cobas® HCV GT

HCV genotyping test
for use on the cobas® 4800 System

For in vitro diagnostic use

cobas® HCV GT	120 Tests	P/N: 06984274190
cobas® HCV GT Control Kit	10 Sets	P/N: 06984339190
cobas® 4800 System Sample Preparation Kit 2	240 Tests 960 Tests	P/N: 06979513190 P/N: 06979521190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Lysis Kit 2	240 Tests 960 Tests	P/N: 06979530190 P/N: 06979548190

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Additional information

Intended use

cobas® HCV GT is an *in vitro* nucleic acid amplification test for the qualitative identification of Hepatitis C Virus (HCV) genotypes 1 to 6 and genotype 1 subtypes a and b in human plasma or serum from individuals with chronic HCV infection, using the **cobas**® 4800 system: the **cobas x** 480 instrument for automated specimen processing and the **cobas z** 480 analyzer for automated amplification and detection. **cobas**® HCV GT is intended for use in selecting individuals with chronic HCV infection for antiviral therapy and in determining the duration of therapy regimens according to the antiviral therapy prescribing information.

Summary and explanation of the test

Background

HCV is considered to be the principal etiologic agent responsible for 90% to 95% of the cases of post-transfusion hepatitis.[1-4] HCV is a single-stranded, positive sense RNA virus with a genome of approximately 9,500 nucleotides coding for 3,000 amino acids. HCV genotypes and subtypes are distributed throughout the world with various genotypes dominating in specific geographic areas. The diversity of HCV genotypes that can be present at a given geographical location requires identification of the infecting genotype before a patient is prescribed antiviral therapy.

Rationale for HCV genotype testing

Historically, patients with chronic HCV infection have been assigned to various treatment durations with pegylated interferon plus ribavirin, after determination of the infecting HCV genotype, based on the likelihood of achieving sustained virological response (SVR) at the end of antiviral treatment.[5] Patients with genotype 2 and 3 were assigned 24 weeks of pegylated interferon plus ribavirin versus patients with genotype 1 that showed higher success rates with 48 weeks of therapy. With the approval of a multitude of direct acting antiviral (DAA) inhibitors since 2011, there are now multiple approved regimen options per genotype[6] with specific drug combination and treatment duration guidance. Use of a specific regimen is more dependent on local, country specific drug registration and medical reimbursement status. It is generally accepted that the HCV genotype and genotype 1 subtype (1a/1b) must be assessed prior to treatment initiation and will determine the choice of therapy.

Explanation of the test

cobas® HCV GT is a qualitative nucleic acid amplification test performed on the **cobas**® 4800 System. **cobas**® HCV GT uses real-time reverse transcription-polymerase chain reaction (RT-PCR) to identify HCV genotypes 1 to 6 and subtypes 1a and 1b through the use of genotype and subtype specific primers and fluorescent dye-labeled oligonucleotide probes. The test also detects HCV, regardless of genotype, using primers and probes in a highly conserved region of the HCV genome, which serves as an internal control to monitor the entire sample preparation and RT-PCR process.

Principles of the procedure

cobas® HCV GT is based on fully automated sample preparation (nucleic acid extraction and purification) followed by real-time RT-PCR amplification and detection. The **cobas**® 4800 System consists of the **cobas x** 480 instrument for automated specimen processing and the **cobas z** 480 analyzer for automated amplification and detection. Automated data management is performed by the **cobas**® 4800 software which assigns test results for all tests as one or more genotypes and

subtypes, Indeterminate (HCV RNA detected but no genotype or subtype identified) or Invalid (HCV RNA not detected). Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acids from patient samples and external controls are released by addition of proteinase and a chaotropic lysis reagent to the sample. The released nucleic acids bind to the silica surface of magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps, and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature.

Each sample is amplified in three real-time RT-PCR reactions. Genotype and subtype specific RT-PCR amplification and detection of HCV genotypes 1 to 6 and subtypes 1a and 1b is achieved through the use of genotype and subtype specific primers and fluorescent dye-labeled probes. Each reaction also includes primers and probes for a highly conserved region of the HCV genome, for amplification and detection of HCV regardless of genotype, as an internal control. The probes are labeled with four different fluorescent reporter dyes, allowing simultaneous detection of HCV and up to three genotypes or subtypes in each reaction.

A thermostable DNA polymerase is used for both reverse-transcription and PCR amplification. The master mixes include deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[7-9] Any contaminating amplicon from previous PCR runs are inactivated as PCR templates by AmpErase, which is present in the master mixes, prior to the first denaturation step of PCR. AmpErase catalyzes the removal of uracil from DNA, but has no activity on RNA or naturally occurring DNA, which does not contain uracil. Amplicon formed during subsequent cycles of PCR are not inactivated since AmpErase is inactive at the annealing and denaturation temperatures of PCR.

Each of the oligonucleotide probes in the **cobas**® HCV GT master mixes is labeled with a non-fluorescent quencher dye and a fluorescent reporter dye. When probes are intact the fluorescence of the reporter dyes is suppressed by the quencher dye. During PCR amplification, the probes hybridize to their target sites between the primer binding sites, and DNA polymerase extends the primers. The 5'-to-3' nuclease activity of the DNA polymerase cleaves the hybridized probes, 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. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes in each cycle of PCR, which is done automatically by the **cobas z** 480 analyzer.

Materials and reagents

Reagents

All unopened reagents and controls shall be stored as recommended in the Reagent storage and handling requirements table.

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HCV GT 120 Tests (P/N: 06984274190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 x 1.75 mL	N/A
	HCV GT MMX R2A (**cobas**® HCV GT Master Mix Reagent 2A) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTP, < 0.01% HCV primers, < 0.01% fluorescent-labeled oligonucleotide probes,< 0.01% oligonucleotide aptamer, < 0.01% polyadenylic acid, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A
	HCV GT MMX R2B (**cobas**® HCV GT Master Mix Reagent 2B) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTP, < 0.01% HCV primers, < 0.01% fluorescent-labeled oligonucleotide probes,< 0.01% oligonucleotide aptamer, < 0.01% polyadenylic acid, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A
	HCV GT MMX R2C (**cobas**® HCV GT Master Mix Reagent 2C) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTP, < 0.01% HCV primers, < 0.01% fluorescent-labeled oligonucleotide probes,< 0.01% oligonucleotide aptamer, < 0.01% polyadenylic acid, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	10 x 0.5 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HCV GT Control Kit 10 Sets (P/N: 06984339190)	**HCV GT (+)C** (**cobas**® HCV GT Positive Control) < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc ; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	10 x 0.75 mL	  Warning H317 May cause an allergic skin reaction. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272 Contaminated work clothing should not be allowed out of the workplace. P280 Wear protective gloves. P333 + P313 If skin irritation or rash occurs: Get medical advice/attention. P362 + P364 Take off contaminated clothing and wash it before reuse. P501 Dispose of contents/container to an approved waste disposal plant.
	(–) C (**cobas**® Negative Control) Normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	10 x 0.75 mL	
cobas® 4800 System Sample Preparation Kit 2 240 Tests (P/N: 06979513190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 x 8 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 x 17 mL	
cobas® 4800 System Sample Preparation Kit 2 960 Tests (P/N: 06979521190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 x 16 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 x 17 mL	
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 x 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 x 200 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 x 1.0 mL	 Danger H302+H332 Harmful if swallowed or if inhaled. H315 Causes skin irritation. H318 Causes serious eye damage. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ eye protection/ face protection. P284 Wear respiratory protection. 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 or doctor/ physician. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician. P362 + P364 Take off contaminated clothing and wash it before reuse.
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 x 27 mL	

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 x 1.0 mL	 Danger H302+H332 Harmful if swallowed or if inhaled. H315 Causes skin irritation. H318 Causes serious eye damage. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P280 Wear protective gloves/ eye protection/ face protection. P284 Wear respiratory protection. 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 or doctor/ physician. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician. P362 + P364 Take off contaminated clothing and wash it before reuse.
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 x 84 mL	

[a] Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagent	Storage Temperature	Storage Time
cobas® HCV GT	2–8°C	Stable until the expiration date indicated
cobas® HCV GT Control Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Sample Preparation Kit 2	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 2	2–8°C	Stable until the expiration date indicated

Do not freeze reagents.

Additional materials required

Materials	P/N
cobas® 4800 System Extraction (deepwell) Plate 2.0 mL	06884008001
cobas® 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.1.0 or higher
cobas® 4800 System **cobas**® HCV GT AP v1.0.0 or higher

Refer to the **cobas**® 4800 System Operator's Manual – Software Version 2.1 or higher for **cobas**® HCV GT for use on the **cobas**® 4800 System (hereafter referred to as the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT) for additional information for primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, tip racks, reagent racks and plate carriers 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 analytical sensitivity of this test, care should be taken to keep reagents, specimens and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- **cobas**® HCV GT is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection.
- 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.[10,11] Only personnel proficient in handling infectious materials and the use of **cobas**® HCV GT and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
- **cobas**® HCV GT Control Kit contains plasma derived from human blood. The source material has been tested by licensed serology tests and found to be non-reactive for antibody to HCV, antibody to HIV-1/2, HBsAg and antibody to HBc. Testing by PCR methods showed no detectable HIV-1, 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.
- Prevent exposure of MGP to sources of magnetic field.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.

- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT. If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT.

Note: *For specific instructions, see "Specimen collection, transport, and storage".*

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens and kit reagents, and after removing the gloves.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

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 bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 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 GT and **cobas**® 4800 Sample Preparation Kit 2 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**® 4800 Lysis Buffer 2, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

- Gloves must be worn and must be changed between handling specimens and **cobas**® HCV GT reagents to prevent contamination. Avoid contaminating gloves when handling specimens and controls. Wear lab gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of specimens is not prevented during specimen handling.

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use disposable items after their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® HCV GT and **cobas**® 4800 System Sample Preparation Kit 2 contain sodium azide (see "**Warnings and precautions**"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: ***For disposal of liquid waste, refer to the appropriate cobas® 4800 System - System Manual.***

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the appropriate **cobas**® 4800 System - System Manual to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or the **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or the **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - System Manual.

Specimen collection, transport, and storage

Note: Handle all specimens as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

Specimen collection

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.

Note: ***The user must follow the guidance provided by the tube manufacturer for serum/plasma preparation.***

Specimen transport storage and stability

- 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 and subsequent testing.
- Plasma/serum samples may be stored in secondary tubes for up to 24 hours at 2°C to 25°C, up to 72 hours at 2°C to 8°C or up to 6 weeks at ≤ -18°C. Separated plasma/serum samples in secondary tubes are stable for up to three freeze/thaw cycles when stored frozen at ≤ -18°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

Running the test

Figure 1: cobas® HCV GT workflow

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Start run
5	Load samples
6	With LIS: confirm work order Without LIS: create work order
7	Load consumables (deepwell plate, microwell plate, tip racks)
8	Load reagents
9	Start sample preparation run
10	Unload and seal microwell plate
11	Load microwell plate into analyzer
12	Remove samples, used reagents, and deepwell plate
13	Review results
14	With LIS: send results to LIS
15	Unload analyzer

Note: *Refer to the cobas® 4800 System Operator's Manual for cobas® HCV GT for detailed operating instructions.*

Run Size

The generic sample preparation reagents (**cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit) are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and specimens to be run. **cobas**® HCV GT is available in one kit size sufficient to test up to 120 (10 × 12) samples, including controls and specimens. The **cobas**® HCV GT Control Kit is available in one kit size of 10 sets of negative and positive control and can support all run configurations. For each test batch, one HCV GT Positive Control and one Negative Control must be used. For a single test run of **cobas**® HCV GT, the maximum number of samples allowed is 30 specimens and 2 controls. The test procedure is described in detail in the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT. Figure 1 summarizes the procedure.

Note: ***For optimal use of reagents, the generic sample preparation reagents can be used for a run containing 1-22 total specimens (10 × 24 test kit size) or 1-30 total specimens (10 × 96 test kit size). Different kit sizes of the cobas® 4800 System Wash Buffer Kit, cobas® 4800 System Sample Preparation Kit 2 and cobas® 4800 System Lysis Kit 2 cannot be combined. For example, if a 96-test Wash Buffer reagent bottle is scanned at the start of the run, 96-test size reagents from the other sample preparation reagent kits must also be used.***

Workflow

cobas® HCV GT is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. The run can be HCV GT only, or in mixed-batch format with tests that share the same automated specimen extraction process and PCR profile for amplification and detection. The software will display tests that are compatible for mixed batching with **cobas**® HCV GT at the test selection step. Refer to the **cobas**® 4800 System Operator's Manual for details.

1. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System - System Manual for **cobas**® HCV GT in the "**Performing Maintenance**" section.
2. Collect all reagents and consumables needed. All reagents except HCV GT MMX R2A, HCV GT MMX R2B, HCV GT MMX R2C and MMX R1 must be at ambient temperature prior to loading on the **cobas x** 480 instrument. The HCV GT MMX R2A, HCV GT MMX R2B, HCV GT MMX R2C and MMX R1 reagents may be taken directly from 2- 8°C storage as they will equilibrate to ambient temperature on board the **cobas x** 480 instrument by the time they are used in the process.

Note: ***All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.***

3. Start a new run and select the workflow type as HCV GT. There are three ways to create a work order:
   - By using the sample editor before the sample rack is loaded into the **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary.
   - By following the software wizard for the new run and loading specimens into **cobas x** 480 instrument when prompted. The specimen barcodes will be automatically scanned, and the requested results for each specimen must be defined.
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT for more details. When selecting the requested results, check "HCV GT".
4. Load samples and define/select work order or use LIS as appropriate. The minimum sample volume depends on the tube type and size. Refer to the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT for more details.
5. Follow the software wizard guide and load consumables. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.

6. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: ***The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once LYS 2 or WB are scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.***

Note: ***To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial immediately prior to dispensing into the reagent reservoir.***

7. Load amplification/detection reagent vials (HCV GT MMX R2A, HCV GT MMX R2B , HCV GT MMX R2C and MMX R1) and control vials [HCV GT(+)C, and (–)C] directly onto the reagent carrier. In order to prevent contamination, it is required to change gloves after handling positive controls.
8. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carriers. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT.
9. Follow the instructions in the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT to seal the microwell plate, transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

Note: ***The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 40 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.***

10. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.
11. Follow the instructions in the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT to review and accept results.

Results

The **cobas**® 4800 System automatically determines the HCV genotype and subtype 1a and 1b for the specimens.

Quality control and validity of results

- One negative control, (–) C, and one positive control, HCV GT(+), are processed with each batch.
- In the **cobas**® 4800 Software and/or report, check for batch validity.
- Invalidation of results is performed automatically by the **cobas**® 4800 Software based on negative and positive control failures.

Control flags

Table 1: Control flags for negative and positive controls

Negative Control	**Flag ID**	**Result**	**Interpretation**
(–) C	R21	Invalid	The negative control is invalid.
Positive Control	**Flag ID**	**Result**	**Interpretation**
HCV GT (+)C	R20	Invalid	The positive control is invalid.

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

Interpretation of results

Note: *All assay and batch validation is determined by the cobas® 4800 Software.*

Note: *A valid batch may include both valid and invalid specimen results.*

For a valid batch, specimen results are interpreted as shown in Table 2.

Table 2: Results and interpretation

cobas® HCV GT	**Result Report and Interpretation**
Invalid	One, two or all three reactions are invalid. All HCV control is not detected in one, two or all three reactions
Indeterminate	All HCV control is detected in all three reactions, but no genotype or subtype is identified
1; 1a; 1b; 2; 3; 4; 5; or 6	Identified genotypes are reported in alphanumeric order, separated by a semicolon.

Procedural limitations

1. **cobas**® HCV GT has been evaluated only for use in combination with the **cobas**® HCV GT Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2, and **cobas**® 4800 System Wash Buffer Kit.
2. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and the appropriate **cobas**® 4800 System Operator's Manual for-**cobas**® HCV GT.
3. This test has been validated only for use with EDTA plasma and serum. Testing of other sample types may result in inaccurate results.
4. Identification of HCV genotypes is dependent on 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.
5. Though rare, mutations within the binding sites for the primers and probes of **cobas**® HCV GT may affect primers and/or probe binding, resulting in the failure to identify the genotype of the sample.
6. The addition of AmpErase enzyme into the **cobas**® HCV GT Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
7. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
8. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
9. 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.
10. Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of **cobas**® HCV GT has been determined in a non-clinical study to be 0.0%. Run to run cross-contamination has not been observed.
11. **cobas**® HCV GT is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection.
12. Identification of genotype 6 by **cobas**® HCV GT is limited to subtypes 6a and 6b.

Non-clinical performance evaluation

Key performance characteristics

Accuracy

The accuracy of **cobas**® HCV GT was evaluated by testing 379 HCV positive samples, and comparing the results to the genotypes determined by nucleic acid sequencing (Table 3). The study was conducted with two lots of **cobas**® HCV GT. Genotype results with **cobas**® HCV GT and nucleic acid sequencing were in agreement for 352 of 353 samples, for an overall genotype accuracy of 99.7%. The accuracy of **cobas**® HCV GT for identification of genotype 1 subtypes 1a and 1b was evaluated on 99 genotype 1a samples and 50 genotype 1b samples (Table 4). The overall subtype accuracy was 100% (148/148).

Table 3: Accuracy for genotypes 1 through 6

HCV Genotype by Sequencing	Number of Tests[a]	Mixed Genotype Results	Indeterminate Results[b]	Included Results[c]	cobas® HCV GT Results in Agreement with Sequencing	Percent Correctly Identified (Accuracy)		95% CI[d]	
								LL	UL
1[e]	151	0	2	149	149	100	(149/149)	97.6	100
2	65	3	0	62	62	100	(62/62)	94.2	100
3[f]	60	1	12	47	46	97.9	(46/47)	88.7	100
4[g]	57	2	0	55	55	100	(55/55)	93.5	100
5	27	0	3	24	24	100	(24/24)	85.8	100
6[h]	19	0	3	16	16	100	(16/16)	79.4	100
Overall	379	6	20	353	352	99.7	(352/353)	98.4	100

[a] Includes all samples with valid **cobas**® HCV GT results.

[b] **cobas**® HCV GT returns a result of Indeterminate if HCV is detected but no genotype is identified.

[c] Number of Tests – (Mixed Genotype Results + Indeterminate Results)

[d] 95% two-sided Confidence Interval.

[e] Includes 99 genotype 1a, 50 genotype 1b and 2 genotype 1l samples by nucleic acid sequencing.

[f] One genotype 3 sample yielded a genotype 1a result with **cobas**® HCV GT.

[g] Includes 43 genotype 4a samples, 9 genotype 4 samples of non-a subtype and 5 genotype 4 samples of unidentified subtype.

[h] Includes 18 genotype 6a samples and one genotype 6b sample.

Table 4: Accuracy for genotype 1 subtypes 1a and 1b

HCV Genotype by Sequencing	Number of Tests[a]	Mixed Subtype Results	Indeterminate and genotype 1 Results[b]	Included Results[c]	cobas® HCV GT Results in Agreement with Sequencing	Percent Correctly Identified (Accuracy)		95% CI[d]	
								LL	UL
1a	99	0	0	99	99	100	(99/99)	96.3	100
1b[e]	50	0	1	49	49	100	(49/49)	92.8	100
Overall	149	0	1	148	148	100	(148/148)	97.5	100

[a] Includes all genotype 1a and 1b samples with valid **cobas**® HCV GT results.

[b] **cobas**® HCV GT returns a result of Indeterminate if HCV is detected but no genotype is identified. For calculation of subtype accuracy, a result of "gt 1" without identification of genotype 1a or genotype 1b was considered Indeterminate.

[c] Number of Tests – (Mixed Genotype Results + Indeterminate Results)

[d] 95% two-sided Confidence Interval.

[e] One genotype 1b sample yielded a result of "gt 1" with **cobas**® HCV GT, without identification of genotype 1a or genotype 1b.

Limit of Detection (LoD)

The limit of detection of **cobas**® HCV GT was determined by testing dilutions of HCV positive specimens prepared in HCV-negative serum or EDTA plasma (Table 5). For each genotype a single HCV specimen was diluted in HCV-negative EDTA plasma or serum to concentrations of 1000, 500, 250, 125, 50 and 25 International Units/mL (for genotype 5, the concentrations were 1000, 750, 500, 250, 125 and 50 IU/mL). Panels containing six concentrations of each genotype and HCV-negative plasma or serum were tested with multiple runs, days, operators and instruments, using three lots of **cobas**® HCV GT, with 20 to 24 measurements per reagent lot, for a total of 60 to 72 measurements per sample. The lowest concentrations that yielded the correct genotype result in ≥ 95% of tests are shown in Table 5.

Table 5: Limit of detection* (IU/mL) of cobas® HCV GT

Sample Type	HCV Genotype						
	1a	1b	2	3	4	5	6
Plasma	125	250	125	125	125	1000	125
Serum	125	125	50	125	125	500	125

* Lowest tested concentrations with correct genotype results in at least 95% of tests

Repeatability

The repeatability of **cobas**® HCV GT was determined by testing a blinded, randomized, 15-member panel, with 2 replicates per run, 2 runs per day, 5 days per reagent lot and 3 reagent lots, using one instrument and one operator, for a total of 60 tests per sample (Table 6). The panel was composed of two dilutions of one clinical specimen of each genotype, prepared in HCV-negative, human serum or EDTA plasma, and an HCV-negative human serum or plasma sample. Each genotype was included in the panel at two concentrations: Low (500 – 1,000 IU/mL) and either Medium (10,000 – 20,000 IU/mL) or High (≥ 100,000 IU/mL). The overall rate of correct results for **cobas**® HCV GT was 99.8% (890/892).

Table 6: Repeatability of cobas® HCV GT

Genotype	Concentration (IU/mL)	Sample Type	Total Number of Results[a]	Number of Correct Results	Percent Correct Results[b]	
1a	910	Serum	60	60[c]	100	(60/60)
1a	330,000	Plasma	60	60	100	(60/60)
1b	970	Serum	58	58	100	(58/58)
1b	350,000	Plasma	59	59	100	(59/59)
2	780	Serum	59	59	100	(59/59)
2	13,000	Plasma	60	60	100	(60/60)
3	570	Plasma	60	60	100	(60/60)
3	96,000	Serum	60	60	100	(60/60)
4	940	Serum	59	59	100	(59/59)
4	180,000	Plasma	59	59	100	(59/59)
5	1,000	Serum	59	57[d]	96.6	(57/59)
5	16,000	Plasma	60	60	100	(60/60)
6	800	Plasma	60	60	100	(60/60)
6	17,000	Serum	59	59	100	(59/59)
HCV negative	0	Plasma	30	30	100	(30/30)
HCV negative	0	Serum	30	30	100	(30/30)
Overall			892	890	99.8	(890/892)

[a] Eight of 900 total tests yielded no result due to clot errors.

[b] Percent Correct Results = Number of Correct Results / Total Number of Results.

[c] 58 of 60 tests yielded a result of 1; 1a, and 2 tests yielded a result of 1a.

[d] One test yielded a result of Indeterminate, and one test yielded a result of 1; 5.

Mixed genotype infections

Identification of mixed genotype infections was evaluated by testing all possible two-genotype combinations of genotypes 1a, 1b and 2 through 6, at three different concentration ratios: 1:1 (1000:1000 IU/mL), 1:1 (1E+06:1E+06 IU/mL), 10:1 (1E+04:1E+03 IU/mL) and 100:1 (1E+05:1E+03 IU/mL). Each sample was tested in triplicate. **cobas**® HCV GT identified both genotypes in 100% (63/63) of tests of 1:1 mixtures at 1E+06 IU/mL and in 95.2% (60/63) of tests of 1:1 mixtures at 1000 IU/mL (Table 7). **cobas**® HCV GT identified the minority genotype in 85.7% (108/126) of

tests of 10:1 mixtures and in 63.5% (80/126) of tests of 100:1 mixtures. **cobas**® HCV GT identified the majority genotype in 100% of tests (252/252) of the 10:1 and 100:1 mixtures.

Table 7: Identification of mixed genotype infections

Concentrations[a] (IU/mL)	Concentration Ratio	Number of Combinations Tested	Total Tests	Identification of Both Genotypes[b]	
$1 \times 10^6 : 1 \times 10^6$	1:1	21	63	100%	(63/63)
$1 \times 10^3 : 1 \times 10^3$	1:1	21	63	95.2%	(60/63)[c]
$1 \times 10^4 : 1 \times 10^3$	10:1	42	126	85.7%	(108/126)[d]
$1 \times 10^5 : 1 \times 10^3$	100:1	42	126	63.5%	(80/126)[e]

[a] The high concentration 1:1 mixtures containing genotype 5 were tested at 1×10^5 IU/mL.

[b] In the mixtures of unequal concentrations (10:1 and 100:1) the majority genotype was identified in 100% (252/252) of tests.

[c] In the 1:1 mixtures at 1×10^3 IU/mL, genotype 3 was identified in 83% (15/18) of tests. All other genotypes were identified in 100% of tests.

[d] In the 10:1 mixtures, genotype 3 was identified in 0/18 tests when it was the minority genotype. All other genotypes were identified in 100% of tests when they were the minority genotype.

[e] In the 100:1 mixtures, genotypes 1b, 2, 3 and 6 were identified in 83% (15/18), 44% (8/18), 0% (0/18) and 17% (3/18) of tests, respectively, when they were the minority genotype. Genotypes 1a, 4 and 5 were identified in 100% of tests when they were the minority genotype.

Specificity

The specificity of **cobas**® HCV GT was determined by testing 52 EDTA plasma samples and 52 serum samples from 104 individual, normal, HCV-negative donors, using two lots of **cobas**® HCV GT. **cobas**® HCV GT is intended for use on individuals with chronic HCV infection. The test detects HCV, regardless of genotype, as an internal control. When HCV is not detected, **cobas**® HCV GT returns a result of Invalid. All 104 tests in the specificity study yielded results of Invalid, as expected for HCV negative samples.

Analytical specificity

The analytical specificity of **cobas**® HCV GT was evaluated by diluting a panel of 27 pathogens (Table 8) with HCV-negative EDTA plasma, and testing the samples with and without HCV genotype 1a added to approximately 1500 IU/mL. HCV genotype 1a was identified in all samples with HCV and the pathogens, demonstrating that the pathogens did not interfere. Results of Invalid (the expected result for HCV negative samples) were obtained with **cobas**® HCV GT for all pathogen samples without HCV, demonstrating that the pathogens do not cross-react with **cobas**® HCV GT. **cobas**® HCV GT detects HCV, regardless of genotype, as an internal control. When HCV is not detected, **cobas**® HCV GT returns a result of Invalid.

Table 8: Pathogens tested for cross-reactivity

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

Analytical specificity – interfering substances

Plasma samples with elevated levels of triglycerides (27.9 - 30.0 g/L), conjugated bilirubin (0.18 - 0.22 g/L), unconjugated bilirubin (0.19 - 0.2 g/L), albumin (57.8 - 60.6 g/L), hemoglobin (1.8 - 2.3 g/L) and human DNA (2 mg/L) were tested with **cobas**® HCV GT in the presence and absence of HCV genotype 1a, added to approximately 1500 IU/mL. HCV genotype 1a was identified in all samples with HCV, and results of Invalid (the expected result for HCV negative samples) were obtained for all samples without HCV, demonstrating that the substances do not interfere with the performance of **cobas**® HCV GT. Plasma samples from HCV negative donors with markers for the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody (ANA) were tested with **cobas**® HCV GT in the presence and absence of HCV genotype 1a, added to approximately 1500 IU/mL, and had no effect on the performance of **cobas**® HCV GT.

Plasma samples containing the drugs listed in Table 9 at three times the C_{max} were tested with **cobas**® HCV GT in the presence and absence of HCV genotype 1a, added to approximately 1500 IU/mL. HCV genotype 1a was detected in all of the samples with HCV, and results of Invalid (the expected result for HCV negative samples) were obtained for all samples without HCV demonstrating that the drugs do not interfere with the performance of **cobas**® HCV GT.

Table 9: Drug compounds tested for interference with genotype identification by cobas® HCV GT

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

Method correlation

Performance evaluation of cobas® HCV GT compared to an *in vitro* diagnostic product bearing the CE mark

The performance of **cobas**® HCV GT and an *in vitro* diagnostic HCV genotyping product bearing the CE mark were compared for identification of HCV genotypes 1 to 6 by testing 379 HCV positive samples with both methods (Table 10). Valid, single-genotype results were obtained with both methods on 334 of 379 samples in the study. Two samples did not yield results with the comparison product due to system errors, and 43 samples yielded indeterminate or mixed genotype results with one or both methods. The percent agreement between **cobas**® HCV GT and the comparison product for identification of HCV genotypes 1 to 6 was 99.7% (333/334). The agreement between the comparison product and **cobas**® HCV GT for identification of subtypes 1a and 1b of genotype 1 was determined from the results on 99 genotype 1a samples and 50 genotype 1b samples by nucleic acid sequencing. Valid results identifying the subtype were obtained with both methods on 142 of 149 samples. One genotype 1a sample and 4 genotype 1b samples (by nucleic acid sequencing) yielded genotype 1 results with the comparison product, without identifying subtype 1a or 1b. One genotype 1b sample (by nucleic acid sequencing) was indeterminate with the comparison product, and one genotype 1b sample yielded a genotype 1 result with **cobas**® HCV GT. The percent agreement between **cobas**® HCV GT and the comparison product for identification of HCV subtype 1a and 1b was 100.0% (142/142).

Table 10: Method comparison of cobas® HCV GT to an IVD device bearing the CE mark, for identification of HCV genotypes 1 to 6

Genotype Result of Comparison Product	Number of Results[a]	cobas® HCV GT Results in Agreement with Comparison Product	Percent Agreement		95% CI[b]	
					LL	UL
1	150[c]	149[c]	99.3	(149/150)	96.3	100
2	52	52	100.0	(52/52)	93.2	100
3	44	44	100.0	(44/44)	92.0	100
4	50	50	100.0	(50/50)	92.9	100
5	24	24	100.0	(24/24)	85.8	100
6	14	14	100.0	(14/14)	76.8	100
Overall	334	333	99.7	(333/334)	98.3	100

[a] Includes all samples with valid, single genotype results with both the comparison product and **cobas**® HCV GT. Samples with failed, Indeterminate or mixed genotype results with either or both methods were excluded.

[b] 95% two-sided Confidence Interval.

[c] One sample with a genotype 1 result with the comparison product, was genotype 6 by **cobas**® HCV GT and nucleic acid sequencing. One sample with genotype 1 results with both the comparison product and **cobas**® HCV GT was genotype 3 by nucleic acid sequencing.

Table 11: Method comparison of cobas® HCV GT to an IVD device bearing the CE mark, for identification of subtypes 1a and 1b of genotype 1

Genotype Result of Comparison Product	Number of Results[a]	cobas® HCV GT Results in Agreement with Comparison Product	Percent Agreement		95% CI[b]	
					LL	UL
1a	98	98	100.0	(98/98)	96.3	100
1b	44	44	100.0	(44/44)	92.0	100
Overall	142	142	100.0	(142/142)	97.5	100

[a] Includes all genotype 1a and 1b samples, by nucleic acid sequencing, with valid results with both the comparison product and **cobas**® HCV GT. Samples with failed, Indeterminate or mixed genotype results with either or both methods were excluded.

[b] 95% two-sided Confidence Interval.

Whole system failure

The rate of whole system failure, leading to false negative results for **cobas**® HCV GT, was determined by testing 200 total replicates of EDTA plasma and serum with HCV genotype 1a added to 375 IU/mL, approximately 3 times the limit of detection for genotype 1a. **cobas**® HCV GT correctly identified genotype 1a in all 200 samples, for a whole system failure rate of 0.0% (upper 1-sided 95% confidence limit of 1.49%).

Cross contamination

The cross-contamination rate for **cobas**® HCV GT was determined by performing eight test batches of **cobas**® HCV GT, each with 15 replicates of EDTA plasma containing the HCV GT positive control armored RNA at a concentration equivalent to 1.0E+08 IU/mL, and 15 replicates of HCV-negative EDTA plasma. The samples were loaded into sample racks in the appropriate positions to yield a checkerboard pattern on the sample extraction plate on the **cobas x** 480 instrument. All 120 replicates of the HCV-negative EDTA plasma yielded Invalid results, the expected result for HCV negative samples, for a cross-contamination rate of 0.0% (upper 1-sided 95% confidence limit of 2.47%).

Additional information

Key assay features

Sample type	EDTA plasma, serum
Minimum amount of sample required	Please refer to the **cobas**® 4800 System Operator's Manual for **cobas**® HCV GT.
Sample processing volume	400 µL
Genotypes and subtypes identified	HCV genotypes 1-6, and genotype 1 subtypes a and b.
Accuracy	For identification of HCV genotypes 1 to 6: 99.7% For identification of HCV genotype 1 subtypes 1a and 1b: 100%
Analytical sensitivity	Serum: 50 to 125 IU/mL (genotypes 1a, 1b, 2, 3, 4 and 6), 500 IU/mL (genotype 5) Plasma: 125 to 250 IU/mL (genotypes 1a, 1b, 2, 3, 4 and 6), 1000 IU/mL (genotype 5)

Symbols

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

Table 12: Symbols used in labeling for Roche PCR diagnostic products

	Ancillary Software		*In Vitro* Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains Sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number

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

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 13: Manufacturer and distributors

Manufactured in the United States

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

D

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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

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

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

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

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

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

Trademarks and patents

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

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IVD

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Document revision

Document Revision Information	
Doc Rev. 2.0 11/2016	Updated note for **Required Instrumentation and Software, Not Provided** table. Added mixing step to **Specimen collection, transport, and storage** section. Removed Telaprevir from **Table 9**. Corrected number of samples in **Method correlation** section. Updated descriptions of the harmonized symbol page. Added Roche web address www.roche.com. Please contact your local Roche Representative if you have any questions.