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: 06997732190
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 06997767190
cobas® NHP Negative Control Kit	P/N: 07002220190

Table of contents

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**® 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**® 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 monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

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 < x < 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 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 96 test cassette (P/N 06997732190)		
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. 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, 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	6 mL

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

cobas® HBV/HCV/HIV-1 Control Kit
Store at 2–8°C
(P/N 06997767190)

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/ fume/ 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

07941714001-04EN

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, 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/ 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 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

* 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 protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338: + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. 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 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	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	30 days from first usage	Max 10 runs	Max 8 hours
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable	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

* 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 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 **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, 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.

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**® 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 (15-30ºC) 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.

Instructions for use

Procedural notes

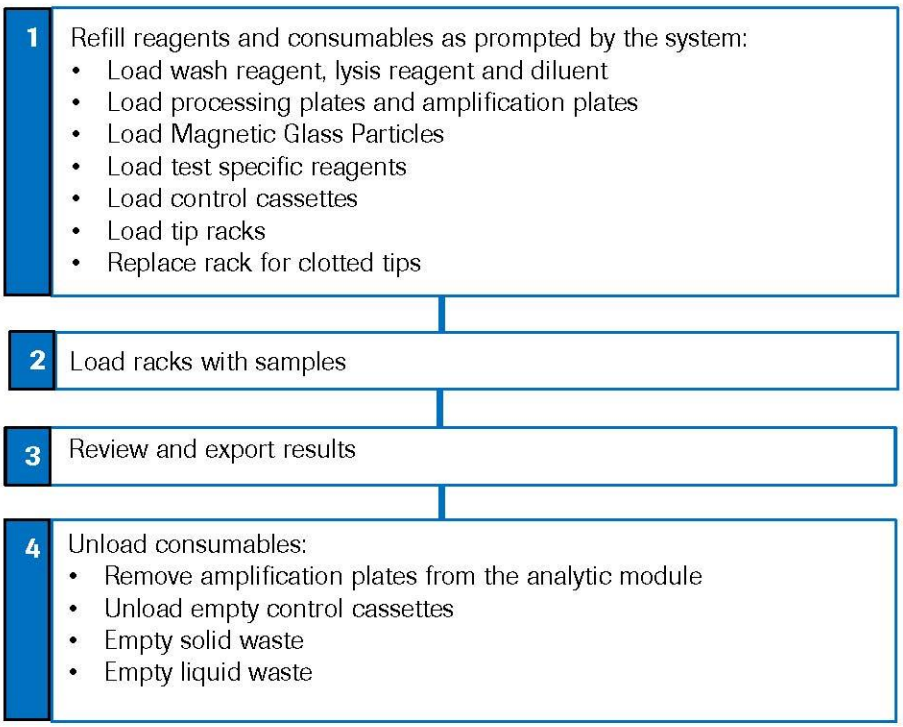
- Do not use **cobas**® HCV test reagents, **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual for proper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

Running cobas® HCV

cobas® HCV can be run with 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 Operator's Manual. Figure 1 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 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.

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

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 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

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.

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.
- 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

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 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 11 to Table 14, 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 11 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 12 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 13 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 14 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 2 to Figure 5 for representative results.

Figure 2 Linearity in EDTA plasma (500 µL)

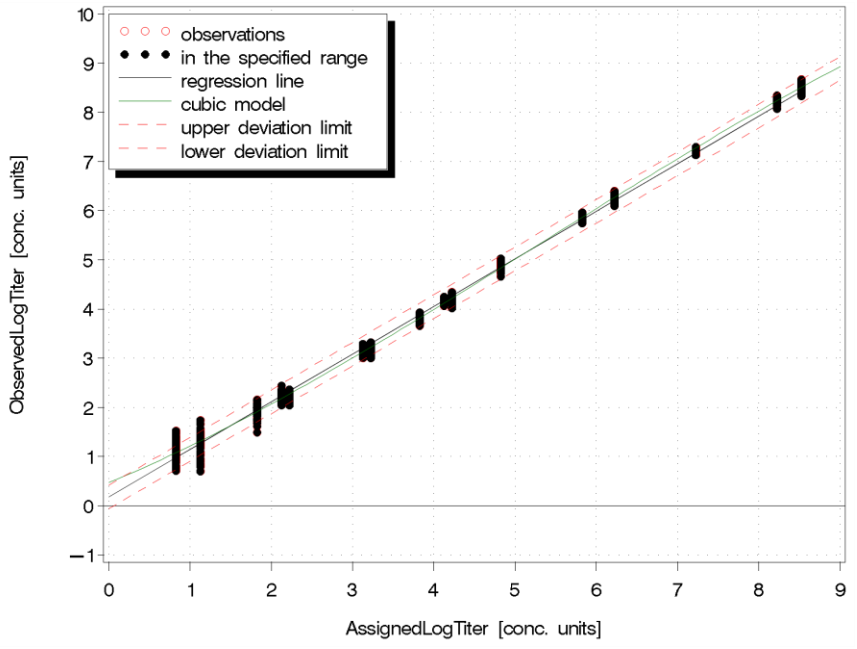


Figure 3 Linearity in serum (500 µL)

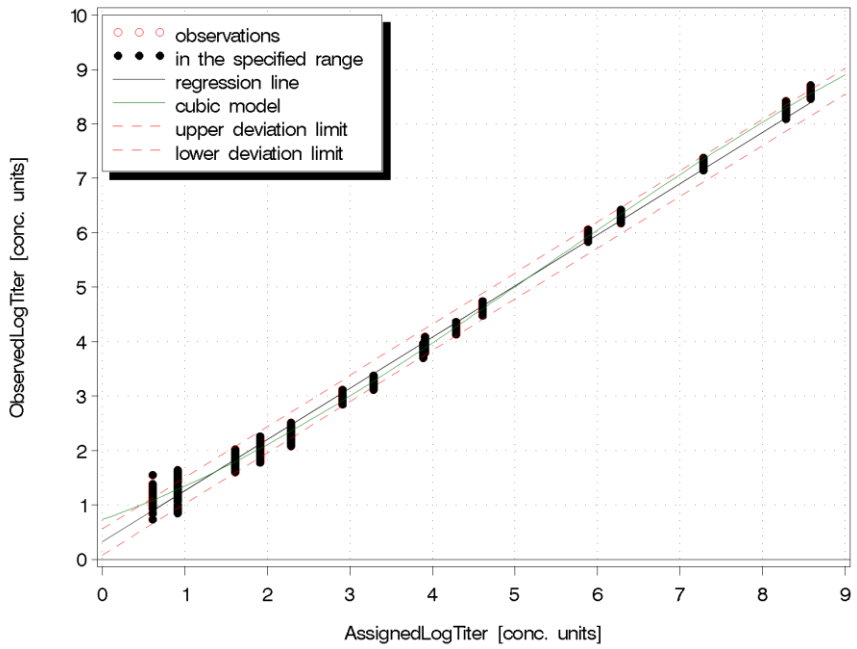


Figure 4 Linearity in EDTA plasma (200 µL)

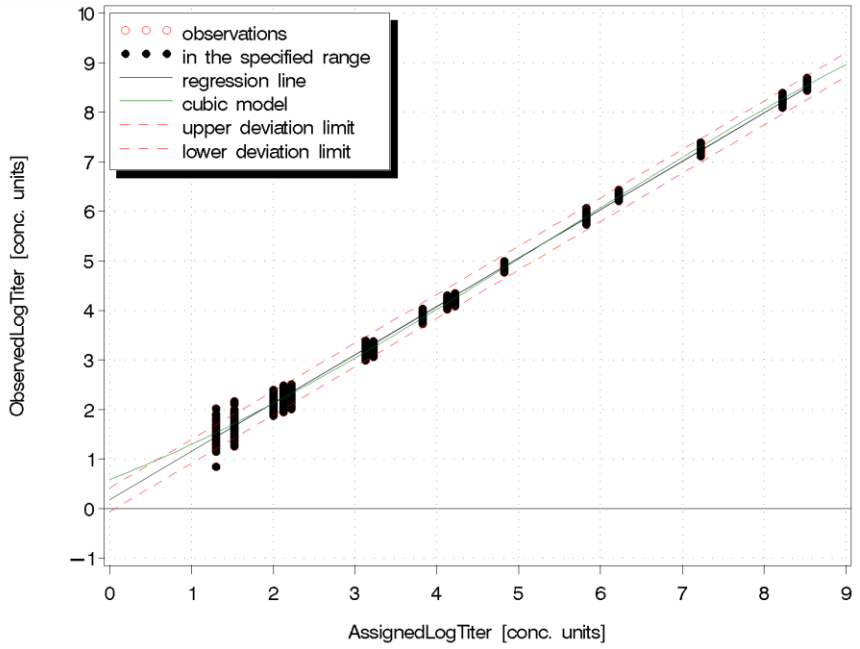
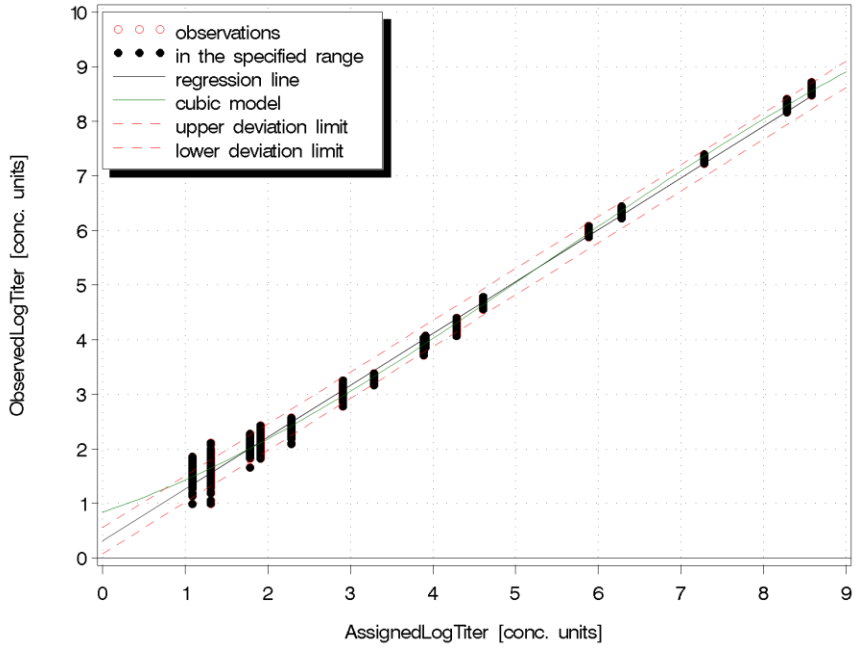


Figure 5 Linearity in serum (200 µL)



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 (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 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 15 to Table 18.

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 15 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 16 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 17 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 18 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 19 and Table 20, respectively. The study demonstrates that **cobas**® HCV detected all HCV genotypes tested with a similar LoD as HCV genotype 1a.

Table 19 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 20 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 21 and Table 22. 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 21 HCV RNA genotype verification of limit of detection in EDTA plasma (200 µL)

	17.5 IU/mL			33 IU/mL			50 IU/mL		
Genotype	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 22 HCV RNA genotype verification of limit of detection in serum (200 µL)

	17.5 IU/mL			33 IU/mL			50 IU/mL		
Genotype	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 23 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 24 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 24 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

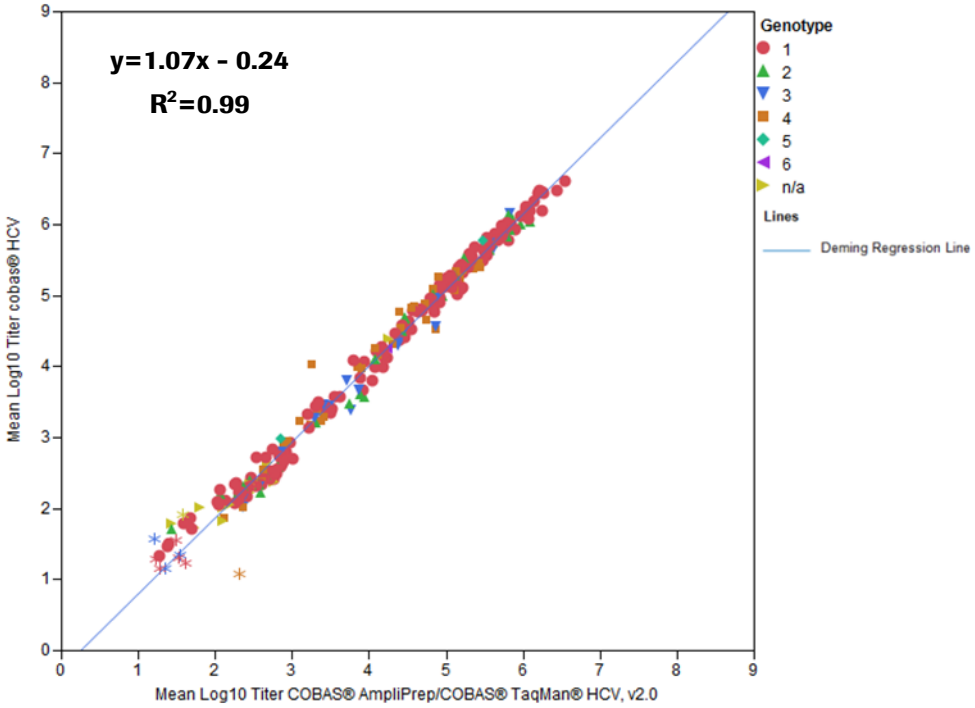
Method correlation

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 6. The symbol ✳ in Figure 6 shows single determination.

Figure 6 Regression analysis of **cobas**® HCV vs TaqMan® HCV Test, v2.0, EDTA plasma and serum samples

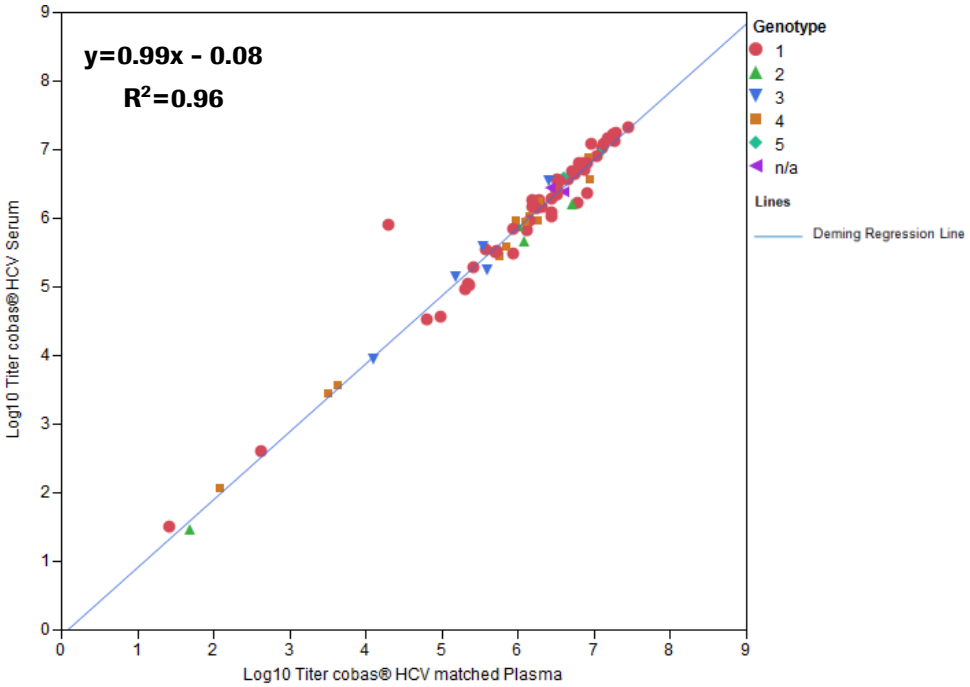


Matrix equivalency – EDTA plasma versus serum

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 7).

Figure 7 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%].

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 diagnostics products.

Table 25 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)	D Distributed by	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	IVD In vitro diagnostic medical device	Temperature limit
REF Catalogue number	LLR Lower Limit of Assigned Range	TDF Test Definition File
CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	Male	This way up
	Manufacturer	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	CONTROL − Negative control	UDI Unique Device Identification
Consult instructions for use	NON STERILE Non sterile	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	Patient Name	Urine Fill Line Urine Fill Line
CONTENT Contents of kit	# Patient number	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Peel here	Use-by date
Date of manufacture	CONTROL + Positive control	
Device for near-patient testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	
Device for self-testing		

Technical support

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

Manufacturer and distributors

Table 26 Manufacturer and distributors

Manufactured in the United States

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

Made in USA

Distributed by

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

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, 8129118, 9963737, 9512494, 10041134, 6727067, and foreign equivalent patents of each.

COBAS, COBAS OMNI, and AMPERASE are trademarks of Roche.

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

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

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

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase® enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

Certain components of this product are covered by one or more US Patents and their foreign equivalents issued to Novartis Vaccines and Diagnostics, Inc. and licensed to Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd. See http://www.roche-diagnostics.us/patents

Copyright

CE 0123

IVD

07941714001-04EN

References

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome [Science 1989;244:359-362]. J Hepatol. 2002;36:582-585.
2. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med. 2006;144:705-714.
3. Rustgi VK. The epidemiology of hepatitis C infection in the United States. J Gastroenterol. 2007;42:513-521.
4. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41-52.
5. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med. 1998;339(21):1485-92.
6. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med. 1998;339:1493-1499.
7. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358):958-965.
8. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347(13):975-982.
9. Hadziyannis SJ, Sette H, Jr., Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346-355.
10. Ghany MG, Strader DB, Thomas DL, Seeff LB; . American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009;49:1335-1374.
11. Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54:1433-1444.
12. Poordad F, McCone J Jr., Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1195-1206.
13. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011;364:2405-2416.
14. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011;364:1207-1217.
15. Zeuzem S, Andreone P, Pol S, t al. Telaprevir for retreatment of HCV infection. N Engl J Med. 2011;364:2417-2428.
16. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med. 2013;368:1878-1887.
17. Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med. 2013;368:1867-1877.
18. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med. 2013;368:1907-1917.

19. Rutter K, Hofer H, Beinhardt S, et al. Durability of SVR in chronic hepatitis C patients treated with peginterferon-alpha2a/ribavirin in combination with a direct-acting anti-viral. Aliment Pharmacol Ther. 2013;38:118-123.

20. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990;93:125-128.

21. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. Nature. 1995;373:487-493.

22. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. Cell. 1995;80:869-878.

23. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY). 1992;10:413-417.

24. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Res. 1996;6:986-994.

25. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.

26. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc. Rev. 4.0 (Mfg-US) 05/2021	Inserted Rx Only symbol on first page. Updated hazard warnings. Updated the harmonized symbol page. Added Technical support section. Added Made in statement. Updated distributors addresses. Updated **Trademarks and patents** section. Please contact your local Roche Representative if you have any questions.