycling denatures the Target and IC amplicon to form single stranded DNA. The specific detection oligonucleotide probes hybridize to the single stranded form of the amplified DNA. Amplification, Hybridization and Detection occur simultaneously.

Detection of PCR Products[27,28]

The **cobas**® TaqScreen MPX Test, v2.0 Master Mix contains detection probes which are specific for HIV-1 (Groups M and O), HIV-2, HCV, HBV or IC nucleic acid. The HIV, HCV, HBV, and IC detection probes are each labeled with 1) one of four fluorescent dyes which act as a reporter and 2) another dye which acts as a quencher. Three unique reporter dyes are associated with the HIV, HCV or HBV specific probes and are measured at defined wavelengths. A fourth reporter dye is associated with the IC specific probe and is measured at a different wavelength. A single type of quencher dye is used in all probes. This system permits simultaneous detection and discrimination of the amplified HIV, HCV and HBV targets, and the IC, using four wavelengths.

Before PCR amplification begins, the probes are intact and the reporter dye fluorescence is suppressed by the quencher dye due to Förster-type energy transfer. During PCR amplification, the probes hybridize to specific single stranded DNA sequences and are cleaved by the 5' to 3' nuclease activity of the Z05D DNA Polymerase at the same time that amplification is occurring. Once the reporter and quencher dyes are separated by this cleavage, the fluorescent activity of the reporter dye is unmasked. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased.

Real time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes representing the viral targets and IC.

Automated Data Management using the Pooling and Data Management (PDM) Software

Roche PDM software allows the user to review and report results. The Roche PDM software assigns test results for all tests as non-reactive, reactive or invalid. In addition to retrieving and examining PCR results, the Roche PDM software allows the operator to print reports, search for results, accept donor results and optionally transmit results to a Laboratory Information System (LIS).

MATERIALS PROVIDED BY ROCHE

Three kits are required for the detection of HIV-1 (Groups M and O), HIV-2 and HCV RNA, and HBV DNA in plasma specimens: 1) **cobas**® TaqScreen MPX Test, v2.0, 2) **cobas**® TaqScreen MPX Control Kit, v2.0 and 3) **cobas**® TaqScreen Wash Reagent. Safety Data Sheets (SDS) are available on request from your local Roche office.

cobas® TaqScreen MPX Test, v2.0 — **MPX v2.0** — **96 Tests**
(P/N: 05969484 190)

MPX2 CS1
(MPX Magnetic Glass Particles Reagent Cassette)

MPX2 CS2
(MPX Lysis Reagent Cassette)

MPX2 CS3
(MPX Multi-Reagent Cassette)

MPX2 CS4
(MPX Test-Specific Reagent Cassette)

cobas® TaqScreen MPX Control Kit, v2.0 — **MPX CTL v2.0** — **6 Sets**
(P/N: 05965390 190)

MPX M(+)C, v2.0
(**cobas**® TaqScreen MPX Multi-Positive Control, v2.0)
(HIV-1 M Positive Control)
(HBV Positive Control)
(HCV Positive Control)

MPX O(+)C, v2.0
(**cobas**® TaqScreen MPX HIV-1 O Positive Control, v2.0)

MPX 2(+)C, v2.0
(**cobas**® TaqScreen MPX HIV-2 Positive Control, v2.0)

MPX (–) C, v2.0
(**cobas**® TaqScreen MPX Negative Control, v2.0)

cobas® TaqScreen Wash Reagent — **TS WR** — **5.1 L**
(P/N: 04404220 190)

TS WR
(**cobas**® TaqScreen Wash Reagent)

Note: For detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA and HBV DNA in cadaveric specimens, the following kit is required and provided, in addition to the kits mentioned above: cobas® TaqScreen Cadaveric Specimen Diluent.

cobas® TaqScreen Cadaveric Specimen Diluent Kit — **CADV SPEC DIL** — **96 Tests**
(P/N: 05002125 190)

CADV SPEC DIL
(**cobas**® TaqScreen Cadaveric Specimen Diluent)

OTHER MATERIALS REQUIRED BUT SOLD SEPARATELY (May Be Purchased From Roche)

This test must be run on the **cobas s** 201 system. The **cobas s** 201 system must be installed by a Roche Diagnostics Field Service Representative, and used as a complete system configuration. Individual **cobas s** 201 system components cannot be used as stand-alone devices, nor may other components be substituted. The **cobas s** 201 system utilizes the components listed below. Please refer to the Product Information Card for additional details.

Instrumentation and Software for cobas s 201 system

- Hamilton MICROLAB STAR/STARlet IVD Pipettor (optional), Pooling Manager workstation and software
- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer
- Docking Station (optional)
- AMPLILINK Data Station and software
- Roche PDM Server, Data Manager workstation and software
- **cobas**® TaqScreen MPX Test, v2.0 Test Definition File (TDF)
- **cobas s 201 system Operator's Manual**
- **cobas s** 201 system Known Issues List

Racks and Disposables

- COBAS® AmpliPrep Sample Racks (SK24) (P/N: 28122172001)
- COBAS® AmpliPrep SPU-racks (P/N: 05471664001)
- COBAS® AmpliPrep Reagent Racks (P/N: 28122199001)
- Sample Processing Units (SPU) (P/N: 03755525001)
- Sample Input Tubes (S-tubes) with Barcode Clips (P/N: 03137040001)
- Racks of K-tips (P/N: 03287343001)
- K-tube Box of 12 x 96 (P/N: 03137082001)
- COBAS® TaqMan® K-carrier (P/N: 28150397001)
- High Volume CO-RE Tips (1000 µL), filter (P/N: 04639642001)
- Deep Well Plates with Barcode Labels (P/N: 04639634001)
- Deep Well Plate Sealing Mats (P/N: 04789288001)
- Sample Carrier for 24 Test Tubes (P/N: 04639502001)
- Sample Carrier for 32 Test Tubes (P/N: 04639529001)
- Tip Carrier (P/N: 04639545001)
- Deep Well Plate Carrier (P/N: 04639553001)
- SK24 Rack Carrier (P/N: 04639600001)
- Microcide SQ™ or Hamilton Disinfectant Spray Kit (P/N: 06254250001)
- Hamilton MICROLAB Detergent Kit (P/N: 06254268001)
- Disposable gloves, powderless

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REAGENTS

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® TaqScreen MPX Test, v2.0 96 Tests (P/N: 05969484 190)	**MPX2 CS1** **MGP** (Magnetic Glass Particles, 2 x 7.0 mL) 93% Isopropanol**	2 x 48 Tests	 **DANGER** H225 Highly flammable liquid and vapour. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, sparks, open flames, and other ignition sources. No smoking. P233 Keep container tightly closed. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ eye protection/ face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish. 67-63-0 propan-2-ol
	MPX2 CS2 **LYS** (Lysis Reagent, 2 x 78 mL) Sodium citrate dihydrate 42.5% Guanidine thiocyanate** < 1% Polydocanol 0.9% Dithiothreitol	2 x 48 Tests	 **WARNING** H302 + H332 Harmful if swallowed or if inhaled. H319 Causes serious eye irritation. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273 Avoid release to the environment. P280 Wear eye protection/ face protection. P304 + P340 + P312 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P337 + P313 If eye irritation persists: Get medical advice/attention. P501 Dispose of contents/ container to an approved waste disposal plant. 593-84-0 guanidinium thiocyanate

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® TaqScreen MPX Test, v2.0 96 Tests (P/N: 05969484 190)	**MPX2 CS3** **Pase** (Proteinase Solution; 2 x 3.8 mL) TRIS buffer < 0.05% EDTA Calcium chloride Calcium acetate ≤ 7.8% Proteinase** Glycerol	2 x 48 Tests	 **DANGER** H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves. P284 Wear respiratory protection. P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor. 39450-01-6 Proteinase, Tritirachium album serine
	EB (Elution Buffer; 2 x 7.0 mL) TRIS buffer EDTA 0.09% Sodium azide ≤ 0.002% Poly rA RNA (synthetic)	2 x 48 Tests	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® TaqScreen MPX Test, v2.0 96 Tests (P/N: 05960484 190)	**MPX2 CS4** **MPX v2.0 MMX-R1** (MPX v2.0 Master Mix Reagent 1; 2 x 3.0 mL) Potassium acetate Manganese acetate Glycerol 14.4% Dimethyl sulfoxide 0.08% Sodium azide Acetic Acid **MPX MMX-R2, v2.0** (MPX Master Mix Reagent 2, v2.0; 2 x 2.5 mL) Tricine buffer Potassium chloride Potassium hydroxide 8% Dimethyl sulfoxide Glycerol EDTA Tween 20 Igepal CA630 < 0.12% dATP, dGTP, dCTP, dUTP < 0.01% Upstream and downstream HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, HBV and Internal Control primers < 0.01% Fluorescent-labeled HIV-1, HIV-2, HCV, HBV probes < 0.01% Fluorescent-labeled Internal Control probe < 0.01% Oligonucleotide aptamer < 0.01% Z05D DNA Polymerase (microbial) < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) 0.09% Sodium azide **MPX IC, v2.0** (MPX Internal Control, v2.0; 2 x 8 mL) TRIS buffer ≤ 0.002% Poly rA RNA (synthetic) EDTA 0.05% Sodium azide < 0.001% Non-infectious, synthetic internal control RNA encapsulated in MS2 bacteriophage coat protein	2 x 48 Tests	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® TaqScreen MPX Control Kit, v2.0 6 Sets (P/N: 05965390 190)	**MPX M(+)C, v2.0** (**cobas®** TaqScreen MPX Multi-Positive Control, v2.0; 6 x 1.8 mL) < 0.001% Non-infectious, synthetic HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein < 0.001% Non-infectious, synthetic HBV DNA encapsulated in Lambda bacteriophage coat protein < 0.001% Non-infectious, synthetic HCV RNA encapsulated in MS2 bacteriophage coat protein Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, HBcAg and HIV p24 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative** **MPX O(+)C, v2.0** (**cobas®** TaqScreen MPX HIV-1 O Positive Control, v2.0; 6 x 1.8 mL) < 0.001% Non-infectious, synthetic HIV-1 Group O RNA encapsulated in MS2 bacteriophage coat protein Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, HBcAg and HIV p24 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative** **MPX 2(+)C, v2.0** (**cobas®** TaqScreen MPX HIV-2 Positive Control, v2.0; 6 x 1.8 mL) < 0.001% Non-infectious, synthetic HIV-2 RNA encapsulated in MS2 bacteriophage coat protein Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, HBcAg and HIV p24 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative** **MPX (–) C, v2.0** 6 x 1.6 mL (**cobas®** TaqScreen MPX Negative Control, v2.0; 6 x 1.8 mL) Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, HBcAg and HIV p24 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA and HBV DNA not detectable by PCR methods 0.1% ProClin® 300 preservative**	6 x 24 Tests	 **WARNING** H317 May cause an allergic skin reaction. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/container to an approved waste disposal plant. 55965-84-9 mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

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Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® TaqScreen Wash Reagent 5.1 L (P/N: 04404220 190)	**TS WR** (**cobas®** TaqScreen Wash Reagent) Sodium citrate dihydrate 0.1% Methylparaben	1 x 96 Tests	N/A
cobas® TaqScreen Cadaveric Specimen Diluent Kit 96 Tests (P/N: 05002125 190)	**CADV SPEC DIL** (**cobas®** TaqScreen Cadaveric Specimen Diluent) EDTA	4 x 100 mL	Hazard labeling is indicated on Kit carton.

* Product safety labeling primarily follows EU GHS guidance.

**Hazardous substance

STORAGE AND HANDLING REQUIREMENTS N/A

A. Room temperature is defined as 15 to 30°C.

B. Do not freeze reagents or controls.

C. Store **MPX2 CS1, MPX2 CS2, MPX2 CS3** and **MPX2 CS4** at 2 to 8°C. Unused, these reagents are stable until the expiration date indicated.

D. After initial use, reagents are stable for 20 days at 2 to 8°C or until the expiration date, whichever comes first.

E. Reagents can be used for up to 5 instrument runs and up to a maximum of 40 cumulative hours on the COBAS® AmpliPrep Instrument. Reagents must be stored at 2 to 8°C between uses. The AMPLILINK software monitors the cumulative hours of the reagent cassettes on the COBAS® AmpliPrep Instrument, and blocks the cassettes from being used once the 40 cumulative hours are reached.

F. Reagents are stable for a total of 24 continuous hours on the COBAS® AmpliPrep Instrument. The AMPLILINK software does not monitor the continuous hours of the reagent cassettes on the COBAS® AmpliPrep Instrument, nor the number of instrument runs the cassettes have been used for. It is the user's responsibility to discard the reagent cassettes once the 24 continuous hours or 5 instrument runs are reached.

G. Store **MPX M(+)C, v2.0, MPX O(+)C, v2.0, MPX 2(+)C, v2.0,** and **MPX (–) C, v2.0** at 2 to 8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

H. Store **TS WR** at 15 to 30°C. Unopened **TS WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days at 15 to 30°C or until the expiration date, whichever comes first.

I. Store unopened **CADV SPEC DIL** at 15 – 30°C. Unopened **CADV SPEC DIL** is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days at 2 – 8°C or until the expiration date, whichever comes first.

PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

A. Specimens may be infectious. Use Universal Precautions when performing the test.[29, 30] Only personnel proficient in the use of the **cobas®** TaqScreen MPX Test, v2.0 and trained in handling infectious materials should perform this procedure. Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (10% dilute bleach). Follow by wiping the surface with 70% Ethanol.

B. **CAUTION: MPX M(+)C, v2.0, MPX O(+)C, v2.0, MPX 2(+)C, v2.0, and MPX (–) C, v2.0 contain plasma derived from human blood. The source material has been tested and found non-reactive for the presence of antibody to HIV-1/2, HCV, HIV p24 antigen, HBsAg, and antibody to HBcAg. Source material has also been tested using the cobas® TaqScreen MPX Test, v2.0. Testing of negative human plasma by PCR methods showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA or HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood-sourced materials should be considered potentially infectious and should be handled with Universal Precautions.** If spillage occurs, immediately disinfect with a freshly prepared solution of dilute bleach or follow appropriate site procedures.

C. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

D. **EB, MPX v2.0 MMX-R1, MPX MMX-R2, v2.0** and **MPX IC, v2.0** contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

E. **Heparin has been shown to inhibit PCR. Do not use heparinized plasma with this procedure.**

F. The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. False positive results may occur if cross contamination of specimens is not prevented during specimen handling and processing.

G. Use only supplied or specified required disposables to ensure optimal test performance.

H. Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross contamination of specimens or controls.

I. Before use, visually inspect each reagent cassette, control tube and Wash Reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.

J. Dispose of all materials that have come in contact with specimens and reagents in accordance with country, federal, state and local regulations.

K. Do not use a **cobas®** TaqScreen MPX Test, v2.0, **cobas®** TaqScreen MPX Control Kit, v2.0, or **cobas®** TaqScreen Wash Reagent Kit or **cobas®** TaqScreen Cadaveric Specimen Diluent Kit after its expiration date. Do not interchange, mix, or combine reagents from different kits or different lots. Do not load mixed reagent lots on the COBAS® AmpliPrep Instrument.

L. Safety Data Sheets (SDS) are available on request from your local Roche office.

M. Avoid contact of reagents with the skin, eyes or mucous membranes. **If contact does occur, immediately wash with large amounts of water, otherwise, burns can occur.** If these reagents are spilled, dilute with water before wiping dry. **Do not allow LYS, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.**

N. 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.

O. The use of excessively hemolyzed living donor specimens should be avoided.

P. Red blood cell contamination of plasma specimens (> 2.5%) may inhibit the **cobas®** TaqScreen MPX Test, v2.0.

Q. Do not use any component with damaged barcode labels at any phase of testing.

R. Store all specimens at specified temperatures since specimen exposure to elevated temperatures may affect virus stability. See SPECIMEN COLLECTION, STORAGE AND POOLING for specific instructions.

CONTROL AND REAGENT PREPARATION

A. Remove the **cobas®** TaqScreen MPX Control Kit, v2.0 and the **cobas®** TaqScreen MPX Test, v2.0, reagents from the refrigerator minimally 30 minutes prior to use.

SPECIMEN COLLECTION, STORAGE AND POOLING

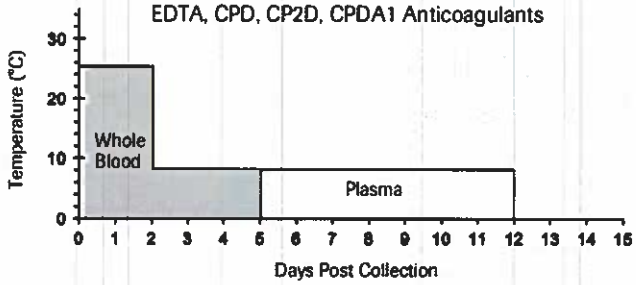
NOTE: Handle all specimens as if they are infectious agents.

Living Donor Specimens

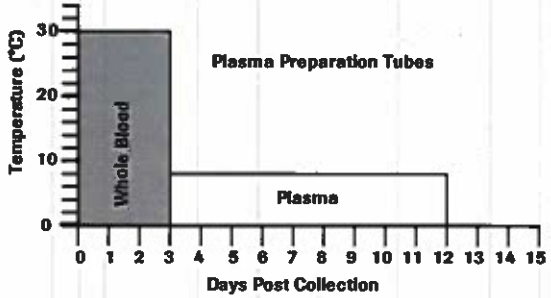
A. Plasma specimens collected using EDTA, CPD, CPDA1, CP2D and 4% Sodium Citrate anticoagulants, and Plasma Preparation Tubes (PPT) may be used with the **cobas®** TaqScreen MPX Test, v2.0. Follow the specimen collection tube/bag manufacturer instructions for handling and centrifugation. Virus stability may be affected by elevated temperatures.

B. Blood collected in EDTA, CPD, CPDA1 or CP2D anticoagulants may be stored for up to 48 hours at 2 – 25°C, and up to 72 hours at 2 – 8°C prior to plasma separation from cells. For storage longer than 5 days, separate the plasma from the red blood cells by centrifugation and remove the separated plasma from the red blood cells prior to storage. Following removal, plasma may be stored

at 2 – 8°C for up to 7 days, and up to 30 days at ≤ -18°C. Plasma may be frozen and thawed up to three times. Plasma in covered Deep Well Plates may be stored at 2 to 8°C for up to 7 days or at ≤ -16°C for up to 7 days.



C. Blood collected in Becton Dickinson Vacutainer® PPT may be stored for up to 72 hours at 2 – 30°C prior to plasma seperation by centrifugation. Gel-separated plasma in PPT may be stored at 2 – 8°C for an additional nine days.[40]



D. Apheresis plasma in 4% Sodium Citrate anticoagulant may be stored for up to 48 hours at 2 – 25°C. Apheresis plasma may then be stored at one of the following two conditions:

- 2 – 8°C for up to 28 days, and ≤ -18°C for 30 days, and thawed up to one (1) time.
- 2 – 8°C for up to 20 days, and ≤ -18°C for 6 months, and thawed up to two (2) times.

Apheresis plasma in deep well plates may be stored for up to 48 hours at 2 – 25°C, and up to 7 days at 2 – 8°C.

E. The following plasma volume guidelines are based on pipetting from 13 x 100 mm plastic donor tubes on the Hamilton MICROLAB STAR/STARlet IVD Pipettor. The listed volumes are for plasma on top of packed red blood cells, and are for use when running the **cobas®** TaqScreen MPX Test, v2.0.

Pool Type	Minimum Plasma Volume
Primary Pool*	3 mL
Repeat Pool	1.5 mL
Resolution Pool	2 mL

**Includes creation of Deep Well Plate*

F. **Do not freeze whole blood.**[40]

G. Allow refrigerated pooled or individual specimens to sit at room temperature for minimally 30 minutes before using.

H. The user must validate other collection and storage conditions. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of specimens and etiologic agents.[31]

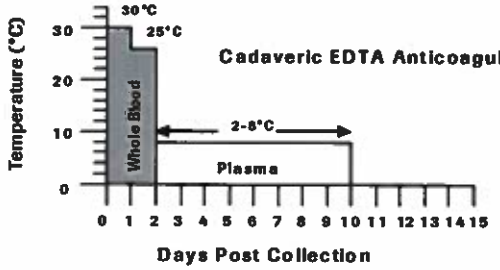
I. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**

Cadaveric Specimens

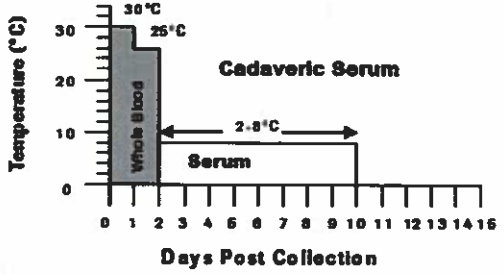
A. Specimens from cadaveric donors displaying a straw to pink color are classified as Moderately Hemolyzed and specimens displaying a red to dark red or brown color are classified as Highly Hemolyzed specimens.

B. Cadaveric blood specimens collected in EDTA anticoagulant tubes or in serum tubes may be used with the **cobas®** TaqScreen MPX Test, v2.0. Follow the tube manufacturer instructions. Specimen stability is affected by elevated temperatures.

C. Cadaveric blood collected in EDTA anticoagulant may be stored for up to 24 hours at 2 – 30°C, and up to 24 hours at 2 – 25°C prior to plasma separation from cells. For storage longer than 48 hours, separate the plasma from the red blood cells by centrifugation and remove the separated plasma from the red blood cells prior to storage. Following removal, plasma may be stored at 2 – 8°C for an additional 8 days. Alternatively, plasma may be stored at ≤ -18°C for up to 30 days after removal from red blood cells. Cadaveric EDTA plasma may be frozen and thawed up to three times.



D. Cadaveric blood collected as serum may be stored for up to 24 hours at 2 – 30°C, and up to 24 hours at 2 – 25°C prior to separation from cells. For storage longer than 48 hours, separate the serum from the clot by centrifugation and remove the separated serum from clot prior to storage. Following removal from cells, serum may be stored at 2 – 8°C for an additional 8 days. Alternatively, the serum may be stored at ≤ -18°C for up to 30 days after removal from the clot. Cadaveric EDTA serum may be frozen and thawed up to three times.



E. Cadaveric blood collected in PPT may be stored for up to 24 hours at 2 – 30°C and up to 24 hours at 2 – 25°C prior to plasma separation by centrifugation. For storage longer than 48 hours, gel separated plasma in PPT may be stored at 2 – 8°C for an additional 8 days.

F. Cadaveric specimens diluted 1:5 in the **cobas®** TaqScreen Cadaveric Specimen Diluent, may be stored for up to 7 days at 2 – 8°C and after remixing (by pipetting up and down four times in each tube), may be tested with the **cobas®** TaqScreen MPX Test, v2.0.

G. The storage of cadaveric specimens diluted 1:5 in the **cobas®** TaqScreen Cadaveric Specimen Diluent at ≤ -18°C has not been assessed.

H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of specimens and etiological agents.[31]

I. **False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.**

SPECIMEN POOLING AND PIPETTING

A. The **cobas s** 201 system utilizes the Hamilton MICROLAB STAR/STARlet IVD Pipettor for pipetting and pooling activities. The Hamilton MICROLAB STAR/STARlet IVD Pipettor performs barcode scanning and pooling operations from specimens to form pools.

B. If reactive pools are detected by the **cobas®** TaqScreen MPX Test, v2.0, the Hamilton MICROLAB STAR/STARlet IVD Pipettor may be used to pipette the individual specimens from either Deep Well Plates or original specimen tubes for secondary testing.

C. The **cobas s** 201 system may be installed without a Hamilton MICROLAB STAR/STARlet IVD Pipettor, which would require manual barcode ID entry. Refer to the ***cobas s 201 system Operator's Manual*** for instructions.

PROCEDURAL NOTES

A. **Equipment**

1. Prepare the **cobas s** 201 system for use according to instructions in the ***cobas s 201 system Operator's Manual***.
2. Perform recommended maintenance on instruments to ensure proper functioning.

B. **Reagents**

1. Remove the **cobas®** TaqScreen MPX Control Kit, v2.0 and the **cobas®** TaqScreen MPX Test, v2.0, reagents from the refrigerator minimally 30 minutes prior to use. The **cobas®** TaqScreen Wash Reagent must be at room temperature before use. See "Storage and Handling Requirements" Section for reagent storage conditions.
2. Each **cobas®** TaqScreen MPX Test, v 2.0 kit contains sufficient material for processing a total of 96 tests which are recommended to be run in batches consisting of up to 24 tests per SK24 rack. One replicate of the negative control (**MPX (–) C, v2.0**) and one replicate of each positive control (**MPX M(+)C, v2.0, MPX O(+)C, v2.0** and **MPX 2(+)C, v2.0**) must be processed with each batch or SK24 rack.
3. Each **cobas®** TaqScreen MPX Cadaveric Specimen Diluent kit contains sufficient material for processing a total of 96 tests which are recommended to be run in batches consisting of up to 24 tests per SK24 rack. One replicate of the negative control (**MPX (–)C, v2.0**) and one replicate of each positive control (**MPX M(+)C, v2.0, MPX O(+)C, v2.0** and **MPX 2(+)C, v2.0**) must be processed with each batch or SK24 rack. Controls are processed in the same way when testing living donor and cadaveric specimens with the **cobas®** TaqScreen MPX Test, v2.0.
4. All controls are for single use only.
5. The system will prevent the use of reagents which have exceeded the allowed usage on the COBAS® AmpliPrep Instrument (more than 40 cumulative hours or 20 days after initial use), reagents which have expired, or mixed cassettes from sets of cassettes previously used on the system.

C. **Specimen Processing**

1. Avoid contaminating gloves when handling specimens and controls.
2. Care should be used to avoid contamination of specimens and **MPX (–) C, v2.0** with Positive Controls.

D. **Individual Viral Target Identification**

The **cobas®** TaqScreen MPX Test, v2.0 simultaneously detects and discriminates the HIV, HCV and HBV targets. The three HIV targets (HIV-1 Group M, HIV-1 Group O and HIV-2) are not discriminated from each other.

INSTRUCTIONS FOR USE

The **cobas s** 201 system includes four major processes: Specimen and Control Pipetting on the optional Hamilton MICROLAB STAR/STARlet IVD Pipettor, Specimen preparation on the COBAS® AmpliPrep Instrument using the **cobas®** TaqScreen MPX Test, v2.0, Amplification/Detection on the COBAS® TaqMan® Analyzer and Data Management.

Each **cobas®** TaqScreen MPX Test, v2.0 kit contains eight cassettes: two **MPX2 CS1** cassettes with Magnetic Glass Particles, two **MPX2 CS2** cassettes with Lysis Reagent, two **MPX2 CS3** cassettes with Proteinase Solution and Elution Buffer, and two **MPX2 CS4** cassettes with MPX Internal Control, v2.0, MPX v2.0 Master Mix Reagent 1 and MPX Master Mix Reagent 2, v2.0. This test kit is to be used in conjunction with the **cobas®** TaqScreen MPX Control Kit, v2.0 and the **cobas®** TaqScreen Wash Reagent, and for processing cadaveric specimens, with the **cobas®** TaqScreen Cadaveric Specimen Diluent Kit.

Note: Do not open the cassettes.

Note: Do not pool reagents from different lots or from different bottles of the same lot.

Note: Do not mix reagents (including cassettes) from different kits. Do not load mixed reagent lots on the COBAS® AmpliPrep Instrument.

Note: Do not separate control tubes from adapters (the plastic control tube holder).

Note: The PDM Software tracks and enforces that a batch is run on a single COBAS® AmpliPrep Instrument and COBAS® TaqMan® Analyzer linked to the same AMPLILINK datastation.

Note: Do not separate batches across more than one COBAS® AmpliPrep Instrument or COBAS® TaqMan® Analyzer.

Perform all required maintenance as described in the ***cobas s 201 system Operator's Manual***.

Refer to the ***cobas s 201 system Operator's Manual*** for detailed instructions for use. It is important that the user follow the instructions in the ***cobas s 201 system Operator's Manual*** for proper performance of the assay.

A. Pipetting Controls and Specimens on the Hamilton MICROLAB STAR/STARlet IVD Pipettor

Note: Avoid contaminating gloves when preparing the specimens and controls.

Note: Mix controls by inversion at least 3 times as specified below.

- ***Inversion each time is defined as turning the control upside down and right side up again.***
- ***Within inversion each time, hold the control for at least 2 seconds in each orientation (i.e. turn the control upside down, and hold for at least 2 seconds. Then, turn the control right side up again, hold for at least 2 seconds.)***

Note: For testing of cadaveric specimens, Deep Well plate usage is disabled at the time of installation of the cobas s 201 system.

Note: The viral targets in controls, pooled, and individual specimens and diluted cadaveric specimens are stable on the system (up to 30°C) for at least 18 hours prior to sample extraction on the COBAS® AmpliPrep Instrument.

A1. Perform startup procedures on the Hamilton MICROLAB STAR/STARlet IVD Pipettor, then start the Roche PDM Pooling Wizard following the on-screen instructions.

A2. Use caution not to damage the identifier barcode on specimen tubes and control tube adapters. If damaged, the system will not be able to recognize the specimens or controls.

A3. Uncap the control tubes and load the specimens, consumables and controls onto the Hamilton MICROLAB STAR/STARlet IVD Pipettor. When the specimens, consumables, and controls have been loaded, the instrument transfers controls and specimens into S-tubes.

A4. For individual cadaveric specimens, pipette 2000 µL of **CADV SPEC DIL** into appropriately labeled 13 x 100mm tubes, add 500 µL of cadaveric specimen to each individual tube and mix each specimen by pipetting up and down four (4) times. Maintain correct sample identification during the manual dilution procedure. Load the diluted cadaveric specimens, consumables and controls onto the Hamilton MICROLAB STAR/STARlet IVD Pipettor. When the diluted cadaveric specimens, consumables and controls have been loaded, the instrument transfers controls and diluted specimens into S tubes.

A5. When the pipetting run is completed, review alarms and print the pooling report(s). Inspect pools and Deep Well Plate wells if used. Invalidate pools and/or wells if red blood cell contamination is observed or if volumes are inconsistent.

A6. Recap the S-tubes and transfer the SK24 rack(s) to the COBAS® AmpliPrep Instrument for nucleic acid extraction. The S-tubes must be recapped for proper operation of the COBAS® AmpliPrep Instrument.

A7. Cover the Deep Well Plates (if plates were created during the pipetting run).

A8. Remove and store the donor tubes. Refer to "Specimen Collection, Storage and Pooling" Section for conditions.

A9. Remove and discard the control tubes. (Control tubes are single use only.)

B. Preparation and Loading of cobas® TaqScreen MPX Test, v2.0 Reagents

Note: Use caution so as to not damage the cassette labels. The barcode reader on the COBAS® AmpliPrep Instrument automatically reads the barcode label of each cassette when the reagent racks are loaded onto the instrument.

B1. Remove reagents from refrigerator minimally 30 minutes prior to the first specimen being processed. No other reagent preparation is required.

B2. Prior to start, a sufficient number of all cassettes must be loaded to accommodate the total number of specimens that will be processed during continuous operation of the COBAS® AmpliPrep Instrument. Each cassette contains enough reagents for 48 tests. Refer to the *cobas s 201 system Operator's Manual* for information regarding loading of reagents for continuous operation.

B3. Place the **MPX2 CS1** cassette into a reagent rack, ensuring the cassette barcode is in line with the rack barcode located to the right side of the rack. **MPX2 CS1** cassettes must be loaded together on a separate reagent rack from the other cassettes.

B4. Load the reagent rack containing **MPX2 CS1** into rack position A. Do not load mixed reagent lots on the instrument.

B5. Place one set of **MPX2 CS2, MPX2 CS3** and **MPX2 CS4** cassettes for each **MPX2 CS1** cassette into a reagent rack(s), ensuring the cassette barcodes are in line with the rack barcode located to the right side of the rack.

B6. Load the reagent rack(s) into rack position B, C, D or E.

B7. LED lights on the COBAS® AmpliPrep Instrument Status bar will turn green when all required kit components are loaded and recognized by the system.

C. Extraction of Nucleic Acids from the Pipetted Specimens and Controls

Note: Perform the following steps on a clean bench surface.

C1. Remove the wrap from Sample Processing Unit (SPU) bundle, leaving tape and plastic cover intact.

C2. With the large tab of the SPU Rack facing toward the operator, insert SPU bundle flush with the right side of the SPU rack.

C3. Remove tape and plastic cover from SPUs seated in the rack. Ensure all SPUs are pressed down, level and fully seated in rack. Elevated SPUs may cause an instrument failure. Do not apply pressure to the S-tip in the SPU.

C4. Slide loaded SPU racks into COBAS® AmpliPrep Instrument SPU positions J, K or L until the rack is inserted completely and recognized. The instrument will hold up to 72 SPUs at a time. Load at least the number of SPUs needed for run or insert more as needed.

C5. Remove cellophane wrapping from manufacturer loaded K-tube and K-tip racks being careful not to tip the racks. Ensure that all are properly seated.

C6. Slide at least the required number of K-tube racks into positions M, N or O and K-tip racks into COBAS® AmpliPrep Instrument positions M, N, O or P.

C7. Load SK24 racks containing Hamilton MICROLAB STAR/STARlet IVD Pipettor pipetted specimens into COBAS® AmpliPrep Instrument Positions F, G or H. Slide in until rack is locked. Check system status Sample window to ensure all specimens on each rack were recognized.

C8. Check AMPLILINK software to ensure adequate reagents and consumables are loaded for desired specimen preparation.

C9. Press Start on AMPLILINK software workstation to begin the COBAS® AmpliPrep Instrument Specimen Preparation Procedure.

D. Amplification and Detection

Note: The Working Master Mix plus processed specimens contained in the SK24 rack has a limited stability. The COBAS® TaqMan® Analyzer must be ready to accept samples as soon as the COBAS® AmpliPrep Instrument is finished with the Specimen Preparation Procedure.

D1. For installations without a Docking Station, transfer the SK24 rack containing processed specimens and mastermix to the COBAS® TaqMan® Analyzer to automatically start amplification and detection. AMPLILINK software tracks each processed specimen as to when Master Mix has been added and will invalidate the specimen if amplification is not started within the defined time in the Test Definition File. For workflow simplicity, transfer the SK24 rack to the COBAS® TaqMan® Analyzer within 1 hour of completion of specimen preparation of that rack.

D2. When the amplification and detection is completed on the COBAS® TaqMan® Analyzer, the analyzed K-tubes are automatically disposed of in the waste bin.

D3. The results are automatically accepted and transferred to the PDM software.

E. Reviewing and Releasing Results

E1. Log on to the Roche Data Manager workstation.

E2. Retrieve Unevaluated Batches on the "Review Batches" tab at the Data Manager workstation.

E3. Review Alarms by highlighting a batch and then clicking "Next".

E4. Review Control Results on the "Controls Review" tab. Refer to the "Quality Control" Section for control validity criteria.

E5. Review Pool Results on the "Pools Review" tab for the selected batch. Non-Reactive pools can be invalidated manually by the user if required. Donor specimens in an invalid pool must be retested.

E6. Review and Release Donors on the "Donor Review" tab for the selected batch.

E7. Print reports and send to Laboratory Information System (LIS), if applicable.

QUALITY CONTROL

1. One replicate of the Negative Control (**MPX (–) C, v2.0**) and one replicate of each of the three Positive Controls (**MPX M(+)C, v2.0, MPX O(+)C, v2.0** and **MPX 2(+)C, v2.0**) must be processed with each batch.
2. **Batch Status:** A Batch Status is assigned "Complete, Valid" when the batch controls are valid. If any control within a batch is invalid, the entire batch is invalid and must be repeated. The invalidation of results based on control failures is performed automatically by the PDM software.
   a. Negative Control

      For a batch to be valid, the Negative Control (**MPX (–) C, v2.0**) must be valid. For the Negative Control to be valid, the interpreted result must be Non-reactive, and the associated Internal Control must be valid. If the interpreted result for the Negative Control is invalid, the entire batch is invalid.

   b. Positive Controls

      For a batch to be valid, the 3 Positive Controls (**MPX M(+)C, v2.0, MPX O(+)C, v2.0** and **MPX 2(+)C, v2.0**) must be valid. For the 3 Positive Controls to be valid, the interpreted result for each Positive Control target must be reactive, and the associated Internal Control must be valid. If the interpreted result for any of the Positive Controls is invalid, the entire batch is invalid.
3. Internal Control for Donor Specimens
   a. For a donor specimen to have a valid non-reactive (-) test result, the associated Internal Control must be valid, otherwise, the non-reactive result is invalid and the donor specimen must be retested.
   b. For a donor specimen to have a valid reactive test result, the associated Internal Control may be either valid or invalid.

RESULTS

1. Specimen Results are valid only if the batch containing them is valid. See "Quality Control" Section for acceptance criteria. Four parameters are measured for each specimen, one for each viral target and another for the Internal Control.
2. Final donor results for the **cobas®** TaqScreen MPX Test, v2.0 are reported by the PDM Software as follows:

Donor Status	Interpretation
Completed Non-Reactive	The final result for each target was determined, and all targets are Non-Reactive.
Accepted Non-Reactive	The Completed Non-Reactive result was accepted.
Completed Reactive	The result for each target was determined and at least one target is Reactive.
Accepted Reactive	The Completed Reactive result was accepted.
Completed Unresolved	The viability time limit expired before the result was accepted and none of the results were Reactive.
Accepted Unresolved	The Completed Unresolved result was accepted.

Repeat for Individual Specimen

Donor tubes with a final result of invalid for one target require repeat testing regardless of the final results for the other targets. However, the user has the option of selecting the "Force Unresolve" button to complete the workflow for a donor. The "Force Unresolve" function assigns an Accepted Unresolved donor status to donors not having a final result of reactive for any target or assigns an Accepted Reactive status to donors having a result of reactive for one or more targets.

Secondary Pooling Test

Donor tubes in a multi-specimen pool with a final result of invalid for one target require repeat testing if the results for the other targets are non-reactive or invalid.

When a multi-specimen pool is reported as reactive for one or more targets, the **cobas s** 201 system assigns all donors within that pool the pooling request for a secondary pooling test. These donor specimens are pipetted by the Hamilton MICROLAB STAR/STARlet IVD Pipettor (from either the Deep Well Plates or from the original donor tubes) into smaller pools or as pools of one, and further tested with the **cobas**® TaqScreen MPX Test, v2.0 as part of the resolution process to identify the reactive individual donor specimens. Refer to the ***cobas s 201 system Operator's Manual*** for specific information on performing resolution testing.

When a subdivided, multi-specimen pool is tested as non-reactive for all targets, the individual specimen(s) are reported as "Completed" with a final result of Non-Reactive.

WARNINGS

Warning: This assay may not detect some HIV positive specimens due to LTR mutations in the primers and/or probe binding regions of the HIV-1 genome. It is estimated that such LTR mutations occur in approximately 1.7% of HIV-1 NAT (+)/Antibody (-) donations, which represents an estimated risk to the blood supply of 1 in 121,176,500 donations.

LIMITATIONS

Procedural Limitations

1. The **cobas**® TaqScreen MPX Test, v2.0 has been evaluated only for use in combination with the **cobas**® TaqScreen MPX Control Kit, v2.0, the **cobas**® TaqScreen Wash Reagent and the **cobas s** 201 system.
2. For testing of cadaveric specimens, the **cobas**® TaqScreen MPX Test, v2.0 has been evaluated only for use with the **cobas**® TaqScreen Cadaveric Specimen Diluent Kit, the **cobas**® TaqScreen MPX Control Kit, v2.0, the **cobas**® TaqScreen Wash Reagent and the **cobas s** 201 system.
3. **Heparin has been shown to inhibit PCR. Do not use heparinized plasma with this procedure.**
4. Reliable results are dependent on adequate specimen collection and proper transport procedures.
5. Only the Hamilton MICROLAB STAR/STARlet IVD Pipettor has been validated for use with the **cobas**® TaqScreen MPX Test, v2.0, for the automated preparation of plasma pools. Adhere to the hardware instructions and safety precautions outlined in the ***cobas s 201 system Operator's Manual*** and the User Manual for the Hamilton MICROLAB STAR/STARlet IVD Pipettor.
6. 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.

Limitations of the Test

1. Detection of HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, storage, patient factors (i.e. age, presence of symptoms), and/or stage of infection and pool size.
2. Though rare, mutations within the highly conserved regions of a viral genome covered by the **cobas**® TaqScreen MPX Test, v2.0 primers and/or probe may result in failure to detect a virus.
3. Some true positive HCV specimens may be reported as "invalid" due to signal spiking, which would require retesting. The estimated frequency of this occurrence in NAT(+)/Antibody(-) donations in the U.S. is 1 in 180 million.
4. Specimens with a high viral load may generate invalid results. Retest in accordance with the ***cobas s 201 system Operator's Manual*** to resolve invalid results.

PERFORMANCE CHARACTERISTICS

LIVING DONOR SPECIMENS

Analytical Sensitivity — WHO International Standards/Roche Standards/CBER Standard

The Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test, v2.0 for HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, and HBV DNA were determined using the following standards: the WHO SECOND International Standard for HIV-1 RNA, Second International Standard (NIBSC code 97/650)[37], the WHO INTERNATIONAL STANDARD FOR HEPATITIS B VIRUS DNA FOR NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) ASSAYS (NIBSC code 97/746)[32], the WHO SECOND INTERNATIONAL STANDARD FOR HEPATITIS C VIRUS RNA FOR GENOMIC AMPLIFICATION TECHNOLOGY ASSAYS (NIBSC code 96/798)[33] and the Roche Primary Standards for HIV-1 Group O and HIV-2. Additionally, the LOD of the **cobas**® TaqScreen MPX Test, v2.0 for HIV-2 was determined using the HIV-2 RNA International Standard (NIBSC code 08/150).[39] No international standard is currently available for HIV-1 Group O RNA. The Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA are commercially available cultured virus stocks, PN 2420

(Boston Biomedica, Inc.) and Cat. No. 10-27-000 (Advanced Biotechnologies, Inc.). The Roche HIV-1 Group O and HIV-2 Standards are traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot 01 and to the CBER HIV-2 RNA Lot Release Panel ISD, respectively.

For the WHO and Roche standards, 3 independent dilution series of each viral standard were prepared with pooled human plasma collected in EDTA anticoagulant. Each dilution series was tested using 3 different lots of the **cobas**® TaqScreen MPX Test, v2.0 kits with approximately 20 replicates per lot, for a total of approximately 180 replicates per concentration. For the HIV-2 International Standard, 10 replicates per lot from 3 independent dilutions and 3 reagent lots were tested, for a total of 90 replicates per concentration. PROBIT analysis on the combined data from all replicates tested for each virus was used to estimate the LOD and 2-sided 95% fiducial confidence intervals (**Table 1**). PROBIT analysis also was used to estimate the 95% LOD and 2-sided 95% fiducial confidence intervals on the individual lot results for each virus (**Table 8** to **Table 10**).

Table 1 to **Table 10** summarizes the overall results of the Analytical Sensitivity Study. The commonly used conversion factors for International Units (IU) to copies for HIV-1 Group M, HCV and HBV are 0.6[34], 2.7[35] and 5.0[36], respectively.

Table 1.

PROBIT Analysis for Viral Standards

Analyte	Standard	Units	Average 95% LOD	95% Lower Limit	95% Upper Limit
HIV-1 Group M	WHO Second International Standard	IU/mL	46.2	35.5	65.9
HIV-1 Group O	Roche Primary Standard	Copies/mL	18.3	13.1	30.9
HIV-2	Roche Primary Standard	Copies/mL	56.1	48.6	66.5
HIV-2	WHO International Standard	IU/mL	7.9	5.6	13.8
HCV	WHO Second International Standard	IU/mL	6.8	5.8	8.3
HBV	WHO International Standard	IU/mL	2.3	2.0	2.8

The **cobas**® TaqScreen MPX Test, v2.0 should be performed with 2 replicates to detect HBV DNA at approximately 2 IU/mL at greater than 95% probability when the assay is used for reentry of donors deferred because of HBV test results. A reactive result in at least 1 of the 2 replicates indicates the sample is reactive for HBV DNA.

Table 2.

Analytical Sensitivity Summary: WHO International Standard for HIV-1 (97/650)

HIV-1 Group M RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
100.00	180	180	100.0%	98.4%
75.00	182	183	99.5%	97.4%
50.00	176	183	96.2%	92.9%
25.00	148	183	80.9%	75.5%
12.50	119	183	65.0%	58.8%
7.50	65	183	35.5%	29.6%
2.50	23	183	12.6%	8.7%

Table 3.

Analytical Sensitivity Summary: Roche Primary Standard for HIV-1 Group O

HIV-1 Group O RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
54.00	177	177	100.0%	98.3%
36.00	178	178	100.0%	98.3%
18.00	172	179	96.1%	92.8%
9.00	145	178	81.5%	76.0%
5.50	101	180	56.1%	49.7%
1.75	45	178	25.3%	20.0%

Table 4.

Analytical Sensitivity Summary: Roche Primary Standard for HIV-2

HIV-2 RNA Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
184.00	184	184	100.0%	98.4%
123.00	183	184	99.5%	97.4%
91.50	182	182	100.0%	98.4%
61.00	172	184	93.5%	89.6%
31.00	153	184	83.2%	77.9%
18.50	133	185	71.9%	65.9%
6.00	52	185	28.1%	22.7%

Table 5.

Analytical Sensitivity Summary: International Standard for HIV-2 (08/150)

HIV-2 RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
17.50	90	90	100.0%	96.7%
12.30	89	90	98.9%	94.8%
7.00	86	90	95.6%	90.1%
3.50	68	90	75.6%	67.0%
2.10	36	90	40.0%	31.3%
0.70	13	90	14.4%	8.8%

Table 6.

Analytical Sensitivity Summary: WHO International Standard for HCV (96/798)

HCV RNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
32.00	180	180	100.0%	98.4%
25.00	182	183	99.5%	97.4%
16.00	183	183	100.0%	98.4%
8.00	179	183	97.8%	95.1%
4.00	158	183	86.3%	81.4%
2.50	127	183	69.4%	63.3%
1.00	79	183	43.2%	37.0%

Table 7.

Analytical Sensitivity Summary: WHO International Standard for HBV (97/746)

HBV DNA Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactives	95% Lower Confidence Bound (one-sided)
6.00	180	180	100.0%	98.4%
5.00	183	183	100.0%	98.4%
3.00	178	183	97.3%	94.3%
1.50	155	183	84.7%	79.6%
1.00	147	183	80.3%	74.9%
0.50	92	183	50.3%	44.0%
0.25	53	183	29.0%	23.5%

Table 8.

Reagent Lot 1 Limits of Detection

Reagent Lot 1	Units	Lowest Observed Conc. With 95% Reactivity	PROBIT 95% LOD	95% Lower Limit	95% Upper Limit
HIV-1 Group M	IU/mL	75.0	48.4	38.3	65.6
HIV-1 Group O	Copies/mL	36.0	21.7	16.7	31.2
HIV-2*	Copies/mL	91.5	53.6	42.7	72.4
HIV-2*	IU/mL	12.3	8.0	6.0	12.4
HCV	IU/mL	8.0	7.2	5.5	10.8
HBV	IU/mL	3.0	2.9	2.3	4.1

Table 9.

Reagent Lot 2 Limits of Detection

Reagent Lot 2	Units	Lowest Observed Conc. With 95% Reactivity	PROBIT 95% LOD	95% Lower Limit	95% Upper Limit
HIV-1 Group M	IU/mL	50.0	48.3	29.1	127.5
HIV-1 Group O	Copies/mL	18.0	18.8	14.6	26.8
HIV-2*	Copies/mL	91.5	62.6	49.5	85.3
HIV-2*	IU/mL	7.0	8.9	6.5	14.4
HCV	IU/mL	8.0	7.0	5.4	10.1
HBV	IU/mL	3.0	2.0	1.6	2.9

Table 10.

Reagent Lot 3 Limits of Detection

Reagent Lot 3	Units	Lowest Observed Conc. With 95% Reactivity	PROBIT 95% LOD	95% Lower Limit	95% Upper Limit
HIV-1 Group M	IU/mL	50.0	41.6	33.1	56.2
HIV-1 Group O	Copies/mL	18.0	14.3	8.6	50.4
HIV-2*	Copies/mL	61.0	51.7	40.3	72.8
HIV-2*	IU/mL	7.0	6.8	5.1	10.5
HCV	IU/mL	8.0	6.2	4.1	15.2
HBV	IU/mL	3.0	2.0	1.6	2.8

** The HIV-2 data using the Roche Standard is shown in Copies/mL.*

** The HIV-2 data using the International Standard 08/150 is shown in IU/mL.*

Analytical Sensitivity – CBER Panels for HIV-1 Group M, HCV, HBV, HIV-1 Group O and CBER HIV-2 Panel Stock

The FDA CBER panels for HIV-1 Group M, HIV-1 Group O, HIV-2 HCV, and HBV were tested with the **cobas**® TaqScreen MPX Test, v2.0. One replicate of each of the FDA CBER panel members was tested across three reagent lots. For the HIV-1 Group M panel, the **cobas**® TaqScreen MPX Test, v2.0 detected all members with titers of 25 to 500 cp/mL, irrespective of reagent lot (**Table 11**). For the HIV-1 Group O panel, the **cobas**® TaqScreen MPX Test, v2.0 detected all members with titers of 25 to 1000 cp/mL, irrespective of reagent lot (**Table 12**). For the HIV-2 panel, the **cobas**® TaqScreen MPX Test, v2.0 detected all members with titers of 5 to 100 cp/mL, irrespective of reagent lot (**Table 13**). For the HCV panel, the **cobas**® TaqScreen MPX Test, v2.0 detected all members with titers of 5 to 500 cp/mL, irrespective of reagent lot (**Table 14**). For the HBV panel, the **cobas**® TaqScreen MPX Test, v2.0 detected all members with titers of 10 to 500 cp/mL, irrespective of reagent lot (**Table 15**).

Table 11.

CBER HIV-1 Group M Panel Results

Panel Member	Titer (Cp/mL)	Expected HIV Result[a]	Observed HIV Result		
			Reagent Lot 1	Reagent Lot 2	Reagent Lot 3
HIV-1 M #1901	0	Non-reactive	Non-reactive	Non-reactive	Non-reactive
HIV-1 M #1902	10	---[b]	Non-reactive	Reactive	Reactive
HIV-1 M #1903	50	---[b]	Reactive	Reactive	Reactive
HIV-1 M #1904	100	Reactive	Reactive	Reactive	Reactive
HIV-1 M #1905	500	Reactive	Reactive	Reactive	Reactive
HIV-1 M #1906	100	Reactive	Reactive	Reactive	Reactive
HIV-1 M #1907	25	---[b]	Reactive	Reactive	Reactive
HIV-1 M #1908	5	---[b]	Reactive	Non-reactive	Reactive

[a] *Expected result when testing the panel at the indicated titer using the* ***cobas***® *TaqScreen MPX Test, v2.0.*

[b] *Results obtained on this panel member were expected to be either reactive or non-reactive and were for information purposes only.*

Table 12.

CBER HIV-1 Group O Panel Results

Panel Member	Titer (Cp/mL)	Expected HIV Result[a]	Observed HIV Result		
			Reagent Lot 1	Reagent Lot 2	Reagent Lot 3
HIV-1 O #NC1	0	Non-reactive	Non-reactive	Non-reactive	Non-reactive
HIV-1 O #01	1000	Reactive	Reactive	Reactive[c]	Reactive
HIV-1 O #02	100	---[b]	Reactive	Reactive	Reactive
HIV-1 O #03	25	---[b]	Reactive	Reactive	Reactive

[a] *Expected result when testing the panel at the indicated titer using the* ***cobas***® *TaqScreen MPX Test, v2.0.*

[b] *Results obtained on this panel member were expected to be either reactive or non-reactive and were for information purposes only.*

[c] *Replicate had a HBV (Channel 2) reactive result. Repeat testing using the COBAS® TaqMan® HBV Test For Use With The High Pure System, produced a non-reactive result.*

Table 13.

CBER HIV-2 Panel Results

Panel Member	Titer (Cp/mL)	Expected HIV Result[a]	Observed HIV Result		
			Reagent Lot 1	Reagent Lot 2	Reagent Lot 3
HIV-2 #1	0	Non-reactive	Non-reactive	Non-reactive[c]	Non-reactive
HIV-2 #2	5	---[b]	Reactive	Reactive	Reactive
HIV-2 #3	10	---[b]	Reactive	Reactive	Reactive
HIV-2 #4	50	Reactive	Reactive	Reactive	Reactive
HIV-2 #5	100	Reactive	Reactive	Reactive	Reactive

[a] *Expected result when testing the panel at the indicated titer using the* ***cobas***® *TaqScreen MPX Test, v2.0.*

[b] *Results obtained on this panel member were expected to be either reactive or non-reactive and were for information purposes only.*

[c] *Replicate had a HCV (Channel 3) reactive result. Repeat testing using the COBAS® TaqMan® HCV Test For Use With The High Pure System, produced a non-reactive result.*

Table 14.

CBER HCV Panel Results

Panel Member	Titer (Cp/mL)	Expected HCV Result[a]	Observed HCV Result		
			Reagent Lot 1	Reagent Lot 2	Reagent Lot 3
HCV #2	0	Non-reactive	Non-reactive	Non-reactive[c]	Non-reactive
HCV #6	500	Reactive	Reactive	Reactive	Reactive
HCV #7	100	Reactive	Reactive	Reactive	Reactive
HCV #8	50	---[b]	Reactive	Reactive	Reactive
HCV #9	10	---[b]	Reactive	Reactive	Reactive
HCV #10	5	---[b]	Reactive	Reactive[d]	Reactive

[a] *Expected result when testing the panel at the indicated titer using the* ***cobas***® *TaqScreen MPX Test, v2.0.*

[b] *Results obtained on this panel member were expected to be either reactive or non-reactive and were for information purposes only.*

[c] *Replicate had a HBV (Channel 2) reactive result. Repeat testing using the COBAS® TaqMan® HBV Test For Use With The High Pure System, produced a reactive result below the tests limit of detection (4.8 IU/mL).*

[d] *Replicate had a HBV (Channel 2) reactive result. Repeat testing using the COBAS® TaqMan® HBV Test For Use With The High Pure System, produced a non-reactive result.*

Table 15.

CBER HBV Panel Results

Panel Member	Titer (Cp/mL)	Expected HBV Result[a]	Observed HBV Result		
			Reagent Lot 1	Reagent Lot 2	Reagent Lot 3
HBV #1	0	Non-reactive	Non-reactive	Non-reactive	Non-reactive
HBV #2	10	---[b]	Reactive	Reactive	Reactive
HBV #3	100	Reactive	Reactive	Reactive	Reactive
HBV #4	50	---[b]	Reactive	Reactive	Reactive
HBV #5	500	Reactive	Reactive	Reactive	Reactive

[a] *Expected result when testing the panel at the indicated titer using the* ***cobas***® *TaqScreen MPX Test, v2.0.*

[b] *Results obtained on this panel member were expected to be either reactive or non-reactive and were for information purposes only.*

Genotype/Subtype Inclusivity

HIV-1 Group M

A total of 77 HIV-1 Group M neat clinical specimens and 81 HIV-1 Group M diluted clinical specimens were tested with the **cobas®** TaqScreen MPX Test, v2.0. For HIV-1 Group M specimens tested neat, the **cobas®** TaqScreen MPX Test, v2.0 detected all 77 specimens (**Table 16**). For 1:6 diluted specimens, all specimens of all subtypes were detected by the **cobas®** TaqScreen MPX Test, v2.0, except one of 10 Subtype B specimens (**Table 17**).

Table 16.
HIV-1 Group M Subtype Specimens Neat

Subtype	Total Number of Specimens	**cobas®** TaqScreen MPX Test, v2.0 Number Reactive
A	7	7
AE	10	10
AG	10	10
B	10	10
C	10	10
D	3	3
E	8	8
F	7	7
G	10	10
G-BG	1	1
J	1	1

Table 17.
HIV-1 Group M Subtype Specimens Diluted

Subtype	Total Number of Specimens	**cobas®** TaqScreen MPX Test, v2.0 Number Reactive
A	7	7
AE	10	10
AG	10	10
B	10[a]	9
C	10	10
D	3	3
E	8	8
F	10	10
G	10	10
G-BG	1	1
J	1	1
BF	1	1

[a] One specimen was confirmed to have a low titer when diluted (below Limit of Detection for MPX v2.0) with the COBAS® AmpliPrep /COBAS® TaqMan® HIV-1 Test, v2.0.

HIV-1 Group O and HIV-1 Group N

A total of nine HIV-1 Group O cultured isolates were tested. Six of these isolates were characterized in particles/mL. For these six HIV-1 Group O isolates, all replicates were detected at the highest concentration, and at least 1 replicate from each of the 6 isolates was detected at 10 particles/mL (**Table 18**). The remaining three HIV-1 Group O isolates were characterized only by serial dilution.

All replicates were detected at a 1 x 10^{-5} dilution, and at least 1 replicate from each of the isolates was detected at a 1 x 10^{-7} dilution (**Table 19**).

One HIV-1 Group N cultured isolate was tested in 24 replicates each at concentrations of approximately 3X and 1X the Limit of Detection for HIV-1 Group M. All replicates were detected at both concentrations (**Table 20**).

Table 18.
HIV-1 Group O Isolates

Subtype	Culture Supernatants	Detected at (Particles/mL)						
		0.001	0.01	0.1	1	10	100	1000
HIV-1 Group O	6	33% (1/3)	100% (3/3)	22% (4/18)	44% (8/18)	89% (16/18)	83% (15/18)	100% (18/18)

Table 19.
HIV-1 Group O Additional Isolates

Subtype	Culture Supernatants	Dilution level				
		1 x 10^{-9}	1 x 10^{-8}	1 x 10^{-7}	1 x 10^{-6}	1 x 10^{-5}
HIV-1 Group O	3	0% (0/9)	0% (0/9)	67% (6/9)	100% (9/9)	100% (9/9)

Table 20.
HIV-1 Group N Cultured Isolates

Subtype	Culture Supernatants	Detected at ~0.3X LOD	Detected at ~1X LOD	Detected at ~3X LOD
HIV-Group N	1	100% (24/24)	100% (24/24)	100% (24/24)

HIV-2

One HIV-2 subtype A and one HIV-2 subtype B cultured isolate were tested. The HIV-2 Subtype A cultured isolate was diluted in pooled virus-negative human plasma and was detected 100% of the time at 91.5 Copies/mL (~12.9 IU/mL after conversion to WHO International units) and was detected as low as 6 Copies/mL (~0.85 IU/mL after conversion to WHO International units). Log dilutions of the HIV-2 Subtype B were prepared in normal, virus-negative human plasma and 3 of 3 replicates of each dilution were detected with the **cobas®** TaqScreen MPX Test, v2.0 in all dilutions to 1 x 10^{-5}. No clinical specimens were available for this study.

HCV

A total of 91 HCV clinical specimens were tested neat and diluted 1:6 the **cobas®** TaqScreen MPX Test, v2.0. For HCV specimens tested neat, the **cobas®** TaqScreen MPX Test, v2.0 detected all specimens of all HCV genotypes except one of the 10 HCV genotype 1b (**Table 21**). For all other 1:6 diluted specimens, the MPX v2.0 detected all specimens of all HCV genotypes tested (**Table 22**).

Table 21.
HCV Genotype Specimens Neat

Genotype	Total Number of Specimens	**cobas**® TaqScreen MPX Test, v2.0 Number Reactive
1a	10	10
1b	10	9
2	10	10
2a	5	5
2b	10	10
3a	10	10
4	10	10
4a	5	5
4acd	4	4
4d	1	1
5a	10	10
6	5	5
6ab	3	3
6c	1	1

Table 22.
HCV Genotype Specimens Diluted

Genotype	Total Number of Specimens	**cobas**® TaqScreen MPX Test, v2.0 Number Reactive
1a	10	10
1b	10	9
2	10	10
2a	5	5
2b	8	8
3a	10	10
4	10	10
4a	5	5
4acd	4	4
4d	1	1
5a	10	10
6	5	5
6ab	2	2
6c	1	1

HBV

A total of 51 HBV clinical specimens were tested neat and diluted 1:6 with the **cobas**® TaqScreen MPX Test, v2.0. The **cobas**® TaqScreen MPX Test, v2.0 detected each HBV genotype tested neat and diluted (**Table 23** and **Table 24**).

Table 23.
HBV Genotype Specimens Neat

Genotype	Total Number of Specimens	**cobas**® TaqScreen MPX Test, v2.0 Number Reactive
A	10	10
B	8	8
C	10	10
D	10	10
E	3	3
F	4	4
Pre-core mutant	6	6

Table 24.
HBV Genotype Specimens Diluted

Genotype	Total Number of Specimens	**cobas**® TaqScreen MPX Test, v2.0 Number Reactive
A	10	10
B	8	8
C	10	10
D	10	10
E	3	3
F	4	4
Pre-core mutant	6	6

Seroconversion Panels

The performance of the **cobas**® TaqScreen MPX Test, v2.0 during seroconversion was determined for HIV-1 Group M, HCV, and HBV using 32 commercially available seroconversion panels. No seroconversion panels are available for HIV-1 Group O and HIV-2.

HIV-1 Seroconversion Panels

Ten commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HIV antibody were tested with the **cobas**® TaqScreen MPX Test, v2.0. Each sample was tested neat and diluted 1:6 to simulate testing in pools of donors. The **cobas**® TaqScreen MPX Test, v2.0 results were compared to the results obtained with the Abbott PRISM® HIV O Plus Assay (US), and the Abbott PRISM anti-HIV 1/2 (CE) (**Table 25**).

The **cobas**® TaqScreen MPX Test, v2.0 on neat samples detected HIV RNA prior to detection of HIV antibody with either the Abbott PRISM HIV O Plus Assay or the Abbott PRISM anti-HIV 1/2 in 10 of 10 panels by an average of 13.9 days each. The **cobas**® TaqScreen MPX Test, v2.0 on 6-fold diluted samples detected HIV RNA prior to detection of HIV antibody with either the Abbott PRISM HIV O Plus Assay (US) or the Abbott PRISM anti-HIV 1/2 (CE) in 10 of 10 panels by an average of 13.4 days each.

Table 25.

Performance of cobas® TaqScreen MPX Test, v2.0 on HIV Seroconversion Panels

HIV Seroconversion Panels	Days Earlier Detection by the **cobas®** TaqScreen MPX Test, v2.0 than by tests for HIV-1/2 Antibody			
	Abbott PRISM HIV O Plus: Neat (US)		Abbott PRISM anti-HIV 1/2: Neat (CE)	
	Neat	1:6	Neat	1:6
1	14	9	14	9
2	14	14	14	14
3	12	12	12	12
4	14	14	14	14
5	11	11	11	11
6	14	14	14	14
7	9	9	9	9
8	14	14	14	14
9	15	15	15	15
10	22	22	22	22

HCV Seroconversion Panels

Twelve commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HCV antibody were tested with the **cobas®** TaqScreen MPX Test, v2.0. Each sample was tested neat and diluted 1:6 to simulate testing in pools of donors. The **cobas®** TaqScreen MPX Test, v2.0 results were compared to the results obtained with the Abbott PRISM® HCV Assay (US) and the ORTHO® HCV Version 3.0 Test System (**Table 26**).

The **cobas®** TaqScreen MPX Test, v2.0 detected HCV RNA in both the neat and dilute specimens on the first draw in 8 of the 12 panels, noted below. Therefore, the values shown for those panels, and the average number of days between NAT and serology detection represents a minimum number of days of window period closure.

The **cobas®** TaqScreen MPX Test, v2.0 on neat samples detected HCV RNA prior to detection of HCV antibody with either the Abbott PRISM HCV Assay (US) or the ORTHO HCV Version 3.0 ELISA Test System (CE) in 12 of 12 panels by an average of at least 21.6 days each. The **cobas®** TaqScreen MPX Test, v2.0 on 6-fold diluted samples detected HCV RNA prior to detection of HCV antibody with either the Abbott PRISM HCV Assay (US) or the ORTHO HCV Version 3.0 ELISA Test System (CE) in 12 of 12 panels by an average of at least 23.6 days each.

Table 26.

Performance of cobas® TaqScreen MPX Test, v2.0 on HCV Seroconversion Panels

HCV Seroconversion Panels	Days Earlier Detection by the MPX v2.0 than by Tests for HCV Antibody			
	Abbott PRISM HCV: Neat (US)		ORTHO HCV Version 3.0 ELISA Test System: Neat (CE)	
	Neat	1:6	Neat	1:6
1[a]	12	12	23	23
2[a]	30	30	32	32
3	23	23	23	23
4[a]	25	25	25	25
5[a]	28	28	28	28
6[a]	4	4	4	4
7[a]	11	11	11	11
8[a]	24	24	24	24
9	7	7	18	18
10	33	33	33	33
11	32	32	32	32
12	30	30	30	30

*[a] The **cobas®** TaqScreen MPX Test, v2.0 neat and 1:6 diluted results were reactive in first bleed of the panel series.*

HBV Seroconversion Panels

Ten commercially available seroconversion panels collected from plasmapheresis donors that seroconverted to HBsAg were tested with the **cobas®** TaqScreen MPX Test, v2.0. Each sample was tested neat and diluted 1:6 to simulate testing in pools of donors. The **cobas®** TaqScreen MPX Test, v2.0 results were compared to the results obtained with the Abbott PRISM® HBsAg Assay and the ORTHO® HBsAg ELISA Test System 3 (**Table 27**).

The **cobas®** TaqScreen MPX Test, v2.0 detected HBV DNA on the first draw in 2 of the 10 panels when tested neat and in 1 of 10 when diluted 6-fold, shaded below. Therefore, the values shown for those panels, and the average number of days between NAT and serology detection represents a minimum number of days of window period closure.

The **cobas®** TaqScreen MPX Test, v2.0 on neat samples detected HBV DNA prior to detection of HBsAg with the Abbott PRISM HBsAg Assay in 9 of 10 panels by an average of at least 13 days. Compared to the ORTHO HBsAg ELISA Test System 3, the **cobas®** TaqScreen MPX Test, v2.0 on neat samples detected HBV DNA prior to detection of HBsAg in 10 of 10 panels by an average of at least 7.5 days.

The **cobas®** TaqScreen MPX Test, v2.0 on 6-fold diluted samples detected HBV DNA prior to detection of HBsAg with the Abbott PRISM HBsAg Assay in 7 of 10 panels by an average of at least 22.3 days. Compared to the ORTHO HBsAg ELISA Test System 3, the **cobas®** TaqScreen MPX Test, v2.0 on 6-fold diluted samples detected HBV DNA prior to detection of HBsAg in 10 of 10 panels by an average of at least 16.8 days.

Table 27.

Performance of cobas® TaqScreen MPX Test, v2.0 on HBV Seroconversion Panels

HBV Seroconversion Panels	Days Earlier Detection by the MPX v2.0 Than by Tests for HBsAg			
	Abbott PRISM HBsAg: Neat (US)		ORTHO HBsAg ELISA Test System 3: Neat (CE)	
	Neat	1:6	Neat	1:6
1[a]	3	0	25	22
2	12	12	19	19
3	8	0	19	11
4	-14	-14	14	14
5	22	13	22	13
6[a, b]	7	7	23	23
7	20	8	20	8
8	11	11	11	11
9	28	14	30	16
10	33	24	40	31

[a] The cobas® TaqScreen MPX Test, v2.0 neat result was reactive in first bleed of the panel series.
[b] The cobas® TaqScreen MPX Test, v2.0 1:6 diluted result was reactive in first bleed of the panel series.

Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the **cobas®** TaqScreen MPX Test, v2.0 was evaluated by testing a panel of 20 microorganisms including 13 viral isolates, 6 bacterial strains and 1 yeast isolate (**Table 28**). The microorganisms were added to normal, virus-negative, human plasma and tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X LOD of the **cobas®** TaqScreen MPX Test, v2.0 for each virus.

Non-reactive results were obtained with the **cobas®** TaqScreen MPX Test, v2.0 on all of the microorganism samples without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV.

The tested microorganisms did not cross-react with the **cobas®** TaqScreen MPX Test, v2.0. Reactive results were obtained on all of the microorganism samples with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV. The tested microorganisms did not interfere with the **cobas®** TaqScreen MPX Test, v2.0 under the conditions tested.

Table 28.

Microorganisms Tested

Analytical Specificity — Microorganisms Tested		
Adenovirus 5	Human Herpes Virus 6 A	*Candida albicans*
Human Cytomegalovirus	Human T-Lymphotrophic Virus Type I	*Propionibacterium acnes*
Epstein Barr Virus	Influenza Virus A	*Staphylococcus epidermis*
Varicella-Zoster Virus	West Nile Virus	*Staphylococcus haemolyticus*
Herpes Simplex Virus Type 1	Dengue-1, Strain Hawaii	*Escherichia coli*
Herpes Simplex Virus Type 2	Chikungunya Virus	*Streptococcus* viridans
Hepatitis G Virus	*Staphylococcus aureus*	

Analytical Specificity — Other Disease States

Plasma specimens from each of the following disease categories (Human Cytomegalovirus, Epstein-Barr Virus, Herpes simplex virus Type I, Herpes simplex virus Type 2, Human T-cell lymphotropic virus Type I, Human T-cell lymphotropic virus Type II, Human T-cell lymphotropic virus Type I/II, Hepatitis A Virus, West Nile virus, and Parvovirus B19) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X the LOD of the **cobas®** TaqScreen MPX Test, v2.0 for each virus. The **cobas®** TaqScreen MPX Test, v2.0 yielded non-reactive results for all of the disease state specimens without added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV. The **cobas®** TaqScreen MPX Test, v2.0 yielded reactive results for all of the disease state specimens with added HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV, except in 1 of 10 HTLV I/II samples in which HCV was invalid. These disease states did not interfere with the sensitivity or specificity of the **cobas®** TaqScreen MPX Test, v2.0 under the conditions tested.

Potentially Interfering Substances

Endogenous Interfering Substances

Plasma specimens with abnormally high levels of triglycerides (up to 3300 mg/dL), hemoglobin (up to 500 mg/dL), unconjugated bilirubin (50 mg/dL), albumin (up to 9.6 g/dL), or human DNA (up to 0.4 mg/dL) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X LOD of the **cobas®** TaqScreen MPX Test, v2.0 for each virus. The listed substances did not interfere with the sensitivity or specificity of the **cobas®** TaqScreen MPX Test, v2.0 under the conditions tested.

Plasma specimens with red blood cells added to abnormally high levels, (up to 10% v/v) were tested with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X the LOD of the **cobas®** TaqScreen MPX Test, v2.0 for each virus. Plasma with red blood cells added to 2.5% (v/v) did not interfere with the viral sensitivity or specificity of the **cobas®** TaqScreen MPX Test, v2.0. Plasma with red blood cells added to 5.0% (v/v) reduced the sensitivity of the **cobas®** TaqScreen MPX Test, v2.0 for detection of HCV. Plasma with red blood cells added to 7.5% (v/v) interfered with sensitivity of the **cobas®** TaqScreen MPX Test, v2.0 for detection of HBV and HCV. Detection of HIV viral targets was 100% in plasma with red blood cells added to 10%. When viral targets were present at 3X LOD, the sensitivity of the **cobas®** TaqScreen MPX Test, v2.0 for detection of the Internal Control (IC) was not reduced in plasma with red blood cells added to 1.0% (v/v). When no viral targets were present, the sensitivity of the **cobas®** TaqScreen MPX Test, v2.0 for detection of the Internal Control (IC) was not reduced in plasma with red blood cells added to 2.5% (v/v). Internal Control (IC) monitors all steps in the testing process (sample preparation, amplification and detection) and does fail under conditions that might affect the performance of the assay.

Exogenous Interfering Substances

Normal, human plasma specimens containing abnormally high concentrations of acetaminophen (1324 µmol/L), acetylsalicylic acid (3.62 mmol/L), ascorbic acid (342 µmol/L), atorvastatin (600 µg Eq/L), fluoxetine (11.2 µmol/L), ibuprofen (2425 µmol/L), loratadine (0.78 µmol/L), nadolol (3.88 µmol/L), naproxen (2170 µmol/L), paroxetine (3.04 µmol/L), phenylephrine HCl (491 µmol/L), and sertraline (1.96 µmol/L), with and without HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, or HBV added to a concentration of 3X the LOD of the **cobas®** TaqScreen MPX Test, v2.0 were tested for each virus. These exogenous substances did not interfere with the sensitivity or specificity of the **cobas®** TaqScreen MPX Test, v2.0 under the conditions tested.

CADAVERIC DONOR SPECIMENS

Note: Although information for HIV-1 Group O RNA and HIV-2 RNA is provided in this section, this test is intended for use to screen blood specimens from cadaveric (non-heart beating) donors for HIV-1 Group M RNA, HCV RNA, and HBV DNA only.

Reproducibility

Twenty individual cadaveric EDTA plasma specimens were spiked with either HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV using clinical specimens for HIV-1 Group M, HBV and HCV, and Roche Primary Standards for HIV-1 Group O and HIV-2, to a final concentration of approximately 3X LOD as appropriate for each virus and hemolysis level for each specimen. The Roche Primary Standards for HIV-1 Group O RNA and HIV-2 RNA are commercially available cultured virus stocks, PN 2420 (Boston Biomedica, Inc.) and Cat. No. 10-27-000 (Advanced Biotechnologies, Inc.). The Roche HIV-1 Group O and HIV-2 Standards are traceable to the CBER HIV-1 Subtype RNA Reference Panel #1 Lot O1 and to the CBER HIV-2 RNA Lot Release Panel ISD, respectively. No international standard is currently available for HIV-1 Group O RNA.

HIV-1 Group M, HCV and HBV were co-formulated, and HIV-1 Group O and HIV-2 were individually formulated. 16 moderately hemolyzed and 4 highly hemolyzed cadaveric specimens were used. Additionally, twenty living donor EDTA specimens were spiked with the same standards to the level that was approximately 3X LOD. The cadaveric specimens were diluted 1:5 with **cobas®** TaqScreen Cadaveric Specimen Diluent to a final concentration of approximately 3X LOD and tested using the cadaveric specimen testing procedure. The living donor specimens were tested neat. Testing was performed using three reagent lots and three instruments.

All valid reproducibility data was analyzed by comparing the reactive rates of living donor and cadaveric specimens spiked with HIV-1 Group M, HIV-1 Group O, HIV-2, HCV or HBV between kit lots (**Table 29**) instruments (**Table 30**) and days (**Table 31**). The exact 2-sided p-value was calculated for the test of statistical significance of the difference between proportions of reactives observed with cadaveric and living donor specimens. No significant differences were observed. Exact 2-sided p-values greater than 0.05 are not considered statistically significant.

Table 29.

Comparison of Cadaveric vs. Living Donor Specimen Results by Reagent Lot

Viral Target	Donor Type	Lot #1	Lot #2	Lot #3	Overall Reactive Rate	2-sided p-value living vs. cadaveric specimens by target
HIV-1 Group M	Cadaveric	120/120 (100.0%)	119/120 (99.2%)	118/120 (98.3%)	357/360 (99.2%)	0.624
	Living	120/120 (100.0%)	120/120 (100.0%)	119/120 (99.2%)	359/360 (99.7%)	
HIV-1 Group O	Cadaveric	120/120 (100.0%)	118/120 (98.3%)	118/120 (98.3%)	356/360 (98.9%)	0.373
	Living	119/119 (100%)	119/120 (99.2%)	120/120 (100.0%)	358/359 (99.7%)	
HIV-2	Cadaveric	119/119 (100%)	119/120 (99.2%)	119/120 (99.2%)	357/359 (99.4%)	0.624
	Living	120/120 (100.0%)	119/120 (99.2%)	120/120 (100.0%)	359/360 (99.7%)	
HBV	Cadaveric	120/120 (100.0%)	120/120 (100.0%)	120/120 (100.0%)	360/360 (100.0%)	0.062
	Living	116/120 (96.7%)	120/120 (100.0%)	119/120 (99.2%)	355/360 (98.6%)	
HCV	Cadaveric	120/120 (100.0%)	120/120 (100.0%)	119/120 (99.2%)	359/360 (99.7%)	0.217
	Living	119/120 (99.2%)	118/120 (98.3%)	118/120 (98.3%)	355/360 (98.6%)	

Table 30.

Comparison of Cadaveric vs. Living Donor Specimen Results by Instrument

Viral Target	Specimen Type	Instrument 1	Instrument 2	Instrument 3	Overall Reactive Rate	2-sided p-value living vs. cadaveric specimens by target
HIV-1 Group M	Cadaveric	119/120 (99.2%)	119/120 (99.2%)	119/120 (99.2%)	357/360 (99.2%)	0.624
	Living-Donor	119/120 (99.2%)	120/120 (100.0%)	120/120 (100.0%)	359/360 (99.7%)	
HIV-1 Group O	Cadaveric	118/120 (98.3%)	119/120 (99.2%)	119/120 (99.2%)	356/360 (98.9%)	0.373
	Living-Donor	119/119 (100.0%)	120/120 (100.0%)	119/120 (99.2%)	358/359 (99.7%)	
HIV-2	Cadaveric	117/119 (98.3%)	120/120 (100.0%)	120/120 (100.0%)	357/359 (99.4%)	0.624
	Living-Donor	120/120 (100.0%)	120/120 (100.0%)	119/120 (99.2%)	359/360 (99.7%)	
HBV	Cadaveric	120/120 (100.0%)	120/120 (100.0%)	120/120 (100.0%)	360/360 (100.0%)	0.062
	Living-Donor	118/120 (98.3%)	118/120 (98.3%)	119/120 (99.2%)	355/360 (98.6%)	
HCV	Cadaveric	120/120 (100.0%)	120/120 (100.0%)	119/120 (99.2%)	359/360 (99.7%)	0.217
	Living-Donor	117/120 (97.5%)	118/120 (98.3%)	120/120 (100.0%)	355/360 (98.6%)	

Table 31.

Comparison of Cadaveric vs. Living Donor Specimen Results by Day

Viral Target	Specimen Type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Overall Reactive Rate	2-sided p-value living vs. cadaveric specimens by target
HIV-1 Group M	Cadaveric	59/60 (98.3%)	58/60 (96.7%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	357/360 (99.2%)	0.624
	Living-Donor	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	59/60 (98.3%)	359/360 (99.7%)	
HIV-1 Group O	Cadaveric	58/60 (96.7%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	59/60 (98.3%)	59/60 (98.3%)	356/360 (98.9%)	0.373
	Living-Donor	59/59 (100.0%)	60/60 (100.0%)	59/60 (98.3%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	358/359 (99.7%)	
HIV-2	Cadaveric	59/59 (100.0%)	59/60 (98.3%)	59/60 (98.3%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	357/359 (99.4%)	0.624
	Living-Donor	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	59/60 (98.3%)	359/360 (99.7%)	
HBV	Cadaveric	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	360/360 (100.0%)	0.062
	Living-Donor	59/60 (98.3%)	58/60 (96.7%)	60/60 (100.0%)	59/60 (98.3%)	60/60 (100.0%)	59/60 (98.3%)	355/360 (98.6%)	
HCV	Cadaveric	59/60 (98.3%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	359/360 (99.7%)	0.217
	Living-Donor	58/60 (96.7%)	60/60 (100.0%)	60/60 (100.0%)	60/60 (100.0%)	58/60 (96.7%)	59/60 (98.3%)	355/360 (98.6%)	

Analytical Sensitivity in Cadaveric Specimens

A limiting dilution study of the **cobas®** TaqScreen MPX Test, v2.0 for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV and HBV viral targets in cadaveric specimens was assessed using the following standards: the Roche Primary Standards for HIV-1 Group O and HIV-2, and Roche Secondary Standards for HIV-1 Group M, HBV, and HCV. The Roche Secondary Standard for HBV DNA is a commercially available, cultured virus stock (Eurohep HBV DNA Standard No. 1 genotype A [adw2]) traceable to the WHO International Standard for HBV DNA for NAT (NIBSC 97/746) 33. HIV-1 Group M, HCV and HBV were co-formulated, and HIV-1 Group O and HIV-2 were individually formulated.

For each target, seven panel members were prepared by dilution of the HIV-1 Group M, HCV and HBV, HIV-1 Group O or HIV-2 virus standards into three unique Moderately Hemolyzed cadaveric EDTA pools tested using three lots of **cobas®** TaqScreen MPX Test, v2.0, and two unique High Hemolyzed cadaveric EDTA pools using one lot of **cobas®** TaqScreen MPX Test, v2.0. Moderately Hemolyzed pools consisted of pools of individual, virus-negative cadaveric specimens having a straw to pink colored appearance. Highly Hemolyzed pools consisted of pools of individual, virus-negative cadavericspecimens having a red to brown colored appearance. The results are summarized in **Table 32** to **Table 41**.

Table 32.

Analytical Sensitivity Summary for HIV-1Group M In Moderately-Hemolyzed Cadaveric Matrix

HIV-1 Group M Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
750	62	63	98.4%	92.7%
500	61	63	96.8%	90.3%
375	62	63	98.4%	92.7%
250	62	64	96.9%	90.5%
125	52	63	82.5%	72.8%
75	42	64	65.6%	54.7%
25	19	63	30.2%	20.7%

Table 33.

Analytical Sensitivity Summary for HIV-1 Group M in Highly-Hemolyzed Cadaveric Matrix

HIV-1 Group M Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
750	40	40	100.0%	92.8%
500	40	40	100.0%	92.8%
375	39	40	97.5%	88.7%
250	40	40	100.0%	92.8%
125	26	40	65.0%	50.8%
75	23	41	59.1%	42.1%
25	17	43	39.5%	27.0%

Table 34.

Analytical Sensitivity Summary for HCV in Moderately-Hemolyzed Cadaveric Matrix

HCV Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
120	62	63	98.4%	92.7%
80	58	63	92.1%	84.0%
60	61	63	96.8%	90.3%
40	52	63	82.5%	72.8 %
20	41	63	65.1%	54.0%
12	22	63	34.9%	25.0%
4	9	63	14.3%	7.7%

Table 35.

Analytical Sensitivity Summary for HCV in Highly-Hemolyzed Cadaveric Matrix

HCV Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
120	40	40	100.0%	92.8%
80	39	40	97.5%	88.7%
60	39	41	95.1%	85.4%
40	37	40	92.5%	81.7%
20	21	40	52.5%	36.5%
12	17	40	42.5%	29.2%
4	6	41	14.6%	6.6%

Table 36.

Analytical Sensitivity Summary for HBV in Moderately-Hemolyzed Cadaveric Matrix

HBV Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
60	63	63	100.0%	95.4%
40	62	63	98.4%	92.7%
30	60	63	95.2%	88.2%
20	52	64	81.3%	71.4%
10	33	63	52.4%	41.3%
6	23	63	36.5%	26.4%
2	12	63	19.0%	11.4%

Table 37.

Analytical Sensitivity Summary for HBV in Highly-Hemolyzed Cadaveric Matrix

HBV Concentration (IU/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
150	40	40	100.0%	92.8%
100	40	40	100.0%	92.8%
75	41	41	100.0%	93.0%
50	39	40	97.5%	88.7%
25	33	40	82.5%	69.6%
15	21	41	51.2%	37.4%
5	13	44	29.5%	18.4%

Table 38.

Analytical Sensitivity Summary for HIV-1 Group O in Moderately-Hemolyzed Cadaveric Matrix

HIV-1 Group O Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Reactive	One-sided Exact Lower 95% Confidence Bound
360	61	63	96.8%	90.3%
240	62	63	98.4%	92.7%
180	64	64	100.0%	95.4%
120	62	63	98.4%	92.7%
60	57	63	90.5%	82.1%
36	58	63	92.1%	84.0%
12	30	63	47.6%	36.7%

Table 39.

Analytical Sensitivity Summary for HIV-1 Group O in Highly-Hemolyzed Cadaveric Matrix

HIV-1 Group O Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Positive	One-sided Exact Lower 95% Confidence Bound
360	40	40	100.0%	92.8%
240	38	40	95.0%	85.1%
180	40	40	100.0%	92.8%
120	38	40	95.0%	85.1%
60	38	40	95.0%	85.1%
36	34	40	85.0%	72.5%
12	8	40	20.0%	10.4%

Table 40.

Analytical Sensitivity Summary for HIV-2 in Moderately-Hemolyzed Cadaveric Matrix

HIV-2 Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Positive	One-sided Exact Lower 95% Confidence Bound
600	63	63	100.0%	95.4%
400	63	64	98.4%	92.8%
300	61	63	96.8%	90.3%
200	60	63	95.2%	88.2%
100	34	63	54.0%	42.9%
60	28	63	44.0%	33.7%
20	12	63	19.0%	11.4%

Table 41.

Analytical Sensitivity Summary for HIV-2 in Highly-Hemolyzed Cadaveric Matrix

HIV-2 Concentration (Copies/mL)	Number of Reactives	Number of Individual Tests	% Positive	One-sided Exact Lower 95% Confidence Bound
600	43	43	100.0%	93.3%
400	40	40	100.0%	92.8%
300	38	40	95.0%	85.1%
200	39	40	97.5%	88.7%
100	27	40	67.5%	53.4%
60	17	40	42.5%	29.2%
20	10	40	25.0%	14.2%

Sensitivity using Clinical Specimens

HIV-1 Group M HCV, HBV

Sixty randomly selected cadaveric EDTA plasma specimens non-reactive for HIV-1 Group M, HIV-1 Group O, HIV-2 HCV RNA and HBV DNA, and classified as either Moderately Hemolyzed (straw to pink colored) or Highly Hemolyzed (red to brown colored), were divided evenly into 5 clinical specimen spiking groups with 12 specimens per group. Each cadaveric specimen within a group was co-spiked with three unique virus containing specimens, one each of HIV-1 Group M, HCV and HBV, singly infected clinical living donor specimens of a known titer, to a final concentration of approximately three times the Limit of Detection (3X LOD) for each respective virus and appropriate hemolysis level. Each cadaveric specimen was manually diluted 1:5 with **cobas®** TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure. Three reagent lots were used for this study and each group was divided between each lot for a total of 20 specimens per reagent kit lot per target. The reactive rate was 98.3% (95% CI: 91.1 – 100%) for HIV-1 Group M, and 100% (95% CI: 94 – 100 %) for HCV and HBV with the **cobas®** TaqScreen MPX Test, v 2.0. A summary of these test results are presented in **Table 42**.

HIV-1 Group O, HIV-2

For the HIV-1 Group O and HIV-2 viral targets, the Roche Primary Standards were used to spike cadaveric EDTA plasma specimens at approximately 3X LOD for each respective virus and appropriate level of hemolysis (Moderately Hemolyzed or Highly Hemolyzed). Consequently, these targets only had one spiking group. Each cadaveric specimen was manually diluted 1:5 with **cobas®** TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure. Three reagent lots were used for this study

and each group was divided between each lot for a total of 12 specimens per reagent kit lot per target. For the twelve specimens tested for HIV-1 Group O and HIV-2, the reactive rate was 91.7% (95% CI: 61.5 – 99.8 %) with the **cobas®** TaqScreen MPX Test, v 2.0. A summary of these test results are presented in **Table 42**.

The exact 2-sided p-value was calculated for the test of statistical significance of the difference between proportions of reactives observed for each target and theoretical 100% reactivity.

Table 42.

Summary of Sensitivity for the cobas® TaqScreen MPX Test, v2.0 with Moderately Hemolyzed (MH) and Highly Hemolyzed (HH) Cadaveric Specimens

Reagent Lot	Hemolysis Level	HIV-1 Group M	HIV-1 Group O	HIV-2	HBV	HCV
Lot 1	MH	13/14	4/4	3/3	14/14	14/14
	HH	6/6	NT	1/1	6/6	6/6
	Total	19/20	4/4	4/4	20/20	20/20
Lot 2	MH	15/15	3/3	3/3	15/15	15/15
	HH	5/5	1/1	1/1	5/5	5/5
	Total	20/20	4/4	4/4	20/20	20/20
Lot 3	MH	16/16	3/4	3/4	16/16	16/16
	HH	4/4	NT	NT	4/4	4/4
	Total	20/20	3/4	3/4	20/20	20/20
Sensitivity	Percent Reactivity	98.3%[a]	91.7%	91.7%	100%	100%
	95% Confidence Interval	91.1% - 100%	61.5% - 99.8%	61.5% - 99.8%	94.0 – 100%	94.0 – 100%
	2-sided p-value	0.5	0.5	0.5	1	1

NT indicates the virus was not tested at the hemolysis level shown. The total for HIV-1 Group O and HIV-2 was maintained at 12 specimens each.

Specificity

Cadaveric Plasma

Sixty individual seronegative living donor (pre-mortem) and sixty cadaveric (39 moderately hemolyzed and 21 highly hemolyzed) EDTA plasma specimens were divided into three groups and each group of specimens was tested with one of three lots of the **cobas®** TaqScreen MPX Test, v2.0. The cadaveric specimens were manually diluted 1:5 with **cobas®** TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure.

For both the cadaveric and living donor specimens, sixty valid replicates were tested using the three kits. One of the living donor samples was initially reactive for HCV. Confirmatory testing using the **cobas®** TaqScreen MPX Test was non-reactive, and the specimen was retained in the analysis. The exact 2-sided p-value was calculated for the test of statistical significance of the difference between proportions of non-reactives observed with cadaveric and living donor specimens. The summary of the specificity test results is presented in **Table 43**.

Table 43.

Summary of MPX Test v2.0 Cadaveric Specificity Results

Reagent Lot	Cadaveric Donor Specimens (Diluted 1 :5 in cobas® TaqScreen Cadaveric Specimen Diluent)		Living Donor Specimens (Neat)	
	Number of Valid Results	Number of Non-reactive Results	Number of Valid Results	Number of Non-reactive Results
Lot # 1	20	20	20	20
Lot # 2	20	20	20	19
Lot # 3	20	20	20	20
Total	60	60	60	59
Specificity	100%		98.3%	
95% Confidence Interval	94.0% - 100.0%		91.1% - 100.0%	
2-sided p-value	0.5			

[a] *Reactive HCV result determined to be a false positive. Two replicates tested with the cobas TaqScreen MPX Test were non-reactive.*

Cadaveric Serum

Sixty individual cadaveric (46 moderately hemolyzed and 14 highly hemolyzed) serum specimens were divided into three groups and each group of specimens was tested with one of three lots of the **cobas®** TaqScreen MPX Test, v2.0. The cadaveric specimens were manually diluted 1:5 with **cobas®** TaqScreen Cadaveric Specimen Diluent and tested using the cadaveric specimen testing procedure. The results for all cadaveric serum specimens were non-reactive. The 95% confidence interval is 94.0% - 100.0%.

Cadaveric Specimen Correlation between the cobas® TaqScreen MPX Test and the cobas® TaqScreen MPX Test, v2.0

The performance of the **cobas®** TaqScreen MPX Test, v2.0 and the **cobas®** TaqScreen MPX Test were evaluated using 50 moderately hemolyzed and 10 highly hemolyzed EDTA plasma cadaveric specimens spiked with Primary or Secondary Standards (previously described) to a final concentration of approximately three times the Limit of Detection (3X LOD) for each respective virus, appropriate hemolysis level and test. HIV-1 Group M, HCV and HBV were individually spiked into cadaveric specimens for testing with one reagent lot of the **cobas®** TaqScreen MPX Test. HIV-1 Group M, HCV and HBV were co-spiked and HIV-1 Group O and HIV-2 were individually spiked into cadaveric specimens for testing with three lots of the **cobas®** TaqScreen MPX Test, v.2.0. The exact 2-sided p-value was calculated for the test of statistical significance of the difference between proportions of reactives observed for each target with the **cobas®** TaqScreen MPX Test and the **cobas®** TaqScreen MPX Test, v2.0. A summary of the results is presented in **Table 44**.

Table 44.

Comparison of the cobas® TaqScreen MPX Test and the cobas TaqScreen MPX Test, v2.0 sensitivity using individual cadaveric specimens at ~3X LOD

Target	Percent Reactivity		2-sided p-value of cadaveric specimens by target
	cobas® TaqScreen MPX Test	cobas® TaqScreen MPX Test, v2.0	
HIV-1 Group M	(60/60) 100%	(59/60) 98%	1
HIV-1 Group O	(59/60) 98%	(60/60) 100%	1
HIV-2	(57/60) 95%	(59/60) 98%	0.6
HBV	(57/60) 95%	(59/60) 98%	0.5
HCV	(59/60) 98%	(59/60) 98%	1

Cadaveric Serum vs. Plasma

Equivalency of the **cobas®** TaqScreen MPX Test, v2.0 performance when testing different cadaveric sample matrices was evaluated by testing twenty pairs of cadaveric specimens, with each set consisting of one cadaveric serum specimen and one cadaveric K2 EDTA plasma specimen from a single donor. Fifteen of the donor sets were moderately-hemolyzed, and five of the donor sets were highly-hemolyzed.

Each pair of cadaveric serum and plasma specimens was spiked with ~3X LOD of either HIV-1 Group M, HIV-1 Group O, or HIV-2, and then co formulated with ~3X LOD of HBV and ~3X LOD of HCV before testing (10 replicates per specimen) with the **cobas**® TaqScreen MPX Test, v2.0. The exact 2-sided p-value was calculated for the test of statistical significance of the difference between proportions of reactives observed with cadaveric serum and plasma specimens. A summary of the results is presented in **Table 45.**

Table 45.

Results Observed for Highly or Moderately-Hemolyzed Serum vs. Plasma Cadaveric Specimens

Target	Hemolysis Level	Type	Number of Tests	Number Reactive	Reactive Rate	2-sided p-value hemolysis and type of cadaveric specimens by target
HIV-1 Group M	Highly Hemolyzed	Plasma	20	20	100%	1
		Serum	20	20	100%	
	Moderately Hemolyzed	Plasma	100	98	98%	0.2
		Serum	100	100	100%	
HIV-1 Group O	Highly Hemolyzed	Plasma	10	10	100%	1
		Serum	10	10	100%	
	Moderately Hemolyzed	Plasma	30	30	100%	1
		Serum	30	30	100%	
HIV-2	Highly Hemolyzed	Plasma	20	20	100%	1
		Serum	20	20	100%	
	Moderately Hemolyzed	Plasma	20	20	100%	1
		Serum	20	20	100%	
HBV	Highly Hemolyzed	Plasma	50	50	100%	1
		Serum	50	50	100%	
	Moderately Hemolyzed	Plasma	150	150	100%	1
		Serum	150	150	100%	
HCV	Highly Hemolyzed	Plasma	50	50	100%	1
		Serum	50	50	100%	
	Moderately Hemolyzed	Plasma	150	149	99.3%	0.5
		Serum	150	150	100%	

The average invalid batch rate across studies using cadaveric specimens was 2.7% after 1:5 dilution, including stability studies for clinical cadaveric specimens and cadaveric specimen diluent.

CLINICAL PERFORMANCE

LIVING DONOR SPECIMENS

Reproducibility

The reproducibility of the **cobas**® TaqScreen MPX Test, v2.0 for use on the **cobas** s 201 system was established by testing a 32-member panel composed of 2 negative plasma samples and 2 positive plasma samples each for HIV-1 Group M, HIV-1 Group O, HIV-2, HCV, and HBV at concentrations of approximately 0.5X, 1.0X and 3.0X the Limit of Detection (LOD) of the **cobas**® TaqScreen MPX Test, v2.0 for each virus.

Operators at each of 3 sites with 1 **cobas** s 201 system per site performed 5 days of testing with each of 3 lots of the **cobas**® TaqScreen MPX Test, v2.0 reagents and two valid panel runs per day to yield up to 180 tests per panel member virus type.

All valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member and the percentage of non-reactive results for the negative control panel member (Table 46). This study demonstrated that the **cobas**® TaqScreen MPX v2.0 Test for use on the **cobas** s 201 system showed reproducible performance across the variables assessed (kit, site, reagent lot, day, run) and for the five analytes tested.

Table 46.

cobas*® *TaqScreen MPX Test, v2.0 – Reproducibility Results

Analyte	Viral Concentration	No. of Tests	No. of Correct Results	Percent Agreement	Exact 95% Confidence Interval
Negative	0	168	167	99.4%	(96.7%, 100.0%)
HIV-1 Group M	0.5 x LOD	173	143	82.7%	(76.2%, 88.0%)
	1.0 x LOD	172	162	94.2%	(89.6%, 97.2%)
	3.0 x LOD	176	174	98.9%	(96.0%, 99.9%)
HIV-1Group O	0.4 x LOD	172	119	69.2%	(61.7%, 76.0%)
	0.7 x LOD	168	145	86.3%	(80.2%, 91.1%)
	1.7 x LOD	171	170	99.4%	(96.8%, 100.0%)
HIV-2	0.5 x LOD	178	149	83.7%	(77.4%, 88.8%)
	1.0 x LOD	173	170	98.3%	(95.0%, 99.6%)
	3.0 x LOD	178	178	100.0%	(97.9%, 100.0%)
HCV	0.5 x LOD	176	142	80.7%	(74.1%, 86.2%)
	1.0 x LOD	171	161	94.2%	(89.5%, 97.2%)
	3.0 x LOD[a]	175	175	100.0%	(97.9%, 100.0%)
HBV	0.5 x LOD	178	131	73.6%	(66.5%, 79.9%)
	1.0 x LOD	177	168	94.9%	(90.6%, 97.6%)
	3.0 x LOD	174	172	98.9%	(95.9%, 99.9%)

[a] *One test was also reactive for HBV.*

Clinical Specificity

Reactivity in Whole Blood Donor Population

Specimens were collected from consented blood donors recruited from 5 test sites, Testing with the **cobas**® TaqScreen MPX Test, v2.0 was done according to two testing algorithms; one for pools of 1 testing (requiring a single level of testing), and one for pools of 6 testing (requiring a single level of testing for primary pools that were non-reactive and 2 levels of testing—primary pool testing and individual donor resolution testing for primary pools that were reactive).

Specificity in Individual Donation Testing

For the individual donation testing a total of 13,306 Whole Blood donations were tested. Of these, 29 specimens were excluded from further calculations because they were from donors who were presumed to be infection status positive due to repeatedly reactive serology test results (13,306-29=13,277). Of the remaining 13,277 donations, 17 were reactive on the **cobas**® TaqScreen MPX Test, v2.0 (13,277-17=13,260). Three of these 17 donations were from donors subsequently shown to be infection status positive, and these three specimens were excluded from the calculation of specificity (13,277-3=13,274) **(Table 47).** The clinical specificity for individual donation testing in this study was 99.895% (13,260/13,274; 95% CI: 99.823% to 99.937%). There were no NAT yield cases identified during this study.

Table 47.

Individual Donation Reactivity in Whole Blood Donors

Category	No. of Specimens	Percentage of Specimens Tested
Individual Donations Tested	13,277	100.00
Non-Reactive Individual Donations	13,260	99.87
Reactive Individual Donations	17	0.13
Reactive Donations with Donor Status Positive (True Positive)	3	0.02
Reactive Donations with Donor Status Negative (False Positive)	14	0.11

Specificity in Pool Testing

The Whole Blood donations were also tested in pools of equal aliquots of not more than six individual donations. Of 10,500 pools tested by the **cobas**® TaqScreen MPX Test, v2.0, 10,471 pools were negative. Of the 29 reactive pools (10,500-10,471=29), 15 pools contained specimens from donors who were shown to be infection status positive (i.e., true positive donor specimens in the pool), and those pools were excluded from the calculation of specificity. The remaining 14 reactive pools contained individual donation specimens that were all

found non-reactive in pool resolution testing; therefore, the test result on the 14 pools was false positive (10,471+14=10485) (**Table 48**). Based on these data the specificity in pool testing was 99.866% (10,471/10,485, 95% CI; 99.78% to 99.92%).

Table 48.

Pool Reactivity in Volunteer Blood Donors

Category	No. of Pools	Percentage of Pools Tested
Pools Tested	10,500	100.00
Non-Reactive pools	10,471	99.72
Reactive pools	29	0.28
Reactive pools with donor status positive	15	0.14
Reactive pools with donor status negative (false positive)	14	0.13

Specificity in Individual Donation Testing and in Pool Resolution Testing

A total of 64,030 donations in pools were tested in this study. Of these, 372 specimens were excluded from further calculations due to infection status positive results or unresolved infection status of the donor. Of the remaining 63,658 donations (64,030-372=63,658), six donations were false positive on the **cobas**® TaqScreen MPX Test, v2.0 after resolution by individual donation testing of the reactive pools (63,658-6=63,652). The clinical specificity for individual donation in pools of not more than 6 in this study was 99.991% (63,652/63,658; 95% CI: 99.979% to 99.996%).

The invalid batch rate for the **cobas**® TaqScreen MPX Test, v2.0 from initial testing of donations in pools of up to six donor specimens and of individual donations was 4.8% and 5.3%, respectively.

Specificity in Source Plasma Donor Samples

A total of 103,981 valid Source Plasma donations which tested negative individually for anti-HCV, anti-HIV-1 and HBsAg, from 14,776 unique donors, were tested in pools of 96 with both the MPX v2 test and MPX test, and individually with the COBAS® AmpliScreen (CAS) tests for HBV, HCV, and HIV. Initial donation-status was assigned on the basis of the results of the MPX test, the CAS tests and a negative serology status.

One HIV-1 window period infection, ten HCV window period infections, and one possible HBV occult infection were identified in the 14,776 unique donors tested in this study. The reactive-NAT-only yield for this study was 1:14,776, 1:1,478, and 1:14,776 for HIV-1, HCV, and HBV, respectively, in Source Plasma donations.

Of the 14,776 unique donors tested, 14,762 were donor infection status negative and 19 had false-reactive results (14,762-19=14,743), resulting in specificity (at the donor level) of 14,743/14,762=99.871% (95% CI: 99.799% to 99.918%).

Specificity in Source Plasma Pool Testing

A total of 1,100 valid pools were tested with the **cobas**® TaqScreen MPX Test, v2.0 of which 1,049 (95.4%) were non-reactive and 51 (4.6%) were reactive. Of the 1,049 non-reactive pools, 1,048 pools contained all infection-status-negative donations and 1 pool contained one donation-status positive donation for HBV (**Table 49**).

For this HBV donation-status positive donation, the pool came from a **cobas**® TaqScreen MPX Test reactive donation that was confirmed with an additional **cobas**® TaqScreen MPX Test, v2.0 and with the COBAS® AmpliScreen HBV Test but the donor declined follow up and could potentially represent a donor with occult HBV infection. The pool that contained this infection-status-positive donation was excluded from the calculation of specificity.

Twenty-one of the 51 reactive pools of 96 were determined to be false positive on the **cobas**® TaqScreen MPX Test, v2.0, as these were resolved to contain all the **cobas**® TaqScreen MPX Test, v2.0 non-reactive donations, following resolution testing using the Pooled Testing Algorithm or follow-up of the donors (i.e., false positive pools) (1,048+21=1,069). The remaining 30 reactive pools that contained at least one donation from a donor with positive infection status were excluded from the calculation of specificity. The clinical specificity (at the pool level) of the **cobas**® TaqScreen MPX Test, v2.0 for testing Source Plasma pools of up to 96 was 1,048/1,069=98.04% (95% CI: 97.02% to 98.71%) in this study.

Table 49.

cobas*® *TaqScreen MPX Test, v2.0 Reactivity with Pools of up to 96 Source Plasma Donations

Category	Number of Pools	Percentage of Pools Tested
Total pools of 96[a] tested:	1,100	100.0
Non-Reactive pools[b]	1,049	95.4
Non-reactive pools with all donations status negative	1,048	95.3
Non-reactive pools with at least one status-positive donation	1	0.1
Reactive pools[b]	51	4.6
Reactive pools with at least one status-positive donation	30	2.7
Reactive pools with all donations status-negative (false reactive pools)	21	1.9

[a] *Note that 299 out of 1100 pools had less than 96 donations. Ninety-seven percent (1071) of these pools had 90 or more donations.*

[b] *Donation status was assigned based on initial* **cobas**® *TaqScreen MPX Test and COBAS*® *AmpliScreen Test results and/or additional testing and follow up.*

Specificity in Individual Donation Testing from Pool Resolution Testing

Of the 103,981 donations tested, 103,950 were assigned a donation status of negative, of which 103,931 were the **cobas**® TaqScreen MPX Test, v2.0 non-reactive, for a clinical specificity (at the donation level) of 103,931/103,950=99.982% (95% CI: 99.971% to 99.988%) in this study.

Studies in High Risk Populations

Samples were collected by a third-party vendor from individuals at high risk for infection with HIV, HCV, and/or HBV. High-risk factors included, but were not limited to, tattoo or body piercing, injection drug use, multiple sex partners, needle stick accidents, blood or blood product transfusion, history of a sexually transmitted disease, and dialysis. A total of 570 specimens from a high risk population were distributed evenly across 4 sites for testing with the **cobas**® TaqScreen MPX Test, v2.0 and the **cobas**® TaqScreen MPX Test.

All samples were prepared as panels at RMS: the diluted samples were manually diluted with pooled human plasma confirmed to be negative for HIV-1/2, HCV, and HBV. At the testing sites, samples were tested neat and diluted (1:6) with the **cobas**® TaqScreen MPX Test, v2.0; and tested neat and diluted (1:6) with the licensed **cobas**® TaqScreen MPX Test. Target resolution testing of specimens that tested reactive with the **cobas**® TaqScreen MPX Test was conducted using the COBAS® AmpliScreen (HIV-1, HCV, HBV) Tests per the Standard Specimen Processing procedure recommended in the **cobas**® TaqScreen MPX Test Package Insert.

There were 567 neat samples with results from the **cobas**® TaqScreen MPX Test, v2.0 and the **cobas**® TaqScreen MPX Test. The **cobas**® TaqScreen MPX Test, v2.0 identified a total of 99 reactive specimens (8 HIV, 87 HCV, and 4 HBV) compared to 87 identified by the **cobas**® TaqScreen MPX Test, (5 HIV, 71 HCV, and 0 HBV, and 11 were unresolved). In this population the **cobas**® TaqScreen MPX Test, v2.0 identified more specimens than the **cobas**® TaqScreen MPX Test. There were 570 diluted (1:6) specimens with results from the **cobas**® TaqScreen MPX Test, v2.0 and the **cobas**® TaqScreen MPX Test. The **cobas**® TaqScreen MPX Test, v2.0 identified a total 80 reactive specimens (4 HIV, 74 HCV, and 2 HBV) compared to 78 identified by the **cobas**® TaqScreen MPX Test (4 HIV, 69 HCV, 0 HBV, and 5 unresolved). There were no observed true reactive specimens from HBV infected individuals from this study for both neat and diluted samples.

Table 50.
The cobas® TaqScreen MPX Test, v2.0 Testing with Specimens from High Risk Populations

Testing Level	Result per Target	cobas® TaqScreen MPX Test, v2.0	cobas® TaqScreen MPX Test
Neat	Nonreactive	468	480
	Total Reactive	99*	87*
	HIV	8	5
	HCV	87	71
	HBV	4[1]	0
	Unresolved	0	11
	Total Tested	567	567
1:6 Diluted	Nonreactive	490	492
	Total Reactive	80**	78**
	HIV	4	4
	HCV	74	69
	HBV	2[2]	0
	Unresolved	0	5
	Total Tested	570	570

** 82 were reactive with both the cobas® TaqScreen MPX Test, v2.0 and cobas® TaqScreen MPX Test.*
*** 73 were reactive with both the cobas® TaqScreen MPX Test, v2.0 and cobas® TaqScreen MPX Test.*
[1] One cobas® TaqScreen MPX Test, v2.0 HBV-reactive, cobas® TaqScreen MPX Test reactive, COBAS® AmpliScreen HIV 1, HCV, HBV Test negative and alternate NAT non-reactive and 3 cobas® TaqScreen MPX Test, v2.0 HBV-reactive, cobas® TaqScreen MPX Test non-reactive, COBAS® AmpliScreen HIV 1, HCV, HBV Test negative and alternate NAT non-reactive.
[2] Two cobas® TaqScreen MPX Test, v2.0 HBV-reactive, cobas® TaqScreen MPX Test non-reactive, and alternate NAT non-reactive.

Studies in Seropositive and NAT Positive Populations

A total of 2,799 HIV, HCV, and HBV NAT known positive by viral load and/or qualitative assays specimens across 4 test sites were tested with the cobas® TaqScreen MPX Test, v2.0 (3 lots of reagents) and the cobas® TaqScreen MPX Test and COBAS® AmpliScreen HIV t Test, v1.5; COBAS® HCV Test, v2.0; and COBAS® AmpliScreen HBV Test These, 2,799 specimens known to be seropositive for HIV (n=1,158), HCV (n=1,137), or HBV (n=504) were tested both neat and diluted (1:6) with the cobas® TaqScreen MPX Test, v2.0 and the cobas® TaqScreen MPX Test. Only neat samples were tested with the licensed COBAS® AmpliScreen HIV 1, HCV, HBV Tests per the Standard Specimen Processing Procedure recommended in the cobas® TaqScreen MPX Test Package Insert at 2 test sites.

HIV Seropositive and HIV NAT Positive Population

There were 1,106 and 1,123 samples with test results, respectively, for neat and 1:6 diluted HIV samples. The cobas® TaqScreen MPX Test, v2.0 was reactive for 1,098 (99.3%) neat samples and 1,086 (96.7%) diluted samples. The cobas® TaqScreen MPX Test was reactive for 1,095 (99.0%) neat samples and 1,078 (96.0%) diluted samples (**Table 51**).

HCV Seropositive and HCV NAT Positive Population

There were 1,137 and 1,122 samples with test results, respectively, for neat and 1:6 diluted HCV samples. The cobas® TaqScreen MPX Test, v2.0 was reactive for 1,117 (98.2%) neat samples and 1,106 (98.6%) diluted samples. The cobas® TaqScreen MPX Test was reactive for 1,118 (98.3%) neat samples and 1,106 (98.6%) diluted samples (**Table 51**).

HBV Seropositive and HBV NAT Positive Population

There were 491 and 498 samples with test results, respectively, for neat and 1:6 diluted HBV samples. The cobas® TaqScreen MPX Test, v2.0 was reactive for 491 (100.0%) neat samples and 493 (99.0%) diluted samples. The cobas® TaqScreen MPX Test was reactive for 491 (100.0%) neat samples and 489 (98.2%) diluted samples (**Table 51**).

Table 51.

Summary of Test Results for Known NAT Positive Specimens

Target	Dilution	Total Tested	cobas® TaqScreen MPX Test, v2.0 Reactive	cobas® TaqScreen MPX Test Reactive
HIV-1 Group M	Neat	1,106	1,098	1,095
	1:6 Diluted	1,123	1,086	1,078
HCV	Neat	1,137	1,117	1,118
	1:6 Diluted	1,122	1,106	1,106
HBV	Neat	491	491	491
	1:6 Diluted	498	493	489
HIV-1 Group O*	Seropositive, diluted	11	8	8
	Cultured, diluted	9	9	9
HIV-2*	Seropositive, Neat	312	181	172
	Seropositive, diluted	318	137	137

** Results for HIV-1 Group O and HIV-2 are discussed below.*

Clinical Sensitivity for HIV-1 Group O and HIV-2 Seropositive Population

HIV-1 Group O Seropositive Population

A total of 11 HIV-1 Group O seropositive specimens were tested after 1:6 dilution using the cobas® TaqScreen MPX Test, v2.0. A total of 8 specimens of the 11 were reactive. The 3 non-reactive specimens had viral loads below the Limit of Detection of the Abbot Real Time HIV-1 Test (< 60 Copies/mL) and none of the three were reactive using the cobas® TaqScreen MPX Test. In addition, a total of nine different HIV-1 Group O cultured isolates were diluted and tested with cobas® TaqScreen MPX Test and cobas® TaqScreen MPX Test, v2.0. All specimens were reactive using both tests (**Table 52**, **Table 53**).

Table 52.

Test Results for HIV-1 Group O Seropositive Specimens

Specimen ID_Dilution	cobas® TaqScreen MPX Test, v2.0	cobas® TaqScreen MPX Test
BSE191_1:6	R	R
HJ1230_1:6	R	R
HJ1357_1:6	NR	NR
HJ162_1:6	R	R
HJ1977_1:6	R	R
HJ367_1:6	NR	NR
HJ736_1:6	NR	NR
HJ2044_1:6	R	R
K1043_1:6	R	R
HJ100_1:6	R	R
HJ1322_1:6	R	R

Note: R = Reactive; NR = Non-reactive

Table 53.

Test Results for HIV-1 Group O Cultured Isolates

Culture ID_Dilution	cobas® TaqScreen MPX Test, v2.0	cobas® TaqScreen MPX Test
60736_1:1000	R	R
BCF02_1:1000	R	R
MVP 5180_1:1000	R	R
BV5003_1:2000	R	R
BV 5051_1:1000	R	R
BV 5024_1:1000	R	R
BCF11_1:2000	R	R
BCF01_1:2000	R	R
BCF06_1:2000	R	R

Note: R = Reactive; NR = Non-reactive

HIV-2 Seropositive Population

A total of 312 HIV-2 seropositive specimens were tested using the **cobas®** TaqScreen MPX Test, v2.0 and **cobas®** TaqScreen MPX Test. A total of 181 specimens of the 312 were reactive using **cobas®** TaqScreen MPX Test, v2.0 compared to 172 for the **cobas®** TaqScreen MPX Test (**Table 54**). Of the 131 non-reactive specimens, none were reactive using an alternate quantitative NAT method (Research Use Only test developed by Dr. Florence Damond, Hopital Bichat Claude Bernard, Paris, France).[39]

Table 54.

Test Results for Neat HIV-2 Seropositive Specimens

cobas® TaqScreen MPX Test, v2.0 (Neat)	cobas® TaqScreen MPX Test (Neat)		Total
	Reactive	Non-Reactive	
Positive	145	36	181
Negative	27	104	131
Total	172	140	312

A comparable rate of detection of the **cobas®** TaqScreen MPX Test, v2.0 and the **cobas®** TaqScreen MPX Test for HIV-2 was also demonstrated when 318 HIV-2 seropositive specimens were diluted 1:6 prior to testing with both tests. Both the **cobas®** TaqScreen MPX Test, v2.0 and the **cobas®** TaqScreen MPX Test detected 137 of 318 diluted specimens (**Table 55**).

Table 55.

Test Results for 1:6 Diluted HIV-2 Seropositive Specimens

cobas® TaqScreen MPX Test, v2.0 (1:6)	cobas® TaqScreen MPX Test (1:6)		Total
	Reactive	Non-Reactive	
Positive	107	30	137
Negative	30	151	181
Total	137	181	318

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