COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, version 2.0

FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0	**HCVQLV2**	72 Tests	P/N: 05480477 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	**PG WR**	5.1 Liters	P/N: 03587797 190

Table of Contents

INTENDED USE

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is a qualitative *in vitro* nucleic acid amplification test for the detection of Hepatitis C Virus (HCV) RNA genotypes 1 to 6 in human EDTA plasma or serum using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer for automated amplification and detection. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is indicated for patients who have clinical and/or biochemical evidence of liver disease and antibody evidence of HCV infection, and who are suspected to be actively infected with HCV. The test can be used to confirm antibody positive specimens. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is not intended for use as a screening test for the presence of HCV in blood or blood products.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C Virus 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]. The global prevalence of HCV infection, as determined by immunoserology, ranges from 0.6% in Canada to 1.5% in Japan[3]. Spontaneous viral clearance rates in exposed individuals are highly variable; between 10 and 60% have been reported as measured clinically by normalization of liver enzymes and clearance of plasma HCV RNA[5].

HCV virus particles cannot be cultured from infected blood samples; hence the presence of anti-HCV antibodies in patients infected with HCV has led to the development of immunoserological assays that are specific for these antibodies. The presence of anti-HCV antibodies, however, is a measure of prior exposure to HCV infection, but cannot be considered a marker for current infection. The measurement of alanine aminotransferase levels (ALT) is considered to be a surrogate indicator of HCV infection, but is not a direct measure of viremia.

Alternatively, detection of HCV RNA by nucleic acid tests may provide evidence for current infection. Using nucleic acid tests, it is possible to detect HCV viremia prior to immunological seroconversion[6,7]. Since nucleic acid tests are able to detect HCV RNA directly, i.e. independent of the immunological status of the patient, a nucleic acid-based test is valuable in detecting HCV RNA in immunocompromised patients[8].

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is a nucleic acid amplification test for the detection of Hepatitis C Virus (HCV) RNA in human serum or EDTA plasma. Specimen preparation is automated using the COBAS® AmpliPrep Instrument with amplification and detection automated using the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer.

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is based on three major processes: (1) specimen preparation to isolate HCV RNA, (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probes specific to the target.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The sample input volume is 650 µL, whereas the procedure processes 500 µL of EDTA plasma or serum. The HCV virus particles are lysed by incubation at elevated temperature with protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released HCV RNA from RNases in serum or EDTA plasma. Protease and a known number HCV Internal Control (IC) RNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the HCV RNA and HCV IC RNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separating the beads and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed specimen, containing the released HCV RNA and HCV IC RNA, is added to the amplification mixture and transferred to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

Reverse Transcription and PCR Amplification

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 uses reverse transcription of HCV RNA to complementary DNA (cDNA) and PCR amplification of cDNA using primers that define a sequence within the highly conserved region of the 5'-untranslated region of the HCV genome[9]. The nucleotide sequence of the primers has been optimized to yield comparable amplification of HCV genotypes 1 to 6. The reverse transcription and PCR amplification reaction is performed with an optimized blend of thermostable recombinant enzymes: Z05 and Z05D DNA Polymerase. In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, Z05 and Z05D have both reverse transcriptase and DNA polymerase activity. This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow a downstream primer to anneal specifically to the HCV target RNA and to the HCV IC RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine triphosphates, Z05 and Z05D polymerases extend the annealed primers forming a DNA strand complementary to the RNA target.

Target Amplification

Following reverse transcription of the HCV target RNA and the HCV IC RNA, the Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the RNA:cDNA hybrid and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target cDNA. The thermostable DNA Polymerases (Z05 and Z05D) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), extend the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to doubling the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the HCV genome between the primers; the entire HCV genome is not amplified.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[10], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55°C, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed throughout the duration of the PCR reaction.

Detection of cleaved dual-labeled probes and HCV RNA

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 utilizes real-time[14,15] PCR technology. The use of dual-labeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HCV and HCV IC-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0, the HCV and HCV IC probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermostable Z05 and Z05D DNA polymerases. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HCV RNA and HCV IC RNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HCV RNA and HCV IC RNA.

In nucleic acid amplification processes, efficiency can be reduced by inhibitors that may be present in the specimen. The HCV IC has been added to the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 to permit identification of processed specimens containing substances that may interfere with PCR amplification. The HCV IC is a non-infectious armored RNA (aRNA) construct that contains fragments of HCV sequences with identical primer binding sites as the HCV target RNA and a unique probe binding region that allows HCV IC amplicon to be distinguished from HCV target amplicon. It serves as an extraction and amplification control for each independently processed specimen.

During the extension phase of the PCR on the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and the filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HCV RNA and HCV IC RNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HCV RNA and the HCV IC RNA. Results are reported as either positive or negative.

REAGENTS

COBAS® AmpliPrep/COBAS® TaqMan® **HCVQLV2** **72 Tests**
HCV Qualitative Test, v2.0
(P/N: 05480477 190)

HCV QL v2.0 CS1 1 x 72 Tests
(HCV Magnetic Glass Particles Reagent Cassette) 1 x 7.0 mL
- Magnetic glass particles
- Tris buffer
- 0.09% Sodium azide
- 0.1% Methylparaben

HCV QL v2.0 CS2 1 x 72 Tests
(HCV Lysis Reagent Cassette) 1 x 78.0 mL
- Sodium citrate dihydrate
- 42.5% Guanidine thiocyanate
- < 6% Polydocanol
- 0.9% Dithiothreitol

HCV QL v2.0 CS3 1 x 72 Tests
HCV Multi-Reagent Cassette containing:

Pase 1 x 3.8 mL
(Proteinase Solution)
- Tris buffer
- < 0.05% EDTA
- Calcium chloride
- Calcium acetate
- ≤ 7.8% Proteinase
- Glycerol

EB 1 x 8.1 mL
(Elution Buffer)
- Tris-base buffer
- 0.09% Sodium azide

HCV QL v2.0 CS4 1 x 72 Tests

HCV Test-Specific Reagent Cassette containing:

IC 1 x 3.6 mL

(HCV Internal Control)
- Tris buffer
- EDTA
- < 0.002% Poly rA RNA (synthetic)
- < 0.001% Armored HCV RNA construct containing HCV primer binding sequences and a unique probe binding region (non-infectious RNA in MS2 bacteriophage)
- 0.05% Sodium azide

MMX 1 x 3.5 mL

(HCV Master Mix)
- Tricine buffer
- Potassium acetate
- Potassium hydroxide
- < 20% Dimethyl sulfoxide
- Glycerol
- < 0.004% dATP, dCTP, dGTP, dUTP
- < 0.002% Upstream and downstream HCV primers to the 5' UTR region of HCV
- < 0.001% Fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Internal Control
- < 0.001% Oligonucleotide aptamer
- < 0.05% Z05 and Z05D DNA polymerase (microbial)
- < 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)
- 0.09% Sodium azide

Mn^{2+} 1 x 19.8 mL

(COBAS® AmpliPrep/COBAS® TaqMan® Manganese Solution)
- < 0.5% Manganese acetate
- Glacial acetic acid
- 0.09% Sodium azide

HCV (+) C, v2.0 6 x 0.85 mL

(HCV Positive Control)
- < 0.001% Armored HCV RNA construct containing HCV sequences (non-infectious RNA in MS2 bacteriophage)
- Negative Human Plasma, non-reactive by tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
- 0.1% ProClin® 300 preservative

CTM (–) C 6 x 1.0 mL

[COBAS® TaqMan® Negative Control (Human Plasma)]
- Negative Human Plasma, non-reactive by tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
- 0.1% ProClin® 300 preservative

HCV (+) C, v2.0 Clip 1 x 6 Clips

(HCV Positive Control Barcode Clip)

HCV (–) C, v2.0 Clip 1 x 6 Clips

(HCV Negative Control Barcode Clip)

PG WR
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)
Sodium citrate dihydrate
< 0.1% N-Methylisothiazolone-HCl

WARNINGS AND PROCEDURAL PRECAUTIONS

As with any test procedure, good laboratory technique 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.

A. FOR *IN VITRO* DIAGNOSTIC USE.

B. This test is for use with human serum or plasma collected in the anticoagulant EDTA.

C. Do not pipet by mouth.

D. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

E. Avoid microbial and ribonuclease contamination of reagents when removing aliquots from control vials.

F. The use of sterile disposable pipets and RNase-free pipet tips is recommended.

G. Do not pool controls from different lots or from different vials of the same lot.

H. Do not mix reagent cassettes or controls from different kits.

I. Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles.

J. Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.

K. Do not use a kit after its expiration date.

L. Safety Data Sheets (SDS) are available on request from your local Roche office.

M. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[11] and in the CLSI Document M29-A3[12]. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

N. **CTM (–) C** and **HCV (+) C, v2.0** contain Human Plasma derived from human blood. The source material has been tested and found non-reactive for the presence of Hepatitis B Surface Antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 Antigen. Testing of Negative Human Plasma by PCR methods showed no detectable HIV-1 RNA, HCV RNA or HBV DNA. No known test methods can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all human sourced material, including **CTM (–) C** and **HCV (+) C, v2.0** should be considered potentially infectious.

O. **MGP, EB, IC, Mn^{2+}** and **MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

P. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. 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 of these reagents occur, dilute with water before wiping dry.

Q. Do not allow **HCV QL v2.0 CS2** and liquid waste including used COBAS® AmpliPrep Sample Processing Units (SPUs) from the COBAS® AmpliPrep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

STORAGE AND HANDLING REQUIREMENTS

A. Store **HCV QL v2.0 CS1, HCV QL v2.0 CS2, HCV QL v2.0 CS3** and **HCV QL v2.0 CS4** at 2-8ºC. Unused, these reagents are stable until the expiration date indicated. Once used, these reagents are stable for 70 days at 2-8°C or until the expiration date, whichever comes first. **HCV QL v2.0 CS1, HCV QL v2.0 CS2, HCV QL v2.0 CS3** and **HCV QL v2.0 CS4** can be used up to a maximum of 96 hours cumulative on board the COBAS® AmpliPrep Instrument. Reagents must be stored at 2-8°C between instrument cycles.

B. Store **HCV (+) C, v2.0** and **CTM (–) C** at 2-8ºC. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

C. Store Barcode clips [**HCV (+) C, v2.0 Clip** and **HCV (–) C, v2.0 Clip**] at 2-30°C.

D. Store **PG WR** at 2-30°C. Unused **PG WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2-30°C or until the expiration date, whichever comes first.

MATERIALS PROVIDED

COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 **HCVQLV2**

HCV QL v2.0 CS1
(HCV Magnetic Glass Particles Reagent Cassette)

HCV QL v2.0 CS2
(HCV Lysis Reagent Cassette)

HCV QL v2.0 CS3
(HCV Multi-Reagent Cassette)

HCV QL v2.0 CS4
(HCV Test-Specific Reagent Cassette)

HCV (+) C, v2.0
(HCV Positive Control)

CTM (–) C
[COBAS® TaqMan® Negative Control (Human Plasma)]

HCV (+) C, v2.0 Clip
(HCV Positive Control Barcode Clip)

HCV (–) C, v2.0 Clip
(HCV Negative Control Barcode Clip)

COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent **PG WR**

PG WR
(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation and Software

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Docking Station (optional)
- **cobas p** 630 Instrument (optional)
- AMPLILINK Software Version 3.3 or Version 3.4 Series
- Control Unit for the AMPLILINK Software, with printer
- Instrument and Software Manuals:
  - COBAS® AmpliPrep Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® Analyzer Instrument Manual for use with AMPLILINK Software Version 3.3 and 3.4 Series
  - COBAS® TaqMan® 48 Analyzer Instrument Manual for use with the AMPLILINK Software Version 3.3 and 3.4 Series
  - AMPLILINK Software Version 3.3 Series Application Manual for use with COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR Analyzer and **cobas p** 630 Instrument

    or
  - AMPLILINK Software Version 3.4 Series Application Manual
  - Optional: **cobas p** 630 Instrument Operator`s Manual Software Version 2.2
- Test Definition File (TDF). See product information card, provided with the kit, for name and current version of the TDF.

Other Materials

- Sample Rack (SK 24 rack)
- Reagent Rack
- SPU rack
- K-carrier
- K-carrier Transporter
- K-carrier rack
- Pipettors with aerosol barrier or positive displacement RNase-free tips (capacity 1,000 µL); Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement RNase-free tips must be used to prevent specimen and amplicon cross-contamination.
- Disposable gloves, powder free
- Vortex mixer

Disposables

- Sample Processing units (SPUs)
- Sample input tube (S-tubes) with barcode clips
- Rack of K-tips
- K-tube Box of 12 x 96

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

NOTE: Handle all specimens and controls as if they are capable of transmitting infectious agents.

Specimen Collection and Storage

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is for use with serum or EDTA plasma specimens. 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 (lavender top) as the anticoagulant. Follow the manufacturer's instructions for handling of collection tubes. Freshly drawn specimens (whole blood) may be stored at 2-25°C for up to 24 hours prior to centrifugation. After centrifugation, transfer serum or EDTA plasma to a sterile polypropylene tube. It is recommended that specimens be stored in approximately 1,000 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as 2 mL screw cap micro tube from Sarstedt). Serum or EDTA plasma specimens may be stored:

- At 2-8°C for up to 72 hours
- At -20°C or -80°C for up to 6 weeks

Serum and EDTA plasma specimens may be frozen and thawed up to five times without loss of HCV RNA.

Specimen Transport

Transportation of whole blood, serum or EDTA plasma must comply with country, federal, state and local regulations for the transport of etiologic agents[13]. Whole blood must be transported at 2-25°C and centrifuged within 24 hours of collection. EDTA plasma or serum may be transported at 2-8°C or frozen at -20°C or -80°C.

INSTRUCTIONS FOR USE

For detailed operating instructions, a description of the possible configurations, printing results and interpreting flags, comments and error messages, refer to AMPLILINK software – Version 3.3 or Version 3.4 Series manuals, as listed in section Instrumentation and Software.

Batch Size and Workflow

Each kit contains reagents sufficient for 72 tests, which may be performed in batches of 12 to 24 tests. At least one of each control [**CTM (–) C** and **HCV (+) C, v2.0**] must be included in each batch (see "*Quality Control*" section). The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation. DO NOT FREEZE or STORE processed specimens and controls at 2-8°C.

Specimen and Control Preparation

If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3-5 seconds before use. Controls should be removed from 2-8°C storage, brought to ambient temperature and vortexed for 3-5 seconds before use.

COBAS® AmpliPrep Instrument Set-up

Part A. Maintenance and Priming

A1. The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.

A2. Turn the Control Unit for the AMPLILINK software **ON**. Prepare the Control Unit as follows:

1. Log onto Microsoft Windows Operating System.
2. Double click the AMPLILINK software icon.
3. Log onto AMPLILINK software by entering the assigned User ID and password.

A3. Check the supply of **PG WR** using the **Status** Screen and replace if necessary.

A4. Perform all Maintenance that is listed in the **Due** Tab. The COBAS® AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

NOTE: All reagent cassettes should be removed from 2-8°C storage, immediately loaded onto the COBAS® AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is to be processed. Do not let reagent cassettes come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels.

B1. Place **HCV QL v2.0 CS1** onto a reagent rack. Place **HCV QL v2.0 CS2, HCV QL v2.0 CS3** and **HCV QL v2.0 CS4** onto a separate reagent rack.

B2. Load the reagent rack containing **HCV QL v2.0 CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument.

B3. Load the reagent rack containing **HCV QL v2.0 CS2, HCV QL v2.0 CS3** and **HCV QL v2.0 CS4** onto rack position **B, C, D** or **E** of the COBAS® AmpliPrep Instrument (please refer to the appropriate Instrument Manuals for additional and detailed information).

Part C. Loading of Disposables

NOTE: Determine the number of COBAS® AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (S-tubes), K-tips and K-tubes needed. One SPU, one Input S-tube, one K-tip and one K-tube are needed for each specimen or control.

Multiple configurations for use of the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer are possible. Depending on the configuration used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks onto the respective rack positions of the COBAS® AmpliPrep Instrument.

C1. Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position **J, K** or **L** of the COBAS® AmpliPrep Instrument.

C2. Depending on the configuration used, load full K-tube rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument.

C3. Load full K-tip rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument.

C4. Depending on the configuration used, load K-carriers on K-carrier rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument.

Part D. Ordering and Loading of Specimens

D1. Prepare sample racks as follows: attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [**CTM (-) C** and **HCV (+) C, v2.0**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

D2. Using the AMPLILINK software, create specimen orders for each specimen and control in the **Orders** window **Sample** folder. Select the appropriate test file and complete by saving.

D3. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be for the rack prepared in Step D1.

D4. Print the **Sample Rack Order** report to use as a worksheet.

D5. Prepare specimen and control racks in the designated area for specimen and control addition as follows: Vortex each specimen and control [**CTM (–) C** and **HCV (+) C, v2.0**] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.

D6. Transfer 650 µL of each specimen and control [**CTM (–) C** and **HCV (+) C, v2.0**] to the appropriate barcode labeled Input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. ***Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube.*** Specimens and controls should be transferred to tube positions as assigned and recorded on the worksheet in step D4. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Assign the right control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.***

D7. If using the **cobas p** 630 Instrument for preparation of specimens, refer to the **cobas p** 630 Instrument Operators Manual.

D8. Depending on the configuration used, load the sample rack(s) filled with Input S-tubes onto rack positions **F, G** or **H** of the COBAS® AmpliPrep Instrument.

D9. Depending on the configuration used, load sample rack(s) with Input S-tubes and K-tubes (one for each Input S-tube, loaded in the right position adjacent to Input S-tubes) onto rack position **F, G** or **H** of the COBAS® AmpliPrep Instrument.

Part E. Start of COBAS® AmpliPrep Instrument Run

E1. Start the COBAS® AmpliPrep Instrument using the AMPLILINK software.

Part F. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (only for manual transfer)

F1. Check for flags or error messages.

F2. Remove processed specimens and controls from the COBAS® AmpliPrep Instrument on either sample racks (for COBAS® TaqMan® Analyzer without Docking Station) or K-carrier racks (for COBAS® TaqMan® 48 Analyzer), depending on the configuration.

F3. Remove waste from the COBAS® AmpliPrep Instrument.

NOTE: All processed specimens and controls should not be exposed to light after completion of specimen and control preparation.

Amplification and Detection

COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Set-up

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation. DO NOT FREEZE or STORE processed specimens and controls at 2-8°C.

Part G. Loading Processed Specimens

G1. Depending on the instrument configuration, perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

Part H. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

H1. Start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer depending on the configuration used.

Part I. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

I1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print Results Report. Check for flags or error messages in the Result report. Specimens with flags and comments are interpreted as described in the Results section. After acceptance, store data in archive.

I2. Remove used K-tubes from the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

QUALITY CONTROL

One COBAS® TaqMan® Negative Control and one HCV Positive Control must be included in each test batch. The batch is valid if no flags appear for any of the controls [**HCV (+) C, v2.0** and **CTM (–) C**].

There are no requirements regarding the position of the controls on the sample rack.

Check the batch printout for flags and comments to ensure that the batch is valid.

Negative Control

The **CTM (–) C** must yield a "Negative" result. If the **CTM (–) C** is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **CTM (–) C** is consistently invalid in multiple batches, contact your local Roche office for technical assistance.

Positive Control

The **HCV (+) C, v2.0** must yield a "Positive" result. If the **HCV (+) C, v2.0** is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **HCV (+) C, v2.0** is consistently invalid in multiple batches, contact your local Roche office for technical assistance.

RESULTS

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the presence of the HCV RNA for the specimens and controls.

AMPLILINK Software:

- Determines the Ct for the HCV RNA and the HCV IC RNA.
- Determines the presence of HCV RNA and HCV IC RNA based upon the Ct values for the HCV RNA and HCV IC RNA.

Batch Validation:

Check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid.

For control orders, a check is made to determine if the Ct value for the control is within its specified range. If the Ct value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [**HCV (+) C, v2.0** and **CTM (–) C**].

The batch is not valid if any of the following flags appear for the HCV Controls:

Negative Control:

Flag	Result	Interpretation
NC_INVALID	Invalid	An invalid result or the result for the negative control is not negative.

HCV Positive Control:

Flag	Result	Interpretation
PC_INVALID	Invalid	An invalid result or the result for the positive control is not positive.

If the batch is invalid, repeat the entire batch including specimen and control preparation, reverse transcription, amplification and detection.

Interpretation of Results:

For a valid batch, check each individual specimen for flags or comments on the result printout.

⇒ A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Result	Interpretation
Negative	Ct value for HCV above the limit for the assay or no Ct value for HCV obtained. Report results as "HCV RNA not detected".
Positive	Report results as "HCV RNA detected".

If specimen result display element is "Failed", "Invalid" or "Aborted" please refer to the AMPLILINK software Version 3.3 or Version 3.4 Series Application Manual as listed in section "Materials required but not provided".

PROCEDURAL LIMITATIONS

1. This test has only been validated for use with human serum or plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.

2. Though rare, mutations within the highly conserved regions of the viral genome covered by the test's primers and/or probes may result in failure to detect the virus.

3. Detection of HCV RNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods, patient factors (e.g. age, presence of symptoms) and/or stage of infection.

4. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.

5. The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Master Mix reduces the risk of amplicon contamination. However, contamination from HCV positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Package Insert.

6. Use of this product should be limited to personnel trained to operate the **cobas p** 630 Instrument (optional), the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer. The operator should have a thorough knowledge of the applications run on the instruments and should follow good laboratory practices.

7. This product can only be used with the **cobas p** 630 Instrument (optional), the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer.

8. 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 assess technology differences.

INTERFERING SUBSTANCES

Elevated levels of triglycerides (3,300 mg/dL), conjugated bilirubin (25 mg/dL) and unconjugated bilirubin (20 mg/dL), albumin (6,000 mg/dL), hemoglobin (200 mg/dL) and human DNA (40 mg/dL) in specimens as well as the presence of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Antinuclear Antibody (ANA) did not interfere with the detection of HCV RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0.

The following drug compounds tested at the Peak Plasma Level (C_{max}) and at 3 times the C_{max} did not interfere with the detection of HCV RNA by the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0:

Nucleotide Reverse Transcriptase and DNA Polymerase Inhibitors Tenofovir Adefovir dipivoxil	**Non-nucleoside Reverse Transcriptase Inhibitors** Efavirenz Nevirapine
HIV Protease Inhibitors Atazanavir Saquinavir Ritonavir Lopinavir/Ritonavir Nelfinavir Darunavir Tipranavir Fosamprenavir	**Nucleoside Reverse Transcriptase Inhibitors** Lamivudine Zidovudine Stavudine Abacavir Didanosine Emtricitabine Entecavir Telbivudine
HIV Fusion Inhibitor Enfuvirtide	**HIV Entry Inhibitor** Maraviroc
Compounds for the Treatment of Herpes Viruses Ganciclovir Valganciclovir Acyclovir	**Immune Modulator** Peginterferon alfa-2b Ribavirin Peginterferon alfa-2a
HIV Integrase Inhibitor Raltegravir	

NON-CLINICAL PERFORMANCE EVALUATION

A. Limit of Detection

The limit of detection of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays, genotype 1a, obtained from NIBSC, in HCV negative human EDTA plasma or serum. Three independent dilution series were analyzed for each matrix. A total of up to 252 replicates per concentration level were tested for each matrix type. The study was performed with three lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents.

The results for EDTA plasma and serum are shown in Tables 1 and 2 and demonstrate that the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 detected HCV RNA at concentrations of 15 IU/mL or greater with a hit rate of ≥ 95%. The difference between serum and EDTA plasma was not statistically significant.

Table 1
Limit of Detection in EDTA Plasma determined with the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays

Input Titer (HCV RNA IU/mL)	Number of Valid Replicates	Number of Positives	Hit Rate in %
50	314	314	100
25	314	313	100
15	314	308	98
10	315	291	92
5	315	228	72
2.5	314	157	50
0	313	0	0
LOD by PROBIT at 95 % Hit Rate	**12 IU/mL 95% confidence range: 10 – 13 IU/mL**		
LOD by Hit Rate	**15 IU/mL**		

Table 2
Limit of Detection in Serum determined with the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays

Input Titer (HCV RNA IU/mL)	Number of Valid Replicates	Number of Positives	Hit Rate in %
50	251	251	100
25	251	250	100
15	252	248	98
10	252	232	92
5	252	185	73
2.5	252	116	46
0	251	0	0
LOD by PROBIT at 95 % Hit Rate	**11 IU/mL 95% confidence range: 10 – 13 IU/mL**		
LOD by Hit Rate	**15 IU/mL**		

B. Precision

Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis C Virus RNA for Nucleic Acid Amplification Technology Assays in HCV negative human EDTA plasma or in serum.

Two concentration levels (5 IU/mL and 50 IU/mL) were tested in up to 168 replicates in 12 runs on 4 days. Each sample was carried through the entire COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 procedure, including specimen preparation, amplification, and detection. The study was performed with three lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents. All valid precision data were evaluated by calculating the Hit Rate in % for each panel member by reagent lot (both matrices combined).

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 demonstrates consistent performance at concentration levels 5 IU/mL and 50 IU/mL for EDTA plasma and serum specimens across all three reagent lots tested (Table 3).

Table 3
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 (EDTA Plasma and Serum Specimens combined)

Nominal Concentration [IU/mL]	Hit Rate in %		
	Lot 1	Lot 2	Lot 3
5	74	70	74
50	100	100	100

C. Inclusivity

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 on HCV genotypes was evaluated by verifying the Limit of Detection for genotypes 1 to 6.

HCV RNA clinical specimens for 8 different genotypes/subtypes (1a, 1b, 2a, 2b, 3, 4, 5 and 6) were diluted to three different concentration levels in EDTA plasma or serum and a Hit Rate determination was performed for each level with up to 63 replicates. The study was conducted with one lot of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents.

The results for EDTA plasma and serum are shown in Tables 4 and 5 and verify that the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 detected HCV RNA for 8 different genotypes/subtypes at concentrations of 15 IU/mL or greater with a hit rate of ≥ 95%. The difference between serum and EDTA plasma was not statistically significant.

Table 4
HCV RNA Genotype Verification of Limit of Detection in EDTA Plasma

Genotype	5 IU/mL			15 IU/mL			45 IU/mL		
	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %
1a	63	44	70	63	63	100	63	63	100
1b	63	47	75	63	62	98	63	63	100
2a	63	43	68	63	61	97	62	61	98
2b	62	57	92	62	62	100	62	62	100
3	62	58	94	63	63	100	62	62	100
4	63	43	68	63	62	98	63	63	100
5	63	46	73	62	62	100	62	62	100
6	63	54	86	62	62	100	63	63	100

Table 5
HCV RNA Genotype Verification of Limit of Detection in Serum

Genotype	5 IU/mL			15 IU/mL			45 IU/mL		
	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %	Number of Valid Replicates	Number of Positives	Hit Rate in %
1a	63	45	71	62	62	100	63	63	100
1b	62	50	81	63	63	100	63	63	100
2a	62	47	76	61	60	98	63	63	100
2b	63	42	67	63	62	98	63	63	100
3	63	58	92	63	63	100	63	63	100
4	63	40	64	63	62	98	63	63	100
5	62	46	74	61	60	98	62	62	100
6	63	51	81	63	63	100	63	63	100

D. Diagnostic Sensitivity

The diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was determined by analyzing individual HCV RNA positive EDTA plasma or serum samples (337 total results) with two lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents. All specimens tested positive for HCV RNA. In this panel, the diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is 100% (one sided lower 95% confidence limit: ≥ 99.1%).

In addition, the diagnostic sensitivity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was evaluated during seroconversion. The members of 10 commercially available HCV seroconversion panels, each collected from an individual plasma donor during a period of HCV antibody seroconversion, were tested with one lot of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents. COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 detected HCV earlier in 10 out of 10 seroconversion panels, if compared to the serology reference Abbott HCV EIA (enzyme immunoassay) 2.0. If compared with another NAT test system, the COBAS® AMPLICOR HCV Test, v2.0, the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 detected HCV RNA earlier (2 out of 10 panels) or at the same day since 1st bleed (8 out of 10 panels).

E. Specificity

The specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was determined by analyzing HCV RNA negative and sero-negative EDTA plasma or serum samples from blood donors. Individual EDTA plasma and serum specimens (500 total results) were tested with two lots of COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 reagents. 499 specimens tested negative for HCV RNA. In this panel, the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 is 99.8% (one sided lower 95% confidence limit: ≥ 99.1%).

F. Analytical Specificity

The analytical specificity of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 was evaluated by diluting high titer stocks of different pathogens (see Table 6) with HCV RNA positive and HCV RNA negative clinical EDTA plasma specimens. None of the non-HCV pathogens interfered with test performance, or showed a false positive result in the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0.

Table 6
Analytical Specificity Specimens

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

G. Performance Compared to COBAS® AMPLICOR HCV Test, v2.0

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 and the COBAS® AMPLICOR HCV Test, v2.0 were compared by analysis of serum and EDTA plasma specimens from HCV infected patients. A total of 463 specimens across genotypes 1 to 6 for EDTA plasma and genotypes 1 – 4 for serum were analyzed in duplicate. Results of 436 EDTA plasma and serum specimens were positive and within the detection range of both tests, leading to a 100% positive agreement between COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 and the COBAS® AMPLICOR HCV Test, v2.0. Results for 27 specimens were excluded from data analysis; 3 specimens had insufficient volume to be tested on COBAS® AMPLICOR HCV Test, v2.0, 4 specimens yielded "target not detected" results in all measurements on both tests, 8 specimens yielded discrepant results between the 2 replicates within each test, 12 specimens yielded discrepant results between the two tests [rationale for exclusion: all these specimens had HCV concentrations below the LOD of the COBAS® AMPLICOR HCV Test, v2.0, LOD (95% detection rate) Plasma 50 IU/ml, LOD Serum: 60 IU/ml]. All negative EDTA plasma and serum specimens tested (200 total results) were valid and returned a negative result, leading to a 100% negative agreement between COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2.0 and the COBAS® AMPLICOR HCV Test, v2.0.

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Consult instructions for use

For IVD Performance Evaluation Only

Contains Sufficient for <*n*> tests

Lower Limit of Assigned Range

Contents of kit

Manufacturer

Distributed by

Store in the dark

In Vitro Diagnostic Medical Device

Temperature Limit

Test Definition File

Use-by date

Upper Limit of Assigned Range

Global Trade Item Number

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