Laboratory Procedure Manual

Analyte:	HCV RNA Quantification Assay for Hepatitis C Virus
Matrix:	**Serum**
Method:	**Roche COBAS® Ampliprep TNAI/ TaqMan® 48 RUO Assay**
As performed by:	*Hepatitis Branch* *Division of Viral Hepatitis* *National Center for Infectious Diseases*
Contact:	*Dr. Ruth Jiles* *Viral Hepatitis, STD and TB Prevention* *Division of Viral Hepatitis* *Hepatitis Reference Laboratory* *Centers for Disease Control and Prevention*

Important Information for Users

The National Center for Infectious Diseases periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Lab Name	Variable Name	Description
HEPC_D	LBXHCR	Hepatitis C RNA (HCV-RNA)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The COBAS® TaqMan® HCV Test, v2.0 is an *in vitro* nucleic acid amplification test for the quantitation of Hepatitis C Virus (HCV) RNA genotypes 1 through 6 in human serum or plasma, using the COBAS® AmpliPrep Total Nucleic Acid Isolation Kit (TNAI) for the preparation of highly purified total nucleic acid from serum or plasma on the COBAS® AmpliPrep Instrument and automated amplification and detection on the COBAS® TaqMan® 48 Analyzer. The COBAS® TaqMan® HCV Test, v2.0 is based on three major processes: (1) automated sample preparation to extract HCV RNA; (2) automated reverse transcription of the target RNA to generate complementary DNA (cDNA); (3) PCR amplification of target cDNA using HCV specific complementary primers, and simultaneous detection of cleaved dual fluorescent dye-labeled oligonucleotide probes that permit quantitation of HCV target amplified product (amplicon). The Master Mix reagent contains primer pairs and probes specific for both HCV RNA and HCV Quantitation Standard RNA. The detection of amplified DNA is performed using target-specific and Quantitation Standard-specific dual labeled oligonucleotide probes that permit independent identification of HCV amplicon and HCV Quantitation Standard amplicon.

The quantitation of HCV viral RNA is performed using the HCV Quantitation Standard. The HCV Quantitation Standard is a non-infectious Armored RNA construct that contains the HCV sequences with identical primer binding sites as the HCV RNA target and a unique probe binding region that allows HCV Quantitation Standard amplicon to be distinguished from HCV target amplicon. The HCV Quantitation Standard is incorporated into each individual sample and control at a known copy number and is carried through the sample preparation, reverse transcription, PCR amplification and detection steps along with the HCV target. The COBAS® TaqMan® 48 Analyzer calculates the HCV RNA titer in the test samples by comparing the HCV signal to the HCV Quantitation Standard signal for each sample and control. The HCV Quantitation Standard compensates for effects of inhibition and controls for the preparation and amplification processes to allow the accurate quantitation of HCV RNA in each sample.

Target Selection

Selection of the target RNA sequence for HCV depends on identification of regions within the HCV genome that show maximum sequence conservation among the various HCV genotypes[3,4]. The 5'-untranslated region of the HCV genome has been shown to have maximum conservation of RNA sequences among known HCV genotypes[5] . The COBAS® TaqMan® HCV Test, v2.0 uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved 5'-untranslated region of the HCV genome.

Sample Preparation

The COBAS AmpliPrep Instrument is designed to process specimens in a continuous workflow. The basic principle of sample preparation is Magnetic Glass particle (MGP) technology.

The COBAS® AmpliPrep Total Nucleic Acid Isolation Kit process consists of the

following five steps: Digestion of proteins by Protease solution to facilitate the release of RNA and DNA. Addition of Lysis Reagent to the sample to result in complete lysis by denaturation of proteins. RNA and DNA are released and simultaneously stabilized. The released nucleic acid binds to the silica surface of the added magnetic glass particles (**TNAI MGP**) due to the chaotropic salt conditions and the high ionic strength of the Lysis Reagent. Wash Reagent removes unbound substances and impurities such as denatured proteins, cellular debris and potential PCR inhibitors such as hemoglobin, etc., and reduces the salt concentration. Purified nucleic acids are eluted at elevated temperature.

The processed samples, containing HCV RNA and HCV Quantitation Standard RNA, is added to the amplification/detection mixture. The HCV target RNA and the HCV Quantitation Standard RNA are then amplified and detected on the COBAS® TaqMan® 48 Analyzer using the amplification and detection reagents provided in the COBAS® TaqMan® HCV Test, v2.0 kit.

Reverse Transcription and PCR Amplification

Reverse Transcription

The reverse transcription, PCR amplification and detection reactions are performed with the thermostable recombinant enzyme *Thermus species* Z05 DNA Polymerase (Z05). In the presence of manganese (Mn2+) and under the appropriate buffer conditions, Z05 has both reverse transcriptase and DNA polymerase activity[6,7]. This allows reverse transcription, PCR amplification, and detection to occur in the same reaction mixture. Processed samples are added to the amplification mixture in amplification tubes (K-tubes) in which both reverse transcription and PCR amplification occurs. The reaction mixture is heated to allow a downstream primer to anneal specifically to the HCV target RNA and to the HCV Quantitation Standard RNA. In the presence of Mn2+ and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine triphosphates, Z05 polymerase extends the annealed primer forming a DNA strand (cDNA) complementary to the RNA target.

Target Amplification

Following reverse transcription of the HCV target RNA and the HCV Quantitation Standard RNA, the reaction mixture is heated to denature the RNA:cDNA hybrid and expose the primer target sequences. As the mixture cools, the upstream primers anneal specifically to the cDNA strand, Z05 extends the primer, and a second DNA strand is synthesized. This completes the first cycle of PCR, yielding a double-stranded DNA copy of the target region of the HCV RNA and the HCV Quantitation Standard RNA. The reaction mixture is heated again to separate the resulting double-stranded DNA and expose the primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05, in the presence of Mn2+ and excess dNTPs, extends the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the 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 sample is achieved in the COBAS® TaqMan® HCV Test, v2.0 by the use of the AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, 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 RNA. Also any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme, thus improving sensitivity and specificity. 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 is inactive at temperatures above 55ºC, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® TaqMan® HCV Test, v2.0 utilizes real-time[8,9] PCR technology. The use of dual-labeled fluorescent probes provides for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HCV and HCV Quantitation Standard-specific oligonucleotides labeled with a reporter dye and a quencher dye. In the COBAS® TaqMan® HCV Test, v2.0, the HCV and HCV Quantitation Standard probes are labeled with different fluorescent reporter dyes. When the dual fluorescent dye-labeled probes are intact, the reporter fluorescence 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 DNA polymerase. 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 Quantitation Standard 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 Quantitation Standard RNA. The intensity of the signals is related to the amount of starting material at the beginning of the PCR.

Fundamentals of COBAS® TaqMan® HCV Test, v2.0 Quantitation

The COBAS® TaqMan® HCV Test, v2.0 accurately provides quantitative results over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HCV titer of a sample, the earlier the fluorescence of the reporter dye of the HCV probe rises above the baseline fluorescence level. Since the amount of HCV Quantitation Standard (QS) RNA is constant between all samples, the fluorescence of the reporter dye of the HCV QS probe should appear at the same cycle for all samples. In cases where the QS amplification and detection is affected

by inhibition or poor sample recovery, the appearance of fluorescence will be delayed, thereby enabling the calculated titer of HCV target RNA to be adjusted accordingly. The appearance of the specific fluorescent signal is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a predetermined threshold (the Assigned Fluorescence Level), and starts the beginning of an exponential growth phase of this signal. A higher Ct value indicates a lower titer of initial HCV target RNA. A 2-fold increase in titer correlates with a decrease of 1 Ct for target HCV RNA, while a 10-fold increase in titer correlates with a decrease of 3.3 Ct.

HCV RNA QUANTITATION

The COBAS® TaqMan® HCV Test, v2.0 quantitates HCV viral RNA by utilizing a second target sequence (HCV Quantitation Standard) that is added to each test sample at a known concentration. The HCV Quantitation Standard is a non-infectious Armored RNA construct, containing fragments of HCV sequences with primer binding regions identical to those of the HCV target sequence. The HCV Quantitation Standard also generates an amplification product of the same length and base composition as the HCV target RNA. The detection probe binding region of the HCV Quantitation Standard has been modified to differentiate HCV Quantitation Standard amplicon from HCV target amplicon. During the annealing phase of the PCR on the COBAS® TaqMan® 48 Analyzer, the samples are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each sample. The readings from each sample 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 Quantitation Standard 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 Quantitation Standard RNA. The lot-specific calibration constants provided with the COBAS® TaqMan® HCV Test, v2.0 are used to calculate the titer value for the samples and controls based upon the HCV RNA and HCV Quantitation Standard RNA Ct values. The COBAS® TaqMan® HCV Test, v2.0 is standardized against the Second WHO International Standard for HCV RNA NAT assays (NIBSC Code 96/798)[10] and titer results are reported in International Units (IU/mL).

The COBAS® TaqMan® 48 Analyzer allows automated amplification and real-time detection of RNA for up to two simultaneous assays.

2. SAFETY PRECAUTIONS

Test kits for the Roche COBAS Ampliprep TNAI/Taqman 48 RUO Assay contain components derived from human serum or plasma. Although various treatments in the manufacturing process are sufficient to inactivate most blood-borne pathogens, there is no assurance that these reagents are entirely noninfectious. Therefore, test kit components should be treated as though they are capable of transmitting HCV. Consider all serum specimens for analysis potentially positive for infectious agents including HIV, hepatitis B virus and HCV. Controls and samples 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-A[12].

Observe universal precautions when performing the assay, thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water, handle samples with extreme care to prevent sample contamination, **use** new, sterile aerosol barrier or positive displacement RNase-free pipette tips and sterile pipettes, wear personal protective apparel, disposable gloves and eyewear during all steps of this method to minimize both infectious and chemical contamination hazards.

Do not eat, drink, smoke, or apply cosmetics in areas where reagents or samples are handled. If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water. Seek medical advice. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Do not use components beyond the expiration date on the kit. Alterations in the physical appearance of kit components may indicate instability or deterioration. Do not mix reagents from different lots. Store the kit away from any source of contaminating DNA, especially amplified nucleic acid. We recommend Biosafety Level 2 containment procedures as described in CDC/NIH publication #93-8395[1] be used by those handling test specimens and kit reagents. Use a Unidirectional work flow proceeding from the sample preparation to the amplification and detection steps. To help prevent laboratory areas from becoming contaminated with amplified RT-PCR product, maximize the physical separation of the pre- and post-amplification steps. Do not return samples, equipment, or reagents to the area where you performed the previous step. If you need to return to a previous work area, first perform the appropriate anti-contamination safeguards. Avoid microbial and RBase contamination of reagents.

Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control.

Material safety data sheets for the Roche COBAS Ampliprep TNAI/Taqman 48 RUO Assay are available through the Hepatitis Reference Laboratory, G drive computer network.

Risk is minimal due to the small quantity of chemicals, the safety of packaging and the limited handling by the operators using the test kits.

3. COMPUTERIZATION; DATA MANAGEMENT SYSTEM

a. AMPLILINK software v 3.2.2 is a custom software running under the Microsoft Windows XP Professional operating system. AMPLILINK software allows operation and data management of various COBAS® instrument configurations The COBAS® AmpliPrep Instrument is connected to the Data Station for AMPLILINK software through an Ethernet LAN. AMPLILINK software supports an interface to an external host for transmission of test results. Test results can be automatically transferred to an external host or they can be transmitted as a request from the host. The Data Station for AMPLILINK software can be connected to a LIS through the laboratory network or a

serial cable to download test orders and upload test results. The run information can be uploaded into the computerized database after the run information is exported from the software to the computerized database. This database was custom-designed for the management of CDC Hepatitis Reference Laboratory (HRL) test results, and functions within SQL Server software (Microsoft, Redmond, WA) with a Visual Basic (Microsoft, Redmond, WA) user interface. Include with every run the HCV Negative control, HCV Low Positive control and HCV High Positive control as a part of the Quality Control. For control orders, IU/mL value for the control must be within a specified range for the run to be valid. After interpretation, format for reporting the results is Quantitative HCV RNA values in IU/mL and appropriate comments. Other information in the database may typically include the HRL identification number, the specimen number, the date collected, the date tested and results of testing for other hepatitis markers. Reporting is done directly from the database in printed form or by electronic transfer. Electronically stored data are backed up routinely.

b. Finished data are reviewed by the lab supervisor. After each NHANES container is completed (i.e., when all clinical evaluations and analyses from each mobile survey site are complete), the supervisor will transmit the results to the SQL Server along with other NHANES IV data.

c. Files stored on the CDC Local Area Network (LAN) are automatically backed up nightly to tape by CDC Data Center staff.

d. Documentation for data system maintenance is maintained with printed copies of data records for 2 years.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. Specimens submitted for testing are handled according to the HRL SOP entitled "Sample Handling" [2].

b. No special instructions such as fasting or special diets are required. Diurnal variation is not a major consideration.

c. Specimens may be serum or plasma only. Blood should be collected in BD SST Serum Separator Tubes or sterile tubes using EDTA (lavender top) as the anticoagulant.

d. Serum or plasma samples are collected aseptically to minimize hemolysis and bacterial contamination.

e. Whole blood is stored at 2-25°C for no longer than 6 hours. Tube manufacturer's instructions are followed to separate serum or plasma from whole blood within 6 hours of collection.

f. Serum or plasma is transferred and stored in sterile 2ml Nalgene cryovials or equivalent.

g. Transportation of whole blood, serum or plasma must comply with country, federal, state

and local regulations for the transport of etiologic agents[13].

h. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma or serum may be transported at 2-8°C or frozen at -20°C to -80°C.

i. Required sample volume is 650 uL for the assay; 2.0 mL will permit repeat analyses as well as other testing.

j. Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder in long-term storage, are indexed in the database for easy retrieval.

k. Specimens should be stored in plastic vials and sealed tightly to prevent desiccation of the sample.

l. Serum and plasma samples may be frozen and thawed up to three times without a loss of HCV RNA.

m. Specimens are rejected if contaminated, hemolyzed, or stored improperly. However, rejection is done only after consultation with NCHS.

n. Avoid multiple freeze/thaw cycles of specimens.

o. Do not use heat-inactivated specimens.

p. Performance has not been established for cadaver specimens or body fluids other than serum or plasma (such as urine, saliva or pleural fluid.)

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

a. Instrumentation and Software

(1) Roche COBAS® Ampliprep Instrument
Distributed by
Roche Diagnostics
Indianapolis, IN 46256, USA
(For Technical Assistance call the Roche Response Center toll-free 1-800 526 1247)

(2) Roche COBAS® TaqMan® 48 Analyzer
Distributed by
Roche Diagnostics
Indianapolis, IN 46256, USA

(For Technical Assistance call the Roche Response Center toll-free 1-800 526 1247)

(3) COBAS® AmpliPrep TNAI Prep File CD ROM

(4) COBAS® TaqMan® 48 HCV, v2.0 Test Definition File RUO CD ROM

(5) Data Station for the AMPLILINK software, with printer

(6) AMPLILINK software, v3.2.2

(7) COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.1.0 Series

(8) AMPLILINK Software Version 3.1 Series Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer

(9) Manual single-channel LTS pipettes, variable volumes L10 (10ul), L20(20ul), L200 (200uL), L1000(1000ul) (Rainin Instrument LLC, Woburn, MA) http://www.rainin.com

b. **Materials**

(1) Deionized water (Continental Water Systems, Inc., San Antonio, TX).

(2) Ethanol (96 - 100%) - meets ACS specifications or better

(3) Lint-free Laboratory tissues from any Vendor

(4) Applicator cotton swabs from any Vendor

(5) DNA AWAY from any Vendor

(6) Pipet tips, Cat No. 99023, Cat No. 98333, Cat No. 98081 (CDC Glassware) with aerosol barrier or positive displacement DNAse/RNase-free tips.

(7) Protective gloves, Latex, small/medium/large Cat No. 99198/99944/99232 (CDC Glassware).

(8) 2 mL cryovials, Cat. no. 5000-0020 (Nalgene Company, Inc., Rochester, NY).

(9) Cryovial boxes, Cat. no. 5026-0909 (Nalgene Company, Inc., Rochester, NY).

(10) 50 mL-polypropylene tubes (Cat No. 95625, Falcon #352098) (CDC Glassware)

(11) 15 mL-polypropylene tubes (Cat No. 96780, Falcon #352099) (CDC Glassware)

(12) Fixed or adjustable pipetting devices capable of delivering 10ul, 20ul, 100ul, 200ul,

1000ul with at least +/- 5% accuracy (i.e., Rainin pipettes models L-10, L-20, L-100, L-200 and L-1000).

(13) 37°C Incubator (Type 37900 Culture Incubator) from any Vendor

(14) Timers (2 hours ± 1 minute) from Fisher Scientific

(15) Vortex mixer from any Vendor

(16) Microcentrifuge from any Vendor

(17) Safe-Lock tubes 2.0mL Eppendorf tubes, Cat. No: 2260004-4

(18) Aluminium foil from any Vendor

(19) Taqman 48 Analyzer S. No. 2416

(20) Ampliprep Instrument S. No. 392412

(21) Cassette opener from Roche

(22) Reagent Rack Cat. No. 28122199001

(23) Reagent Rack Barcode Labels, 001-020, Cat. No. 28048398001

(24) Reagent Rack Labels, 1-20, Cat. No. 28073112001

(25) Sample Rack (SK 24 rack), Cat. No: 28122172001

(26) Sample Rack Barcode Labels, 001-020, Cat. No. 28136289001

(27) Sample Rack Labels, 1-20, Cat. No. 28048355001

(28) SPU Rack, Cat. No: 28122806001

(29) SPUs, Cat. No: 03755525001

(30) Sample Input Tubes with Barcode Clips, Cat. No: 0313704001

(31) Racks of Output Tubes, Cat. No: 03137058001

(32) Racks of K-tips, Cat. No: 03287343001

(33) Reagent Tip, Cat. No: 28173362001

(34) Syringe 2.5ml, Cat. No: 28122911001 and Syringe Plunger, Cat. No: 28153744001

(35) Seal Cap pipettes, Cat. No: 28136815001

(36) Seal Tip Grippers, Cat. No: 28154104001

(37) UV Tube Light, Cat. No: 28127328001

(38) K-trays, Cat. No: 03343146001

(39) K-Carrier, Cat. No: 28150397001

(40) K-Carrier Holder, Cat. No: 03287696001

(41) K-Carrier Transporter, Cat. No: 03517519001

(42) K-tray Capping Tool, Cat. No: 03339904001

(43) Fan Filters, Cat. No: 28035792001

(44) Power Supply Air Filter, Cat. No: 03132307001

(45) Electronic Rack Filter, Cat. No: 03132307001

(46) Halogen Lamp, Cat. No: 28051763001

c. **Reagents**

The Roche COBAS® Ampliprep TNAI/Taqman 48 RUO Assay contains the following reagents prepared by the manufacturer.

The Roche COBAS® Ampliprep Total Nucleic Acid Isolation Kit (TNAI) (P/N: 03337928) contains sufficient reagents and materials to perform 48 tests, including controls and samples.

(1) TNAI CS1 Magnetic glass particles glass particles (**TNAI MGP**) cassette, 1 x 48 Tests
Contents: 60 g/L suspension of magnetic glass particles glass particles and Isopropanol

(2) TNAI CS2 Lysis Reagent (**TNAI LYS**) cassette, 1 x 48 Tests
Contents: Sodium citrate buffer, 473 g/L Guanidine thiocyanate, Polydocanol and 9 g/L Dithiothreitol

(3) TNAI CS3 Multi-Reagent cassette, 1 x 48 Tests
Contents: 1 x 3.8 mL Protease Solution (TNAI Pase), 1 x 19 mL 90 g/L Proteinase Prep, PCR-grade, 1 x 4 mL vial Elution buffer (TNAI EB)
General Purpose Vial (GPV) for preparation of working IC/QS

(4) TNAI CS4 Specimen Diluent (**TNAI SD**) cassette, 1 x 48 Tests

Contents: Phosphate buffer, Sodium chloride and 0.09% Sodium azide

(5) Internal Control /Quantitation Standard Diluent (**TNAI IC/QS DIL**)
Contents: 1 x 5.3 mL Tris-HCl buffer, EDTA, Poly rA RNA and 0.05% Sodium azide

(6) General Purpose Vial - 3 vials - Vial for preparation of working (GPV) IC/QS

TNAI Wash Reagent Cat. No: 03337936190
Contents: 1 x 5.1 L Sodium citrate buffer and 0.05% N-Methylisothiazolone

The Roche COBAS® TaqMan® HCV Test, v2.0 (P/N: 04878477 190) contains sufficient reagents and materials sufficient for three 16-test runs, which may be performed separately or simultaneously. One replicate each of the **CTM (-) C**; **HCV L(+)C, v2.0** and the **HCV H(+)C, v2.0** must be included in each test run of up to 24 tests. The Amplification and Detection Reagents are packaged in 24-test, dual-use bottles. For the most efficient use of reagents, samples and controls should be processed in batches that are multiples of 16.

(1) HCV QS 2 x 1.0 mL
(COBAS® TaqMan® HCV Quantitation Standard)
Sodium phosphate buffer
EDTA
< 0.005% Poly rA RNA (synthetic)
< 0.001% Armored RNA construct containing an insert with HCV primer binding sequences and a unique probe binding region (non-infectious RNA in MS2 bacteriophage)
Amaranth dye
0.1% Proclin 300

(2) HCV High (+) Control, v2.0 2 x 1.0 mL
< 0.001% Armored 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

(3) HCV Low (+) Control, v2.0 2 x 1.0 mL
< 0.001% Armored 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

(4) CTM Negative (-) Control, 4 x 1.0 mL
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

(5) HCV MMX, 2 x 24 Tests
(COBAS® TaqMan® HCV Master Mix) 2 x 1.4 mL
Tricine buffer
Potassium hydroxide
Potassium acetate
< 20% Dimethyl sulfoxide
Glycerol
< 0.001% dATP, dCTP, dGTP, dUTP
< 0.001% Upstream and downstream primers to the 5'-untranslated region of HCV
< 0.001% Fluorescent-labeled oligonucleotide probes specific for HCV and the HCV Quantitation Standard
< 0.05% Z05 DNA Polymerase (microbial)
< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)
0.09% Sodium azide

(6) CTM Mn2+ 2 x 24 Tests
(COBAS® TaqMan® Manganese Solution) 2 x 1.0 mL
< 1.2% Manganese acetate
Glacial acetic acid
0.09% Sodium azide

d. **Reagent Preparation**

Remove reagent cassettes **TNAI CS1, TNAI CS2, TNAI CS3,** from storage and load immediately. Insert these reagents immediately from refrigerator into COBAS® AmpliPrep® Instrument and equilibrate in the instrument for 30 minutes. Equilibrate **TNAI CS4** at 20-30°C for 12 hours or at 37°C for one hour prior to use. After equilibration, mix the content of **TNAI CS4** by shaking for 10 seconds.

Place the controls and QS at room temperature until completely thawed and vortex for 5-10 seconds before use. If using frozen serum or plasma samples, place the samples at room temperature until completely thawed and vortex for 5 - 10 seconds before use. Equilibrate one vial of **HCV MMX** and one vial of **CTM Mn2+** at ambient temperature for at least 30 minutes. Briefly vortex **CTM Mn2+** before use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. **Calibration Curve**

The kit lot specific calibration coefficients information are entered from the *COBAS® TaqMan® HCVTest, v2.0 Controls Value Card* supplied with the kit using the keypad or barcode scanner.

b. **Verification**

Not Applicable

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. **Preliminaries**

(1) The Ampliprep instrument should not be turned off for more than two days. If the instrument is not in use, leave it in **Standby** mode and check the wash reagent containers and waste reservoirs bi-weekly, since priming actions are automatically performed at the beginning of the day (BOD).

(2) Do not pool controls from different lots or different kits.

(3) Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove or add bottles. Do not mix reagent cassettes from different kit lots or different kits.

(4) Check expiration date of reagents prior to loading on the COBAS® AmpliPrep Instrument. Do not use kits after its expiration date. The reagent cassettes are stable until expiration date (checked by Software). The software contains an optional feature that does allow kits to be used up to 60 days longer, but results are flagged: reagent expired after 60 days the cassettes are blocked and can no longer be used. Once used, these reagents are stable for 30 days at 2-8°C or until the expiration date, whichever comes first.

(5) Store the reagent cassettes at 2-8°C. Store partially used sample preparation reagents at 2-8°C between instrument runs.

(6) **TNAI CS1, TNAI CS2, TNAI CS3, TNAI CS4** and **TNAI IC/QS DIL** can be used for a maximum of 4 instrument cycles, either continuously on board or with storage at 2-8°C between cycles, up to a maximum of 72 hours cumulative on board on the COBAS® AmpliPrep Instrument.

(7) The negative control, Low Positive control and High Positive control should be assayed with each series of patient/donor specimens. The positive and negative controls should be treated exactly as patient/donor specimens throughout the assay procedure.

(8) Controls should be removed from 2-8°C storage and equilibrated to ambient temperature (15° to 30°C) until completely thawed before use. Shake controls gently and vortex for 5-10 seconds before use.

(9) If using frozen serum or plasma samples, place the samples at room temperature until completely thawed and vortex for 5 - 10 seconds before use.

(10)The minimum run size is 4 tests (including a Negative control, Low Positive control and High Positive control).

(11) The COBAS® AmpliPrep Instrument can be operated continuously. Once the run is started, samples, reagents (except magnetic glass particles/magnetic particles), and consumables can be replaced during operation. A rack can be removed from the COBAS® AmpliPrep Instrument if the rack LED is green (ready), orange (barcode reading failure, no available consumables, run completes, or not in use). If the LED is red (in use), the rack is locked.

(12) Store **TNAI WR** at 2-30°C. **TNAI WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days at 2-30°C or until the expiration date, whichever comes first.

(13) Remove the sample rack when sample preparation completes on the Ampliprep instrument.

(14)Store **HCV MMX**; **CTM (-) C**; **HCV L(+)C, v2.0**; **HCV H(+)C, v2.0**; **HCV QS** and **CTM Mn2+** at 2 - 8ºC. Unopened, these reagents are stable until the expiration date indicated. Once opened, **HCV MMX**; **HCV L(+)C, v2.0**; **HCV H(+)C, v2.0**; **HCV QS** and **CTM Mn2+** are stable at 2 - 8°C for 28 days or until the expiration date, whichever comes first. Once opened, any unused portion of **CTM (-) C** must be discarded.

(15)Equilibrate one vial of **HCV MMX** and one vial of **CTM Mn2+** at ambient temperature for at least 30 minutes.

(16)Working Master Mix must be prepared after completion of sample and control preparation.

(17)Vortex CTM Mn2+ briefly before use. Working Master Mix (prepared by the addition of **CTM Mn2+** to **HCV MMX**) must be stored at 2- 8ºC in the dark before use.

(18)Prepared samples and controls must be added within 1 hour of preparation of Working Master Mix.

(19) Do not freeze or store processed samples and controls at 2-8°C.

(20) COBAS® TaqMan® HCV Master Mix (HCV MMX), “Working Master Mix” (Working MMX) and Working MMX plus processed samples and controls are light sensitive. All processed samples and controls should not be exposed to light after completion of sample and control preparation.

(21) The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of sample and control Preparartion.

(22) Remove used K-trays from the COBAS® TaqMan® Analyzer or COBAS®

TaqMan® 48 Analyzer after completion of the run.

b. **Sample Preparation**

(1) Bring serum specimens to 20-25°C. While one box or rack of samples is being pipetted, the other racks should be refrigerated.

(2) Serum and plasma samples may stratify when frozen or stored at 4-8°C for extended periods. Mix specimens gently before testing.

Best Practices for Preparing Primary Samples

Primary samples should be handled in a dedicated area of the room, preferably in a laminar flow hood.
The work area and pipettes should be cleaned immediately before transferring primary samples to S-tubes according to your standard laboratory practices. Change gloves after cleaning the work area and pipettes and before handling samples. This cleaning procedure should be repeated after all samples have been transferred to S-tubes.
If the COBAS AmpliPrep is located in a separate lab from the sample processing area: Change lab coats and gloves after the samples have been transferred from primary tubes to S-tubes and the sample handling area has been cleaned.

Put on a clean lab coat and new gloves before loading samples on the COBAS AmpliPrep instrument.

c. **Amplification Procedure**

Ensure all the components used for amplification are RNase free.

Store **HCV MMX** and **CTM Mn2+** at 2 - 8ºC. Unopened, these reagents are stable until the expiration date indicated. Once opened remove an aliquot for a specific batch size, store remaining **HCV MMX** and **CTM Mn2+** at 2 - 8ºC. Once opened, **HCV MMX** and **CTM Mn2+** are stable at 2 - 8°C for 28 days or until the expiration date, whichever comes first.

Prepare Working MMX after completion of Sample and Control Preparation.

Volume of CTM Mn2+ is specific to the COBAS® TaqMan® HCV Test, v2.0.

Equilibrate one vial of **HCV MMX** and one vial of **CTM Mn2+** at ambient temperature for at least 30 minutes before use.

Briefly vortex CTM Mn2+ briefly before use. Working Master Mix (prepared by the addition of **CTM Mn2+** to **HCV MMX**) must be stored at 2- 8ºC in the dark before use.

Prepare the Working MMX as follows:

For 12 tests, remove 669 µL of **HCV MMX** and place in a 2 mL tube. Add 81 µL of

CTM Mn2+ to the 2 mL tube containing **HCV MMX**, cap the tube and mix well by inverting 10 times.

For 24 tests, add 170 µL of **CTM Mn2+** to one vial of **HCV MMX**. Cap the bottle and mix well by inverting 10 times.

Do not vortex the Working MMX.

Pipette 50 µL of Working MMX into each K-tube of a K-tray. K-tray has a unique barcode and is disposable. The K-tray is setup on a K-carrier. The K-carrier is setup on a K-carrier holder.

Prepared samples and controls must be added within 1 hour of preparation of Working Master Mix.

Vortex the prepared sample for couple of seconds and add 50 µL of each prepared sample and control to the appropriate K-tube containing Working MMX using a micropipettor with an aerosol barrier or positive displacement tip.

Gently mix each sample or control up and down three times with the micropipettor without generating bubbles.

Cap the K-tray with K-tray cap array. Use the K-Tray Capping Tool for capping.

Amplification must be started within 2 hours from the time that the processed samples and controls are added to the Working Master Mix.

K-tubes with prepared sample and Master Mix set up in K-carriers are manually loaded into the instrument using the K-carrier transporter.

The K-carrier transporter is a device used to lift the K-carrier loaded with tubes into the thermal cycler. Since the block of the thermal cycler is usually hot, the K-carrier transporter must be used to transfer the K-carrier to prevent burns.

The barcoded K-carrier ID is read by a barcode scanner when loaded into the instrument.

Remove used K-tray from the thermal cycler after detection, discard the K-tray with the amplicon in a closed plastic bag in an autoclave pan.

c. **Instrument Setup**

COBAS® AmpliPrep Instrument Setup

Part A. Maintenance and Priming

The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.
Turn the Data Station for the AMPLILINK software **ON**. Prepare the Data Station as follows:

Log onto Windows® XP.
Double click the AMPLILINK software icon.
Log onto AMPLILINK software by entering the assigned User ID and password.

Check the supply of **TNAI WR** using the **Status** Screen and replace if necessary. Perform all Maintenance that is listed in the Due Tab, as outlined in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2. The COBAS® AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

Place **HCV CS1** onto a reagent rack. Place **HCV CS2, HCV CS3** and **HCV CS4** onto a separate reagent rack.

Load the reagent rack containing **HCV CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2.

Load the reagent rack containing **HCV CS2, HCV CS3** and **HCV CS4** onto rack position **B, C or D** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2.

Load the sample rack (rack of 24) onto rack position **E, F, G or H** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2.

Part C. **Loading of Disposables**

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

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 as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software Version 3.2.2.

Load full Output Sample tubes rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2.

Load full K-tip rack(s) onto rack position **M, N, O** or **P** of the COBAS® AmpliPrep Instrument as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2.

Part D. Ordering and Loading of Samples

Prepare sample racks as follows: Attach a barcode label clip to each sample rack position where a sample (S-tube) is to be placed. Place one Input S-tube into each position containing a barcode label clip.
Using the AMPLILINK software, create orders for each control and sample by clicking on the **Sample Rack** tab in the **Orders** window. In the Sample Rack window click **New**. Enter a Sample Rack number. Select the test **TNAI500** from the test panel at the lower portion of the window. Assign control and sample orders to sample rack positions in the Order/Lot Number column in the **Sample Rack** tab. The sample rack number must be for the rack prepared. Save the Sample Rack Order. Print the **Sample Rack Order** report to use as a worksheet.

Prepare Sample racks in the designated area for control and sample addition as follows: Vortex each control [**CTM (–) C, HCV L(+)C** and **HCV H(+)C**] and sample for 3 to 5 seconds.

Avoid contaminating gloves when manipulating the samples and controls.
Avoid transferring particulates and/or fibrin clots from the original sample to the Input S-tube.
Avoid contaminating the upper part of the S-tubes with samples or controls.

Controls and samples should be transferred to tube positions as assigned and recorded on the worksheet.

Load the sample rack(s) filled with Input S-tubes onto rack positions **E, F, G** or **H** of the COBAS® AmpliPrep Instrument.

Part E. Start of COBAS® AmpliPrep Instrument Run

Start the COBAS® AmpliPrep Instrument using the AMPLILINK software as described in the AMPLILINK Software Version 3.2.2 Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer.

Part F. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer

Check for flags or error messages in the system screen as described in the COBAS® AmpliPrep Instrument Manual for use with the COBAS® TaqMan® 48 Analyzer and the AMPLILINK Software, Version 3.2.2 and the AMPLILINK Software Version 3.2.2 Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer.

Remove processed samples and controls from the COBAS® AmpliPrep Instrument on sample racks for amplification and detection on the COBAS® TaqMan® 48 Analyzer. Remove waste from the COBAS® AmpliPrep Instrument..

COBAS® TaqMan® 48 Analyzer Set-up

Turn on the COBAS® TaqMan® 48 Analyzer instrument using the power switch located at the left rear of the instrument. The color of the status LED indicates instrument status. The color of the status LED is green indicating that the system is in standby mode after the analyzer completes initialization.

Part G. Ordering and Loading Processed Samples

Enter Quality Control information by selecting the **Quality Control** tab in the **Orders** window. Click the **New** button and enter the information from the *COBAS® TaqMan® HCV Test, v2.0 Controls Value Card* supplied with the kit using the keypad or barcode scanner. Enter the COBAS® TaqMan® HCV Test, v2.0 lot number, expiration date, Low (+) and High (+) Control ranges as well as lot-specific calibration coefficients in the designated spaces. Click "**OK**."

Assign a K-carrier number for the run by clicking on the **K-Carrier** tab in the **Orders** window. In the **K-Carrier** window, click **New**. In the cell to the right of "**K-Carrier ID**," enter the K-carrier number from the barcode on the K-carrier using the keypad or barcode scanner. Select the test **HCV-HPS** from the test panel at the lower portion of the window.

In the **Worklist**, select the first row of the Type (T) column. Highlight this field to access the pull down menu and then select the required control type. Next, double click the sample ID field for the same row. The **LookUp Control** window will be displayed with all available controls. When the control is selected, the corresponding calibration and control values will be displayed in the lower right Information panel. Repeat this process for all required controls. Enter the sample ID's in the Order/Lot Number column. Click **Save** to save the K-carrier order assignment

Part H. Reverse Transcription, Amplification and Detection

Select the **Systems** icon in the System Tab; click **Open** to open the Thermal Cycler. When the Thermal Cycler Cover has completely opened and "**Ready to Load**" is seen in the **Systems** window, lift and hold the Thermal Cycler lid open. Using the K-carrier Transporter, transfer the loaded K-carrier containing the capped K-tubes with Working Master Mix and controls and samples into the Thermal Cycler according to the AMPLILINK Software Version 3.2.2 Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer. Close the Thermal Cycler lid.

Click **Start** on the **Systems** window below the TC icon to close the Thermal Cycler Cover and start the run.

Reverse transcription, amplification and detection are automatically performed by the COBAS® TaqMan® 48 Analyzer.

Part I. End of COBAS® TaqMan® Analyzer Run

At the completion of the COBAS® TaqMan® 48 Analyzer run, print Results Report after accepting the results. Check for flags or error messages in the Result report as described

in AMPLILINK Software, Version 3.2.2, the COBAS® TaqMan® 48 Analyzer Instrument Manual for use with AMPLILINK Software, Version 3.2.2 Series and the AMPLILINK Software Version 3.2.2 Series Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer. Samples with flags and comments are interpreted as described in the Results section. After acceptance, store data in archive.

Remove used K-tubes from the COBAS® TaqMan® 48 Analyzer and discard it in a plastic bag in an autoclave pan.

c. **Operation of Assay Procedure**

The COBAS AmpliPrep Instrument is designed to process specimens in a continuous flow. Operator attendance is not required after a run is started. If needed, processed samples can be removed for testing and reagent kit racks can be replaced without interrupting the run. Sample processing is confined to a self contained, disposable sample processing unit (SPU) with a dedicated plugged sample tip, to control cross-contamination. The first sample is completed within one hour of commencing operation. Subsequent samples are completed within three to four minutes per sample. The processed samples are then amplified and detected automatically using the COBAS® TaqMan® 48 Analyzer.

The operator prepares the run by transferring patient specimens and controls to sample tubes (S-tubes) and loading them. Reagents and consumables are loaded, the appropriate orders are entered, and the run is started.

All COBAS AmpliPrep reagents are ready to use and are contained in barcoded cassettes. Reagent identification and expiration date are automatically read by the instrument. Delivery of reagents and transfer of samples, S-tubes and SPUs is carried out by two *x*, *y*, *z* transfer mechanisms. Separation and washing of the magnetic particles is done in an automatic separation station.

After the run is completed, the processed samples are removed for amplification and detection and the used consumables are removed and discarded.
AMPLILINK software supports an interface to an external host for transmission of test results. Test results can be automatically transferred to an external host, or they can be transmitted as a result of a request from Host.

d. **Recording of Data**

The COBAS® TaqMan® 48 Analyzer automatically determines the HCV RNA titer for the sample or control. **The HCV RNA titer is expressed in International Units (IU)/mL in accordance with the Second WHO International Standard for HCV RNA NAT assays (NIBSC Code 96/798)10.**
The COBAS® TaqMan® 48 Analyzer:
- Determines the Cycle Threshold value (Ct) for the HCV RNA and the HCV Quantitation Standard RNA.
- Determines the HCV RNA titer based upon the Ct values for the HCV RNA and HCV

Quantitation Standard RNA and the lot-specific calibration coefficients.
- Determines that the calculated IU/mL titers for HCV L (+)C, v2.0 and HCV H(+)C, v2.0 fall within the established ranges.

If **Negative Control, HCV Low Positive Control or HCV High Positive Control** is invalid then the entire run is invalid, repeat the entire run including control and sample preparation, reverse transcription, amplification and detection.

A valid run may include both valid and invalid sample results depending on whether flags and/or comments are obtained for the individual samples.

Titer	Interpretation
Target Not Detected	**Ct value for HCV above the limit for the assay or no Ct value for HCV obtained. Report results as "HCV RNA not detected."**
< 50 IU/mL	**Calculated IU/mL are below the range of the assay. Report results as "HCV RNA detected, less than 50 HCV RNA IU/mL."**
≥ 50 IU/mL and ≤ 50,000,000 IU/mL	**Calculated results greater than or equal to 50 IU/mL and less than or equal to 50,000,000 IU/mL are within the range of the assay.**
> 50,000,000 IU/mL	**Calculated IU/mL are above the range of the assay. Report results as "greater than 50,000,000 HCV RNA IU/mL." If quantitative results are desired, the original sample should be diluted with HCV-negative human serum or plasma, depending on the matrix of the original sample and the test repeated. Multiply the original result by the dilution factor.**

Note: Samples above the range of the assay may also produce an Invalid result with a flag "QS_INVALID". If quantitative results are desired, the original sample should be diluted with HCV-negative human plasma or serum in accordance with the matrix of the original sample, and the test repeated. Multiply the reported result by the dilution factor.

f. **Periodic Maintenance and Replacement of Key Components**

(1) Daily Maintenance: COBAS® AmpliPrep Instrument

Daily Maintenance should be performed at the beginning of each workday. The following instructions replace the Daily Maintenance Service Actions described in the AMPLILINK software:

a. Switch off the COBAS AmpliPrep instrument.
b. Empty the waste container after removing the black holder for the sensor. Rinse both the waste container and the black holder with deionized or distilled water. Thoroughly wipe the mouth of the waste container, the handle, and the black holder for the sensor with 70% ethanol.
c. Wipe the inside of the main cover with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.
d. Wipe the initialization posts with a new lint-free tissue moistened with deionized

or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

e. Wipe the plastic panel at the initialization post and the plastic panel in the rear with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

f. Wipe the top surface of the rack platform with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

g. Wipe the surface of the rack loading area with a new lint- free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

h. Wipe the SPU incubator surfaces with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol. It is not necessary to clean inside the slit and holes of the SPU incubators.

i. Wipe the empty slots of the output rack platform, the separation area, and then the target release incubator with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

j. Move the transfer heads to the middle of the instrument. Wipe the surface of both wash towers with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

k. If K-carriers are left in the K-carrier park positions, remove them and clean the K-carriers with DNA AWAY surface decontaminant, then with deionized water, followed by 70% ethanol.

l. Wipe the K-carrier park positions with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

m. Wipe the reagent tips with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol. Make sure any crystals or droplets are removed.

n. Wipe the tube handler front part with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

o. Wipe the SPU gripper with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

p. Wipe the SPU gripper with a cotton swab moistened with deionized or distilled water, then wipe with a new cotton swab moistened with 70% ethanol.

q. The sensors and light barriers on both transfer heads should be cleaned at least once a month or whenever signs of spillage are visible. New lint-free tissues moistened with **deionized or distilled water only** should be used for cleaning. Wipe dry with a new lint-free tissue.

r. Open the loading panel and wipe it with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.

s. Close the main cover of the instrument.

t. Wipe the outside of the instrument, especially the ouside of the main cover and loading panel with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol. The rear

outside of the instrument should be excluded.

u. Switch the instrument on.
v. After initialization is completed, inspect syringes and tubing for leakage and bubbles. If bubbles are visible, perform an extended prime to eliminate them.
w. Update the daily maintenance service counters by selecting the daily maintenance service actions within the AMPLILINK Service Due tab and click the Done button.

At the end of the day or before performing daily maintenance, all racks used on the COBAS AmpliPrep instrument and COBAS® TaqMan® analyzer should be cleaned with DNA AWAY and thoroughly rinsed with deionized or distilled water followed with 70% ethanol.

Normal system priming needs to be performed every day.

Weekly Maintenance

Wiping the outside of the instrument may not need to be performed every day, but should be performed at least once a week.

Extended system priming needs to be performed once a week.

Cleaning Waste container needs to be performed once a week.

Cleaning

Changing filters for fans 120mm to be performed every 365 days.
Cleaning wash towers to be performed every 30 days.

Replacement

Electronic fan filter to be replaced every 180 days
Gripper O-rings to be replaced every 60 days
Reagent tips to be replaced every 10,000 tests
Syringe assembly to be replaced every 730 days
Syringe plunger to be replaced every 180 days
UV lamp to be replaced every 1000 days

The UV light in the COBAS AmpliPrep instrument should be turned on at the end of each work day. If the instrument is not used every day, the UV light should be turned on at least twice a week.

COBAS® TaqMan® 48 Analyzer
Daily Maintenance

Daily Maintenance should be performed at the beginning of each workday. The following instructions replace the Periodic Maintenance Service Actions described in the AMPLILINK software:

1. Discard used K-tubes into a plastic bag and seal or secure the contents. Dispose the bag in accordance with local regulations. If possible, immediately place the waste

outside of the lab. Change gloves before proceeding to the next step.
2. Close the thermal cyclers and turn off the analyzer.
3. Clean K-carriers with DNA AWAY, then with deionized or distilled water, then with 70% ethanol.
4. Clean K-carrier racks and K-carrier holders with DNA AWAY, then with deionized or distilled water, then with 70% ethanol.
5. Wipe the K-carrier transporter with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.
6. Wipe all accessible surfaces around the thermal cyclers, including the lids with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.
7. Wipe the surfaces of the analyzer with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol. The rear outside of the analyzer must be excluded as the power supply is accessible.
8. Wipe the bench surfaces around the COBAS® TaqMan® 48 analyzer with a new lint-free tissue moistened with deionized or distilled water, then wipe with a new lint-free tissue moistened with 70% ethanol.
9. Switch on the analyzer.

Weekly Maintenance

Wiping the surfaces of the analyzer may not need to be performed every day, but should be performed at least once a week.

Replacement

Electronic Fan Filter – to be replaced every 365 days
Power Supply Filter – to be replaced every 365 days
Halogen Lamp – to be replaced every 1000 hours

Best Practices for Performing a Run and Cleaning Up at the End of the Day

New gloves must be worn when loading reagents and samples on the COBAS AmpliPrep instrument.

Load the COBAS AmpliPrep instrument with the quantity of consumables needed for the run. Do not load extra SPU sets, K-tip racks, or K-tube racks.
Immediately discard all used SPUs and S-tubes according to local requirements. Care should be taken to prevent splashing when S-tubes are discarded into waste bags or containers.

Unused SPUs should be covered with a white SPU cover (the cover that is on them when they are delivered) and stored in an area cleaned according to your standard laboratory practices. These SPUs may be used in future runs.

Racks containing unused K-tips and Output-tubes may be left on the COBAS AmpliPrep

instrument at the end of the day.

At the end of each day or before performing daily maintenance, all racks used on the COBAS AmpliPrep instrument and the COBAS® TaqMan® analyzer should be cleaned with DNA AWAY and thoroughly rinsed with deionized or distilled water followed by 70% ethanol.

Cleaned SPU racks, reagent racks, and sample racks should be stored in a clean, dedicated, closed storage space.

g. **Calculations**

Not Applicable

h. **Special Procedure Notes**

None

9. REPORTABLE RANGE OF RESULTS

Calculated results greater than or equal to 50 IU/mL and less than or equal to 50,000,000 IU/mL are reportable range of results for the assay.

10. QUALITY CONTROL (QC) PROCEDURES

The assay controls supplied with the test kit must be included with each run, regardless of the number of specimens tested.

One replicate each of the COBAS® TaqMan® Negative Control, the COBAS® TaqMan® HCV Low (+) Control, v2.0 and the COBAS® TaqMan® HCV High (+) Control, v2.0 must be included in each test run.

As with any new laboratory procedure, the use of additional in-house controls is recommended.

There are no requirements regarding the position of the positive controls in the K-carrier. Refer to the AMPLILINK Software Version 3.2.2 Application Manual for use with the COBAS® AmpliPrep Instrument and COBAS® TaqMan® 48 Analyzer for printing results and interpreting flags and comments.

Negative Control CTM (-) C must yield a **"Target Not Detected"** result, i.e., the Ct value for HCV RNA was above the limit for the assay or no Ct value for HCV RNA was obtained, but a valid Ct value was obtained for the HCV Quantitation Standard RNA. If **CTM (-) C** does not meet this criteria, the entire run is invalid. Repeat the entire process (sample and control preparation, amplification and detection).
If **CTM (-) C** is consistently not valid, contact your local Roche office for technical assistance.

Positive Controls

The established ranges for **HCV L(+)C, v2.0** and **HCV H(+)C, v2.0** are provided on the *COBAS® TaqMan® HCV Test, v2.0 Controls Value Card* supplied in the kit. These ranges are entered into the Data Station for the AMPLILINK software using the COBAS® TaqMan® 48 Analyzer barcode scanner or keypad.

The HCV RNA IU/mL for both the **HCV L(+)C, v2.0** and **HCV H(+)C, v2.0** must fall within the range indicated on the *COBAS® TaqMan® HCV Test, v2.0 Controls Value Card* supplied in the kit. If one or both of the positive controls does not meet this criteria, then the entire run is invalid. Repeat the entire process (sample and control preparation, amplification and detection). If the calculated HCV RNA titer of one or both of the positive controls is consistently outside the assigned range, contact your local Roche office for technical assistance.

In our Laboratory Roche recommended the use of COBAS® AmpliPrep Total Nucleic Acid Isolation Kit (automated sample preparation) replacing the High Pure System kit (manual sample preparation) with the COBAS® TaqMan® HCV Test, v2.0 RUO assay, so we have established our own ranges for the HCV H(+) control, v2.0 since the HCV H(+) control values does not fall within the range assigned in the *COBAS® TaqMan® HCV Test, v2.0 Controls Value Card,* these values for the HCV H(+) control on the Card were assigned for the High Pure system kit.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Repeat run for individual sample as described above.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. The values assigned to the calibration coefficients are lot specific and specific to the COBAS® TaqMan® HCV Test, v2.0 For Use With The High Pure System. These values may not be valid when used with alternative sample preparation methods.

b. This test has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Testing of other sample types may result in incorrect results, false negative or false positive results.

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

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

e. Reliable results are dependent on adequate sample collection, transport, storage and processing procedures.

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

g. Use of this product should be limited to personnel trained in the techniques of PCR.

h. This product can only be used with the COBAS® TaqMan® 48 Analyzer.

13. REFERENCE RANGES (NORMAL VALUES)

All normal noninfected humans should have negative values for HCV RNA.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may remain at 20-25 °C during preparation and testing only.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

Other FDA-licensed tests for HCV RNA may be substituted but must be accompanied by validation data to show substantial equivalence with this assay. Substitution of test methods may not be done without approval from the NCHS.

Alternate storage is not recommended.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Test results are documented through the lab management database (Section 3). Generally, a CDC epidemiologist communicates the findings to other participants in the study. Final reports may be electronic or in printed form. All electronically held data are backed up routinely.

Specimens in long-term storage are arranged by study group. The storage location of each sample is listed with the test data.

REFERENCES

[1] Biosafety in Microbiological and Biomedical Laboratories. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health. Fifth Edition, 2007. U. S. Government Printing Office, Washington: 2007

[2] Hepatitis Reference Laboratory, Centers for Disease Control and Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, Division of Viral Hepatitis, Laboratory Branch. SOP Title: Sample Handling. Effective Date: 06/2008. Approved by: Saleem Kamili, PhD and Jan Drobeniuc, MD, PhD

[3] Stuyver, L., Rossau, R., Wyseur, A. et al. 1993. Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. Journal of General Virology **74**:1093-1102

[4] Machida, A., Ohnuma, H., Tsuda, F. et al. 1992. Two distinct subtypes of hepatitis C virus defined by antibodies directed to the putative core protein. Hepatology **16**:886-891

[5] Bukh, J., Purcell, R. H., and Miller, R.H. 1992. Sequence analysis of the 5' noncoding region of hepatitis C virus. Proceedings of the National Academy of Science, USA **89**:4942-4946

[6] Meng Q, Wong C, Rangachari A, Tamatsukuri S, Sasaki M, Fiss E, Cheng L, Ramankutty T, Clarke D, Yawata H, Sakakura Y, Hirose T, Impraim C. 2001. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type 1 RNA. J Clin Microbiol **8**:2937-45

[7] Smith, E.S., Li, A.K., Wang, A.M., Gelfand, D.H., Myers, T.M. 2003. Amplification of RNA: High-Temperature Reverse Transcription and DNA Amplification with a Magnesium-Activated Thermostable DNA Polymerase. In *PCR Primer: A LaboratoryManual*, 2nd Edition, Dieffenbach C.W. and Dveksler G.S., Eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 211-219

[8] Higuchi, R., Dollinger, G., Walsh, P.S., and Griffith, R. 1992. Simultaneous amplification and detection of specific DNA sequences. Bio/Technology **10**:413-417

[9] Heid, C.A., Stevens, J., Livak, J.K., and Williams, P.M. 1996. Real time quantitative PCR. Genome Research **6**:986-994

[10] Saldanha, J., Heath, A., Aberham, C., Albrech, J., Gentili, G., Gessner, M. and Pisani, G. 2005. World Health Organization collaborative study to establish a replacement WHO international standard for hepatitis C virus RNA nucleic acid amplification technology assays. Vox Sanguinis **88**:202-204

[11] Richmond, J.Y. and McKinney, R.W. eds. 1999. *Biosafety in Microbiological and Biomedical Laboratories*. HHS Publication Number (CDC) 93-8395

[12] CLSI. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue. Approved Guideline. CLSI Document M29-A Villanova, PA:CLSI, 1997