



Rx Only

cobas[®] HCV

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

For in vitro diagnostic use

cobas[®] HCV

P/N: 06998798190

cobas[®] HBV/HCV/HIV-1 Control Kit

P/N: 06998887190

cobas[®] NHP Negative Control Kit

P/N: 07002220190

Table of contents

Intended use	4
Summary and explanation of the test.....	4
Reagents and materials.....	6
cobas® HCV reagents and controls	6
cobas omni reagents for sample preparation.....	9
Reagent storage and handling requirements.....	10
Additional materials required	11
Instrumentation and software required	11
Precautions and handling requirements	12
Warnings and precautions.....	12
Reagent handling	12
Good laboratory practice	13
Sample collection, transport and storage	13
Samples.....	13
Instructions for use.....	14
Procedural notes	14
Running cobas® HCV	14
Results.....	15
Quality control and validity of results.....	15
Interpretation of results	16
Procedural limitations	17
System equivalency	17
Non-clinical performance evaluation	18
Key performance characteristics	18
Limit of Detection (LoD)	18
Linear range	20
Precision – within laboratory.....	22
Performance with HCV negative specimens.....	24
Analytical specificity/cross-reactivity.....	24

Analytical specificity – interfering substances	25
Matrix equivalency – EDTA plasma versus serum	26
Cross contamination	26
Clinical performance evaluation	27
Lot-to-lot variability and reproducibility	27
Lot-to-lot variability	27
Reproducibility	30
Comparison between cobas® 6800 and cobas® 8800 Systems - lot-to-lot variability and reproducibility	33
Clinical utility	33
Prediction of response to antiviral therapy	35
Comparison between cobas® 6800 and cobas® 8800 Systems – clinical utility	36
Diagnostic utility	37
Cross-reactivity in subjects with non-HCV related liver disease	39
Comparison between cobas® 6800 and cobas® 8800 Systems for diagnosis	42
Conclusion	42
Additional information	43
Key test features	43
Symbols	44
Manufacturer and distributors	45
Trademarks and patents	45
Copyright	45
References	46
Document revision	48

Intended use

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

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.

cobas® HCV is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay can be used to measure HCV RNA levels at baseline, during treatment, at the end of treatment, and at the end of follow up of treatment to determine sustained or non-sustained viral response. The results must be interpreted within the context of all relevant clinical and laboratory findings.

cobas® HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.

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

Summary and explanation of the test

Background

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.^{4,5}

Detection of antibodies to HCV (anti-HCV) indicates prior exposure to hepatitis C but does not distinguish between cleared or active infection (i.e. where the virus is still replicating). Detection of HCV RNA with the detection of anti-HCV identifies an active hepatitis C infection. The results of HCV RNA testing together with other biochemical and clinical information, may be used to confirm an active HCV infection, measure the level of virus in the blood and assist in HCV prevention counseling, medical care and treatment decision making.

Quantitation of HCV RNA for measuring baseline viral loads and for on-treatment viral loads have been well established in demonstrating the efficacy of antiviral response to pegylated interferon plus ribavirin (pegIFN/RBV) combination therapy.⁶⁻¹⁰ More recently direct acting antiviral combination therapies are prescribed, consisting of a nucleotide analogue viral polymerase inhibitor (NS5B) and a viral protease (NS3) or viral replicase inhibitor (NS5A) agent and lists of preferred first-line anti-HCV therapies per HCV genotype have been established.^{11,12} Current guidelines for the management and treatment of HCV^{13,14} recommend quantitative testing for HCV RNA before the start of antiviral therapy, and at 12 weeks or later, following the end of treatment. Additional time points may be recommended per therapy type, see current guidelines.¹¹

An HCV RNA level below 25 IU/mL, 12 weeks after the end of treatment, is the goal of treatment and indicates that a sustained virologic response (SVR) has been achieved.¹³

Explanation of the test

cobas® HCV is a quantitative test performed on the cobas® 6800 System and 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 assess substantial failures during the sample preparation and PCR amplification processes. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

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

Nucleic acid from patient samples, external controls and added armored RNA-QS molecules are simultaneously extracted 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 deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).¹⁵⁻¹⁷ 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.^{18,19} 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

Refer to the Reagents section and Warning and Precautions section for the hazard information for the product.

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 96 test cassette (P/N 06998798190)		
Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase* EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin, 9014-01-1. May produce an allergic reaction.	13 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	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
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, dUTPs, < 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	6 mL

* Hazardous substance

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

Store at 2–8°C

(P/N 06998887190)

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% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	  WARNING H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant.
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C)	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	  WARNING H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant.

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

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

Store at 2-8°C

(P/N 07002220190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV 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.

* 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. H318: Causes serious 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. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. 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. P501: Dispose of contents/ container to an approved waste disposal plant.
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2L	Not applicable

* These reagents are not included in the cobas® HCV test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

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

When reagents are not loaded on the 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

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 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® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the cobas® 6800/8800 Systems

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

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

Additional materials required

Table 7 Materials and consumables for use on **cobas®** 6800/8800 Systems

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

Instrumentation and software required

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

Table 8 Instrumentation

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

Refer to the **cobas®** 6800/8800 Systems Operator's Manual for additional information for primary and secondary sample tubes accepted on the instruments.

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

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use only.
- **cobas® HCV** has not been approved 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.^{20,21} Only personnel proficient in handling infectious materials and the use of **cobas® HCV** and **cobas® 6800/8800** Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **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, HBV DNA, HEV RNA, WNV RNA, and CMV 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.
- Handle all samples according to good laboratory practice in order to prevent carryover of samples.

Reagent handling

- Handle all reagents and controls according to good laboratory practice in order to prevent carryover of reagents 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. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution or acids. 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® 6800/8800 instrument, follow the instructions in the cobas® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport and storage

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

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

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

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 samples or serum may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at $\leq -18^{\circ}\text{C}$. For long-term storage (up to 6 months), temperatures at $\leq -60^{\circ}\text{C}$ are recommended.
- Plasma/serum samples are stable for up to four freeze/thaw cycles when frozen at $\leq -18^{\circ}\text{C}$.
- Ensure sufficient whole blood collection to allow usage of the processing volume for EDTA plasma or serum of 500 μL (for a total minimum sample requirement of 650 μL).
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

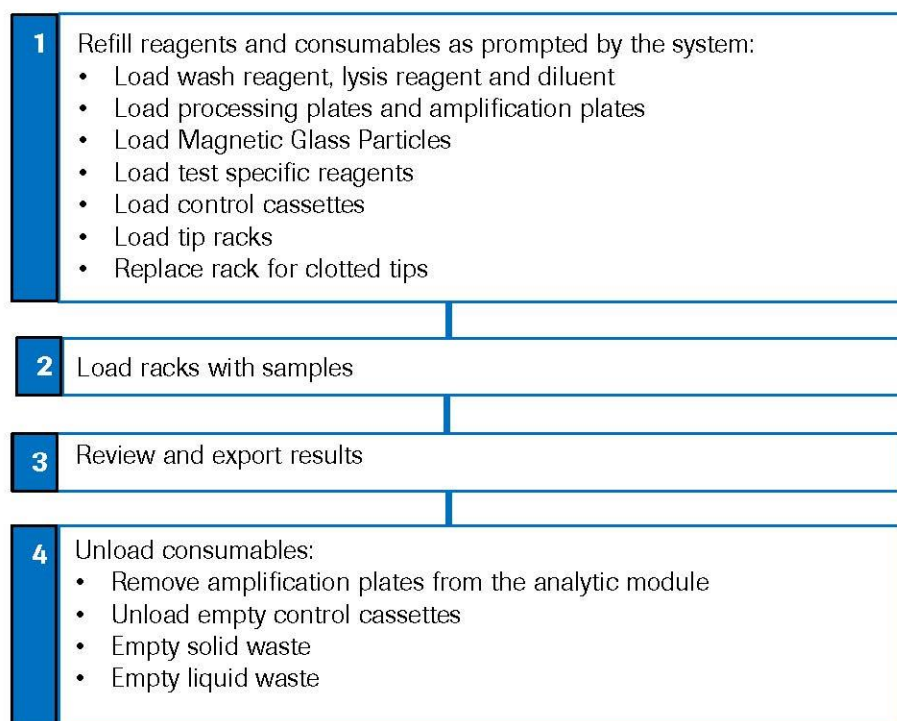
Procedural notes

- 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® 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Running cobas® HCV

cobas® HCV can be run with required sample volumes of 650 µL (the 500 µL sample workflow). The test procedure is described in detail in the cobas® 6800/8800 Systems Operator's Manual. Figure 1 below summarizes the procedure.

Figure 1 cobas® HCV test procedure



Results

The 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

- 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.
- In the cobas® 6800/8800 software and/or report, check for batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: 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.

Validation of results is performed automatically by the cobas® 6800/8800 Systems software based on negative and positive controls.

Control flags

Table 9 Control flags for negative and positive controls

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

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

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

Interpretation of results

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

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

Table 10 Target results for individual target result interpretation

Result Read-Out from cobas® System	Analytical Interpretation	Clinical Interpretation
Target Not Detected	HCV RNA not detected. Report results as “HCV not detected.”	No current HCV infection For HCV Diagnosis: No further testing indicated.* For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
< Titer Min	HCV RNA detected but not quantified. 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	Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection. For HCV Diagnosis: Results must be interpreted within the context of all relevant clinical and laboratory findings.* For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.
15 IU/mL ≤ Titer < 25 IU/mL	HCV RNA detected and quantified. Calculated titer is within the Linear Range of the assay – greater than or equal to 15 IU/mL and less than 25 IU/mL. Report results as “(Titer) of HCV detected”.	Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection.* For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
25 IU/mL ≤ Titer ≤ Titer Max	HCV RNA detected and quantified Calculated titer is within the Linear Range of the assay – greater than or equal to 25 IU/mL and less than or equal to Titer Max. Report results as “(Titer) of HCV detected”.	Current HCV Infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.
> Titer Max	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	Current HCV Infection. For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.

* Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

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® 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.
- Drug interference studies were performed in vitro and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.
- 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 has not been approved for use as a screening test for the presence of HCV in blood or blood products.

System equivalency

System equivalency was demonstrated of the cobas® 6800 System and cobas® 8800 System via the following studies: LoD, LLoQ, Linearity, Precision, and Reproducibility. In addition, a subset of samples from the clinical studies were also tested on both the cobas® 6800 and cobas® 8800 Systems. The results presented in the package insert support equivalent performance for both systems.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

The limit of detection (LoD) of cobas® HCV was determined for WHO International Standard (genotype 1a) and for genotype 1b through 6. The overall LoD was 12.0 IU/mL for EDTA plasma, 13.7 IU/mL for serum.

WHO International Standard

The limit of detection of cobas® HCV for the WHO International Standard 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 NIBSC, in HCV-negative human EDTA plasma and serum using sample processing volumes of 500 µL. The minimum sample requirement was 650 µL to be processed by cobas® 6800/8800 Systems. Panels of six concentration levels plus a negative were tested over three lots of cobas® HCV test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum for both the cobas® 6800 and cobas® 8800 System are shown in Table 11 and Table 12, respectively.

Table 11 HCV RNA WHO International Standard limit of detection in EDTA plasma

Instrument	LoD by PROBIT at 95% hit rate	95% confidence interval
cobas® 6800 System	8.5 IU/mL	7.5-9.79 IU/mL
cobas® 8800 System	8.3 IU/mL	7.28-9.55 IU/mL

Table 12 HCV RNA WHO International Standard limit of detection in serum

Instrument	LoD by PROBIT at 95% hit rate	95% confidence interval
cobas® 6800 System	9.6 IU/mL	8.70-10.95 IU/mL
cobas® 8800 System	12.4 IU/mL	10.93- 14.69 IU/mL

Genotypes 1b through 6

The limit of detection of cobas® HCV for genotypes 1b through 6 was determined by analysis of serial dilutions of each genotype, in HCV-negative human EDTA plasma and serum on the cobas® 6800/8800 Systems. Panels of six concentration levels plus a negative sample were tested using four lots of cobas® HCV test reagents, over multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum are shown in Table 13 and Table 14, respectively.

Table 13 HCV RNA genotype 1b through 6 limit of detection in EDTA plasma

	cobas® 6800 System		cobas® 8800 System	
Genotype	95% LoD by Probit	95% Confidence Interval	95% LoD by Probit	95% Confidence Interval
GT 1b	11.4 IU/mL	9.82 – 14.24 IU/mL	10.2 IU/mL	8.68 – 13.04 IU/mL
GT 2	9.3 IU/mL	8.09 – 11.49 IU/mL	9.5 IU/mL	8.43 – 11.55 IU/mL
GT 3	8.5 IU/mL	7.30 – 10.73 IU/mL	9.2 IU/mL	7.65 – 11.98 IU/mL
GT 4	12.0 IU/mL	10.30 – 15.33 IU/mL	11.4 IU/mL	9.86 – 14.28 IU/mL
GT 5	10.5 IU/mL	8.93 – 13.65 IU/mL	8.3 IU/mL	7.32 – 10.19 IU/mL
GT 6	11.9 IU/mL	9.36 – 17.55 IU/mL	10.6 IU/mL	9.09 – 13.48 IU/mL

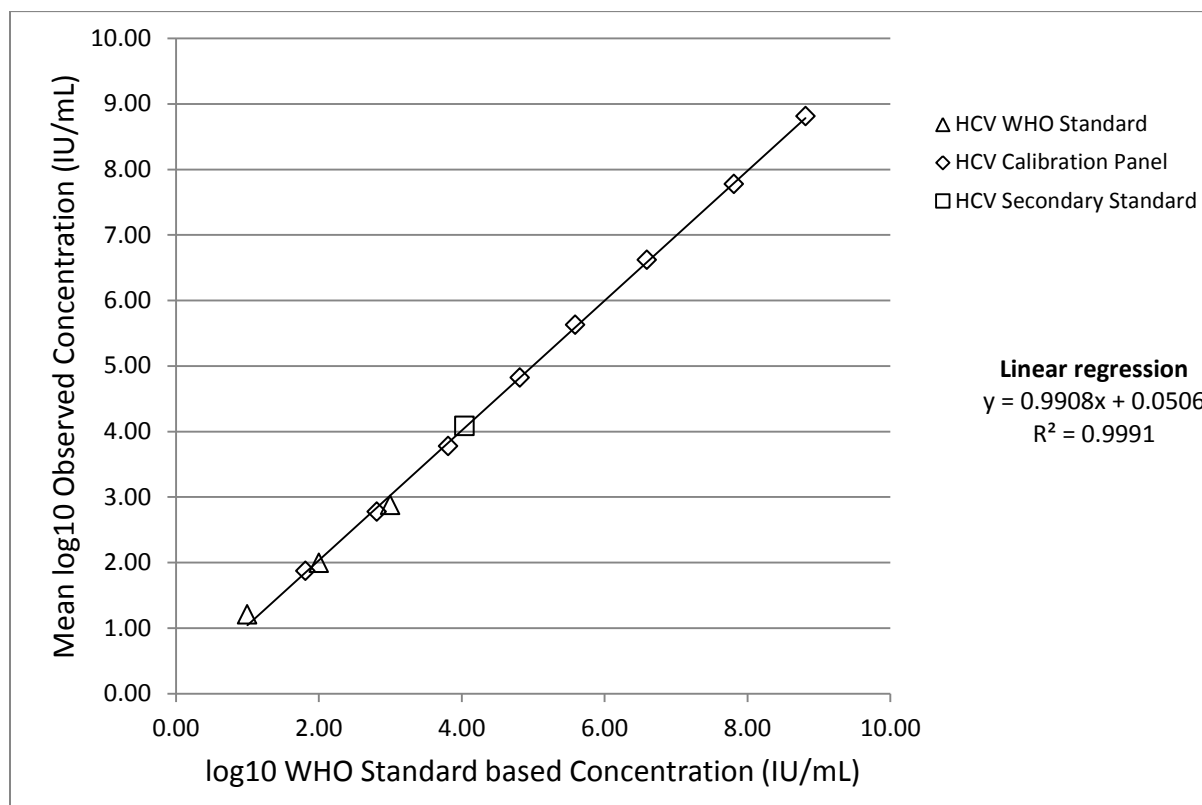
Table 14 HCV RNA genotype 1b through 6 limit of detection in serum

	cobas® 6800 System		cobas® 8800 System	
Genotype	95% LoD by Probit	95% Confidence Interval	95% LoD by Probit	95% Confidence Interval
GT 1b	13.7 IU/mL	11.45 – 18.34 IU/mL	9.9 IU/mL	8.65 – 12.05 IU/mL
GT 2	11.5 IU/mL	9.67 – 15.22 IU/mL	11.8 IU/mL	10.02 – 15.17 IU/mL
GT 3	6.8 IU/mL	5.90 – 8.50 IU/mL	8.0 IU/mL	6.73 – 10.13 IU/mL
GT 4	11.3 IU/mL	9.90 – 13.90 IU/mL	13.7 IU/mL	11.26 – 18.95 IU/mL
GT 5	11.9 IU/mL	9.94 – 15.79 IU/mL	13.1 IU/mL	10.78 – 17.94 IU/mL
GT 6	10.5 IU/mL	9.16 – 12.92 IU/mL	10.4 IU/mL	8.89 – 13.26 IU/mL

Traceability to the WHO Standard

Several standards and controls have been used during development of this test to provide traceability to the WHO standard.²² The standards used during development of the test include the HCV WHO Standard, the RMS HCV Secondary Standard, and the RMS HCV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the HCV WHO Standard was from 1.00E+01 IU/mL to 1.00E+03 IU/mL (1.00 – 3.00 log₁₀ IU/mL), the RMS HCV Secondary Standard was tested at 1.10E+04 IU/mL (4.04 log₁₀ IU/mL), and the RMS HCV Calibration Panel was tested from 6.50E+01 to 6.50E+08 IU/mL (1.81 – 8.81 log₁₀ IU/mL).

All materials behaved similarly and demonstrated co-linear dilution performance across the linear range of cobas® HCV (Figure 2). Based on these results, the calibration and standardization process of cobas® HCV provides quantitation values for the RMS HCV calibration panel, the RMS HCV Secondary Standard, and the HCV WHO Standard that are similar to the expected values with deviation of not more than 0.21 log₁₀ IU/mL. The maximum deviation was obtained around the test LLoQ using a combined regression analyses for the RMS HCV Calibration Panel the RMS HCV Secondary Standard and the HCV WHO Standard.

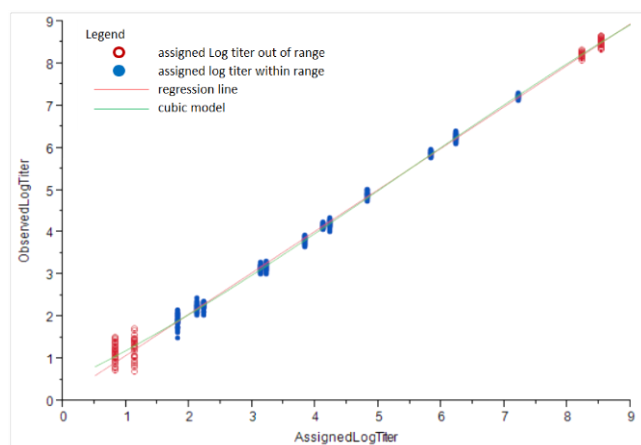
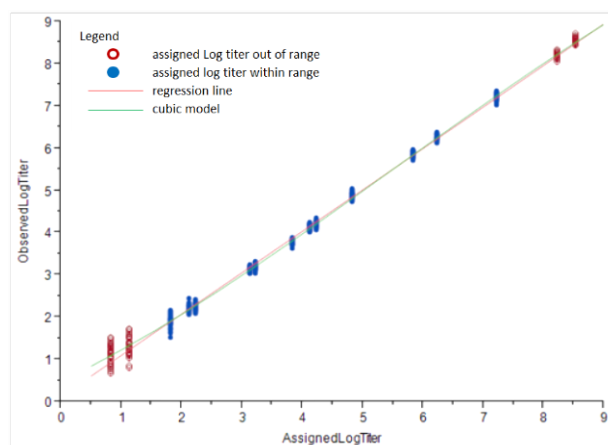
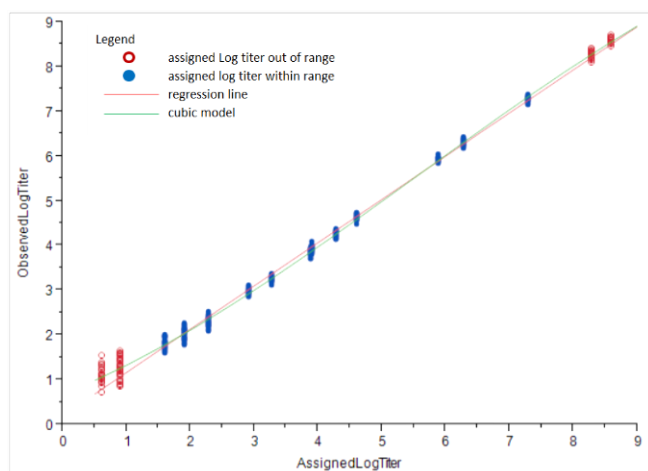
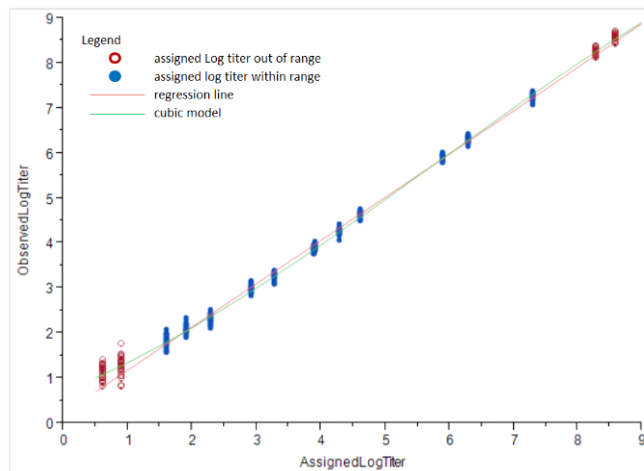
Figure 2 Traceability to WHO International Standard (mean observed $\log_{10}$ titer versus $\log_{10}$ WHO standard based titer) using cobas® HCV

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 samples (CS). The titers of the arRNA stocks and the clinical samples have been verified against a secondary standard which is directly traceable to the HCV WHO standard. The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The expected linear range of cobas® HCV is from LLoQ (15 IU/mL) to ULoQ (1.00E+08 IU/mL). 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 are given.

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 $\pm 0.24 \log_{10}$. Across the linear range, the accuracy of the test was within $\pm 0.24 \log_{10}$.

See Figure 3 and Figure 4 for representative results.

Figure 3 Linearity in EDTA plasma**cobas® 6800 System****cobas® 8800 System****Figure 4** Linearity in serum**cobas® 6800 System****cobas® 8800 System**

Linearity for genotypes 2 through 6

The dilution series used in the 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 clinical specimens. The linearity panel was designed to have an approximately 2 log₁₀ titer overlap between the armored RNA HCV (arRNA) and clinical specimen panel dilutions. The linear range of cobas® HCV was explored between the LLoQ (15 IU/mL) and the ULoQ (1.00E+08 IU/mL) and included multiple medical decision points. Testing was conducted with three lots of cobas® HCV reagent; 15 replicates per level were tested in EDTA plasma or serum on the cobas® 6800 and cobas® 8800 Systems.

The linearity within the linear range of cobas® HCV was verified for all five genotypes (2, 3, 4, 5, and 6) on the cobas® 6800/8800 Systems. The results are summarized in Table 15.

Table 15 cobas® HCV linearity using HCV genotypes 2 through 6

GT	EDTA plasma		Serum	
	Linear Equation HCV Genotype Linearity Study	Maximum Difference Between 1 st Order Model and Higher Order Model (log ₁₀ IU/mL)	Linear Equation HCV Genotype Linearity Study	Maximum Difference Between 1 st Order Model and Higher Order Model (log ₁₀ IU/mL)
2	$y = 0.9601x + 0.1827$	0.15	$y = 0.9758x + 0.2241$	0.20
3	$y = 0.9807x + 0.0920$	0.09	$y = 0.9432x + 0.2217$	0.11
4	$y = 0.9814x + 0.1570$	0.18	$y = 0.9834x + 0.0068$	0.08
5	$y = 0.9788x + 0.1595$	0.12	$y = 0.9410x + 0.2800$	0.19
6	$y = 0.9809x + 0.1990$	0.14	$y = 0.9498x + 0.3068$	0.17

Precision – within laboratory

Precision of cobas® HCV was determined by analysis of serial dilutions of clinical HCV (Genotype 1) samples (CS) or of arRNA 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 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 16 and Table 17.

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.

Table 16 Within-laboratory precision of cobas® HCV (EDTA plasma samples)*

Nominal concentration (IU/mL)	Assigned concentration [IU/mL]	Source material	EDTA plasma							
			cobas® 6800				cobas® 8800			
			Lot1	Lot 2	Lot 3	All Lots	Lot1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD	SD	SD	SD	Pooled SD
1.00E+07	1.67E+07	arRNA	0.04	0.05	0.03	0.04	0.04	0.09	0.04	0.06
1.00E+06	1.67E+06	arRNA	0.05	0.05	0.06	0.05	0.04	0.05	0.04	0.04
4.00E+05	6.69E+05	arRNA	0.03	0.04	0.05	0.04	0.05	0.06	0.04	0.06
5.00E+04	6.69E+04	CS	0.08	0.06	0.06	0.06	0.04	0.08	0.06	0.06
1.00E+04	1.67E+04	arRNA	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05
1.00E+04	1.34E+04	CS	0.03	0.06	0.05	0.05	0.06	0.07	0.06	0.06
4.00E+03	6.69E+03	arRNA	0.05	0.06	0.06	0.06	0.06	0.05	0.06	0.05
1.00E+03	1.34E+03	CS	0.05	0.06	0.05	0.05	0.05	0.06	0.04	0.05
1.00E+03	1.67E+03	arRNA	0.05	0.07	0.05	0.06	0.06	0.08	0.06	0.06
1.00E+02	1.34E+02	CS	0.06	0.09	0.05	0.07	0.06	0.08	0.08	0.07
1.00E+02	1.67E+02	arRNA	0.10	0.06	0.06	0.08	0.07	0.08	0.07	0.07
5.00E+01	6.69E+01	CS	0.09	0.17	0.10	0.13	0.17	0.15	0.08	0.14
1.00E+01	1.34E+01	CS	0.26	0.21	0.13	0.21	0.21	0.26	0.17	0.22

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

Table 17 Within-laboratory precision of cobas® HCV (serum samples)*

Nominal concentration (IU/mL)	Assigned concentration [IU/mL]	Source material	Serum							
			cobas® 6800				cobas® 8800			
			Lot1	Lot 2	Lot 3	All Lots	Lot1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD	SD	SD	SD	Pooled SD
1.00E+07	1.92E+07	arRNA	0.03	0.07	0.04	0.05	0.05	0.08	0.04	0.06
1.00E+06	1.92E+06	arRNA	0.05	0.06	0.04	0.05	0.06	0.06	0.04	0.05
4.00E+05	7.69E+05	arRNA	0.03	0.07	0.03	0.05	0.05	0.06	0.03	0.05
5.00E+04	4.05E+04	CS	0.07	0.06	0.04	0.06	0.05	0.06	0.06	0.06
1.00E+04	1.92E+04	arRNA	0.06	0.06	0.04	0.05	0.05	0.06	0.06	0.06
1.00E+04	8.11E+03	CS	0.05	0.06	0.04	0.05	0.04	0.06	0.04	0.05
4.00E+03	7.69E+03	arRNA	0.04	0.08	0.04	0.06	0.06	0.05	0.04	0.05
1.00E+03	8.11E+02	CS	0.05	0.06	0.06	0.05	0.05	0.09	0.07	0.07
1.00E+03	1.92E+03	arRNA	0.06	0.05	0.05	0.05	0.05	0.07	0.04	0.05
1.00E+02	8.11E+01	CS	0.10	0.18	0.10	0.13	0.07	0.11	0.09	0.09
1.00E+02	1.92E+02	arRNA	0.07	0.08	0.09	0.08	0.08	0.10	0.07	0.09
5.00E+01	4.05E+01	CS	0.09	0.14	0.18	0.14	0.09	0.12	0.09	0.10

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

Performance with HCV negative specimens

The performance of cobas® HCV with HCV RNA negative samples 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 results of all specimens tested with cobas® HCV was 100% “Target Not Detected” (two-sided 95% confidence limit: 99.4% - 100%).

Analytical specificity/cross-reactivity

The analytical specificity of cobas® HCV was evaluated by diluting a panel of microorganisms with HCV RNA positive and HCV RNA negative EDTA plasma (Table 18). The microorganisms were added to normal, virus-negative human EDTA plasma and tested with and without 50 IU/mL 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. Microorganisms were present at a concentration of 1×10^6 particles, copies, IU, genome equivalents, or CFU/mL. Furthermore, the mean log₁₀ titer of each of the positive HCV samples containing potentially cross-reacting organisms was within ± 0.3 log₁₀ of the mean log₁₀ titer of the respective positive spike control.

Table 18 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 (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples were tested in the presence and absence of 50 IU/mL HCV RNA. The tested endogenous substances 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.

An initial set of specimens from patients diagnosed with autoimmune diseases (22 ANA, 6 SLE, 7 RF) showed interference in at least one of the 3 replicates tested of two SLE donors, one RF donor, and four ANA donors with cobas® HCV when tested at 50 IU/mL. Although a root-cause investigation into the observed interference did not reveal the source of the interference, a second set of samples was tested (16 ANA, 15 SLE, 15 RF), and no interference in the presence of autoimmune disease states was observed. 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 $\pm 0.3 \log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

In addition, the drug compounds listed in Table 19 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 $\pm 0.3 \log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 19 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	Ribavirin
	Peginterferon α-2b	
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

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 5 in the measuring 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). The results show a correlation of $y=0.99x-0.08$ with an $R^2 = 0.96$.

Cross contamination

The cross-contamination rate for cobas® HCV was determined by testing 240 replicates of a normal, virus-negative (HIV-1, 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 20 through Table 23 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 20 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected log₁₀ HCV RNA concentration for the cobas® 6800 System.

Table 20 Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration (log₁₀ 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

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
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
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

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
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(%) = $\sqrt{10^{[SD^2 \cdot \ln(10)]} - 1} \cdot 100$

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

In Table 21 below, the negative percent agreement (NPA) for the cobas® 6800 System using negative panel member tests was 99.54%.

Table 21 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 22 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected log₁₀ HCV RNA concentration on the cobas® 6800 System.

Table 22 Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration (log₁₀ 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

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
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.

^aCalculated using the SAS MIXED procedure.

^bNumber of valid tests with detectable viral load.

^cCalculated using the total variability from the SAS MIXED procedure.

^dLognormal CV(%) = $\sqrt{10^{[SD^2 \cdot \ln(10)]} - 1} \cdot 100$

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 23 below.

Table 23 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)

^aNegative Percent Agreement = (number of negative results / total number of valid tests in negative panel member) * 100.

^bCalculated 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 24 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 24 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
$1.0 \text{ E}+01 \leq X < 1.0 \text{ E}+02$	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)
$1.0 \text{ E}+02 \leq X < 1.0 \text{ E}+03$	-	0.11 (72)	-	-	0.12 (48)	-
$1.0 \text{ E}+03 \leq X < 1.0 \text{ E}+04$	0.14 (72)	0.07 (72)	0.08 (72)	0.13 (48)	0.07 (48)	0.08 (48)
$1.0 \text{ E}+04 \leq X < 1.0 \text{ E}+05$	0.17 (72)	0.10 (72)	0.08 (72)	0.11 (48)	0.06 (48)	0.08 (48)
$1.0 \text{ E}+05 \leq X < 1.0 \text{ E}+06$	0.15 (72)	0.08 (72)	0.08 (72)	0.11 (48)	0.07 (47)	0.10 (48)
$1.0 \text{ E}+06 \leq X < 1.0 \text{ E}+07$	0.10 (72)	0.12 (72)	0.11 (71)	0.09 (48)	0.13 (48)	0.11 (48)
$1.0 \text{ E}+07 \leq X < 1.0 \text{ E}+08$	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.

^aPrecision Standard Deviation in $\log_{10}$ units

^bNumber 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 direct acting antiviral (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 25 below shows the demographic and baseline characteristics of subjects whose samples were tested on the cobas® 6800 and the cobas® 8800 Systems.

The demographic distribution of the subjects in this study was consistent with that of chronic HCV patients in the US,²³ the majority being 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 25 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 (log₁₀ 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 log₁₀ 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 VR 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 26). 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 (NPV)s of 87.5% and 100%, respectively (Table 26). Additional analysis of Week 2 VL to predict SVR12 shows a PPV of 79.4% but a low NPV of 29.9%. The most recent AASLD guidelines from 2014 have not included any earlier virologic decline assessments than Week 4.²⁴

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 26).

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

Treatment Plan	Genotype	On-Treatment Visit	Eligible Subjects	PPV (%)		NPV (%)		OR
				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.

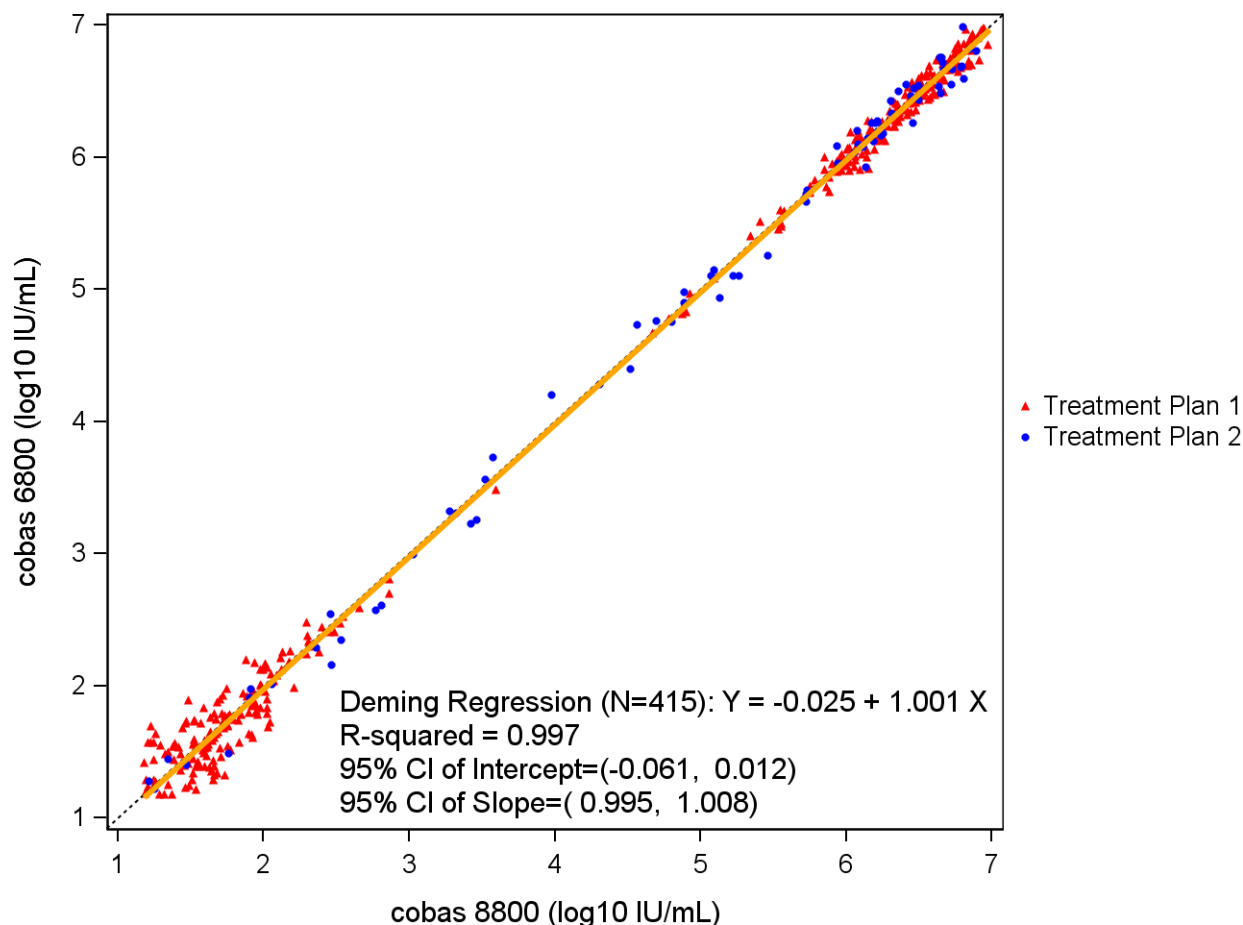
Odds Ratio (OR) = (TP • TN) / (FP • FN)

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 5 below show a Deming regression plots of VLs (log₁₀ IU/mL) greater than 15 IU/mL at all applicable time points on treatment.

Figure 5 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 27 below show the demographic and clinical characteristics of subjects whose samples were tested on the cobas® 6800 System and cobas® 8800 System.

Table 27 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 28). According to current AASLD Guidelines¹¹, an FDA-approved quantitative or qualitative NAT with a detection level of 25 IU/mL or lower should be used to detect HCV RNA. 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 28).

Table 28 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 29, Table 30, Table 31).

Table 29 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 Biliary 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 30 Number of HCV RNA negative samples on the cobas® 6800 System with non HCV-related liver diseases within test result categories by clinical condition

Clinical Condition	Number of Valid Tests						Specificity ^a % (95% CI) ^b
	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	
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 31 Number of HCV RNA negative samples on the cobas® 8800 System with non HCV-related liver diseases within test result categories by clinical condition

Clinical Condition	Number of Valid Tests						Specificity ^a % (95% CI) ^b
	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	
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.

When highly sensitive real-time quantitative PCR assays such as cobas® HCV are used to aid in the diagnoses of HCV infection, a cut-off of 25 IU/mL should be applied to distinguish between non-active and active HCV infection. The HCV RNA concentration, together with other markers of active liver disease, need to be evaluated if antiviral treatment is being considered.²⁴

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.

Additional information

Key test features

Sample type	EDTA plasma, serum	
Minimum amount of sample required	650 µL	
Sample processing volume	500 µL	
Analytical sensitivity	EDTA plasma	12.0 IU/mL
	Serum	13.7 IU/mL
Linear range	15 IU/mL – 1.0E+08 IU/mL	
Specificity	100% (two-sided 95% confidence limit: 99.4% – 100%)	
Genotypes detected	HCV genotypes 1-6	

Symbols

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

Table 32 Symbols used in labeling for Roche PCR diagnostics products



Ancillary Software



In Vitro Diagnostic Medical Device



Authorized Representative
in the European community



Lower Limit of Assigned Range



Barcode Data Sheet



Manufacturer



Batch code



Store in the dark



Biological Risks



Contains sufficient for <n> tests



Catalogue number



Temperature Limit



Consult instructions for use



Test Definition File



Contents of kit



Upper Limit of Assigned Range



Distributed by



Use-by date



For IVD Performance Evaluation
Only



Global Trade Item Number



This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 33 Manufacturer and distributors

Manufactured in the United States



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



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

Trademarks and patents

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

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Document revision

Document Revision Information	
Doc Rev. 2.0 08/2016	Update to current SDS information, including additional change to sodium azide warning in Reagent handling section. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 11/2016	Added mixing step to Specimen collection, transport, and storage section. Clarified sample storage in secondary tubes upon separation in Samples section. Added Roche web address www.roche.com . Please contact your local Roche Representative if you have any questions.