WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: cobas HCV (Quantitative nucleic acid test for use on the cobas 5800/6800/8800 Systems)
WHO reference number: PQDx 0465-046-00

cobas HCV (Quantitative nucleic acid test for use on the **cobas** 5800/6800/8800 Systems) with product codes 06997732190 and 09040765190, manufactured by Roche Molecular Systems, Inc., CE-mark regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 9 March 2021.

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

	Date	Outcome
Prequalification listing	9 March 2021	listed
Dossier review	abridged	N/A
Onsite Assessment of the quality management system	11 July 2022	MR
Product performance evaluation	2nd and 3rd quarters of 2020	MR

MR: Meet Requirements
N/A: Not Applicable

Report amendments and product changes

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

Version	Summary of amendment	Date of report amendment
2.0	1. Change of Legal Manufacturer 2. Change of Notified Body 3. Addition of the 192T Kit size 3.1. Addition of the 192T Size, Onboard & Open Reagent Stability. 3.2. Addition of the 192T Size: Clinical Performance Data 4. Addition of cobas 5800 Instrument.	4 September 2023

Intended use:

According to the claim of intended use from Roche Diagnostics GmbH, "*cobas HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of hepatitis C (HCV) RNA, genotypes 1 to 6, in human EDTA plasma or serum of HCV-infected individuals.*
cobas HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.
The test is intended for use in the management of patients with chronic HCV in conjunction with clinical and laboratory markers of infection. The test can be used to predict the probability of sustained virologic response (SVR) early during a course of antiviral therapy, and to assess viral response to antiviral treatment (response guided therapy) as measured by changes of HCV RNA levels in serum or EDTA plasma. The results must be interpreted within the context of all relevant clinical and laboratory finding."

Assay Description:

According to the claim of assay description from Roche Diagnostics GmbH, *"cobas HCV is a quantitative test performed on the cobas 5800 System, cobas 6800 System, or cobas 8800 System. cobas HCV enables the detection and quantitation of HCV RNA in EDTA plasma or serum of infected patients. Dual probes are used to detect and quantify, but not discriminate genotypes 1-6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to HCV WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HCV WHO International Standard."*

Test kit contents:

Test kit contents:

cobas (HCV 96 test cassette)	**Product code 06997732190**
Proteinase Solution (PASE)	13 mL vessel x 1
RNA Quantitation Standard (RNA-QS)	13 mL vessel x 1
Elution Buffer (EB)	13 mL vessel x 1
Reagent 1 (MMX-R1) Manganese acetate	5.5 mL vessel x 1
Mix Reagent 2 (HCV MMX-R2)	6 mL vessel x 1

Tricine buffer,	
cobas (HCV 192 test cassette)	**Product code 09040765190**
Proteinase Solution (PASE)	22.3 mL
RNA Quantitation Standard (RNA-QS)	21.2 mL
Elution Buffer (EB)	21.2 mL
Master Mix Reagent 1 (MMX-R1)	7.5 mL
HCV Master Mix Reagent 2 (HCV MMX-R2)	9.7 mL

Items required but not provided:

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

Item	**Description**
cobas omni reagents for sample preparation	
cobas omni MGP Reagent (MGP)	P/N 06997546190, 480 tests
cobas omni Specimen Diluent (SPEC DIL)	P/N 06997511190, 4 x 875 mL
cobas omni Lysis Reagent (LYS)	P/N 06997538190, 4 x 875 mL
cobas omni Wash Reagent (WASH)	P/N 06997503190, 4.2 L
Additional Materials for cobas 5800 System	
cobas omni Processing Plate 24	P/N 08413975001
cobas omni Amplification Plate 24	P/N 08499853001
cobas omni Liquid Waste Plate 24	P/N 08413983001
Tip CORE TIPS with Filter, 1mL	P/N 04639642001
Tip CORE TIPS with Filter, 300uL	P/N 07345607001
cobas omni Liquid Waste Container	P/N 07094388001
cobas omni Lysis Reagent	P/N 06997538190
cobas omni MGP Reagent	P/N 06997546190

cobas omni Specimen Diluent	P/N 06997511190
cobas omni Wash Reagent	P/N 06997503190
Solid Waste Bag or **Solid Waste Bag With Insert**	P/N 07435967001 or P/N 08030073001
Additional Materials for cobas 6800/8800 Systems	
cobas omni Processing Plate	P/N 05534917001
cobas omni Amplification Plate	P/N 05534941001
cobas omni Pipette Tips	P/N 05534925001
cobas omni Liquid Waste Container	P/N 07094388001
cobas omni Lysis Reagent	P/N 06997538190
cobas omni MGP Reagent	P/N 06997546190
cobas omni Specimen Diluent	P/N 06997511190
cobas omni Wash Reagent	P/N 06997503190
Solid Waste Bag	P/N 07435967001 and 07094361001
Solid Waste Container	P/N 08030073001 and 08387281001

Instrumentation and software required

The **cobas** 5800 System software and cobas HCV analysis package for the cobas 5800 System shall be installed on the cobas 5800 instrument. The Data Manager software and PC for the **cobas** 5800 System will be provided with the system.

The **cobas** 6800/8800 Systems software and cobas HCV analysis package for the cobas 6800/8800 Systems shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Equipment	**Product Number (P/N)**
cobas 5800 System	08707464001
cobas 6800 System (Option Moveable)	05524245001 and 06379672001
cobas 6800 System (Fix)	05524245001 and 06379664001
cobas 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the cobas 5800 System or cobas 6800/8800 Systems User Assistance and/or User Guides for additional information.

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

Storage:

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

Shelf-life upon manufacture:

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

Warnings/limitations:

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

Prioritization for Prequalification
Based on the established eligibility criteria, **cobas** HCV (Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems) was given priority for WHO prequalification assessment.

Product dossier assessment

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

Manufacturing site inspection

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

https://www.who.int/diagnostics_laboratory/evaluations/PQDxSiteInspection/en/

All published WHOPIRs are with the agreement of the manufacturer.

The onsite inspection was accepted on 21 February 2023.

Product performance evaluation

cobas HCV (Quantitative nucleic acid test for use on the **cobas** 6800/8800 Systems) was evaluated by the National Serology Reference Laboratory (NRL); Melbourne, Australia, on behalf of WHO in the 2nd and 3rd quarters of 2020, according to protocol PQDx_225, version 4.

Clinical performance evaluation

In accordance with the WHO procedure for prequalification assessment and given the fact that cobas HCV is used as the comparator assay in the WHO evaluation protocol PQDx_225 for the performance evaluation of HCV molecular assays, the performance evaluation of this product was limited to the evaluation of analytical performance and operational characteristics. The evaluation of clinical performance was not conducted.

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD)	The LoD was estimated at 29.5 IU/mL (95% CI: 15.9-54.9) using the 500 µL workflow. This was slightly higher than the LoD claimed by the manufacturer for the 500µL workflow (8.46 IU/mL; 95% CI: 7.50-9.79) but was considered acceptable.
Within-run precision (repeatability)	At 2.0 $\log_{10}$ IU/mL, CV% were < 5% At 4.0 $\log_{10}$ IU /mL, CV% were < 2.5% Using the formula for log-transformed data, the within-run CV% ranged from 20.9% to 23.3%
Within-laboratory precision (reproducibility)	At 2.0 $\log_{10}$ IU/mL, CV% were < 5% At 4.0 $\log_{10}$ IU /mL, CV% were < 3% Using the formula for log-transformed data, the within-laboratory CV% ranged from 23.3% to 25.7%
Genotype detection using the 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques (NIBSC ref 14/290) and NRL HCV Mixed Genotype panel (genotypes 1a, 1b, 2, 3, 3a, 3b, 5, 6)	All 7 specimens included in the 4th HCV Genotype panel were detected. All 15 specimens included in the NRL HCV Mixed Genotype panel were detected.

Cross-contamination/carry-over	No cross-contamination was observed when high positive and negative specimens were tested together.

Operational characteristics and ease of use

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

The assay was found easy to use by the operators performing the evaluation.

Key operational characteristics	
Number of steps for one specimen*	6 steps in total No step with precision pipetting
Number of steps for instrument management**	7 steps per run
Time to result for one run	2 hours and 30 minutes
Operator hands-on time for one run	• Checking and loading reagents: 15 minutes • Checking and loading supplies (solutions and inventory) and replacing waste: 20 minutes • Creating orders and loading specimens: 30 minutes • Unloading specimens: 30 minutes • Result review: 25 minutes
Level of automation	Fully automated (extraction and amplification)
Quality controls	QCs are provided by the manufacturer and should be purchased separately. Three controls (Low Positive, High Positive and Negative Control) should be tested with each run.
Operating temperature	15-32 °C

Result display and connectivity	Results are displayed on the system screen. They may be exported or printed as a report. The results can be exported to the laboratory information system.
Power sources	Main power The use of a UPS is recommended, as stable electricity is required.
Biosafety *(outside of infectious specimen handling)*	Operators reported biosafety considerations. They noted that care is required when handling the reagents and removing used amplification plates, solid and liquid waste. In addition, the cobas HCV Lysis Reagent contains guanidine thiocyanate and should not come into contact with sodium hypochlorite solutions as it can produce a highly toxic gas.
Waste	The volume of waste is approx. 2 L per run. Waste disposal requires specific measures in addition to usual laboratory biohazard waste disposal procedures: the lysis reagent has code H412 (harmful to aquatic life with long-lasting effects).
Calibration	Assay calibration is not required.
Maintenance	Weekly maintenance is required.
Other specific requirements	Floor stability must be formally assessed prior to installation.

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

*** Steps for instrument management: each action required daily or per run to set up and shut down the instrument, e. g. switch on the instrument, login, maintain supplies, maintain reagents, discard liquid waste, discard solid waste, archive results, switch off instrument (8 steps)*

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

Labelling

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

1. Labels

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

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

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

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

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

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

Kvantitativ nukleinsyretest til brug på ***cobas®*** *6800/8800 Systems*

Kvantitativt nukleinsyretest för användning med ***cobas®*** *6800/8800 Systems*

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

Kvantitativny test nukleových kyselín na použitie v ***cobas®*** *6800/8800 Systems*

cobas® HCV

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

REF P/N: 06997732190

Σ 96

2°C 8°C

IVD

Roche

cobas®

Cont.

HCV Cassette	x 1
MMX-R1	5.5 mL
HCV MMX-R2	6 mL
EB	13 mL
RNA QS	13 mL
PASE	13 mL

EUH210 Safety data sheet available on request./ Sicherheitsdatenblatt auf Anfrage erhältlich./ Fiche de données de sécurité disponible sur demande./ Scheda dati di sicurezza disponibile su richiesta./ Puede solicitarse la ficha de datos de seguridad./ Ficha de segurança fornecida a pedido./ Sikkerhedsdatablad kan på anmodning rekvireres./ Säkerhetsdatablad finns att rekvirera./ Karta charakterystyki dostępna na żądanie./ Na požiadanie možno poskytnúť kartu bezpečnostných údajov.

EUH208 Contains Subtilisin, 9014-01-1. May produce an allergic reaction./ Enthält Subtilisin, 9014-01-1. Kann allergische Reaktionen hervorrufen./ Contient de la subtilisine, 9014-01-1. Peut produire une réaction allergique./ Contiene Subtilisina, 9014-01-1. Può provocare una reazione allergica./ Contiene subtilisina, 9014-01-1. Puede provocar una reacción alérgica./ Contém subtilisina, 9014-01-1. Pode provocar uma reacção alérgica./ Indeholder subtilisin, 9014-01-1. Kan udløse allergisk reaktion./ Innehåller subtilisin, 9014-01-1. Kan orsaka en allergisk reaktion./ Zawiera subtylizynę, 9014-01-1. Może powodować wystąpienie reakcji alergicznej./ Obsahuje subtilizín, 9014-01-1. Môže vyvolať alergickú reakciu.

07941668001-03

HCV

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

D

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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

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

Roche Diagnostics
2, Avenue du Vercors
38240 Meyfan, France

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

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

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

 Made in USA

cobas® HCV

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

Roche

cobas®

HCV

CE
0123

UDI label here

Drawing: CAM12010

1.1 Outside packaging box

cobas® HCV

*For use on the **cobas**® 6800/8800 Systems*

REF P/N: 09040765190

IVD

Σ 192

2°C – 8°C

Rx Only

Roche

cobas®

*For use on the **cobas**® 6800/8800 Systems*
*Zur Verwendung auf den **cobas**® 6800/8800 Systemen*
*À utiliser avec les systèmes **cobas**® 6800/8800*
*Per l'uso con i sistemi **cobas**® 6800/8800*
*Para uso en sistemas **cobas**® 6800/8800*
*Para ser utilizado nos sistemas **cobas**® 6800/8800*
*Til brug på **cobas**® 6800/8800-systemerne*
*För användning på **cobas**® 6800/8800-systemen*
*Do użytku z systemami **cobas**® 6800/8800*
*Určené na použitie v systémoch **cobas**® 6800/8800*

EUH210
Safety data sheet available on request.
Sicherheitsdatenblatt auf Anfrage erhältlich.
Fiche de données de sécurité disponible sur demande.
Scheda dati di sicurezza disponibile su richiesta.
Puede solicitarse la ficha de datos de seguridad.
Ficha de segurança fornecida a pedido.
Sikkerhedsdatablad kan på anmodning rekvireres.
Säkerhetsdatablad finns att rekvirera.
Karta charakterystyki dostępna na żądanie.
Na požiadanie možno poskytnúť kartu bezpečnostných údajov.

EUH208
Contains Subtilisin. May produce an allergic reaction.
Enthält Subtilisin. Kann allergische Reaktionen hervorrufen.
Contient de la subtilisine. Peut produire une réaction allergique.
Contiene Subtilisina. Può provocare una reazione allergica.
Contiene subtilisina. Puede provocar una reacción alérgica.
Contém subtilisina. Pode provocar uma reacção alérgica.
Indeholder subtilisin. Kan udløse allergisk reaktion.
Innehåller subtilisin. Kan orsaka en allergisk reaktion.
Zawiera subtylizynę. Może powodować wystąpienie reakcji alergicznej.
Obsahuje subtilizín. Môže vyvolať alergickú reakciu.

CONTENT

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

For USA*

HCV Cassette x 1

PASE 22.3 mL
(Proteinase Solution)
Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% (w/v) Proteinase

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

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

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

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

Refer to the product specific Instructions For Use for detailed information regarding hazards.

*Composition information required for US only.

09112243001-02

HCV

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

Distributed by/ Vertrieb/ Distributeur/ Distribuito da/ Distribuido por/ Distribuído por/ Distribueret af/ Distribueras av/ Dystrybucja/ Distribútor

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Made in USA

cobas® HCV

*For use on the **cobas**® 6800/8800 Systems*

Roche

cobas®

HCV

EC REP
Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

CE 2797

UDI label here

Drawing: CAM12010

2. Instructions for use[1]

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

Rx Only

cobas® HCV

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

For in vitro diagnostic use

cobas® HCV	P/N: 09040765190
For use on the cobas® 5800 System	
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 09051554190
For use on the cobas® 6800/8800 Systems	
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 06997767190 or P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 07002220190 or P/N: 09051554190

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Additional information 50

Intended use

cobas® HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of hepatitis C (HCV) RNA, genotypes 1 to 6, in human EDTA plasma or serum of HCV-infected individuals.

cobas® HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.

The test is intended for use in the management of patients with chronic HCV in conjunction with clinical and laboratory markers of infection. The test can be used to predict the probability of sustained virologic response (SVR) early during a course of antiviral therapy, and to assess viral response to antiviral treatment (response guided therapy) as measured by changes of HCV RNA levels in serum or EDTA plasma. The results must be interpreted within the context of all relevant clinical and laboratory finding.

Summary and explanation of the test

Background

Hepatitis C virus (HCV) is considered to be the principal etiologic agent responsible for 90% to 95% of the cases of post-transfusion hepatitis.[1-4] HCV is a single-stranded, positive sense RNA virus with a genome of approximately 9,500 nucleotides coding for 3,000 amino acids. As a blood-borne virus, HCV can be transmitted by blood and blood products. Widespread adoption of HCV blood screening measures has markedly lowered the risk of transfusion-associated hepatitis. The incidence of HCV infection is highest in association with intravenous drug abuse and to a lesser extent with other percutaneous exposures.[4]

Quantitation of HCV RNA for measuring baseline viral loads and for on-treatment monitoring has been well established in demonstrating the efficacy of antiviral response to pegylated interferon plus ribavirin (pegIFN/RBV) combination therapy.[5-9] Guidelines for the management and treatment of HCV[10,11] recommend quantitative testing for HCV RNA before the start of antiviral therapy, at specified time intervals during therapy (response-guided therapy, RGT), and at 12 weeks or later, following the end of treatment.

Absence of detectable HCV RNA by a sensitive test, 12 weeks after the end of treatment, is the goal of treatment and indicates that a sustained virologic response (SVR) has been achieved.[10]

Determining the viral kinetics during therapy has been used to further personalize treatment duration with the more recently approved direct-acting antiviral agents (DAAs), the protease inhibitors telaprevir and boceprevir.[12-15]

Rationale for HCV testing

With the very dynamic and extensive drug discovery pipeline for future HCV therapies, viral load monitoring remains the main laboratory test to confirm that SVR has been achieved with DAAs, such as second generation protease inhibitors, nucleoside inhibitors of HCV polymerase and other mechanisms of antiviral action.[16-19]

In summary, **cobas**® HCV for use on the **cobas**® 5800/6800/8800 Systems is a quantitative test for HCV RNA and viral kinetics, for use in laboratories that support clinical trials as well as the management of HCV patients in routine clinical practice.

Explanation of the test

cobas® HCV is a quantitative test performed on the **cobas**® 5800 System, **cobas**® 6800 System, or **cobas**® 8800 System. **cobas**® HCV enables the detection and quantitation of HCV RNA in EDTA plasma or serum of infected patients. Dual probes are used to detect and quantify, but not discriminate genotypes 1-6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to HCV WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HCV WHO International Standard.

Principles of the procedure

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

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

Selective amplification of target nucleic acid from the patient sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HCV. Selective amplification of RNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HCV genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[20-22] Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® HCV master mix contains dual detection probes specific for the HCV target sequences and one for the RNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of HCV target and RNA-QS in two different target channels.[23,24] When not bound to the target sequence, the fluorescent signal of the intact probe is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time

detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS.

Reagents and materials

cobas® HCV reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® HCV

cobas® HCV

Store at 2-8°C
192 test cassette (P/N 09040765190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheets available on request. EUH208: May produce an allergic reaction. Contains: Subtilisin, 9014-01-1	22.3 mL
RNA Quantitation Standard (**RNA-QS**)	Tris buffer, < 0.05% EDTA, < 0.001% non-HCV related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HCV Master Mix Reagent 2 (HCV MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, and dUTP, < 0.01% upstream and downstream HCV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

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

cobas® HBV/HCV/HIV-1 Control Kit

Store at 2–8°C

For use on the **cobas**® 5800 System (P/N 09040773190)

For use on the **cobas**® 6800/8800 Systems (P/N 06997767190 and P/N 09040773190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+)C)	< 0.001% HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein armored, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1)
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C)	< 0.001% high titered synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fumes/gas/ mist/ vapors/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

09198873001-03EN

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

cobas® **NHP Negative Control Kit**

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051554190)

For use on the **cobas**® 6800/8800 Systems (P/N 07002220190 and P/N 09051554190)

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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

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

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

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

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

Reagent handling requirements for cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® HCV – 192	Date not passed	90 days from first usage	Max 40 runs	Max 36 days*
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days*
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days*
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

[a] Single use reagents

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

Reagent handling requirements for cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® HCV	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

[a] Single use reagents

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

Additional materials required for cobas® 5800 System

Table 8 Material and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas omni Processing Plate 24	08413975001
cobas omni Amplification Plate 24	08499853001
cobas omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300uL	07345607001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001

Additional materials required for cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

The **cobas**® 5800 System software and **cobas**® HCV analysis package for the **cobas**® 5800 System shall be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 Systems software and **cobas**® HCV analysis package for the **cobas**® 6800/8800 Systems shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 10 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems - User Assistance and/or User Guides for additional information .

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

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use only.
- **cobas**® HCV has not been evaluated for use as a screening test for the presence of HCV in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[25,26] Only personnel proficient in handling infectious materials and the use of **cobas**® HCV and the **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® HBV/HCV/HIV-1 Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Do not use 200 µL sample input volume if the viral load is expected to be < 100 IU/mL.
- Inform your local competent authority about any serious incidents which may occur when using this assay.

Reagent handling

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

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

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® HCV kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 instrument, follow the instructions in the **cobas**® 5800 System User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

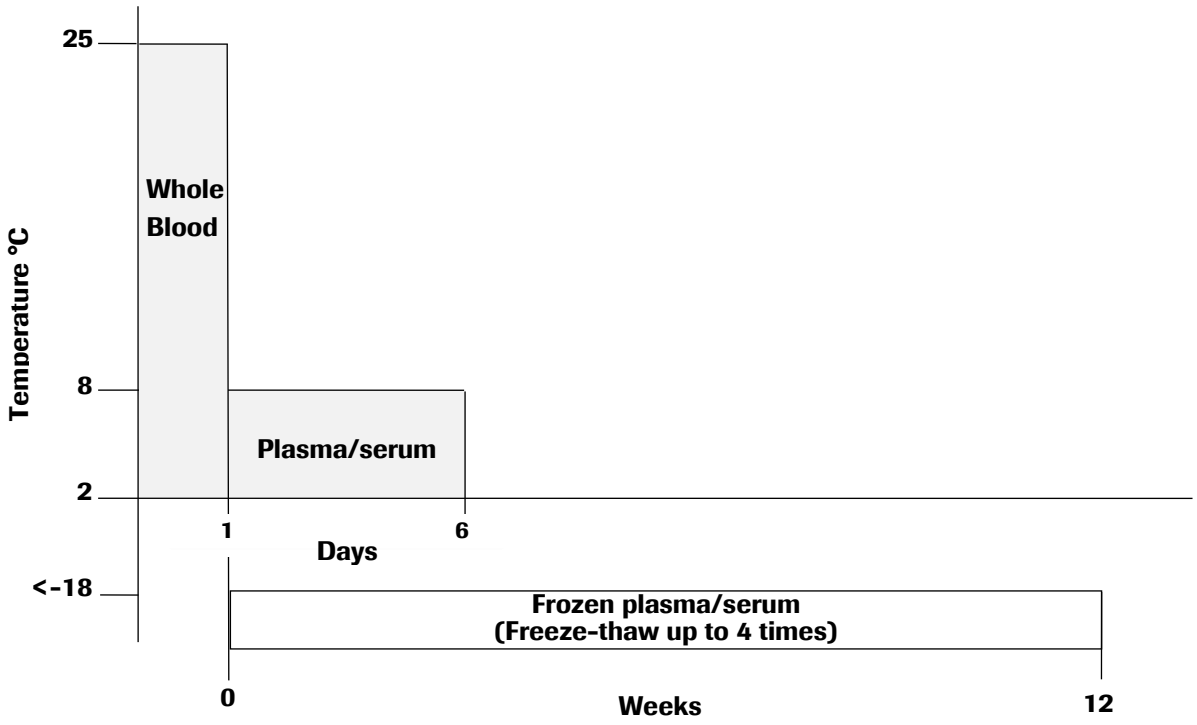
Samples

Blood should be collected in SST™ Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions.

- Whole blood collected in SST™ Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma/serum preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation EDTA plasma or serum samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. For long-term storage, temperatures at ≤ -60°C are recommended.

- Plasma/serum samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.
- Ensure sufficient whole blood collection to allow usage of the preferred processing volume for EDTA plasma or serum of 500 µL (for a total minimum sample requirement of 650 µL) if possible.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Figure 1 Sample storage conditions



Instructions for use

Procedural notes

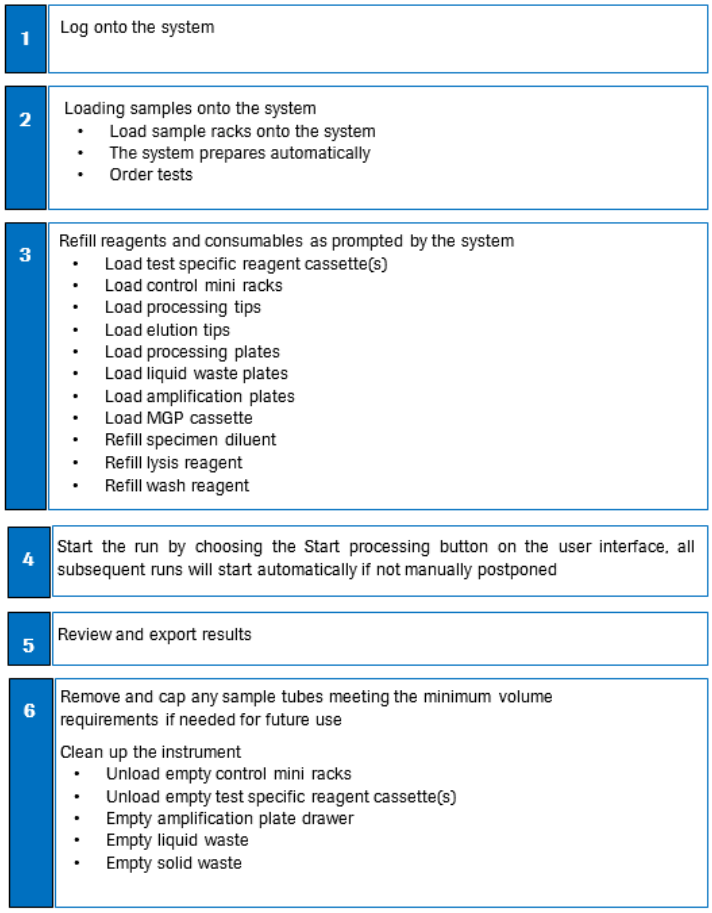
- Do not use **cobas**® HCV test reagents, **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems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

Running cobas® HCV on cobas® 5800 System

cobas® HCV can be run with two required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the **cobas**® 5800 System User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

- **Note: Do not use the 200 µL sample workflow if the viral load is expected to be ≤ 100 IU/mL. Sufficient blood volume should be collected to allow usage of the preferred processing volume for EDTA plasma or serum of 500 µL (for a total minimum sample requirement of 650 µL).**

Figure 2 **cobas**® HCV test procedure on **cobas**® 5800 System

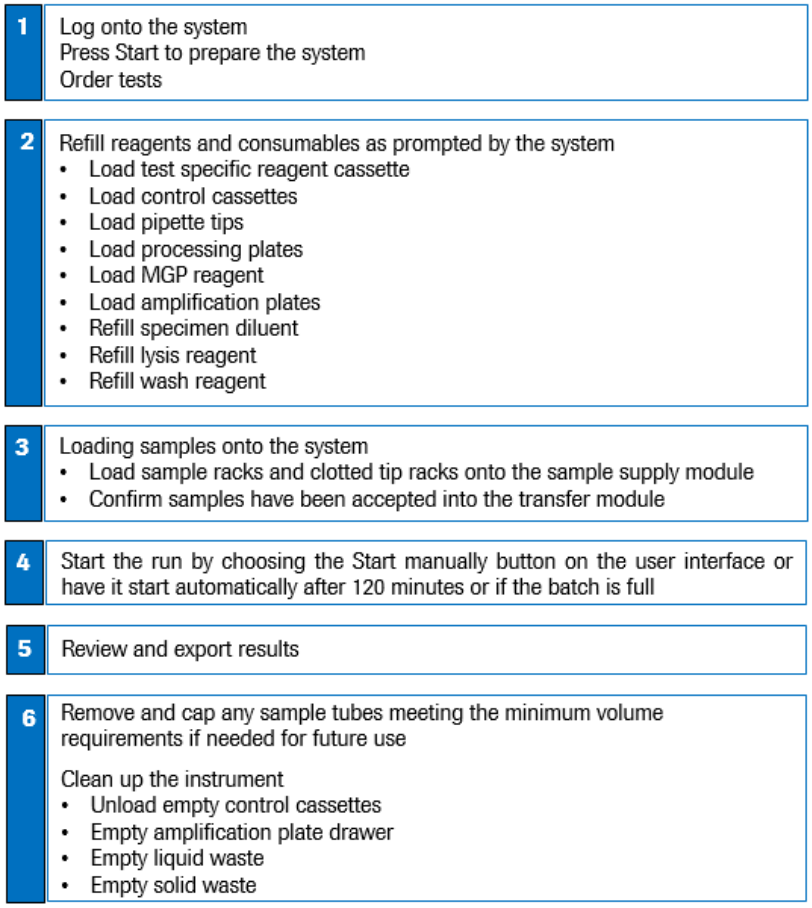


Running cobas® HCV on the cobas® 6800/8800 Systems

cobas® HCV can be run with two required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide. Figure 3 below summarizes the procedure.

- **Note: Do not use the 200 µL sample workflow if the viral load is expected to be ≤ 100 IU/mL. Sufficient blood volume should be collected to allow usage of the preferred processing volume for EDTA plasma or serum of 500 µL (for a total minimum sample requirement of 650 µL).**

Figure 3 **cobas**® HCV test procedure on **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 Systems automatically determine the HCV RNA concentration for the samples and controls. The HCV RNA concentration is expressed in International Units per milliliter (IU/mL).

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

- One negative control [(–) C] and two positive controls, a low positive control [HCV L (+) C] and a high positive control [HCV H (+) C] is processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 System software and/or report, check for flags and their associated results to ensure batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HCV L (+) C, HCV H (+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative and positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the positive controls is invalid, repeat testing of the all positive controls and all associated samples. If the negative control is invalid, repeat testing of all controls and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One negative control [(–) C] and two positive controls, a low positive control [HCV L (+) C] and a high positive control [HCV H (+) C], are processed with each batch running on the **cobas**® 6800/8800 Systems.
- In the **cobas**® 6800/8800 Systems softwares and/or reports, check for flags and their associated results to ensure batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HCV L (+) C, HCV H (+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

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

Control flags on cobas® 6800/8800 Systems

Table 11 Control flags for negative and positive controls

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

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

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

Interpretation of results

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

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

Table 12 Target results for individual target result interpretation

Results	Interpretation
Target Not Detected	HCV RNA not detected. Report results as “HCV not detected.”
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “HCV detected, less than (Titer Min)” Titer min = 15 IU/mL (500 µL) Titer min = 40 IU/mL (200 µL)
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as “(Titer) of HCV detected”.
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “HCV detected, greater than (Titer Max).” Titer max = 1.00E+08 IU/mL (500 µL and 200 µL)

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

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

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

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D : Result validation failure because of an invalid positive control
  - Q06D :Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 12 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 Systems

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

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 12 above,

Procedural limitations

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

Non-clinical performance evaluation

Key performance characteristics performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

WHO International Standard

The limit of detection of **cobas**® HCV was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays (4th WHO International Standard) genotype 1a obtained from National Institute for Biological Standards and Control (NIBSC), in HCV-negative human EDTA plasma and serum using sample processing volumes of 500 µL and 200 µL. The minimum sample requirement was 650 µL and 350 µL respectively to be processed by **cobas**® 6800/8800 Systems. Panels of six concentration levels plus a negative were tested for 500 µL sample processing volume and seven concentration levels for 200 µL sample processing volume over three lots of **cobas**® HCV test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum from both sample processing volumes are shown in Table 13 to Table 16, respectively. The study demonstrates that **cobas**® HCV detected HCV RNA at a concentration of 8.46 IU/mL with a 95% confidence range of 7.50-9.79 IU/mL for the 500 µL sample processing volume in EDTA plasma, and at a concentration of 9.61 IU/mL with a 95% confidence range of 8.70-10.95 IU/mL for the 500 µL sample processing volume in serum. The study demonstrated that **cobas**® HCV detected HCV RNA at a concentration of 24.93 IU/mL with a 95% confidence range of 22.51-28.35 IU/mL for the 200 µL sample processing volume in EDTA plasma, and at a concentration of 33.25 IU/mL with a 95% confidence range of 29.94-37.94 IU/mL for the 200 µL sample processing volume in serum. The difference between EDTA plasma and serum using sample processing volumes of 500 µL and 200 µL was not statistically significant.

Table 13 Limit of detection in EDTA plasma (500 µL)

Input titer concentration (HCV RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
30	189	189	100.00
20	188	186	98.94
15	189	187	98.94
10	189	183	96.83
8	188	182	96.81
5	188	155	82.45
0	189	1*	0.53
LoD by PROBIT at 95% hit rate	8.46 IU/mL 95% confidence range: 7.50-9.79 IU/mL		

*Samples confirmed negative by alternative analytical methods.

Table 14 Limit of detection in serum (500 µL)

Input titer concentration (HCV RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
30	188	187	99.47
20	189	189	100.00
15	189	187	98.94
10	189	184	97.35
8	189	171	90.48
5	189	141	74.60
0	189	0	0.00
LoD by PROBIT at 95% hit rate	9.61 IU/mL 95% confidence range: 8.70-10.95 IU/mL		

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

Input titer concentration (HCV RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
80	189	189	100.00
60	189	189	100.00
50	188	187	99.47
40	189	185	97.88
25	189	179	94.71
20	189	177	93.65
12	188	136	72.34
0	189	1*	0.53
LoD by PROBIT at 95% hit rate	24.93 IU/mL 95% confidence range:22.51-28.35 IU/mL		

*Samples confirmed negative by alternative analytical methods.

Table 16 Limit of detection in serum (200 µL)

Input titer concentration (HCV RNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
80	189	189	100.00
60	189	188	99.47
50	189	186	98.41
40	189	184	97.35
25	189	167	88.36
20	189	156	82.54
12	189	125	66.14
0	189	0	0.00
LoD by PROBIT at 95% hit rate	33.25 IU/mL 95% confidence range: 29.94-37.94 IU/mL		

Linear range

Linearity study of **cobas**® HCV was performed with a dilution series consisting of 16 panel members spanning the intended linear range for the predominant genotype (GT 1). High titer panel members were prepared from a high titer armored RNA (arRNA) stock whereas the lower titer panel members were prepared from clinical sample (CS). The linearity panel was designed to have an approximately 2 $\log_{10}$ titer overlap between the two material sources. The expected linear range of **cobas**® HCV is from LLoQ (15 IU/mL in 500 µL process volume and 40 IU/mL in 200 µL process volume) to ULoQ (1.00E+08 IU/mL in both process volumes). The linearity panel was designed to range from one concentration below LLoQ (e.g. 7.5 IU/mL) to one concentration level above ULoQ (e.g. 2.0E+08 IU/mL) and to include medical decision points. Moreover, the linearity panel was designed to partly support steps of 1.0 $\log_{10}$ throughout the linear range. For each panel member the nominal concentration in IU/mL and the source of the HCV RNA were given.

With 500 µL processing volume, **cobas**® HCV is linear for EDTA plasma and serum from 15 IU/mL to 1.00E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.24 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$.

With 200 µL processing volume, **cobas**® HCV is linear for EDTA plasma and serum from 40 IU/mL to 1.00E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.24 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$ in plasma and ± 0.27 $\log_{10}$ in serum.

See Figure 4 to Figure 7 for representative results.

Figure 4 Linearity in EDTA plasma (500 µL)

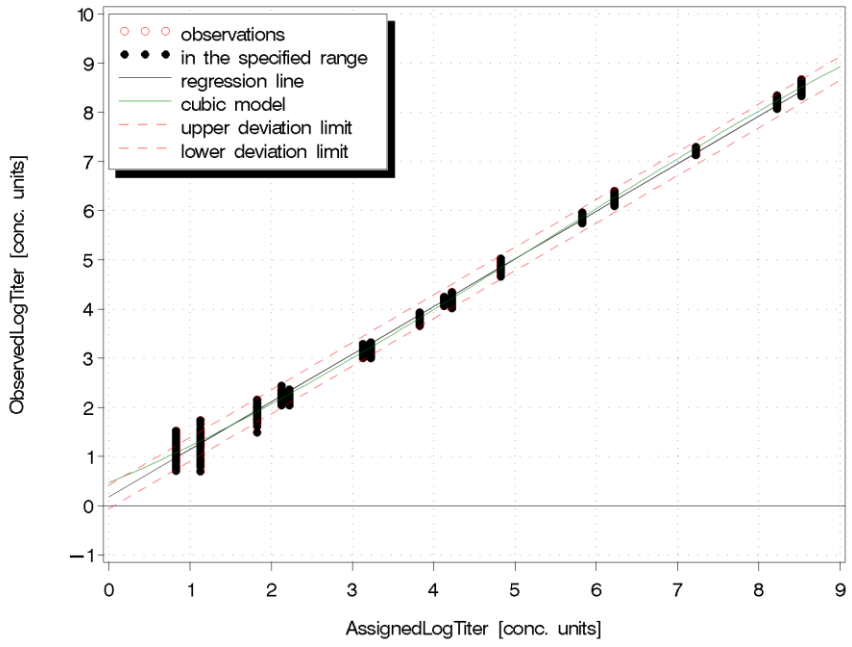


Figure 5 Linearity in serum (500 µL)

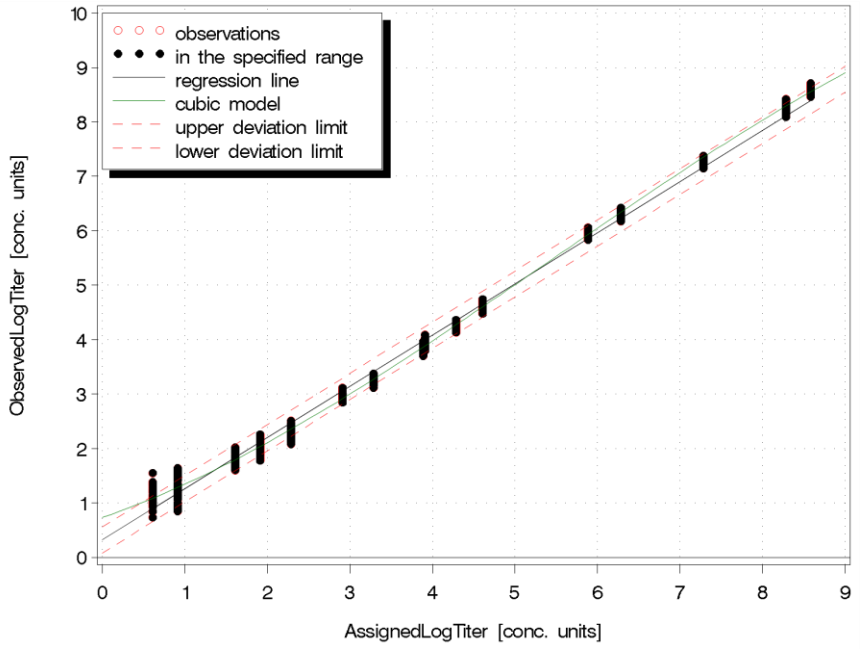


Figure 6 Linearity in EDTA plasma (200 µL)

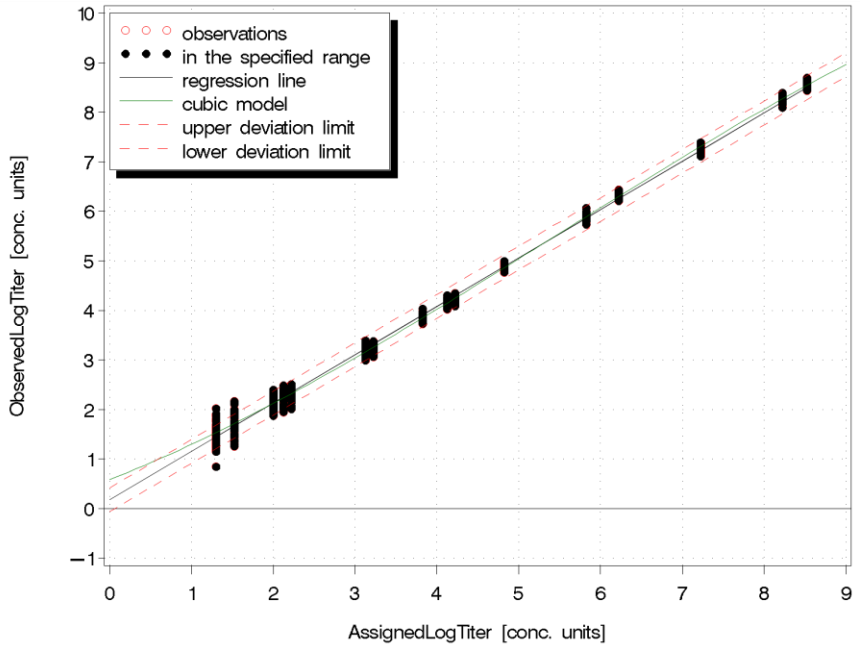
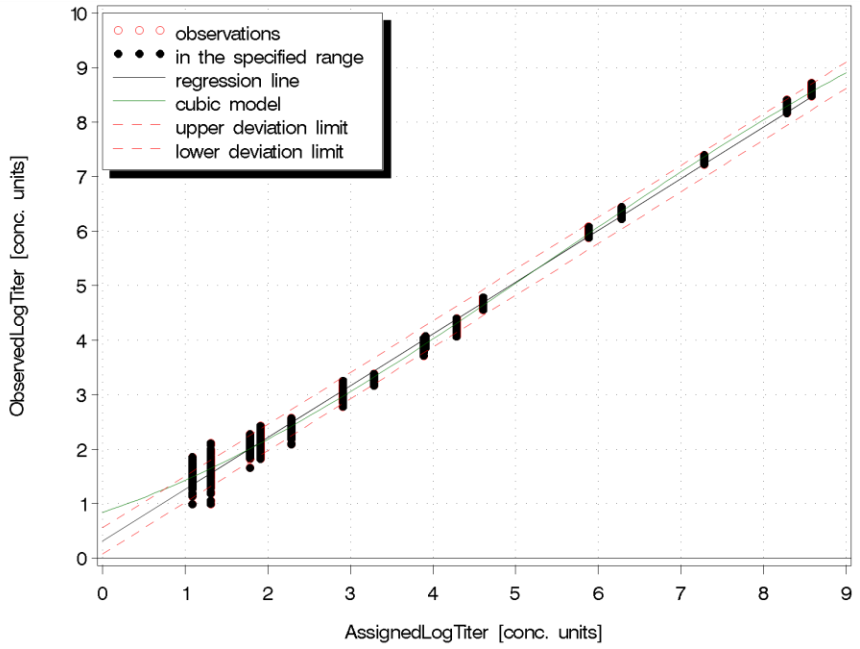


Figure 7 Linearity in serum (200 µL)



Precision – within laboratory

Precision of **cobas**® HCV was determined by analysis of serial dilutions of clinical HCV (Genotype 1) samples (CS) or of armored RNA HCV in HCV-negative EDTA plasma or in serum. Thirteen dilution levels were tested in plasma and 12 levels were tested in serum in two replicates for each level in two runs across 12 days adding up to a total of 48 replicates per concentration. Each sample was carried through the entire **cobas**® HCV test procedure on a fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed with three lots of **cobas**® HCV test reagents. The results are shown in Table 17 to Table 20.

cobas® HCV showed high precision for three lots of reagents tested across a concentration range of 1.00E+01 IU/mL to 1.0E+07 IU/mL with 500 µL sample processing volume and 2.50E+01 IU/mL to 1.0E+07 IU/mL with 200 µL sample processing volume.

Table 17 Within laboratory precision of **cobas**® HCV (EDTA plasma samples – processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	1.67E+07	arRNA	0.04	0.05	0.03	0.04
1.00E+06	1.67E+06	arRNA	0.05	0.05	0.06	0.05
4.00E+05	6.69E+05	arRNA	0.03	0.04	0.05	0.04
5.00E+04	6.69E+04	CS	0.08	0.06	0.06	0.06
1.00E+04	1.67E+04	arRNA	0.05	0.05	0.04	0.05
1.00E+04	1.34E+04	CS	0.03	0.06	0.05	0.05
4.00E+03	6.69E+03	arRNA	0.05	0.06	0.06	0.06
1.00E+03	1.34E+03	CS	0.05	0.06	0.05	0.05
1.00E+03	1.67E+03	arRNA	0.05	0.07	0.05	0.06
1.00E+02	1.34E+02	CS	0.06	0.09	0.05	0.07
1.00E+02	1.67E+02	arRNA	0.10	0.06	0.06	0.08
5.00E+01	6.69E+01	CS	0.09	0.17	0.10	0.13
1.00E+01	1.34E+01	CS	0.26	0.21	0.13	0.21

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

Table 18 Within-laboratory precision of **cobas**® HCV (serum samples – processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Serum			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	1.92E+07	arRNA	0.03	0.07	0.04	0.05
1.00E+06	1.92E+06	arRNA	0.05	0.06	0.04	0.05
4.00E+05	7.69E+05	arRNA	0.03	0.07	0.03	0.05
5.00E+04	4.05E+04	CS	0.07	0.06	0.04	0.06
1.00E+04	1.92E+04	arRNA	0.06	0.06	0.04	0.05
1.00E+04	8.11E+03	CS	0.05	0.06	0.04	0.05
4.00E+03	7.69E+03	arRNA	0.04	0.08	0.04	0.06
1.00E+03	8.11E+02	CS	0.05	0.06	0.06	0.05
1.00E+03	1.92E+03	arRNA	0.06	0.05	0.05	0.05
1.00E+02	8.11E+01	CS	0.10	0.18	0.10	0.13
1.00E+02	1.92E+02	arRNA	0.07	0.08	0.09	0.08
5.00E+01	4.05E+01	CS	0.09	0.14	0.18	0.14

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

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

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	1.67E+07	arRNA	0.04	0.06	0.05	0.05
1.00E+06	1.67E+06	arRNA	0.04	0.03	0.05	0.04
4.00E+05	6.69E+05	arRNA	0.04	0.06	0.03	0.04
5.00E+04	6.69E+04	CS	0.05	0.06	0.05	0.06
1.00E+04	1.67E+04	arRNA	0.05	0.05	0.05	0.05
1.00E+04	1.34E+04	CS	0.07	0.06	0.05	0.06
4.00E+03	6.69E+03	arRNA	0.05	0.06	0.05	0.05
1.00E+03	1.34E+03	CS	0.08	0.08	0.06	0.07
1.00E+03	1.67E+03	arRNA	0.04	0.07	0.05	0.05
1.00E+02	1.34E+02	CS	0.11	0.15	0.13	0.13
1.00E+02	1.67E+02	arRNA	0.10	0.10	0.13	0.11
7.50E+01	1.00E+02	CS	0.15	0.12	0.11	0.13
2.50E+01	3.34E+01	CS	0.19	0.20	0.22	0.21

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

Table 20 Within laboratory precision of **cobas**® HCV (serum – processing volume of 200 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Serum			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+07	1.92E+07	arRNA	0.02	0.06	0.03	0.04
1.00E+06	1.92E+06	arRNA	0.03	0.06	0.04	0.04
4.00E+05	7.69E+05	arRNA	0.04	0.09	0.04	0.06
5.00E+04	4.05E+04	CS	0.05	0.06	0.06	0.06
1.00E+04	1.92E+04	arRNA	0.05	0.07	0.04	0.06
1.00E+04	8.11E+03	CS	0.04	0.05	0.05	0.05
4.00E+03	7.69E+03	arRNA	0.04	0.07	0.04	0.05
1.00E+03	8.11E+02	CS	0.10	0.09	0.08	0.09
1.00E+03	1.92E+03	arRNA	0.05	0.07	0.04	0.05
1.00E+02	8.11E+01	CS	0.17	0.30	0.17	0.22
1.00E+02	1.92E+02	arRNA	0.13	0.13	0.09	0.12
7.50E+01	6.08E+01	CS	0.11	0.16	0.12	0.13

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

Genotype verification

The performance of **cobas**® HCV on HCV genotypes was evaluated by:

- Determination of the limit of detection for genotypes 1b through 6 tested in 500 µL sample processing volume
- Verification of the limit of detection for genotypes 1b through 6 tested in 200 µL sample processing volume
- Verification of the linearity for genotypes 2 through 6.

Limit of detection for genotypes 1b through 6

The limit of detection of **cobas**® HCV for genotypes 1b through 6 was determined by analysis of serial dilutions from each genotype, in HCV-negative human EDTA plasma and serum using sample processing volumes of 500 µL. Panels of six concentration levels plus a negative were tested using three lots of **cobas**® HCV test reagents, over multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum for 500 µL processing volume are shown in Table 21 and Table 22, respectively. The study demonstrates that **cobas**® HCV detected all HCV genotypes tested with a similar LoD as HCV genotype 1a.

Table 21 HCV RNA genotype limit of detection in EDTA plasma (500 µL)

Genotype	95% LoD by Probit	95% Confidence Interval
GT 1b	11.32 IU/mL	9.72-14.52 IU/mL
GT 2	9.10 IU/mL	7.83-11.80 IU/mL
GT 3	8.68 IU/mL	7.30-11.51 IU/mL
GT 4	12.78 IU/mL	10.69-17.20 IU/mL
GT 5	11.63 IU/mL	9.66-15.98 IU/mL
GT 6	12.58 IU/mL	9.78-20.10 IU/mL

Table 22 HCV RNA genotype limit of detection in serum (500 µL)

Genotype	95% LoD by Probit	95% Confidence Interval
GT 1b	15.24 IU/mL	12.40-21.58 IU/mL
GT 2	12.51 IU/mL	10.25-17.63 IU/mL
GT 3	7.21 IU/mL	6.10-9.50 IU/mL
GT 4	11.62 IU/mL	9.92-15.02 IU/mL
GT 5	13.06 IU/mL	10.64-18.68 IU/mL
GT 6	11.15 IU/mL	9.54-14.40 IU/mL

Verification of limit of detection for genotypes 1b through 6

HCV RNA clinical specimens for six different genotypes (1b, 2, 3, 4, 5, 6) were diluted to three different concentration levels in EDTA plasma and serum. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® HCV reagents. The results from EDTA plasma and serum using 200 µL are shown in Table 23 and Table 24. These results verify that **cobas**® HCV detected HCV RNA for the six different genotypes at concentrations of 33 IU/mL with a hit rate of ≥ 90.5% with an upper one-sided 95% confidence interval of ≥ 95.8%.

Table 23 HCV RNA genotype verification of limit of detection in EDTA plasma (200 µL)

Genotype	17.5 IU/mL			33 IU/mL			50 IU/mL		
	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
1b	63	50	79.4	63	61	96.8	63	63	100.0
2	63	51	81.0	63	62	98.4	63	62	98.4
3	63	56	89.0	63	58	92.1	63	63	100.0
4	63	54	85.7	63	57	90.5	63	63	100.0
5	63	57	90.5	63	61	96.8	63	63	100.0
6	63	47	74.6	63	57	90.5	63	62	98.4

* Upper one-sided 95% confidence interval

Table 24 HCV RNA genotype verification of limit of detection in serum (200 µL)

Genotype	17.5 IU/mL			33 IU/mL			50 IU/mL		
	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
1b	63	52	82.5	63	61	96.8	63	63	100.0
2	63	46	73.0	63	62	98.4	63	59	93.7
3	63	58	92.1	63	63	100.0	63	63	100.0
4	63	49	77.8	63	59	93.7	63	63	100.0
5	63	46	73.0	63	59	93.7	63	62	98.4
6	63	44	69.8	63	61	96.8	63	61	96.8

* Upper one-sided 95% confidence interval

Linearity for genotypes 2 through 6

The dilution series used in the verification of genotypes linearity study of **cobas**® HCV consists of nine panel members spanning the intended linear range. High titer panel members were prepared from a high titer arRNA stock whereas the lower titer panel members were made from a high titer clinical sample (CS). The linearity panel was designed to have an approximately 2 $\log_{10}$ titer overlap between the two material sources. The linear range of **cobas**® HCV spanned from the LLoQ (15 IU/mL for a sample processing volume of 500 µL, 40 IU/mL for a process volume of 200 µL) to the ULoQ (1.00E+08 IU/mL for both process volumes) and included at least one medical decision point. Testing was conducted with three lots of **cobas**® HCV reagent; 15 replicates per level were tested in EDTA plasma.

The linearity within the linear range of **cobas**® HCV was verified for all five genotypes (2, 3, 4, 5, and 6). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.24 $\log_{10}$.

Specificity

The specificity of **cobas**® HCV was determined by analyzing HCV negative EDTA plasma and serum samples from individual donors. Three hundred individual EDTA plasma and 300 individual serum samples (600 total results) were tested with two lots of **cobas**® HCV reagents. All samples tested negative for HCV RNA. In the test panel the specificity of **cobas**® HCV was 100% (95% confidence limit: ≥ 99.5%).

Analytical specificity

The analytical specificity of **cobas**® HCV was evaluated by diluting a panel of microorganisms with HCV RNA positive and HCV RNA negative EDTA plasma. The microorganisms were added to normal, virus-negative human EDTA plasma and tested with and without HCV RNA. Negative results were obtained with **cobas**® HCV for all microorganism samples without HCV target and positive results were obtained on all of the microorganism samples with HCV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HCV samples containing potentially cross-reacting organisms was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 25 Microorganisms tested for cross-reactivity

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

Analytical specificity – interfering substances

Elevated levels of triglycerides (34.5g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples were tested in the presence and absence of HCV RNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® HCV.

Moreover, the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid factor (RF) and antinuclear antibody (ANA) were tested.

With regards to sensitivity, in the case of two SLE donors, one RF donor and four ANA donors, individual samples showed interference with **cobas**® HCV. A root cause investigation showed that the test overcame the interference from the affected SLE and RF donors when tested in the presence of 75 IU/mL HCV RNA.

The four ANA donors showing interference with **cobas**® HCV when tested with 50 IU/mL HCV RNA also showed interference when tested with 75 IU/mL HCV RNA. To assess if the observed interference was ANA specific, or donor specific, an additional 15 ANA donors were tested in the presence of 50 IU/mL and 75 IU/mL HCV RNA. None of the additional donors showed any interference with **cobas**® HCV, for both concentrations tested, with regards to sensitivity/quantitation.

In addition, the drug compounds listed in Table 26 were tested at three times the C_{max}. All drug compounds tested were shown not to interfere with the specificity and quantitation of HCV RNA by **cobas**® HCV.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® HCV for all samples without HCV target and positive results were obtained on all of the samples with HCV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HCV samples containing potentially interfering substances was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 26 Drug compounds tested for interference with the quantitation of HCV RNA by **cobas**® HCV

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

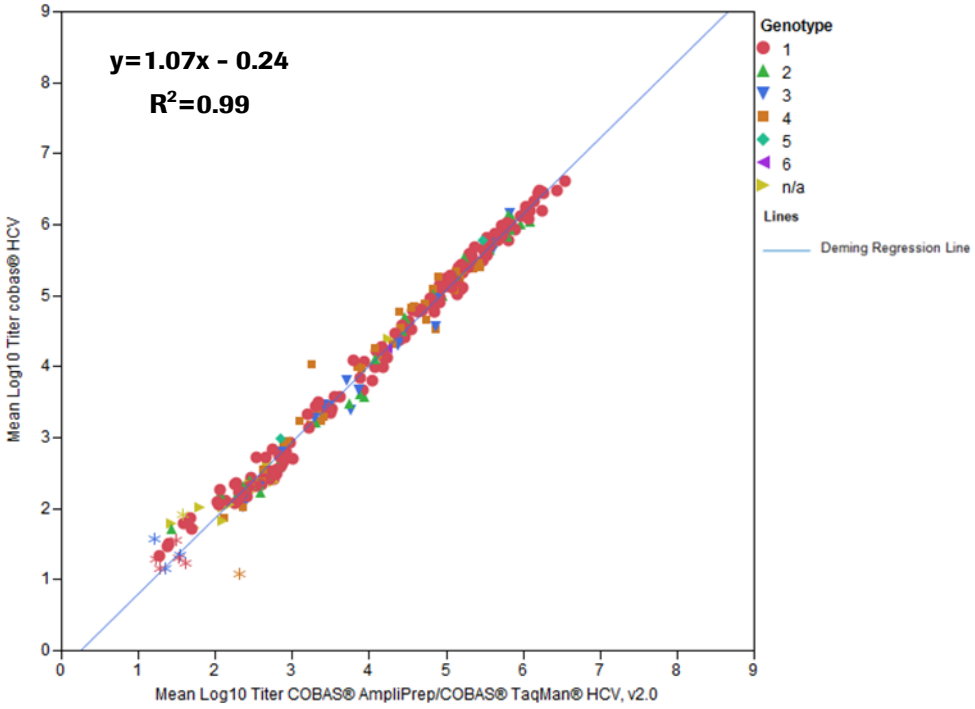
Method correlation

Performance evaluation of cobas® HCV compared to the COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0

The performance of **cobas**® HCV and the COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0 (TaqMan® HCV Test, v2.0) were compared by analysis of serum and EDTA plasma specimens from HCV-infected patients. A total of 149 EDTA plasma and 122 serum specimens across all HCV genotypes, analyzed in duplicate, were valid and within the quantitation range of both tests. Deming regression analysis was performed. The mean titer deviation of the samples tested with the two tests was 0.02 $\log_{10}$ (95% Confidence Interval: 0.00; 0.04).

The Deming regression results are shown in Figure 8. The symbol ✳ in Figure 8 shows single determination.

Figure 8 Regression analysis of **cobas**® HCV vs TaqMan® HCV Test, v2.0, EDTA plasma and serum samples

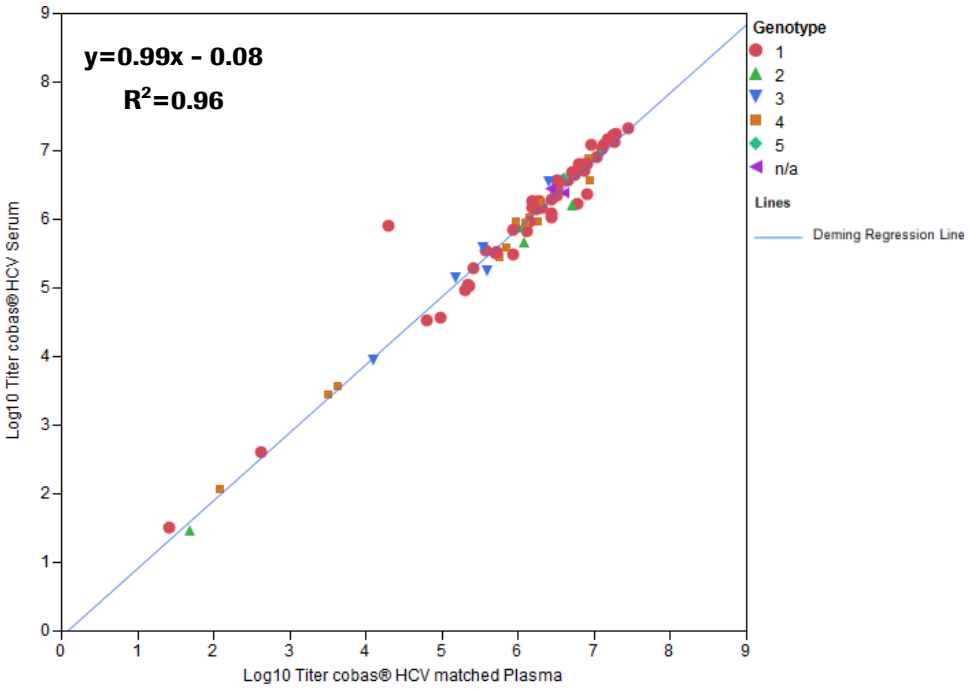


Matrix equivalency – EDTA plasma versus serum

One hundred ninety paired EDTA plasma and serum samples were analyzed for matrix equivalency. Of these, 73 paired samples were HCV positive samples. The HCV positive samples covered genotypes 1 to 4 across the linear range.

The mean titer deviation measured for the matching EDTA plasma and serum samples was -0.13 $\log_{10}$ (95% Confidence Interval: -0.19; -0.07) (Figure 9).

Figure 9 Matrix equivalency performance between EDTA plasma and serum



Whole system failure

The whole system failure Rate for **cobas**® HCV was determined by testing 100 replicates of EDTA plasma and 100 replicates of serum spiked with HCV target. These samples were tested at a target concentration of approximately 3 x LoD.

The results of this study determined that all replicates were valid and positive for HCV, resulting in a Whole System Failure Rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.62% for the upper bound for each matrix [0%: 3.62%].

Cross contamination

The cross-contamination rate for **cobas**® HCV was determined by testing 240 replicates of a normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma sample and 225 replicates of a high titer HCV sample at 4.0E+07 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

Two hundred thirty-nine of 240 replicates of the negative samples were valid and detected negative, resulting in a Cross-Contamination Rate of 0.42%. The two-sided 95% exact confidence interval was 0.01% for the lower bound and 2.3% for the upper bound [0%: 2.3%].

Clinical performance evaluation

Lot-to-lot variability and reproducibility

The lot-to-lot variability and reproducibility of **cobas**® HCV were evaluated in EDTA plasma on the **cobas**® 6800 System using a mixed model to estimate the total variance.

The results are summarized in Table 27 through Table 30 below.

Lot-to-lot variability

Lot-to-lot variability testing was performed for genotypes 1 through 6 at one test site, using three reagent lots. Two operators at the site tested each lot for 6 days. Two runs were performed each day.

Table 27 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected log_{10} HCV RNA concentration for the **cobas**® 6800 System.

Table 27 Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration (log_{10} IU/mL) by genotype and positive panel member on the **cobas**® 6800 System (lot-to-lot)

Geno-type	HCV RNA Concentration			No. of Tests[b]	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
	Expected IU/mL	Expected log_{10} IU/mL	Mean[a] log_{10} IU/mL		Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
1	30	1.477	1.482	68	0% (0.00)	0% (0.00)	0% (0.00)	25% (22.14)	75% (39.26)	0.1899	45.91
	100	2.000	1.890	72	8% (10.98)	1% (3.68)	0% (0.00)	10% (12.12)	81% (35.75)	0.1672	39.97
	5,000	3.699	3.457	72	0% (0.00)	0% (0.00)	0% (0.00)	82% (32.85)	18% (14.84)	0.1531	36.38
	50,000	4.699	4.443	72	3% (7.26)	0% (0.00)	0% (0.00)	86% (37.29)	11% (12.88)	0.1693	40.51
	500,000	5.699	5.552	72	0% (0.00)	0% (0.00)	0% (0.00)	83% (33.86)	17% (14.96)	0.1570	37.36
	5,000,000	6.699	6.453	71	47% (17.58)	0% (0.00)	0% (0.00)	25% (12.71)	28% (13.35)	0.1100	25.74
	50,000,000	7.699	7.103	72	54% (28.85)	0% (0.00)	0% (0.00)	24% (19.14)	22% (18.00)	0.1670	39.92
2	30	1.477	1.611	72	5% (9.52)	0% (0.00)	8% (11.25)	0% (0.00)	87% (39.60)	0.1776	42.67
	100	2.000	2.125	72	0% (0.00)	0% (0.00)	0% (0.00)	25% (12.12)	75% (21.10)	0.1047	24.47
	5,000	3.699	3.714	72	9% (5.63)	0% (0.00)	0% (0.00)	47% (12.66)	44% (12.17)	0.0798	18.53
	50,000	4.699	4.743	72	0% (0.00)	0% (0.00)	0% (0.00)	54% (16.10)	46% (14.97)	0.0949	22.12
	500,000	5.699	5.806	72	7% (4.24)	0% (0.00)	0% (0.00)	22% (7.39)	71% (13.32)	0.0684	15.85
	5,000,000	6.699	6.187	72	41% (20.03)	0% (0.00)	0% (0.00)	17% (12.73)	42% (20.44)	0.1348	31.80
	50,000,000	7.699	7.080	72	40% (17.99)	1% (2.73)	0% (0.00)	0% (0.00)	59% (21.87)	0.1223	28.73

Geno-type	HCV RNA Concentration			No. of Tests[b]	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
	Expected IU/mL	Expected log_{10} IU/mL	Mean[a] log_{10} IU/mL		Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
3	30	1.477	1.474	72	0% (0.00)	3% (8.35)	0% (0.00)	43% (32.35)	54% (36.31)	0.2084	50.89
	100	2.000	1.946	72	13% (13.11)	0% (0.00)	0% (0.00)	49% (25.49)	38% (22.49)	0.1562	37.16
	5,000	3.699	3.636	72	14% (6.76)	0% (0.00)	0% (0.00)	27% (9.30)	59% (13.76)	0.0776	18.01
	50,000	4.699	4.597	72	0% (1.38)	0% (0.00)	0% (0.00)	52% (14.95)	47% (14.24)	0.0894	20.80
	500,000	5.699	5.504	72	0% (0.00)	1% (1.62)	0% (0.00)	43% (13.51)	57% (15.54)	0.0893	20.77
	5,000,000	6.699	6.451	72	28% (14.47)	0% (0.00)	3% (5.08)	0% (0.00)	69% (23.03)	0.1189	27.91
	50,000,000	7.699	7.149	71	21% (18.47)	0% (0.00)	8% (11.62)	0% (0.00)	71% (34.88)	0.1747	41.90
4	30	1.477	1.358	69	7% (14.37)	0% (0.00)	1% (5.44)	0% (0.00)	91% (53.25)	0.2269	56.03
	100	2.000	1.827	72	10% (9.40)	0% (0.00)	1% (2.80)	8% (8.35)	81% (27.09)	0.1283	30.21
	5,000	3.699	3.416	72	20% (7.82)	0% (0.00)	0% (0.00)	42% (11.23)	38% (10.61)	0.0750	17.40
	50,000	4.699	4.405	72	22% (8.06)	0% (0.00)	0% (0.00)	13% (6.30)	65% (14.06)	0.0752	17.46
	500,000	5.699	5.069	71	5% (8.88)	0% (0.00)	24% (19.47)	13% (14.23)	57% (30.31)	0.1699	40.66
	5,000,000	6.699	6.070	72	27% (23.68)	0% (0.00)	12% (15.28)	34% (26.55)	27% (23.52)	0.1940	47.00
	50,000,000	7.699	6.930	72	37% (30.60)	0% (0.00)	22% (23.53)	11% (16.70)	30% (27.73)	0.2149	52.68
5	30	1.477	1.575	72	5% (8.30)	0% (0.00)	0% (0.00)	10% (11.53)	85% (35.32)	0.1611	38.42
	100	2.000	2.049	72	9% (7.51)	0% (0.00)	0% (0.00)	0% (0.00)	91% (24.38)	0.1093	25.57
	5,000	3.699	3.606	72	4% (3.63)	0% (0.00)	0% (0.00)	59% (14.11)	38% (11.28)	0.0797	18.51
	50,000	4.699	4.616	72	20% (8.86)	0% (0.00)	0% (0.00)	37% (12.19)	43% (13.21)	0.0867	20.17
	500,000	5.699	5.678	72	7% (4.63)	0% (0.00)	0% (0.00)	33% (10.36)	60% (13.93)	0.0777	18.04
	5,000,000	6.699	6.505	71	54% (19.49)	0% (0.00)	19% (11.53)	0% (0.00)	27% (13.77)	0.1143	26.79
	50,000,000	7.699	7.592	72	35% (11.59)	1% (2.25)	12% (6.72)	4% (3.94)	47% (13.37)	0.0842	19.58

Geno-type	HCV RNA Concentration			No. of Tests[b]	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
	Expected IU/mL	Expected log_{10} IU/mL	Mean[a] log_{10} IU/mL		Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
6	30	1.477	1.494	70	0% (0.00)	0% (0.00)	0% (0.00)	3% (7.34)	97% (47.65)	0.1990	48.33
	100	2.000	1.940	72	9% (9.29)	0% (0.00)	0% (0.00)	2% (4.14)	90% (30.32)	0.1361	32.13
	5,000	3.699	3.417	72	0% (0.00)	0% (0.00)	0% (0.00)	81% (37.28)	19% (17.38)	0.1737	41.64
	50,000	4.699	4.541	72	0% (0.00)	0% (0.00)	0% (0.00)	70% (26.40)	30% (17.27)	0.1351	31.88
	500,000	5.699	5.611	72	0% (0.00)	0% (0.00)	0% (0.00)	74% (22.82)	26% (13.36)	0.1136	26.62
	5,000,000	6.699	6.414	72	49% (22.99)	0% (0.00)	9% (10.03)	16% (12.88)	26% (16.83)	0.1413	33.42
	50,000,000	7.699	7.529	71	48% (19.63)	1% (2.67)	2% (4.25)	22% (13.15)	28% (14.96)	0.1225	28.78

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

[a] Calculated using the SAS MIXED procedure.

[b] Number of valid tests with detectable viral load.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100

CV(%) = percent coefficient of variation; HCV = hepatitis C virus; No. = number; RNA = ribonucleic acid; SD = standard deviation; sqrt = square root.

In Table 28 below, the negative percent agreement (NPA) for the **cobas**® 6800 System using negative panel member tests was 99.54%.

Table 28 Negative percent agreement using the negative panel member on the **cobas**® 6800 System (lot-to-lot)

Expected HCV RNA Concentration	No. of Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	216	1	215	99.54	(97.45, 99.99)

[a] Negative Percent Agreement = (number of negative results / total number of valid tests in negative panel member) * 100.

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

CI = confidence interval; HCV = hepatitis C virus; No. = number; RNA = ribonucleic acid.

Reproducibility

Reproducibility testing was performed at three sites for genotypes 1 through 3, using one reagent lot. Two operators at each site tested for 6 days. Two runs were performed each day.

Table 29 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected log_{10} HCV RNA concentration on the **cobas**® 6800 System.

Table 29 Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration (log_{10} IU/mL) by genotype and positive panel member on the **cobas**® 6800 System (reproducibility)

Geno-type	HCV RNA Concentration			No. of Tests[b]	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
	Expected IU/mL	Expected log_{10} IU/mL	Mean[a] log_{10} IU/mL		Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
1	30	1.477	1.373	68	1% (6.43)	0% (0.00)	0% (0.00)	20% (25.63)	78% (52.96)	0.2437	60.84
	100	2.000	1.866	72	4% (7.25)	0% (0.00)	0% (0.00)	17% (15.81)	79% (34.64)	0.1644	39.24
	5,000	3.699	3.466	72	0% (0.00)	0% (0.00)	0% (0.00)	83% (29.77)	17% (13.35)	0.1391	32.87
	50,000	4.699	4.444	72	7% (10.74)	0% (0.00)	0% (0.00)	83% (37.40)	9% (12.16)	0.1721	41.24
	500,000	5.699	5.579	72	4% (6.84)	0% (0.00)	0% (0.00)	74% (30.53)	22% (16.27)	0.1504	35.70
	5,000,000	6.699	6.439	72	52% (16.35)	9% (6.91)	0% (0.00)	9% (6.74)	30% (12.36)	0.0979	22.84
	50,000,000	7.699	7.091	72	76% (45.80)	0% (0.00)	0% (0.00)	7% (12.87)	17% (20.92)	0.2170	53.25
2	30	1.477	1.631	72	10% (11.41)	0% (0.00)	0% (0.00)	0% (0.00)	90% (35.77)	0.1586	37.77
	100	2.000	2.096	72	2% (3.71)	0% (0.00)	0% (0.00)	35% (14.49)	63% (19.44)	0.1057	24.70
	5,000	3.699	3.699	72	4% (3.47)	0% (0.00)	0% (0.00)	49% (11.99)	47% (11.76)	0.0742	17.22
	50,000	4.699	4.745	72	0% (0.00)	0% (0.00)	0% (0.00)	59% (17.39)	41% (14.45)	0.0975	22.75
	500,000	5.699	5.824	72	19% (7.91)	0% (0.00)	0% (0.00)	24% (8.99)	57% (13.89)	0.0794	18.43
	5,000,000	6.699	6.177	72	51% (20.74)	0% (1.59)	0% (0.00)	9% (8.47)	40% (18.27)	0.1246	29.30
	50,000,000	7.699	7.069	72	17% (13.08)	0% (0.00)	0% (0.00)	0% (0.00)	83% (29.26)	0.1367	32.28
3	30	1.477	1.457	72	0% (0.00)	0% (0.00)	0% (0.00)	34% (24.33)	66% (34.06)	0.1776	42.67
	100	2.000	1.911	72	16% (13.76)	0% (0.00)	0% (0.00)	27% (18.01)	58% (26.79)	0.1504	35.70
	5,000	3.699	3.628	72	10% (6.12)	0% (0.00)	0% (0.00)	18% (8.09)	71% (16.06)	0.0821	19.07
	50,000	4.699	4.587	72	2% (2.23)	0% (0.00)	0% (0.00)	55% (13.21)	44% (11.85)	0.0774	17.96
	500,000	5.699	5.524	72	0% (0.00)	0% (0.00)	0% (0.00)	44% (12.53)	56% (14.30)	0.0822	19.10
	5,000,000	6.699	6.442	71	22% (11.89)	0% (0.00)	0% (0.00)	0% (0.00)	78% (22.66)	0.1100	25.73
	50,000,000	7.699	7.109	71	10% (13.36)	0% (0.00)	21% (19.65)	0% (0.00)	69% (35.94)	0.1827	44.01

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

[a] Calculated using the SAS MIXED procedure.

[b] Number of valid tests with detectable viral load.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100

CV(%) = percent coefficient of variation; HCV = hepatitis C virus; No. = number; RNA = ribonucleic acid; SD = standard deviation; sqrt = square root.

The NPA was 100% using negative panel member tests on the **cobas**® 6800 System as presented in Table 30 below.

Table 30 Negative percent agreement using the negative panel member (reproducibility) on the **cobas**® 6800 System

Expected HCV RNA Concentration	No. of Tests	Positive Results	Negative Results	Negative Percentage Agreement[a]	95% CI[b]
Negative	108	0	108	100.00	(96.64, 100.00)

[a] Negative Percent Agreement = (number of negative results / total number of valid tests in negative panel member) * 100.

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

CI = confidence interval; HCV = hepatitis C virus; No. = number; RNA = ribonucleic acid.

Comparison between cobas® 6800 and cobas® 8800 Systems - lot-to-lot variability and reproducibility

An identical sample set was tested for lot-to-lot variability and reproducibility of **cobas**® HCV on the **cobas**® 8800 System. The performance of the two systems is comparable. Table 31 lists the precision performance achieved in the reproducibility portion of the study for both the **cobas**® 6800 and **cobas**® 8800 Systems across the linear range of **cobas**® HCV.

Table 31 Comparison of precision standard deviation of HCV RNA concentration (log_{10}IU/mL) for Genotypes 1 - 3 on **cobas**® 6800 and **cobas**® 8800 Systems (reproducibility)

Concentration Level (IU/mL)	Precision Standard Deviation[a] (No. of Tests[b])					
	cobas® 6800 System			cobas® 8800 System		
	Genotype 1	Genotype 2	Genotype 3	Genotype 1	Genotype 2	Genotype 3
1.0E+01 ≤ X < 1.0E+02	0.24 (68) 0.16 (72)	0.16 (72)	0.18 (72) 0.15 (72)	0.23 (47) 0.15 (47)	0.14 (48)	0.17 (47) 0.17 (48)
1.0E+02 ≤ X < 1.0E+03	-	0.11 (72)	-	-	0.12 (48)	-
1.0E+03 ≤ X < 1.0E+04	0.14 (72)	0.07 (72)	0.08 (72)	0.13 (48)	0.07 (48)	0.08 (48)
1.0E+04 ≤ X < 1.0E+05	0.17 (72)	0.10 (72)	0.08 (72)	0.11 (48)	0.06 (48)	0.08 (48)
1.0E+05 ≤ X < 1.0E+06	0.15 (72)	0.08 (72)	0.08 (72)	0.11 (48)	0.07 (47)	0.10 (48)
1.0E+06 ≤ X < 1.0E+07	0.10 (72)	0.12 (72)	0.11 (71)	0.09 (48)	0.13 (48)	0.11 (48)
1.0E+07 ≤ X < 1.0E+08	0.22 (72)	0.14 (72)	0.18 (71)	0.16 (48)	0.10 (48)	0.19 (48)

Note: Grouping of observed precisions to concentration levels are based on the median test results on the untransformed scale (IU/mL). The table only includes results with detectable viral load. SD = standard deviation.

'-' Indicates no applicable results for this level.

[a] Precision Standard Deviation in log_{10} units

[b] Number of valid tests with detectable viral load.

Clinical utility

The study was designed to evaluate the ability of the assay to predict clinical outcome.

Treatment Plan 1 included four treatment regimens, containing a combination of DAA compounds with or without pegIFN/RBV. Subjects were infected with HCV genotype 1 and were partial or null responders during a previous course of pegIFN/RBV combination therapy.

Treatment Plan 2 included subjects infected with genotype 2 or 3, who were treatment naïve and received a course of pegIFN/RBV combination therapy.

Testing with **cobas**® HCV was performed at four sites. Three sites were equipped with one **cobas**® 6800 System. Two sites were equipped with **cobas**® 8800 System. One site tested on both the **cobas**® 6800 and 8800 Systems. Three kit lots of reagents were used in the study; each sample was tested with one kit lot.

Table 32 below shows the demographic and baseline characteristics of subjects whose samples were tested on the **cobas**® 6800 and the **cobas**® 8800 Systems. The majority of subjects were male, over 40 years of age, and infected with HCV genotype 1. Subjects with HCV genotypes 1, 2, and 3 were enrolled. HCV infection with genotypes 4, 5, and 6 is rare in the US.

Table 32 Demographics and baseline characteristics of subjects for the **cobas**® 6800 and **cobas**® 8800 Systems

Characteristics	cobas® 6800 System		cobas® 8800 System	
	Statistics	**Subjects**	**Statistics**	**Subjects**
Total	N	401	N	353
Treatment Plan				
1	n (%)	307 (76.6%)	n (%)	287 (81.3%)
2	n (%)	94 (23.4%)	n (%)	66 (18.7%)
Age Category (years)				
< 40	n (%)	90 (22.4%)	n (%)	81 (22.9%)
≥ 40	n (%)	311 (77.6%)	n (%)	272 (77.1%)
Age (years)				
	Mean ± SD	49 ± 11.1	Mean ± SD	49 ± 11.2
	Median	52	Median	52
	Range	20 - 76	Range	20 – 71
Gender				
Male	n (%)	276 (68.8%)	n (%)	245 (69.4%)
Female	n (%)	125 (31.2%)	n (%)	108 (30.6%)
Race / Ethnicity				
Asian	n (%)	3 (0.7%)	n (%)	2 (0.6%)
African American	n (%)	13 (3.2%)	n (%)	12 (3.4%)
White/Caucasian	n (%)	357 (89.0%)	n (%)	318 (90.1%)
Other	n (%)	28 (7.0%)	n (%)	21 (5.9%)
Genotype				
1A	n (%)	174 (43.4%)	n (%)	159 (45.0%)
1B	n (%)	133 (33.2%)	n (%)	128 (36.3%)
Overall 1	**n (%)**	307 (76.6%)	n (%)	287 (81.3%)
2	n (%)	31 (7.7%)	n (%)	22 (6.2%)
3	n (%)	63 (15.7%)	n (%)	44 (12.5%)
Overall Non-1	**n (%)**	94 (23.4%)	n (%)	66 (18.7%)
Baseline HCV RNA (log_{10} IU/mL)				
	Mean ± SD	6.32 ± 0.58	Mean ± SD	6.33 ± 0.56
	Median	6.41	Median	6.41
	Range	2.57 - 7.52	Range	2.77 - 7.52
HCV RNA Category at Baseline				
< 400,000 IU/mL	n (%)	36 (9.0%)	n (%)	32 (9.1%)
≥ 400,000 IU/mL	n (%)	363 (90.5%)	n (%)	304 (86.1%)
Missing	n (%)	2 (0.5%)	n (%)	17 (4.8%)

HCV = hepatitis C virus; RNA = ribonucleic acid; SD = standard deviation.

Prediction of response to antiviral therapy

Assay performance characteristics have been established for individuals treated with certain DAA regimens. No information is available on the assay's predictive value when other DAA combination therapies are used.

Definitions:

- Week 2 viral load (VL)=HCV RNA <LLoQ=LoD = 15 IU/mL at Week 2 of antiviral therapy
- Week 2 VL: HCV RNA<LoD = LLoQ of 15 IU/mL
- Week 4 VL: HCV RNA<LLoQ at Week 4 of antiviral therapy
- Week 8 VL: HCV RNA<LLoQ at Week 8 of antiviral therapy
- Week 12 VL: Either at least a 2 $\log_{10}$ drop in HCV RNA level compared to baseline or HCV RNA <LLoQ at Week 12 of antiviral therapy
- Week 24 VL (End of Treatment [EOT]): HCV RNA < LLoQ at Week 24 of antiviral therapy.
- Sustained Virologic Response (SVR)12: HCV RNA < LLoQ at Week 12 after completion of antiviral therapy measured with an independent HCV RNA test.

Predictive value of Virological Response to success of antiviral therapy

In this study, the positive predictive value (PPV) for Week 4 VL to predict SVR12 was 78.1% (95% CI: 72.7 to 82.8%) in genotype 1 subjects and 84.7% (95% CI: 73.5 to 91.8%) in subjects with non-1 genotypes (Table 33). Therefore, VR at Week 4 measured by **cobas**® HCV was a useful predictor of SVR 12.

For Treatment Plan 1, as a representative of a DAA containing regimen, a Week 12 VL or Week 24 VL on **cobas**® HCV predicts SVR12 in genotype 1 subjects, with PPVs of 77.0% and 78.6%, respectively. The absence of Week 12 VL or Week 24 VL predicts non-response, with negative predictive values (NPVs) of 87.5% and 100%, respectively (Table 33). Additional analysis of Week 2 VL to predict SVR12 shows a PPV of 79.4% but a low NPV of 29.9%.

In Treatment Plan 2, Week 12 VL using **cobas**® HCV in genotype 2 and 3 was predictive of SVR12, with a PPV of 75.3%. Due to the rarity of non-response, absence of Week 12 VL is not a useful measure of outcome in this population. The NPV was 50% and the number of non-responders was small in this study (Table 33).

Overall, this study demonstrated the clinical utility of **cobas**® HCV and the continued value of the assessment of Week 4, Week 12, and Week 24 HCV RNA responses in patients undergoing treatment for chronic HCV infection.

Table 33 Probability of achieving Sustained Virological Response (SVR12) given virologic response (<15 IU/mL) at a specific on-treatment visit for the **cobas**® 6800 System

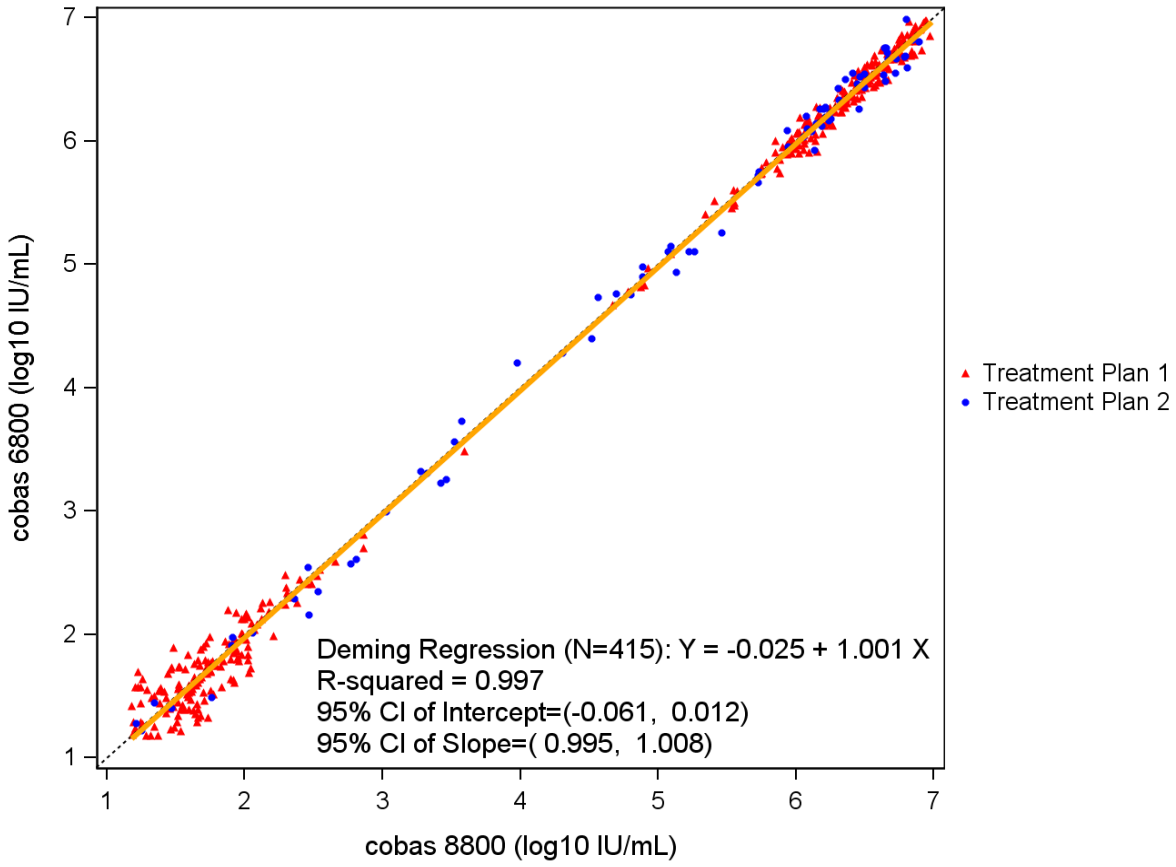
				PPV (%)		**NPV (%)**		**OR**
Treatment Plan	**Genotype**	**On-Treatment Visit**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
1	1	Week 2	290	79.4 (71.5, 85.5)	100 / 126	29.9 (23.4, 37.3)	49 / 164	1.64 (0.95, 2.83)
		Week 4	290	78.1 (72.7, 82.8)	200 / 256	50.0 (34.1, 65.9)	17 / 34	3.57 **(1.71, 7.45)**
		Week 8	285	76.8 (71.5, 81.4)	212 / 276	66.7 (35.4, 87.9)	6 / 9	6.63 **(1.61, 27.24)**
		Week 12	286	77.0 (71.7, 81.5)	214 / 278	87.5 (52.9, 97.8)	7 / 8	23.41 **(2.83,193.80)**
		Week 24	282	78.6 (73.4, 83.0)	217 / 276	100.0 (61.0, 100.0)	6 / 6	47.52 **(2.64,855.66)**
2	Non-1	Week 4	82	84.7 (73.5, 91.8)	50 / 59	47.8 (29.2, 67.0)	11 / 23	5.09 **(1.72, 15.04)**
		Week 12	83	75.3 (64.9, 83.4)	61 / 81	50.0 (9.5, 90.5)	1 / 2	3.05 (0.18, 51.04)

Notes: Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being an SVR12 given the subject was a viral responder at a specific visit. SVR12 is achieved if the subject has HCV RNA < 15 IU/mL at 12 weeks after the last dose.
Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being an SVR12 given the subject was not a viral responder at a specific visit.
Odds Ratio (OR) = (TP • TN) / (FP • FN)
CI = confidence interval; FN = false negative; FP = false positive; HCV = hepatitis C virus; SVR12 = sustained virological response 12 weeks after the last dose; TN = true negative; TP = true positive.

Comparison between cobas® 6800 and cobas® 8800 Systems – clinical utility

An identical sample set was tested for the clinical utility of **cobas**® HCV on the **cobas**® 8800 System. The systems demonstrate highly correlated performance that were not significantly different. Figure 10 below show a Deming regression plots of VLs (log_{10} IU/mL) greater than 15 IU/mL at all applicable time points on treatment.

Figure 10 Deming linear regression plot of viral loads (log_{10} IU/mL) from Baseline, Week 2 and Week 4 (**cobas**® 6800 System vs **cobas**® 8800 System)



CI = confidence interval.

Diagnostic utility

The study was designed to evaluate the ability of the assay to correctly diagnose anti-HCV positive subjects with active HCV infection.

Table 34 below show the demographic and clinical characteristics of subjects whose samples were tested on the **cobas**® 6800 System and **cobas**® 8800 System.

Table 34 Demographic and clinical characteristics by system (HCV antibody positive subjects)

Characteristics	cobas® 6800 System	cobas® 8800 System
Total, N	235	230
Clinical Condition		
HCV Antibody Positive [a], n(%)		
HCV RNA Positive	154 (65.5%)	150 (65.2%)
HCV RNA Negative	81 (34.5%)	80 (34.8%)
Age (years)		
Mean ± SD	48 ± 11.9	49 ± 11.9
Median	50	50
Range	20 - 88	20 - 88
Gender, n(%)		
Male	132 (56.2%)	127 (55.2%)
Female	103 (43.8%)	103 (44.8%)
Race, n(%)		
Black / African-American	49 (20.9%)	48 (20.9%)
White / Caucasian	183 (77.9%)	179 (77.8%)
Other	3 (1.3%)	3 (1.3%)
Risk Factor, n(%)		
Baby Boomers (Born: 1945--1965) only	114 (48.5%)	112 (48.7%)
IVD Users only	22 (9.4%)	22 (9.6%)
Baby Boomers and IVD Users	23 (9.8%)	22 (9.6%)
Undisclosed, HCV antibody positive *	76 (32.3%)	74 (32.2%)

[a] VERSANT HCV Test result was used to determine HCV RNA status. For subjects whose VERSANT HCV Test result was not available, the APTIMA HCV Test result was used. If both Versant and Aptima results were not available then COBAS® AMPLICOR® HCV Test, v2.0 result was used.

* Undisclosed includes those subjects for whom both the risk factors are either missing or 'No', or those for whom one risk factor is missing and the other has a value of 'No'.

APTIMA = Aptima HCV RNA Qualitative Assay; HCV = hepatitis C Virus; IVD = Intravenous Drug Use.

SD = standard deviation; VERSANT= VERSANT HCV RNA Qualitative Assay.

The sensitivity of **cobas**® HCV was evaluated in subjects who had previous exposure to HCV and tested positive for HCV antibody on both **cobas**® 6800 /8800 Systems (Table 35). The agreement of **cobas**® HCV with patient infection status was determined using a cutoff of < 25 IU/mL to define the absence of active HCV infection (Table 35).

Table 35 Agreement of **cobas**® HCV on the **cobas**® 6800 and the **cobas**® 8800 System with the patient infection status using a cutoff of 25 IU/mL

cobas® **HCV**	**Patient Infected Status (PIS)**					
	cobas® **6800 System**			**cobas**® **8800 System**		
	HCV Positive	**HCV Negative**	**Total**	**HCV Positive**	**HCV Negative**	**Total**
HCV RNA Detected Above 25 IU/mL	152	0	152	149	1	150
HCV RNA not Detected or detected below 25 IU/mL	0	81	81	0	79	79
Total	152	81	233	149	80	229
Positive Percent Agreement (95% score CI)	100.0 % (97.5, 100.0)	NA	NA	100.0 % (97.5, 100.0)	NA	NA
Negative Percent Agreement (95% score CI)	NA	100.0 % (95.5, 100.0)	NA	NA	98.8 % (93.3, 99.8)	NA

Note: Only valid results from **cobas**® HCV among the HCV Antibody Positive specimens are included in this table.

CI = confidence interval; **cobas**® HCV = **cobas**® HCV for use on the **cobas**® 6800/8800 systems; HCV = hepatitis C virus; NA = not applicable.

This study demonstrates the clinical utility of **cobas**® HCV to correctly diagnose subjects with ongoing active HCV RNA infection and to distinguish them from subjects with inactive infections in a population with prior exposure to HCV (HCV antibody-positive serology).

Cross-reactivity in subjects with non-HCV related liver disease

The cross-reactivity of **cobas**® HCV was evaluated with specimens that represented a variety of liver diseases for which active HCV infection was not the underlying cause. **cobas**® HCV demonstrated the ability to determine absence of active HCV infection in subjects with a range of liver diseases due to causes other than HCV (Table 36**,** Table 37**,** Table 38).

Table 36 Demographic and clinical characteristics by system

Characteristics	**cobas® 6800 System**	**cobas® 8800 System**
Total, N	247	181
Clinical Condition		
HCV RNA Negative, n(%)		
Alcoholic Liver Disease	33 (13.4%)	20 (11.0%)
Autoimmune Hepatitis	37 (15.0%)	32 (17.7%)
Chronic HBV	30 (12.1%)	30 (16.6%)
Fatty Liver Disease	66 (26.7%)	38 (21.0%)
Non-Alcoholic Steatohepatitis (NASH)	41 (16.6%)	30 (16.6%)
Nonspecific Cirrhosis	6 (2.4%)	3 (1.7%)
Primary Billiary Cirrhosis	33 (13.4%)	28 (15.5%)
Unknown [a]	1 (0.4%)	
Age (years)		
Mean ± SD	54 ± 13.1	54 ± 13.5
Median	56	56
Range	20 - 81	20 - 81
Gender, n(%)		
Male	71 (28.7%)	44 (24.3%)
Female	104 (42.1%)	74 (40.9%)
Unknown	72 (29.1%)	63 (34.8%)
Race, n(%)		
Asian	11 (4.5%)	1 (0.6%)
Black / African-American	13 (5.3%)	11 (6.1%)
White / Caucasian	70 (28.3%)	48 (26.5%)
Other	7 (2.8%)	1 (0.6%)
Unknown	146 (59.1%)	120 (66.3%)
Baby Boomers (Born: 1945-1965), n(%)		
Yes	80 (32.4%)	63 (34.8%)
No	64 (25.9%)	53 (29.3%)
Undisclosed	103 (41.7%)	65 (35.9%)

Table 37 Number of HCV RNA negative samples on the **cobas**® 6800 System with non HCV-related liver diseases within test result categories by clinical condition

	Number of Valid Tests						
Clinical Condition	**Target Not Detected**	**< 1.50E+01 IU/mL**	**1.50E+01 ≤ x < 2.50E+01 IU/mL**	**2.50E+01 ≤ x ≤ 1.00E+08 IU/mL**	**> 1.00E+08 IU/mL**	**Total**	**Specificity[a] % (95% CI)[b]**
Alcoholic Liver Disease	33	0	0	0	0	33	100.0 (89.4, 100.0)
Autoimmune Hepatitis	37	0	0	0	0	37	100.0 (90.5, 100.0)
Chronic HBV	30	0	0	0	0	30	100.0 (88.4, 100.0)
Fatty Liver Disease	66	0	0	0	0	66	100.0 (94.6, 100.0)
NASH	40	1 *	0	0	0	41	97.6 (87.1, 99.9)
Nonspecific Cirrhosis	6	0	0	0	0	6	100.0 (54.1, 100.0)
Primary Billiary Cirrhosis	33	0	0	0	0	33	100.0 (89.4, 100.0)
Total	245	1 *	0	0	0	246	99.6 (97.8, 100.0)

Note: Only valid results from **cobas**® HCV among the HCV Antibody negative specimens (non-HCV-related liver disease) are included in this table.

The single subject with Hepatic Steatosis liver disease was excluded.

[a] Clinical Specificity: percentage of number of RNA negative result to the total number of HCV Antibody negative specimens among valid test results.

[b] 95% CI: 95% exact confidence interval.

* Sample reported <LLOQ, HCV RNA detected at ~ 1.5 IU/mL.

CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; NASH = non-alcoholic steatohepatitis.

Table 38 Number of HCV RNA negative samples on the **cobas**® 8800 System with non HCV-related liver diseases within test result categories by clinical condition

	Number of Valid Tests						
Clinical Condition	**Target Not Detected**	**< 1.50E+01 IU/mL**	**1.50E+01 ≤ x < 2.50E+01 IU/mL**	**2.50E+01 ≤ x ≤ 1.00E+08 IU/mL**	**> 1.00E+08 IU/mL**	**Total**	**Specificity[a] % (95% CI)[b]**
Alcoholic Liver Disease	20	0	0	0	0	20	100.0 (83.2, 100.0)
Autoimmune Hepatitis	32	0	0	0	0	32	100.0 (89.1, 100.0)
Chronic HBV	30	0	0	0	0	30	100.0 (88.4, 100.0)
Fatty Liver Disease	38	0	0	0	0	38	100.0 (90.7, 100.0)
NASH	30	0	0	0	0	30	100.0 (88.4, 100.0)
Nonspecific Cirrhosis	3	0	0	0	0	3	100.0 (29.2, 100.0)
Primary Billiary Cirrhosis	28	0	0	0	0	28	100.0 (87.7, 100.0)
Total	181	0	0	0	0	181	100.0 (98.0, 100.0)

Note: Only valid results from the **cobas**® HCV among the HCV Antibody negative specimens (non-HCV-related liver disease) are included in this table.

[a] Clinical Specificity: percentage of number of RNA negative result to the total number of HCV Antibody negative specimens among valid test results.

[b] 95% CI: 95% exact confidence interval.

CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; NASH = non-alcoholic steatohepatitis.

Comparison between cobas® 6800 and cobas® 8800 Systems for diagnosis

A subset of the samples was tested for confirmation of active HCV infection of **cobas**® HCV on the **cobas**® 8800 System. The specificity of **cobas**® HCV, in a variety of liver diseases for which active HCV infection was not the underlying cause, was also 100%. The agreement of **cobas**® HCV on the **cobas**® 8800 System with patient infection status, using a cutoff of < 25 IU/mL to define absence of active HCV infection, was 99.6%. These results indicate that the **cobas**® 6800 and **cobas**® 8800 Systems are comparable for diagnosis of active HCV using **cobas**® HCV.

Conclusion

cobas® HCV can quantitate the level of HCV RNA to assess treatment and predict response to antiviral therapy. The results of this study demonstrate the clinical utility of this test for determining early on-treatment response to therapy in the management of patients with chronic HCV infection.

Additionally, **cobas**® HCV can be used as an aid in the diagnosis of active HCV infection in HCV-antibody-positive patients.

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

Sample type	EDTA plasma, serum
Minimum amount of sample required	650 µL or 350 µL
Sample processing volume	500 µL or 200 µL
Analytical sensitivity	15 IU/mL (500 µL) 40 IU/mL (200 µL)
Linear range	500 µL: 15 IU/mL – 1.0E+08 IU/mL
	200 µL: 40 IU/mL – 1.0E+08 IU/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%)
Genotypes detected	HCV genotypes 1-6

Symbols

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

Table 39 Symbols used in labeling for Roche PCR diagnostics products

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

Technical Support

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

Manufacturer and importer

Table 40 Manufacturer and importer

Roche Molecular Systems, Inc.
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Made in USA

Roche Diagnostics GmbH
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68305 Mannheim, Germany

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Document revision

Document Revision Information	
Doc Rev. 3.0 09/2022	Updated front page and **Table 2** and **Table 3** with additional P/N for the control kits. Updated **Trademarks and patents** section, including the link. Please contact your local Roche Representative if you have any questions.