# cobas® HCV

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

For in vitro diagnostic use

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

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# Intended use

**cobas® HCV**

**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 or from a **cobas®** Plasma Separation Card (PSC) dried plasma spot 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 findings. **cobas®** PSC dried plasma spots may be used in accordance with clinical practice guidelines and the assay's performance characteristics.

**cobas® HBV/HCV/HIV-1 Control Kit**

**cobas®** HBV/HCV/HIV-1 Control Kit is intended for use as a positive run/batch control on the **cobas®** 5800/6800/8800 systems with the **cobas®** HBV, **cobas®** HCV, and **cobas®** HIV-1 tests.

# 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]

The World Health Organization (WHO) now also now recommends the use of dried spot specimens to expand the reach of viral load testing in resource limited settings without ready access to phlebotomy services or robust EDTA plasma sample transportation capabilities. A PSC dried plasma spot, which also stabilizes the HIV RNA in dried plasma can improve viral load testing coverage in these settings by enabling sample transportation over longer distances and harsher environmental conditions than EDTA plasma.[20]

Specifically, the latest WHO Hepatitis C guidelines include new protocols on the use of dried spot specimens for HCV RNA testing and new data to inform the limit of detection for HCV RNA assays as a test of cure, in addition to their use for diagnosis. Dried spot specimens may be considered to improve access to HCV viral load testing in resource limited settings without ready access to nearby laboratory facilities or NAT or robust sample transportation capabilities, or in persons with poor venous access.[20] Based on the latest HCV clinical practice guidelines by the European Association for the Study of the Liver (EASL), in low-to middle-income areas, as well as in specific settings in high-income countries, a lower limit of detection of ≤1,000 IU/mL (3.0 $\log_{10}$ IU/mL) can be used to broaden access to HCV diagnosis and care.[21]

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

## Explanation of the test

**cobas**® HCV is a quantitative test performed on the **cobas**® 5800 system, **cobas**® 6800 system, or **cobas**® 8800 system. **cobas**® HCV enables the detection and quantitation of HCV RNA in EDTA plasma or serum or from a PSC dried plasma spot of infected patients. Dual probes are used to detect and quantify, but not discriminate genotypes 1-6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to HCV 2$^{nd}$ WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HCV 2$^{nd}$ WHO International Standard (NIBSC code 96/798).

## Principles of the procedure

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

Nucleic acid from patient samples, external controls and added armored RNA-QS molecules is simultaneously extracted. In summary, viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as

denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash buffer steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the patient sample is achieved by the use of target virus-specific forward and reverse primers which are selected from the highly conserved 5'-untranslated region (5'-UTR) 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).[22-24] 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.[25,26] 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

The materials provided for **cobas**® HCV can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 5, Table 12 and Table 13.

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

**Table 1 cobas**® HCV

**cobas® HCV**

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

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

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

**cobas® HBV/HCV/HIV-1 Control Kit**

Store at 2–8°C

For use on the **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09040773190)

For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 06997767190 and P/N 09040773190)

| Kit components | Reagent ingredients | Quantity per kit | Safety symbol and warning* |
|---|---|---|---|
| **HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+)C)** | < 0.001% HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein armored, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods.<br>< 0.1% ProClin® 300 preservative** | 5.2 mL (8 x 0.65 mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>H412: Harmful to aquatic life with long lasting effects.<br>P261: Avoid breathing mist or vapours.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (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,<br>< 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.<br>< 0.1% ProClin® 300 preservative** | 5.2 mL (8 x 0.65 mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>H412: Harmful to aquatic life with long lasting effects.<br>P261: Avoid breathing mist or vapours.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1) |

* 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

For use on the **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09051554190)
For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 07002220190 and P/N 09051554190)

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

* Product safety labeling primarily follows EU GHS guidance
**Hazardous substance

# cobas® omni reagents for sample preparation

**Table 4 cobas® omni** reagents for sample preparation

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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

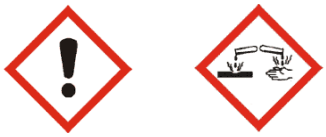
# cobas® Specimen Pre-Extraction Reagent

Note: This reagent is optional and should only to be used in conjunction with the **cobas**® PSC to generate dried plasma spot samples. See PSC Method Sheet ms_09411763190.

**Table 5** **cobas**® Specimen Pre-Extraction Reagent

**cobas® Specimen Pre-Extraction Reagent**

Store at 2-8°C
(P/N 08064695190)

| Reagent | Reagent ingredients | Quantity per kit | Safety symbol and warning* |
|---|---|---|---|
| **cobas® Specimen Pre-Extraction Reagent (SPER)** | 28% (w/w) guanidine thiocyanate**, 6% (w/v) polydocanol**, 1% (w/v) dithiothreitol**, dihydro sodium citrate | 600 mL<br>(15 x 40 mL) |  <br>**DANGER**<br>H302: Harmful if swallowed.<br>H314: Causes severe skin burns and eye damage.<br>H412: Harmful to aquatic life with long lasting effects.<br>EUH032: Contact with acids liberates very toxic gas.<br>EUH071: Corrosive to the respiratory tract.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.<br>P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.<br>P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.<br>P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.<br>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.<br>593-84-0 Guanidinium thiocyanate<br>9002-92-0 Polidocanol |

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

## Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 6, Table 7 and Table 8.

**cobas**® Specimen Pre-Extraction Reagent, used in the PSC dried plasma spot workflow, shall be stored and handled as specified in Table 10 and Table 11. **cobas**® PSC storage and handling requirements are specified in the **PSC Method Sheet ms_09411763190**.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 systems, store them at the corresponding temperature specified in Table 6.

**Table 6** Reagent storage (when reagent is not on the system)

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

## Reagent handling requirements for the cobas® 5800 system and cobas® 6800/8800 systems

Reagents loaded onto the **cobas**® 5800 system or **cobas**® 6800/8800 systems are stored at appropriate temperatures, their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 7, Table 8 and Table 9 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit uses information for assay specific reagents is accessible through the system user interface.

**Table 7** Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

| Reagent | Open-kit stability | Number of kit uses | On-board stability |
|---|---|---|---|
| **cobas**® HCV – 192 | 90 days from first usage | 40 | 36 days from loading |
| **cobas**® HBV/HCV/HIV-1 Control Kit | single use vial | 8 | 36 days from loading |
| **cobas**® NHP Negative Control Kit | single use vial | 16 | 36 days from loading |

[a] Single use reagents

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

**Table 8** Reagent expiry conditions enforced by the **cobas**® 6800/8800 systems

| Reagent | Open-kit stability | Number of kit uses | On-board stability (outside on board refrigerator) |
|---|---|---|---|
| **cobas**® HCV | 90 days from first usage | Max 40 runs | 40 hours from loading |
| **cobas**® HBV/HCV/HIV-1 Control Kit | single use vial | 8 | 8 hours from loading |
| **cobas**® NHP Negative Control Kit | single use vial | 16 | 10 hours from loading |

Table 9 shows the open-kit stability of the **cobas**® **omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

**Table 9** **cobas**® **omni** reagent expiry condition monitored and enforced by the **cobas**® 5800/6800/8800 systems

| Reagent | Open-kit stability |
|---|---|
| **cobas**® **omni** Lysis Reagent | 30 days from loading |
| **cobas**® **omni** MGP Reagent | 30 days from first usage |
| **cobas**® **omni** Specimen Diluent | 30 days from loading |
| **cobas**® **omni** Wash Reagent | 30 days from loading |

Store **cobas**® Specimen Pre-Extraction Reagent (used in PSC workflow) at the corresponding temperature specified in Table 10.

**Table 10** **cobas**® Specimen Pre-Extraction Reagent storage

| Reagent | Storage temperature |
|---|---|
| **cobas**® Specimen Pre-Extraction Reagent | 2–8°C |

**cobas**® Specimen Pre-Extraction Reagent is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days when stored at 2-8°C including cumulative 13 hours at 30°C or until expiration date, whichever comes first, as specified in Table 11.

**Table 11** **cobas**® Specimen Pre-Extraction Reagent expiry conditions

| Reagent | Open-kit stability | Stability at 30°C outside refrigerator (cumulative time) |
|---|---|---|
| **cobas**® Specimen Pre-Extraction Reagent | 30 days from first usage | 13 hours |

# Additional materials required for cobas® 5800/6800/8800 systems

**Table 12** Materials for use on the **cobas**® 5800/6800/8800 systems

| Material | P/N |
|---|---|
| **cobas**® **omni** Lysis Reagent | 06997538190 |
| **cobas**® **omni** MGP Reagent | 06997546190 |
| **cobas**® **omni** Specimen Diluent | 06997511190 |
| **cobas**® **omni** Wash Reagent | 06997503190 |

**Table 13** Consumables for use on the **cobas**® 5800 system

| Material |
|---|
| **cobas**® **omni** Processing Plate 24 |
| **cobas**® **omni** Liquid Waste Plate 24 |
| **cobas**® **omni** Amplification Plate 24 |
| Tip CORE TIPS with Filter, 1ml |
| Tip CORE TIPS with Filter, 300⌠l |
| **cobas**® **omni** Liquid Waste Container |
| Solid Waste Bag or Solid Waste Bag With insert |

*For Part Numbers please refer to the **cobas**® 5800 system User Assistance

**Table 14** Consumables for use on the **cobas**® 6800/8800 systems*

| Material |
|---|
| **cobas**® **omni** Processing Plate |
| **cobas**® **omni** Amplification Plate |
| **cobas**® **omni** Pipette Tips |
| **cobas**® **omni** Liquid Waste Container |
| Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer |

*For Part Numbers please refer to the **cobas**® 6800/8800 systems User Assistance.

**Table 15** Other materials and consumables required for PSC dried plasma spot application only

| **Materials** |
|---|
| **cobas**® Plasma Separation Card (P/N 09411763190)* |
| Sterile or disposable forceps or tweezers** |
| 3 x 140 µL EDTA coated capillaries with dispenser |
| Single Use lancing device (e.g. Greiner Bio-one: MiniCollect® Safety Lancet penetration depth 2.00 mm)* |
| Sample bag (plastic transparent resealable ziplock) and silica gel desiccant sachets (for a total of 4 grams)<br>(for **cobas**® PSC storage and delivery, see **PSC Method Sheet ms_09411763190** for more information) |
| Transport bag (e.g., Wicoseal 180 x 60 x 240 mm) |
| Pipette (e.g., Multistepper pipette) |
| Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes |
| Tubes, 5 mL, internal thread, 12.5 mm diameter, polypropylene (i.e., Greiner Bio-one Cryo.s™) with caps |

* See **PSC Method Sheet ms_09411763190** for more information about the PSC sample collection.

**To prevent cross-contamination, use only one pair of forceps for each patient! The usage of metal forceps that are autoclaved after single use is recommended.

## Instrumentation and software required

The **cobas**® 5800 system software, the **cobas**® 6800/8800 systems software, **cobas**® HCV analysis package (ASAP) and dried PSC plasma spot application analysis package (**cobas**® HCV-PSC ASAP) for the **cobas**® 5800/6800/8800 systems shall be installed.

For **cobas**® 5800 and **cobas**® 6800/8800 systems with software 2.0 or higher, the x800 Data Manager software and PC (or server) will be provided with the system.

For the **cobas**® 6800/8800 systems with software version 1.4, the Instrument Gateway (IG) server will be provided with the systems.

**Table 16** Instrumentation

| **Equipment** | **P/N** |
|---|---|
| **cobas**® 5800 system | 08707464001 |
| **cobas**® 6800 system | 05524245001 and 09575154001 |
| **cobas**® 8800 system | 05412722001 and 09575146001 |
| Sample Supply Module for **cobas**® 6800/8800 systems | 06301037001 and 09936882001 |

Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems - User Assistance for additional information.

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

# Precautions and handling requirements

## Warnings and precautions

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

- For in vitro diagnostic use only.
- **cobas**® HCV has not been evaluated for use as a screening test for the presence of HCV in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[27,28] Only personnel proficient in handling infectious materials and the use of **cobas**® HCV and the **cobas**® 5800/6800/8800 systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
    - If spillage of PSC dried plasma spot samples in **cobas**® Specimen Pre-Extraction Reagent (which contain guanidine thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- **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.
- Refer to **PSC Method Sheet ms_09411763190** for additional warnings and precautions.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- **cobas**® Specimen Pre-Extraction Reagent is light sensitive and shipped in light protective bottles.
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Do not use 200 µL sample input volume if the viral load is expected to be < 100 IU/mL.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

## Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® HCV kits, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® **omni** Lysis Reagent or **cobas**® Specimen Pre-Extraction Reagent, which contains guanidine thiocyanate, to contact sodium or potassium hypochlorite 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 or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 5800 or **cobas**® 6800/8800 systems User Assistance to properly clean and decontaminate the surface of instrument(s).

# Sample collection, transport and storage

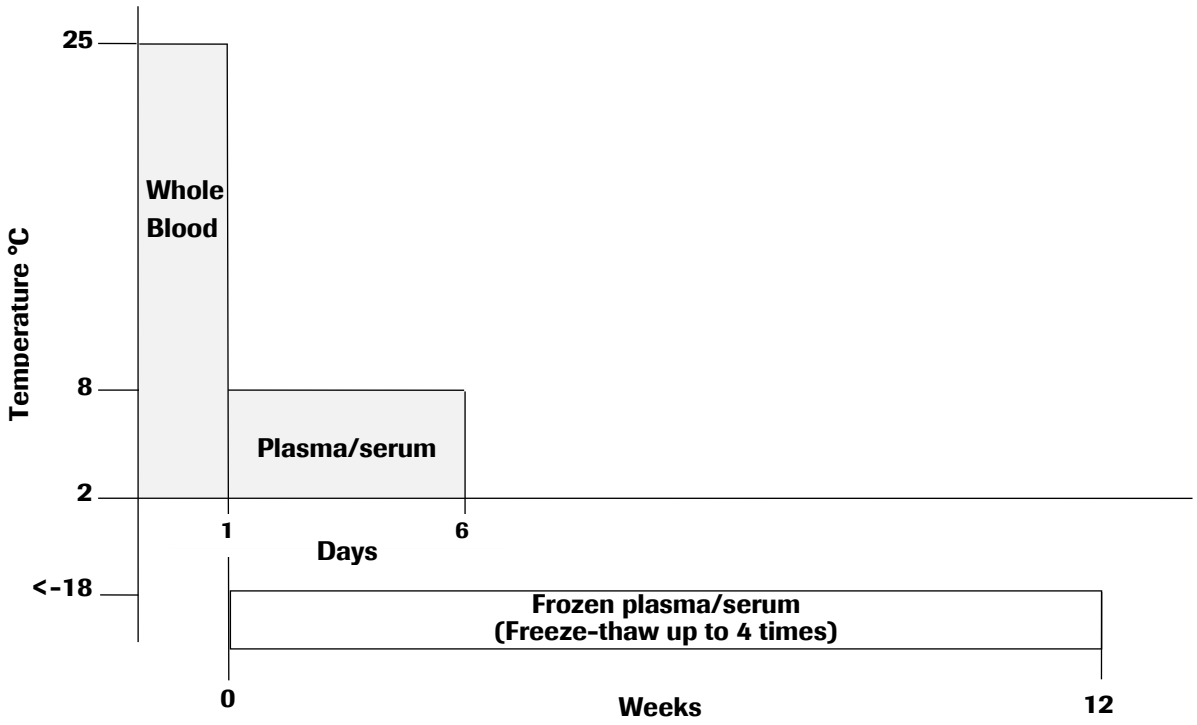
**Note: Handle all samples and controls as if they are capable of transmitting infectious agents.**
Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely 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.

## EDTA plasma and serum samples

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

- Whole blood collected in SST™ Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma/serum preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation EDTA plasma or serum samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C. For long-term storage, temperatures at ≤ -60°C are recommended.
- Plasma/serum samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.
- Ensure sufficient whole blood collection to allow usage of the preferred processing volume for EDTA plasma or serum of 500 µL (for a total minimum sample requirement of 650 µL) if possible.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

**Figure 1** Sample storage conditions

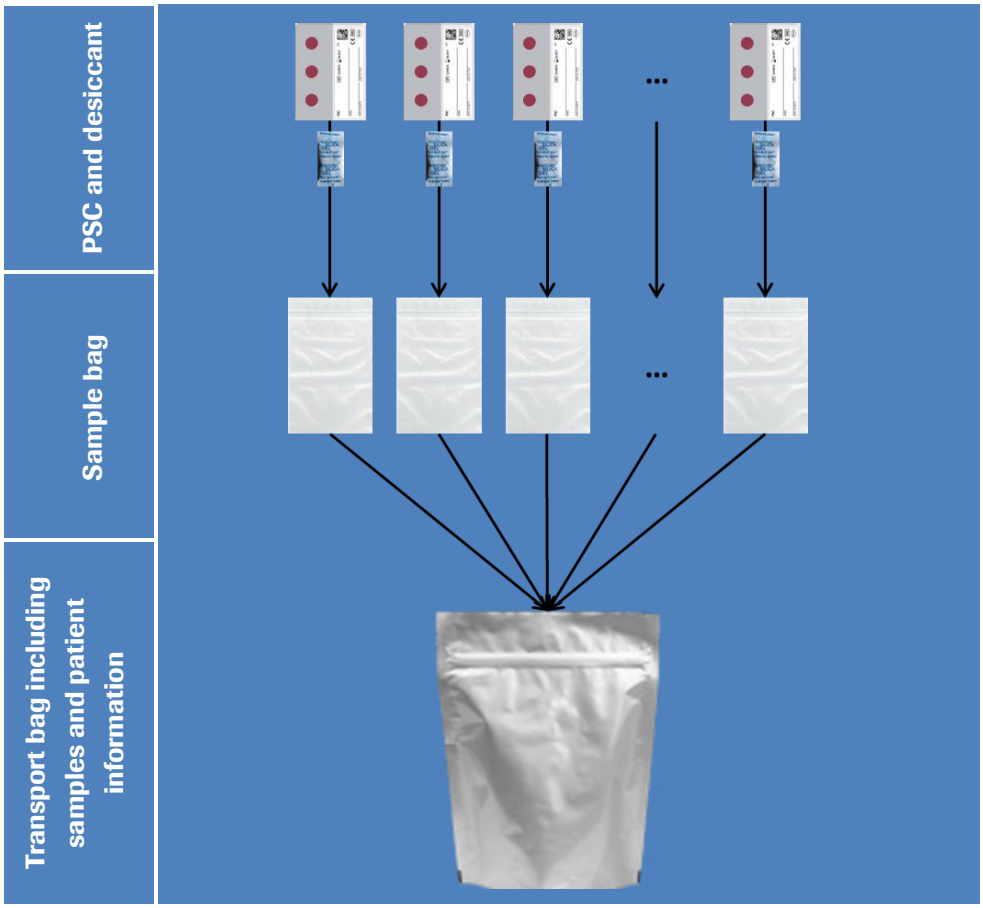



## PSC dried plasma spot samples

- Collect PSC dried plasma spot samples using appropriate clinical procedures (refer to **PSC Method Sheet ms_09411763190**).
- Check the expiry date of the PSC and proceed only if the PSC has not expired yet.
- Make sure that the bag in which the PSC is sealed is completely closed and intact.
- Label the PSC with patient's name, date of birth, date and time of sample collection.
- Apply 140 µL of whole blood on each circle of the PSC membrane delineated by the spotting layer using an appropriate capillary and a dispenser. It is recommended to fill all three spots on the PSC, in order to allow retesting.

- Do not apply samples from more than one patient on the same PSC.
- Do not allow the membranes to get in contact with gloves, tools or any potentially contaminated surfaces during this process.
- Ensure that BOTH sides of the PSC spots (front: membrane with blood; back: spot with plasma) are saturated after 5 minutes. Check the back side through the transparent back layer.
- Allow the PSC to dry at room temperature for at least 4 hours (to maximum overnight), protecting it from direct sunlight.
- Do not remove the spotting layer. This will be done at the laboratory, prior to sample extraction.
- After drying, store the PSC in an individual sample bag with 4 grams of desiccant and seal the bag. The collected sample bags must be packed in a transport bag together with their relative Patient Information Sheet. It is recommended to pack a maximum number of 25 PSCs per transport bag (see Figure 2 for an overview).

**Figure 2** Overview of the packaging concept for PSC transport




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. PSCs may be transported for a period of 28 days before being analyzed at 18-45°C and up to 85% humidity. PSCs in individual sample bags with 4 grams of desiccant, within a transport bag may be stored after transportation at room temperature (18-30°C), at 2- 8°C or at ≤ -10°C for up to 56 days (with and without layer separation).

# 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.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of RD5 or MPA sample racks. Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper barcode specifications and additional information on loading sample tubes.

## Running cobas® HCV on the cobas® 5800/6800/8800 systems

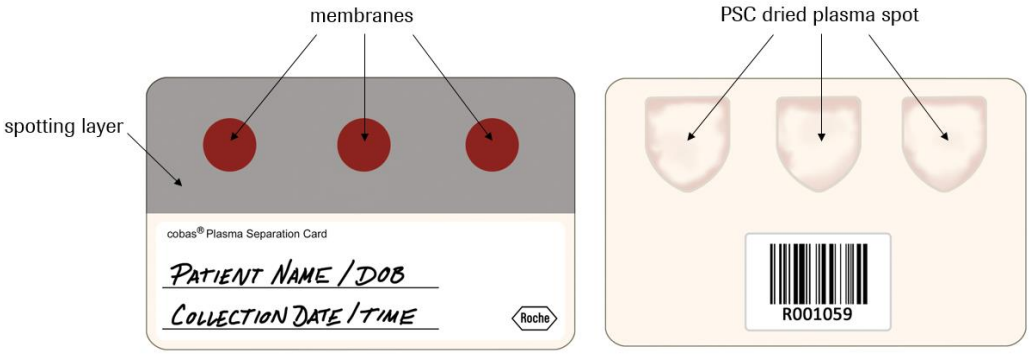
- The operation of the instruments is described in detail in the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance.
- Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- For samples collected with the **cobas**® PSC, verify that the correct sample type (**PSC**) and the correct ASAP is used (**cobas**® HCV-PSC ASAP) before starting the test procedure.
- **cobas**® HCV can be run with two required sample volumes for plasma and serum of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). Figure 14 and Figure 15 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).**
- **cobas**® HCV can be run with 1300 µL **cobas**® Specimen Pre-Extraction Reagent (for the 850 µL PSC sample workflow). Please note, with **cobas**® 6800/8800 System Software 1.4, **cobas**® HCV PSC workflow cannot run in mixed batch mode with plasma or serum samples, however it can be run in mixed batch mode with **cobas**® HIV-1 PSC, **cobas**® HIV-1/ HIV-2 Qualitative Dried Blood Spot.
  Mixed batch testing is working on **cobas**® 5800 system and **cobas**® 6800/8800 systems with software version 2.0 or higher. Figure 14 and Figure 15 summarizes the procedure also for PSC dried plasma spot samples.

## PSC dried plasma spot sample preparation and pre-analytic procedure

- Check the integrity of the transport bag before opening. Proceed only if the transport bag is completely sealed.
- Open the transport bag and, for each sample bag, proceed only if:
    - The laboratory request form is completely filled out.
    - The barcode of the laboratory request form and the PSC match.
    - The sample bag is completely closed and contains a PSC with 4 grams of desiccant.
    - The sample collection date is available, and the sample collection occurred in the past 28 days, and before the expiration date of the PSC (see Figure 3).

- The PSC is not expired.
- The PSC dried plasma spot looks homogeneous on the front side and looks completely covered with plasma when observed from the backside (see Figure 3).

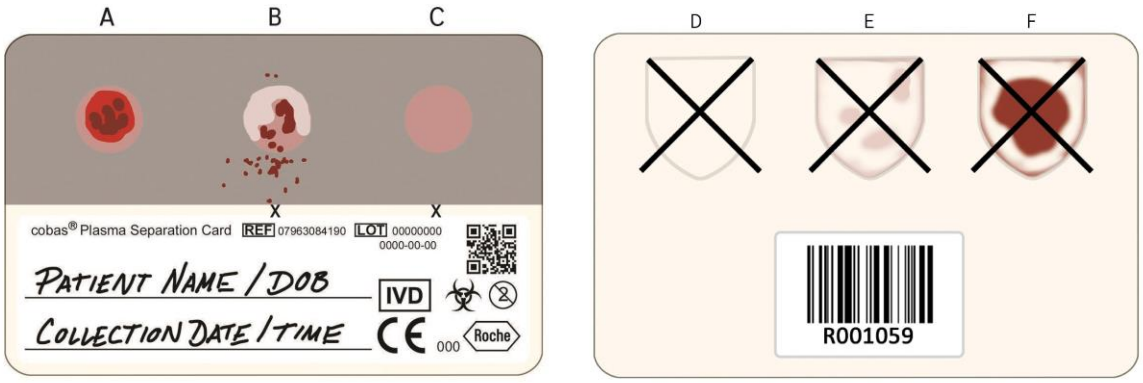
**Figure 3** PSC dried plasma spots to be processed (left: front side. right: back side)




- Acceptability of PSC dried plasma spots:
  - accept circles with small blood coagulates/clots (Figure 4 A)
  - reject if blood spills are visible (Figure 4 B&E)
  - reject if only an incomplete amount of blood was applied to the spot due to air bubbles in the capillary or blood spills (Figure 4 C&D)
  - reject if the membrane is damaged and the card shows dark red-brownish back side (Figure 4 F)

**Note: The three spots are meant for retesting. A PSC could contain a bad spot, but still provide a good sample for testing. Properly mark the bad spots, in order to be recognizable. Avoid marking them on the spotting layer. Always compare the three spots between each other to evaluate their quality.**

**Figure 4** Acceptance criteria for PSC spots (left: front side; right: back side). Spots with small blood coagulates/clots (A) are accepted. Spots with spills (B&E), with incomplete amount of blood applied (C&D) or visibly damaged membranes (F) should not be processed. Spots should be clearly marked when rejected.




- Label a tube (5 mL, internal thread, 12.5 mm diameter, polypropylene [i.e., Greiner Bio-one Cryo.s™]) for each PSC with its corresponding barcode of the laboratory request form (Figure 5) and place them into a rack. Transfer

tubes into a laminar flow hood together with the sample bags containing the PSCs. Refer to the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance for proper barcode labeling.

**Figure 5** Labeling of the tube with the corresponding barcode of the PSC


- Uncap the tubes within the laminar flow hood.
- For the PSC selected, open the sample bag and remove the spotting layer (Figure 6).

**Figure 6** Removal of spotting layer

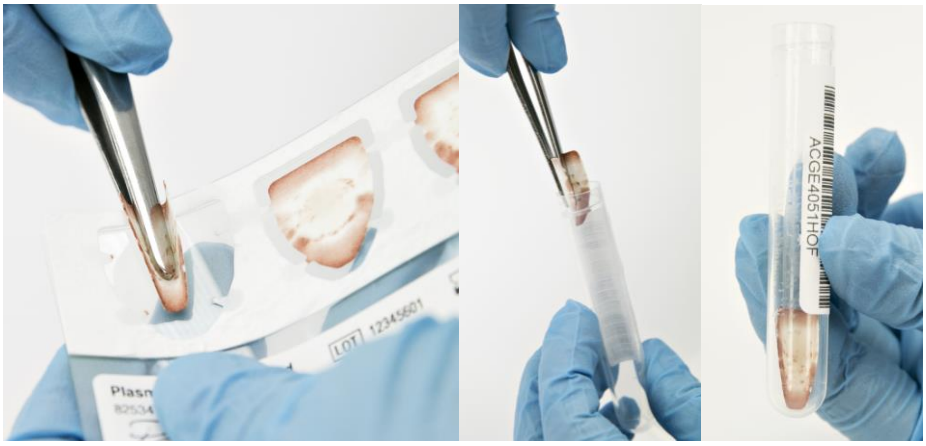
- Slightly bend the PSC and remove one dried plasma spot with sterile forceps or tweezers by pulling it up. Bend the removed dried plasma spot on the PSC to facilitate tube insertion (Figure 7). Use one pair of forceps or tweezers per patient.

**Note: Dried plasma spots may become brittle upon storage. See storage requirements for PSC. Handle them carefully while inserting into the tube.**

**Figure 7** Removal of the PSC dried plasma spot and bending

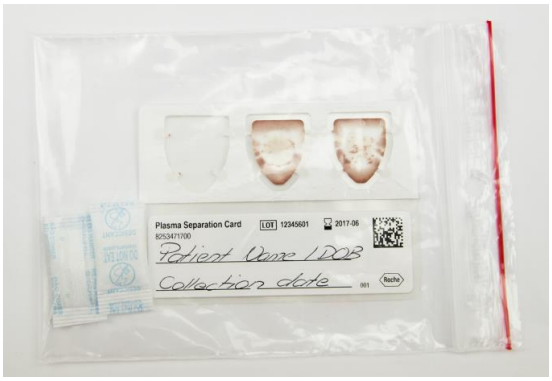
- Transfer one pre-bent PSC dried plasma spot into the corresponding tube so that the lowest tip of the dried PSC dried plasma spot reaches the bottom of the tube and is attached to the tube wall to prevent pipetting errors (Figure 8). Adjust the position of the PSC dried plasma spot with a sterile pipette tip, if necessary. Ensure the tube and the PSC of the transferred PSC dried plasma spot have the same barcode.

**Figure 8** Transfer of the PSC dried plasma spot into the tube




- Place PSC with remaining dried plasma spots back to its original sample bag containing 4 grams of fresh desiccant for retesting, if required (Figure 9). PSCs can be stored for a period of 56 days after transport with and without spotting layer separation (at 18-30°C, or at 2-8°C, or at ≤ -10°C).

**Figure 9** PSC in sample bag for potential retest

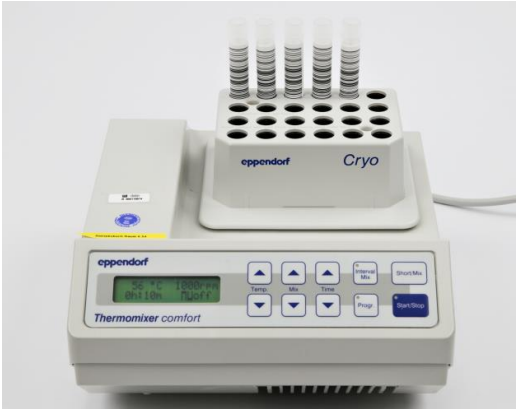



- Allow **cobas**® Specimen Pre-Extraction Reagent (SPER) to equilibrate to ambient temperature before use. Pipette 1300 µL of SPER into the tubes containing the PSC dried plasma spots (Figure 10) and cap the tubes. Make sure the tubes are properly capped to prevent evaporation.

**Figure 10** 1300 µL SPER addition

- Place tubes in each of the positions 1 - 24 on a preheated Eppendorf Thermomixer® (e.g., model R 5355 or C or equivalent) with Thermoblock for 24 cryo tubes and incubate for 10 minutes, at 56°C and 1000 rpm to extract the virus from the dried plasma (Figure 11). Start the incubation right after the addition of SPER.

**Figure 11** Incubation

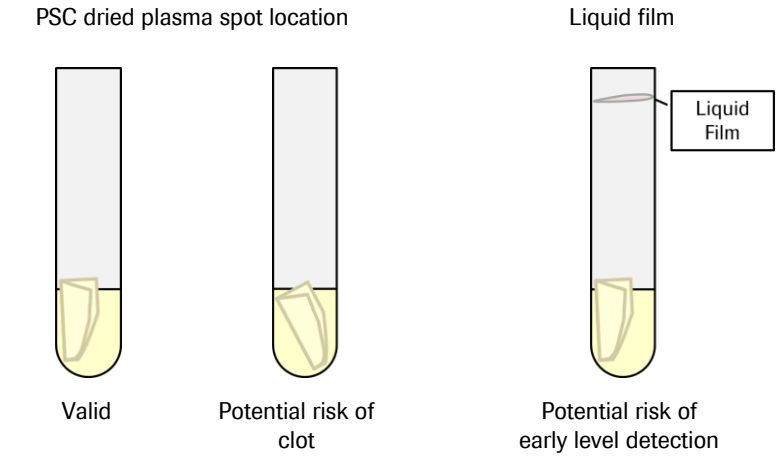



- Transfer the tubes onto a sample rack and uncap the tubes one by one to minimize cross-contamination (Figure 12). Change gloves after removing the caps.

**Figure 12** Uncapping of the tubes

- Ensure the PSC dried plasma spot is correctly placed along the tube walls (Figure 13) to avoid sample clots. Adjust the position of the PSC dried plasma spot with a sterile pipette tip, if necessary.
- Eliminate any potential liquid film located above the liquid level using a sterile pipette tip (to avoid early level detection).
- Load the tubes onto the **cobas**® 5800 system or **cobas**® 6800/8800 systems.

**Figure 13** PSC dried plasma spot preparation before the analytic workflow




**Note: Please be sure to remove any liquid film created during the process.**

**Figure 14** **cobas**® HCV test procedure on **cobas**® 5800 system

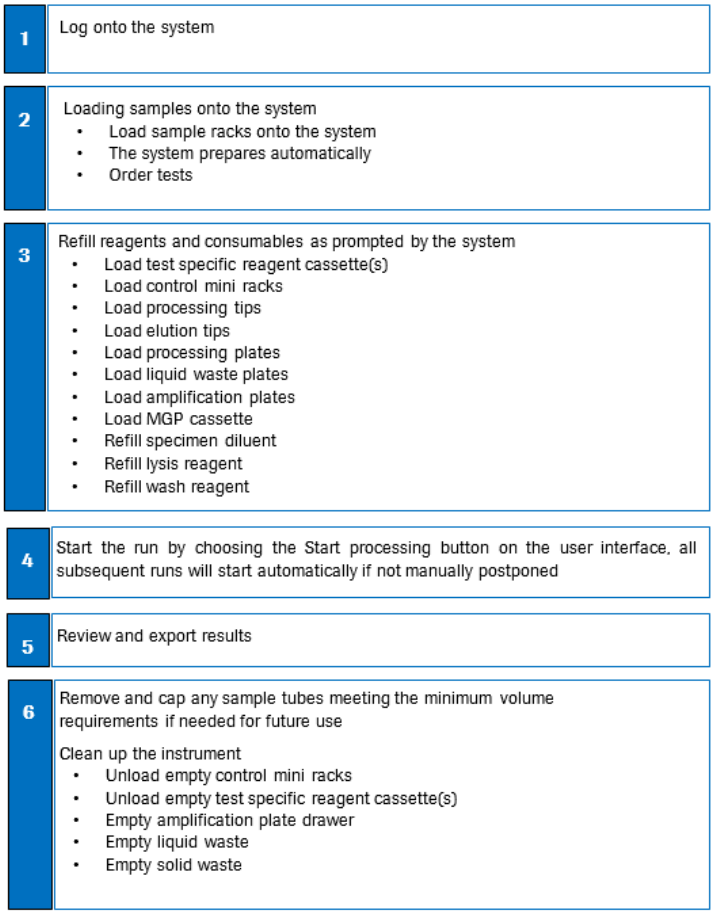
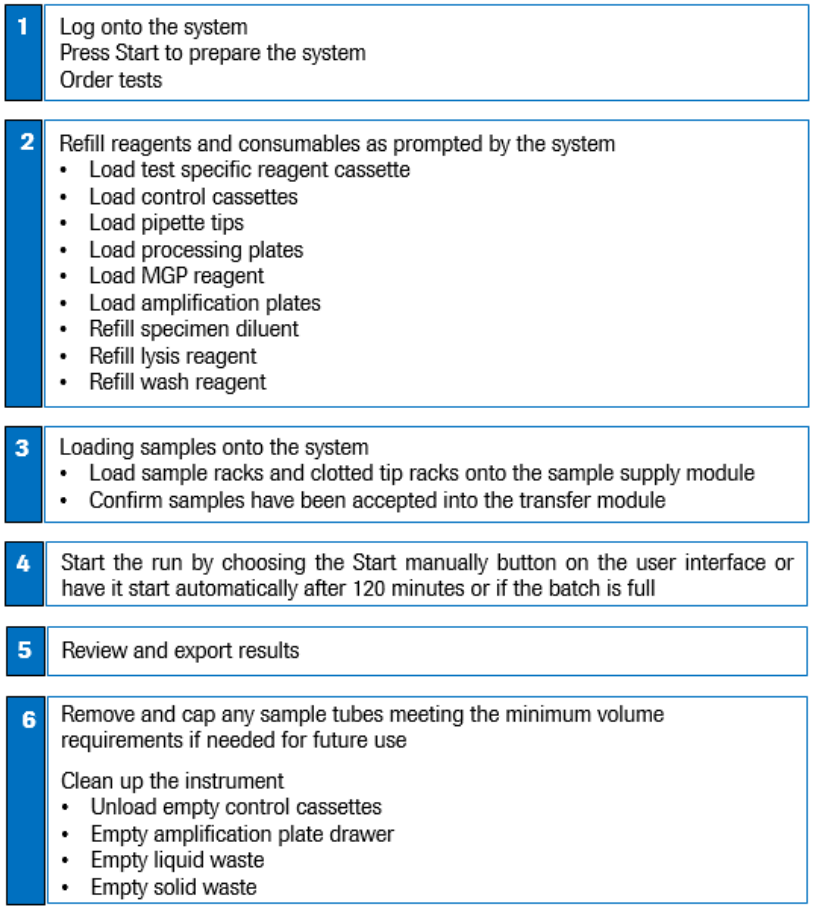


**Figure 15** **cobas**® HCV test procedure on **cobas**® 6800/8800 systems



# Results

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

## Quality control and validity of results on the cobas® 5800 system and the cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas**® NHP Negative Control [(–) C] and two **cobas**® HBV/HCV/HIV Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV H (+) C] is processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the software and/or report, check for flags and their associated results to ensure batch validity (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- The results of the controls are shown in the "Controls" app of the software.
- Controls are marked with 'Valid' in the column "Control result" if the respective target of the controls are reported valid. Controls are marked with 'Invalid' in the column "Control result" if the respective Targets of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information will be shown in the detail view.
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Validation of results is performed automatically by the instrument software based on control results.

**NOTE:** The **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

## Quality control and validity of results on the cobas® 6800/8800 systems with software version 1.4

- One **cobas**® NHP Negative Control [(–) C] and two **cobas**® HBV/HCV/HIV Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV H (+) C], are processed with each batch.
- In the software and/or report, check for flags and their associated results to ensure batch validity.
- All flags are described in the **cobas**® 6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based on control results.

## Control flags on the cobas® 6800/8800 systems with software version 1.4

**Table 17** Control flags for negative and positive controls

| Negative Control | Flag | Result | Interpretation |
|---|---|---|---|
| (-) C | Q02<br>(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<br>(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<br>(Control batch failed) | Invalid | An invalid result or the calculated titer result for the high positive control is not within the assigned range. |

## Interpretation of results for the cobas® 5800/6800/8800 systems

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

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

**Table 18** Target results for individual target result interpretation

| Results | Interpretation |
|---|---|
| Target Not Detected | HCV RNA not detected.<br>Report results as “HCV not detected.” |
| < Titer Min | Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay.<br>Report results as “HCV detected, less than (Titer Min)”<br>Titer min = 15 IU/mL (500 µL plasma/serum)<br>Titer min = 40 IU/mL (200 µL plasma/serum)<br>Titer min = 880 IU/mL (PSC) |
| 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.<br>Report results as “(Titer) of HCV detected”. |
| > Titer Max[a] | Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay.<br>Report results as “HCV detected, greater than (Titer Max).”<br>Titer max = 1.00E+08 IU/mL (500 µL and 200 µL and PSC) |

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

## Interpretation of results on the cobas® 5800 system and the cobas® 6800/8800 systems with software version 2.0 or higher

The results of the samples are shown in the in the "Results" app of the software.

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

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if the respective Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if the respective Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D: Result validation failure because of an invalid positive control
  - Q06D: Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 18 above.
- If one or more sample targets are marked with "Invalid" the software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

## Interpretation of results on the cobas® 6800/8800 systems with software version 1.4

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

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 18 above,

## Procedural limitations

- **cobas**® HCV has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma and serum and dried plasma spot samples from **cobas**® PSC. 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

## System equivalency / system comparison

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

## Key performance characteristics for EDTA plasma and serum samples

## 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, NIBSC code 06/102) genotype 1a obtained from National Institute for Biological Standards and Control (NIBSC), in HCV-negative human EDTA plasma and serum using sample processing volumes of 500 µL and 200 µL. The minimum sample requirement was 650 µL and 350 µL respectively to be processed by **cobas**® 6800/8800 systems. Panels of six concentration levels plus a negative were tested for 500 µL sample processing volume and seven concentration levels for 200 µL sample processing volume over three lots of **cobas**® HCV test reagents, multiple runs, days, operators, and instruments.
The results for EDTA plasma and serum from both sample processing volumes are shown in Table 19 to Table 22, 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 19** 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<br>95% confidence range: 7.50-9.79 IU/mL | | |

*Samples confirmed negative by alternative analytical methods.

**Table 20** 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<br>95% confidence range: 8.70–10.95 IU/mL | | |

**Table 21** 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<br>95% confidence range:22.51–28.35 IU/mL | | |

*Samples confirmed negative by alternative analytical methods.

**Table 22** 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<br>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 16 to Figure 19 for representative results.

**Figure 16** Linearity in EDTA plasma (500 µL)

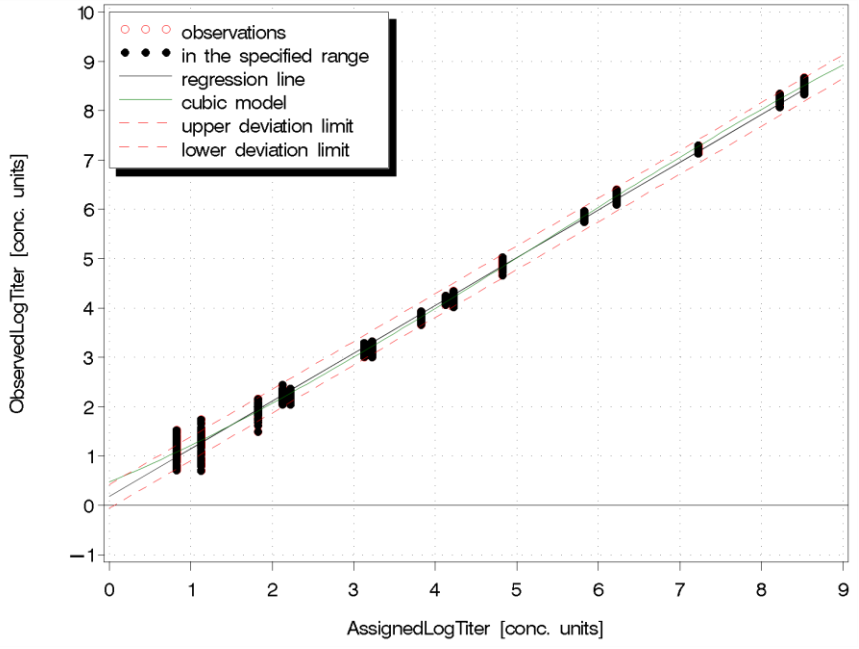



**Figure 17** Linearity in serum (500 µL)

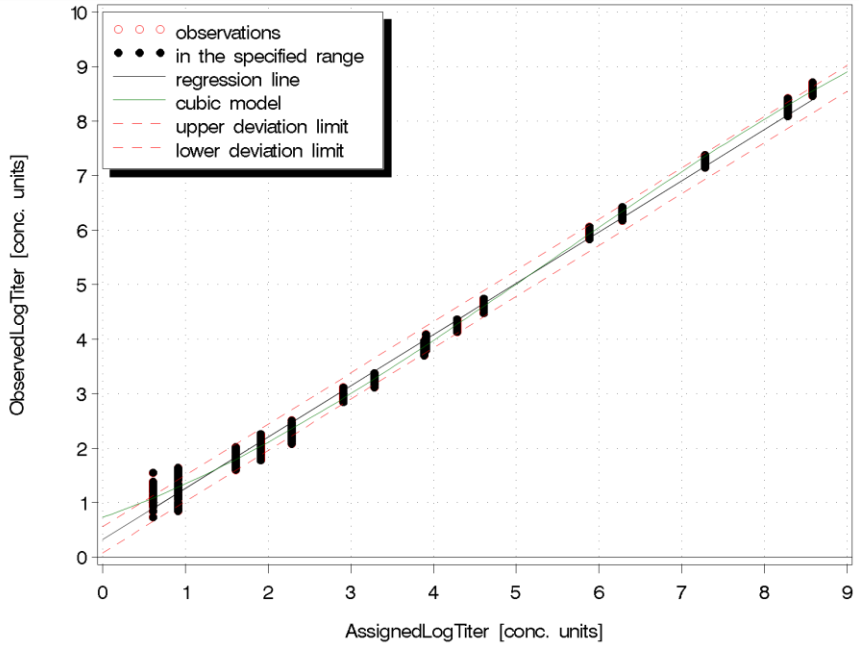


**Figure 18** Linearity in EDTA plasma (200 µL)

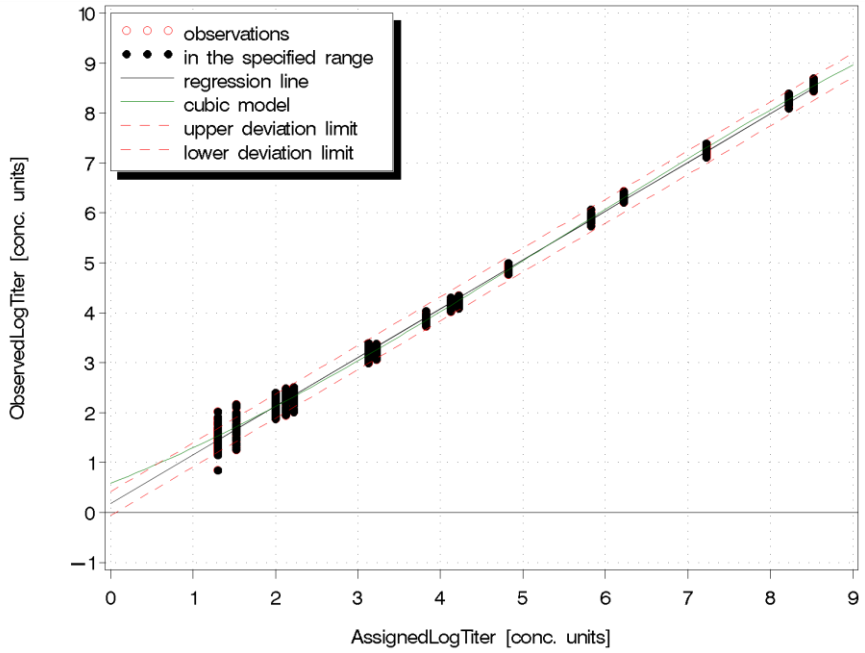
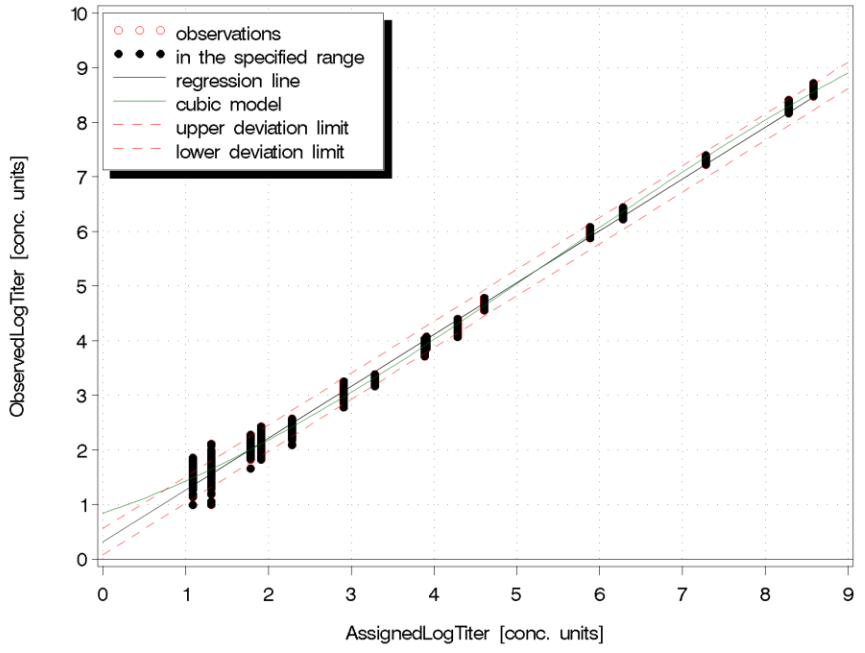



**Figure 19** Linearity in serum (200 µL)



## Precision – within laboratory

Precision of **cobas**® HCV was determined by analysis of serial dilutions of clinical HCV (Genotype 1) samples (CS) or of armored RNA HCV in HCV-negative EDTA plasma or in serum. Thirteen dilution levels were tested in plasma and 12 levels were tested in serum in two replicates for each level in two runs across 12 days adding up to a total of 48 replicates per concentration. Each sample was carried through the entire **cobas**® HCV test procedure on a fully automated **cobas**® 6800/8800 systems. Therefore, the precision reported here represents all aspects of the test procedure. The study was performed with three lots of **cobas**® HCV test reagents. The results are shown in Table 23 to Table 26.

**cobas**® HCV showed high precision for three lots of reagents tested across a concentration range of 1.0E+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 23** 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 24** 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 25** 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 26** 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 27 and Table 28, respectively. The study demonstrates that **cobas**® HCV detected all HCV genotypes tested with a similar LoD as HCV genotype 1a.

**Table 27** 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 28** 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 29 and Table 30. 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 29** HCV RNA genotype verification of limit of detection in EDTA plasma (200 µL)

| Genotype | 17.5 IU/mL | | | 33 IU/mL | | | 50 IU/mL | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) |
| 1b | 63 | 50 | 79.4 | 63 | 61 | 96.8 | 63 | 63 | 100.0 |
| 2 | 63 | 51 | 81.0 | 63 | 62 | 98.4 | 63 | 62 | 98.4 |
| 3 | 63 | 56 | 89.0 | 63 | 58 | 92.1 | 63 | 63 | 100.0 |
| 4 | 63 | 54 | 85.7 | 63 | 57 | 90.5 | 63 | 63 | 100.0 |
| 5 | 63 | 57 | 90.5 | 63 | 61 | 96.8 | 63 | 63 | 100.0 0 |
| 6 | 63 | 47 | 74.6 | 63 | 57 | 90.5 | 63 | 62 | 98.4 |

* Upper one-sided 95% confidence interval

**Table 30** HCV RNA genotype verification of limit of detection in serum (200 µL)

| Genotype | 17.5 IU/mL | | | 33 IU/mL | | | 50 IU/mL | | |
|---|---|---|---|---|---|---|---|---|---|
| | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) | Number of valid replicates | Number of positives | Hit rate in % (95% CI*) |
| 1b | 63 | 52 | 82.5 | 63 | 61 | 96.8 | 63 | 63 | 100.0 |
| 2 | 63 | 46 | 73.0 | 63 | 62 | 98.4 | 63 | 59 | 93.7 |
| 3 | 63 | 58 | 92.1 | 63 | 63 | 100.0 63063 | 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 31** 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 32 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 32** 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<br>Peginterferon α-2b<br>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 |

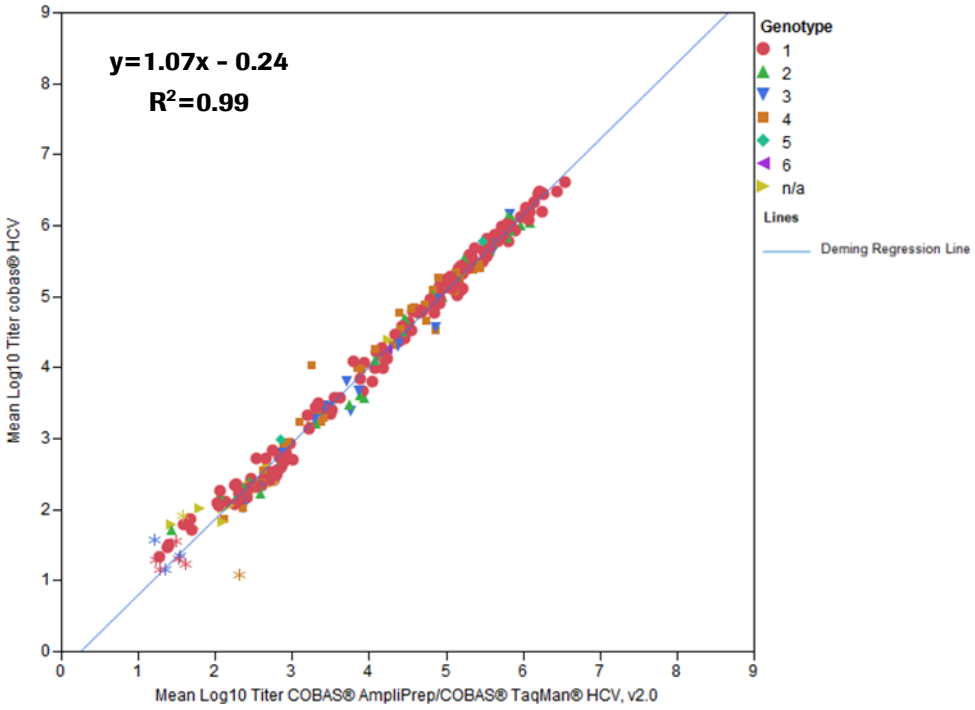
## Method correlation

### 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 20. The symbol ✳ in Figure 20 shows single determination.

**Figure 20** Regression analysis of **cobas**® HCV vs TaqMan® HCV Test, v2.0, EDTA plasma and serum samples

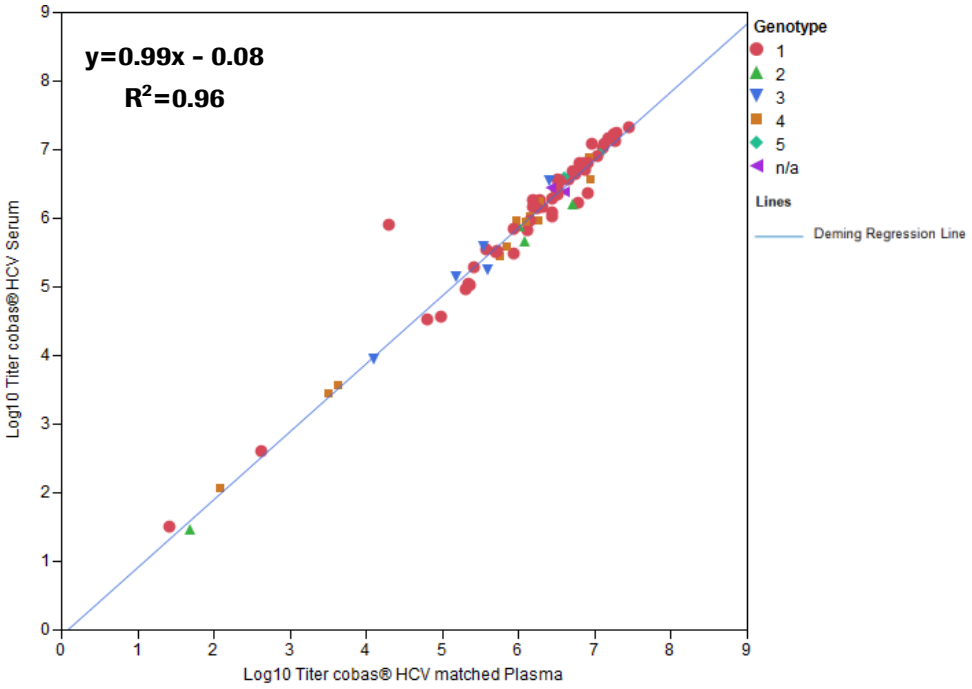



## Matrix equivalency – EDTA plasma versus serum

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 21).

**Figure 21** 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%].

# Key performance characteristics for PSC dried plasma spot samples

## Limit of Detection (LoD) using cobas® PSC

The plasma limit of detection of **cobas**® HCV in combination with **cobas**® PSC was determined by analysis of plasma titers assigned to serial dilutions of a HCV positive clinical specimen, in HCV-negative human whole blood. Panels of six concentration levels plus a negative donor sample were tested over three lots of PSCs and one lot of **cobas**® HCV test reagents, multiple runs, days, operators, and instruments.

The combined results from three **cobas**® PSC lots, negative donors and dilution series with individual plasma titer assignments (OPN) are shown in Table 33. The study demonstrates that **cobas**® HCV in combination with the **cobas**® PSC detected HCV RNA at a concentration of 534.4 IU/mL with a hit rate of 95% as determined by Probit analysis.

**Table 33** Limit of detection in combination with the PSC

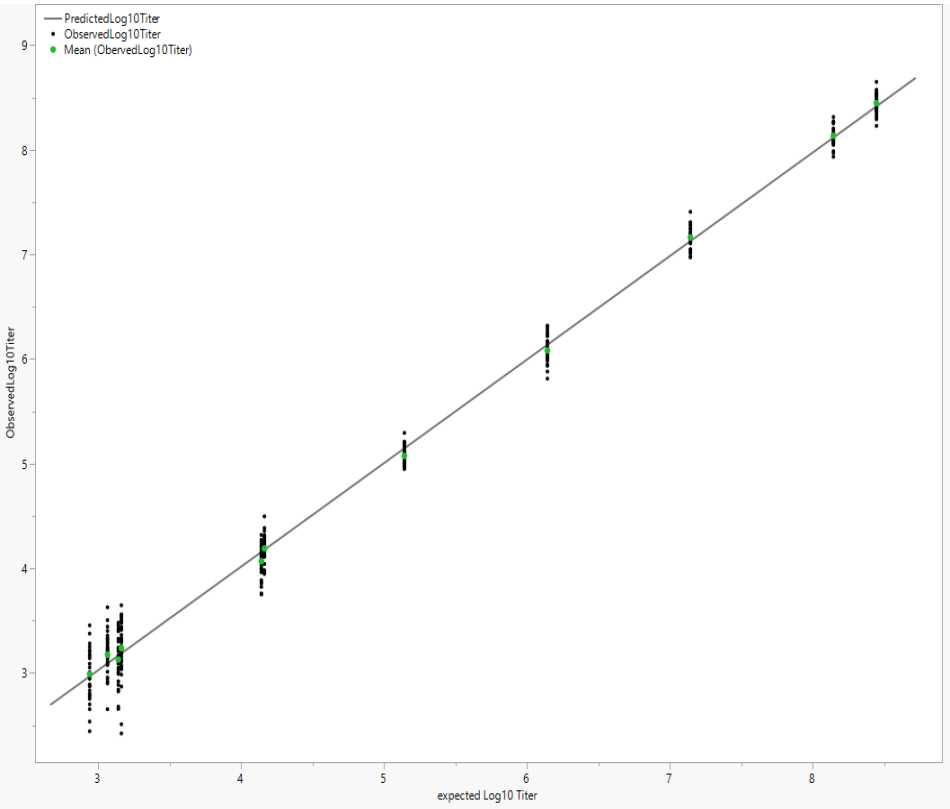
| Assigned plasma titer concentration (HCV RNA IU/mL) | Number of valid replicates | Number of positives | Hit rate in % |
|---|---|---|---|
| 3939.0 | 63 | 63 | 100.0 |
| 3514.8 | 63 | 63 | 100.0 |
| 3706.9 | 63 | 63 | 100.0 |
| 1969.5 | 63 | 63 | 100.0 |
| 1757.4 | 63 | 63 | 100.0 |
| 1853.5 | 63 | 63 | 100.0 |
| 984.8 | 63 | 63 | 100.0 |
| 878.7 | 63 | 63 | 100.0 |
| 926.7 | 63 | 63 | 100.0 |
| 492.4 | 63 | 60 | 95.2 |
| 439.4 | 63 | 57 | 90.5 |
| 463.4 | 63 | 59 | 93.7 |
| 246.2 | 63 | 43 | 68.3 |
| 219.7 | 63 | 42 | 66.7 |
| 231.7 | 63 | 43 | 68.3 |
| 123.1 | 63 | 21 | 33.3 |
| 109.8 | 63 | 30 | 47.6 |
| 115.8 | 63 | 33 | 52.4 |
| 0 | 189 | 0 | 0.0 |
| LoD by PROBIT at 95% hit rate | 534.4 IU/mL; 95% confidence range: (460.0 – 648.3 IU/mL) | | |

## Linear range using cobas® PSC

The linearity study of **cobas**® HCV in combination with **cobas**® PSC was performed with a dilution series consisting of 11 panel members spanning the linear range. 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. Three PSC lots and two reagent lots were analyzed on two **cobas**® 6800/8800 systems and three **cobas**® 5800 systems, by three operators and in total 36 replicates per panel member.

In combination with **cobas**® PSC, **cobas**® HCV is linear from 880 IU/mL to 1.00E+08 IU/mL and shows a maximum deviation from linearity of less than ± 0.3 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.3 $\log_{10}$.

**Figure 22** Linear range determination using **cobas**® PSC



## Precision – within laboratory using cobas® PSC

Precision of **cobas**® HCV in combination with **cobas**® PSC was determined by analysis of serial dilutions of an HCV high positive sample (high titer HCV armored RNA) in HCV-negative EDTA whole blood. Five dilution levels were tested in 72 replicates for each level across three lots of PSC and two lots of **cobas**® HCV test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire PSC workflow and **cobas**® HCV procedure on the fully automated **cobas**® 6800/8800 systems and **cobas**® 5800 system. The precision results reported here represent all aspects of the test procedure. The results are shown in Table 34.

**cobas**® HCV in combination with **cobas**® PSC showed high precision for three lots of PSC, two lots of reagents and three different **cobas**® 5800/6800/8800 systems tested across a concentration range of 1.75+03 IU/mL to 8.74E+07 IU/mL.

**Table 34** Within laboratory precision of **cobas**® HCV in combination with **cobas**® PSC*

| Measured HCV armored RNA concentration (IU/mL in centrifuged EDTA plasma) | Source material | Pooled SD |
|---|---|---|
| 8.74E+07 | aRNA | 0.08 |
| 8.74E+05 | aRNA | 0.09 |
| 8.74E+04 | aRNA | 0.09 |
| 8.74E+03 | aRNA | 0.14 |
| 1.75E+03 | aRNA | 0.17 |

*PSC = Plasma Separation Card

## Whole system failure using cobas® PSC

The whole system failure rate for **cobas**® HCV in combination with **cobas**® PSC was determined by testing 100 replicates of EDTA whole blood spiked with a HCV positive clinical specimen. 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 the HCV target, resulting in a whole system failure rate of 0%.

## Performance of PSC dried plasma spot samples compared to EDTA plasma samples

The performance of **cobas**® HCV in combination with PSC dried plasma spot samples from capillary (PSC capillary) blood for the quantitative determination of HCV RNA compared to EDTA plasma was evaluated in clinical specimens.

A total of n=299 subjects were enrolled in an external multicenter study including 3 collection sites and 3 testing sites. Out of the n=299 subjects, n=105 were subjects with active HCV infection (detectable HCV RNA in EDTA plasma (>LLOQ on **cobas**® HCV) and positive anti-HCV status). Correlation of **cobas**® HCV results between PSC capillary vs. EDTA plasma was performed by analysis of n=93 matched clinical specimens. Only HCV RNA results within the overlapping linear range of **cobas**® HCV for both sample matrices (880 IU/mL to 1.00 E+08 IU/mL) tested on the **cobas**® 6800 system were included in this analysis.

An overview of the correlation between **cobas**® PSC capillary and EDTA plasma for the quantification of HCV RNA on **cobas**® HCV for use on the **cobas**® 5800/6800/8800 systems using Deming regression and Bland-Altman analysis is shown in Table 35.

**Table 35** Correlation of **cobas**® HCV results between **cobas**® PSC capillary and EDTA plasma using Bland-Altman and Deming Regression Analysis

| Sample Type Comparison | Number of paired Samples (n) | Bland-Altman Mean of paired Difference HCV RNA ($log_{10}$ IU/mL) (95% CI) [a] | Bland-Altman 95% limits of agreement [b] | Deming Regression Intercept (95% CI) | Deming Regression Slope (95% CI) | Deming Regression R-Squared |
|---|---|---|---|---|---|---|
| PSC Capillary vs. EDTA plasma | 93 | 0.002 (-0.089, 0.092) | (-0.857, 0.860) | 0.82 (-0.08, 1.71) | 0.87 (0.73, 1.00) | 0.77 |

[a] The mean of paired difference is calculated as the sum of (HCV RNA results [$log_{10}$ IU/mL] in PSC minus the HCV RNA results [$log_{10}$ IU/mL] in EDTA plasma), divided by the total number of paired samples.

[b] 95% limits of agreement is calculated as the mean of paired differences ± 1.96 standard deviation of the observed differences.

# 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 36 through Table 39 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 36 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $\log_{10}$ HCV RNA concentration for the **cobas**® 6800 system.

**Table 36** Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration ($\log_{10}$ IU/mL) by genotype and positive panel member on the **cobas**® 6800 system (lot-to-lot)

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

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

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

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

[a] Calculated using the SAS MIXED procedure.

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

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

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

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

In Table 37 below, the negative percent agreement (NPA) for the **cobas**® 6800 system using negative panel member tests was 99.54%.

**Table 37** 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 38 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $log_{10}$ HCV RNA concentration on the **cobas**® 6800 system.

**Table 38** Attributable percentage of total variance, total precision standard deviation and lognormal CV(%) of HCV RNA concentration ($log_{10}$ IU/mL) by genotype and positive panel member on the **cobas**® 6800 system (reproducibility)

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

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

[a] Calculated using the SAS MIXED procedure.

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

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

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

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

The NPA was 100% using negative panel member tests on the **cobas**® 6800 system as presented in Table 39 below.

**Table 39** Negative percent agreement using the negative panel member (reproducibility) on the **cobas**® 6800 system

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

[a] Negative Percent Agreement = (number of negative results / total number of valid tests in negative panel member) * 100.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; HCV = hepatitis C virus; No. = number; RNA = ribonucleic acid.

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

An identical sample set was tested for lot-to-lot variability and reproducibility of **cobas**® HCV on the **cobas**® 8800 system. The performance of the two systems is comparable. Table 40 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 40** Comparison of precision standard deviation of HCV RNA concentration ($log_{10}$IU/mL) for Genotypes 1 - 3 on **cobas**® 6800 and **cobas**® 8800 systems (reproducibility)

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

Note: Grouping of observed precisions to concentration levels are based on the median test results on the untransformed scale (IU/mL). The table only includes results with detectable viral load. SD = standard deviation.
'-' Indicates no applicable results for this level.
[a] Precision Standard Deviation in $log_{10}$ units
[b] Number of valid tests with detectable viral load.

## Clinical utility with EDTA plasma and serum samples

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

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

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

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

Table 41 below shows the demographic and baseline characteristics of subjects whose samples were tested on the **cobas**® 6800 and the **cobas**® 8800 systems. The majority of subjects were male, over 40 years of age, and infected with HCV genotype 1. Subjects with HCV genotypes 1, 2, and 3 were enrolled. HCV infection with genotypes 4, 5, and 6 is rare in the US.

**Table 41** Demographics and baseline characteristics of subjects for the **cobas**® 6800 and **cobas**® 8800 systems

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

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

## Prediction of response to antiviral therapy

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

**Definitions:**

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

**Predictive value of Virological Response to success of antiviral therapy**

In this study, the positive predictive value (PPV) for Week 4 VL to predict SVR12 was 78.1% (95% CI: 72.7 to 82.8%) in genotype 1 subjects and 84.7% (95% CI: 73.5 to 91.8%) in subjects with non-1 genotypes (Table 42). Therefore, VR at Week 4 measured by **cobas**® HCV was a useful predictor of SVR 12.

For Treatment Plan 1, as a representative of a DAA containing regimen, a Week 12 VL or Week 24 VL on **cobas**® HCV predicts SVR12 in genotype 1 subjects, with PPVs of 77.0% and 78.6%, respectively. The absence of Week 12 VL or Week 24 VL predicts non-response, with negative predictive values (NPVs) of 87.5% and 100%, respectively (Table 42). Additional analysis of Week 2 VL to predict SVR12 shows a PPV of 79.4% but a low NPV of 29.9%.

In Treatment Plan 2, Week 12 VL using **cobas**® HCV in genotype 2 and 3 was predictive of SVR12, with a PPV of 75.3%. Due to the rarity of non-response, absence of Week 12 VL is not a useful measure of outcome in this population. The NPV was 50% and the number of non-responders was small in this study (Table 42).

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

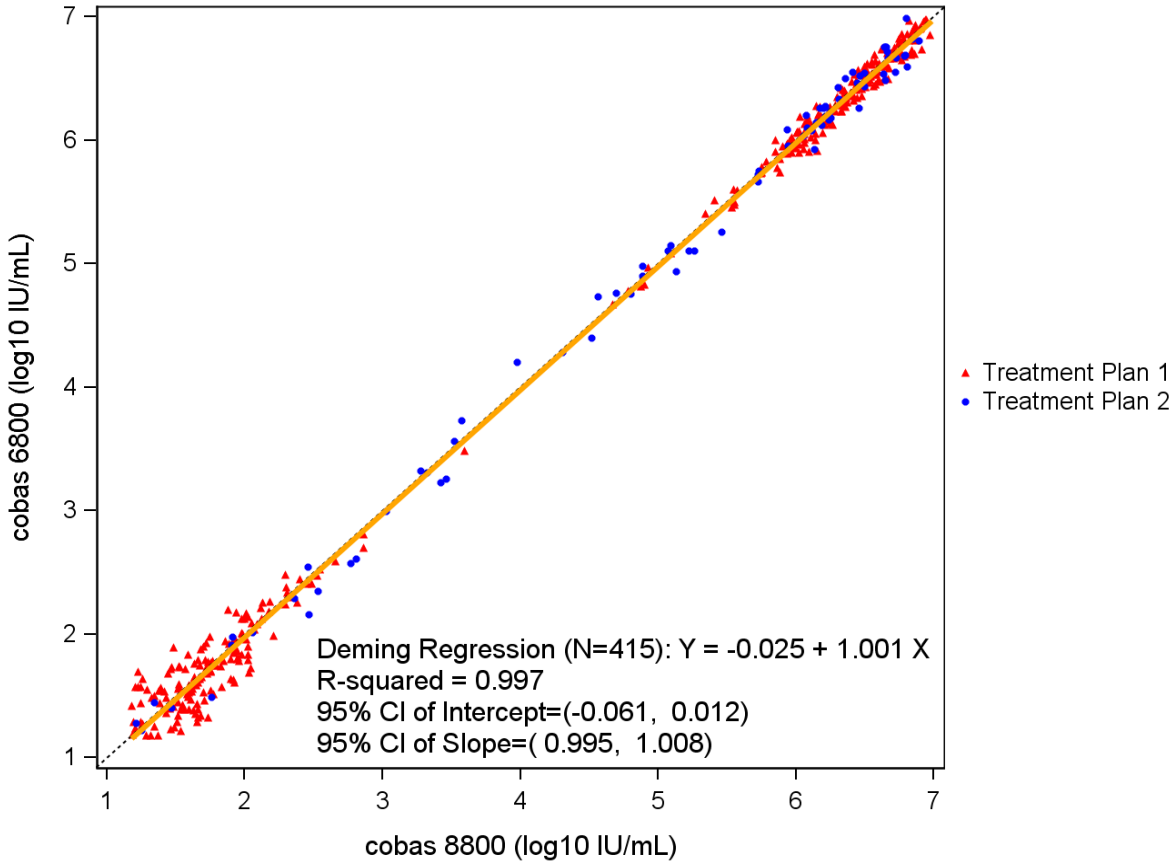
| | | | | PPV (%) | | NPV (%) | | OR |
|---|---|---|---|---|---|---|---|---|
| **Treatment Plan** | **Genotype** | **On-Treatment Visit** | **Eligible Subjects** | **Estimate (95% CI)** | **n / N** | **Estimate (95% CI)** | **n / N** | **Estimate (95% CI)** |
| 1 | 1 | Week 2 | 290 | 79.4 (71.5, 85.5)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

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

## Comparison between cobas® 6800 and cobas® 8800 systems – clinical utility

An identical sample set was tested for the clinical utility of **cobas**® HCV on the **cobas**® 8800 system. The systems demonstrate highly correlated performance that were not significantly different. Figure 23 below show a Deming regression plots of VLs ($log_{10}$ IU/mL) greater than 15 IU/mL at all applicable time points on treatment.

**Figure 23** 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 43 below show the demographic and clinical characteristics of subjects whose samples were tested on the **cobas**® 6800 system and **cobas**® 8800 system.

**Table 43** 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 44). 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 44).

**Table 44** 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 45**,** Table 46**,** Table 47).

**Table 45** Demographic and clinical characteristics by system

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

**Table 46** Number of HCV RNA negative samples on the **cobas**® 6800 system with non HCV-related liver diseases within test result categories by clinical condition

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

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

The single subject with Hepatic Steatosis liver disease was excluded.

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

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

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

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

**Table 47** Number of HCV RNA negative samples on the **cobas**® 8800 system with non HCV-related liver diseases within test result categories by clinical condition

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

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

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

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

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

## Comparison between cobas® 6800 and cobas® 8800 systems for diagnosis

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

# Conclusion

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

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

# 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)<br>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 |

* Dead volume of 150 µL identified for the **cobas**® omni Secondary tubes. Other tubes used for testing may have different dead volume and require more or less minimum volume. Contact your local Roche service representative for further information.

## Key test features for PSC dried plasma spot samples

| | |
|---|---|
| **Sample type** | Dried plasma spot coming from Plasma Separation Card |
| **Minimum amount of sample required** | 140 µL whole blood |
| **Sample processing volume** | 850 µL |
| **Analytical sensitivity** | 534.4 IU/mL |
| **Linear range** | 880 IU/mL – 1.0E+08 IU/mL |

# Symbols

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

**Table 48** Symbols used in labeling for Roche PCR diagnostics products

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

## Technical support

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

## Manufacturer and importer

**Table 49** Manufacturer and importer

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

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

## Trademarks and patents

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

## Copyright



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

CE 2797

IVD

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# Document revision

| Document Revision Information | |
|---|---|
| Doc Rev. 3.1<br>12/2024 | Added system software version 2.0 information for **cobas**® 6800/8800 systems.<br>Please contact your local Roche Representative if you have any questions. |
| Doc Rev. 4.0<br>02/2025 | Removed **cobas**® 6800/8800 systems software version 2.0 information introduced under IVDD.<br>Revised to comply with IVDR, including use of EU Importer and summary of safety and performance.<br>Updated the HxV Control kits hazard information.<br>Updated the harmonized symbol page.<br>Corrected typographical errors.<br>Added **cobas**® 5800 specific information.<br>Added intended use for **cobas**® HBV/HCV/HIV-1 Control Kit.<br>Updated **cobas**® branding.<br>Addition of new sample type **cobas**® Plasma Separation Card<br>Removed Rx Only from front page.<br>Updated **cobas**® branding.<br>Updated References.<br>Please contact your local Roche Representative if you have any questions. |
| Doc Rev. 5.0<br>03/2025 | Added system software version 2.0 information for **cobas**® 6800/8800 systems.<br>P/Ns of consumables removed, detailed information on consumables are referenced in the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance.<br>Updated the harmonized symbol page.<br>Added IVD symbol.<br>Please contact your local Roche Representative if you have any questions. |

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