RAS Lifesciences Pvt Ltd
Striving for better health

Amplisure™ HCV Quantitative Kit

(Real Time PCR Kit)

QT-HCV-25	**: 25 rxns**
QT-HCV-50	**: 50 rxns**
QT-HCV-100	**: 100 rxns**

IVD

Product Insert

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Index **Page No.**

Introduction

Hepatitis C virus (HCV) is a hepatotropic virus of family Flaviviridae and genus Hepacivirus with a single strand positive RNA genome. It is a leading cause of chronic liver disease and has already infected at least 170 million people worldwide. In India, there are about 12-13 million HCV carriers and modeling data predict that the burden of disease could soon increase substantially. Till date, six different HCV genotypes and more than 70 subtypes have been identified based on the nucleic acid sequences. Although most chronic HCV patients have mild chronic hepatitis, it is a progressive disease and can lead to cirrhosis or hepatocellular carcinoma. A large number of genotypes have been identified among hepatitis C virus isolates from all over the world. It has been suggested that different genotypes have different clinical outcomes with regard to disease severity and response to interferon therapy.

Intended Use

The Amplisure™ HCV quantitative kit is used to detect the genome of HCV genotypes(1-6) and quantify HCV genome in plasma , using Real-Time PCR after nucleic acid extraction. The viral load is measured using a range of four quantification standards provided in the kit. Combined with other methods of biological investigation (clinical picture and other laboratory markers), the results obtained with the Amplisure™ HCV quantitative kit are used to monitor HCV infection .

This kit cannot be used for screening blood from donors or blood products.

Product Description

Amplisure™ HCV quantitative kit is an *in-vitro* diagnostic kit for quantitation of Hepatitis C Virus (1-6 genotypes) in human plasma. The kit contains the necessary reagents for performing HCV quantitation by Real-time PCR.

Pathogen detection by Real Time polymerase chain reaction (PCR) is based on the amplification of specific region (5'UTR) of the pathogen genome. The assay principle is based on Taqman probes which allow higher specificity and sensitivity.

In addition, the Amplisure™ HCV quantitative kit contains a second amplification system to identify possible PCR inhibition by using an internal control (IC), without affecting the analytical sensitivity of the assay. External quantitation standards calibrated with WHO control are supplied, which allow the determination of the amount of Hepatitis C Viral RNA.

Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) Pre-PCR area/room - for setting up PCR reaction
c) PCR area/room – for performing PCR using the thermocyclers/real time PCR

As part of Good Laboratory Practices (GLP), it is recommended to have dedicated areas to avoid cross contamination.

General Precautions

Precautions while extracting Nucleic acid

Always wear proper attire (powder free gloves, facemask and Head cap) before starting the nucleic acid extraction procedure. During preparation of samples, open one sample tube at a time and close it before opening another tube, follow it at every step of isolation. Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of Nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with Nucleic acid.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area. Sample Preparation Area must have dedicated laboratory coats, pipettes, pipette tips and cyclo-mixer and not to be moved to other areas. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Sample preparation should be performed in a Biosafety cabinet using aerosol free tips.

Precautions while setting up a PCR reaction

PCR assay is sensitive and any accidental introduction of product from previous amplification reactions can lead to incorrect results. Hence measures to reduce the risk of contamination in the laboratory should include physically separating the activities involved in performing PCR and complying with good laboratory practices. PCR hood should be used to avoid contamination.

It is recommended to have proper cleaning procedures to minimize the risk of cross contamination and carry over contamination (e.g. DNA OUT™, RNase OUT™, 0.1% Sodium Hypochlorite, Fumigation etc.).

It is recommended that areas should be defined in Pre-PCR room for preparation of mastermix and addition of templates. Laboratory coats and equipment used in the Pre-PCR Area must remain in this area and should not be moved to the Sample Preparation Area.

Precautions for post PCR or equipment area/room

The Real time PCR instrument/s should be kept in a separate segregated area away from Sample preparation area and Pre-PCR area.

Precautions after completion of Real time PCR assay

The reaction tubes or strips should be properly discarded without opening the caps, after the completion of run to avoid carry over contamination.

Usage Limitations

1. All the kit components are for *in-vitro* diagnostics only.
2. The product is to be used by personnel specially trained in the *in-vitro* diagnostics procedures only.
3. Follow the product insert strictly for optimal results.
4. Do not use the kit beyond the expiry date mentioned on the box.
5. Follow the guidelines provided in product insert for sample collection, storage and transport.
6. For ideal performance, store the kit under recommended conditions only.

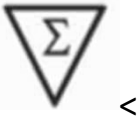
Safety Precautions

1. All patient specimens should be considered as potentially infectious and handled in a BSL2 biosafety hood with BSL3 practices.
2. Wear personal protective equipment, including gloves, head cap, face mask and lab coats when handling kit reagents/sample extraction. Wash hands thoroughly using detergents before and after performing the test.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens as per regulatory guidelines.

Storage Conditions and Product Stability

1. All the kit reagents should be stored at -20 °C. Replace all the kit components immediately at -20 °C after usage.
2. Repeated thawing and freezing (more than 6 x) of all kit reagents should be avoided, as it reduces assay sensitivity. If needed, make aliquots of the kit reagents according to the volume used in the protocol prior to freezing.
3. Allow reagents to be thawed completely on Ice/4 °C prior to use.
4. Kit reagents are stable through the end of the expiration date indicated on the box when stored at -20 °C.

Symbols

Description of Symbol	Denotation
	*in-vitro* Diagnostic medical device
	Consult Instruction manual (Product Insert) for use
	Lot Number of the kit or Kit contents
	Catalogue number of Kit
 <N>	Contains sufficient for <N> reactions (Pack Size)
	Manufacturer
	Temperature limitation (Storage Condition)
	Use by MMM-YYYY (Expiry Date)
	Biological risk (handle carefully)
	Important Note

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-HCV-25)	50 rxns (QT-HCV-50)	100 rxns (QT-HCV-100) 2 x 50 rxns
Yellow	RAS qRNA PCR Mix	DNA Amplification Reagent	1 x 375 µL	1 x 750 µL	2 x 750 µL
Red	RAS RT Mix	cDNA synthesis Reagent	1 x 175 µL	1 x 350 µL	2 x 350 µL
Brown	RAS HCV PPM	HCV Primer-Probe Mix	1 x 50 µL	1 x 100 µL	2 x 100 µL
Lilac	RAS HCQS1 (2×10^4 IU/ µL)	HCV Quantitation Standards	1x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HCQS2 (2×10^3 IU/ µL)		1x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HCQS3 (2×10^2 IU/ µL)		1x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HCQS4 (2×10^1 IU/ µL)		1x30 µL	1 x 60 µL	2 x 60 µL
Natural	RAS IC PCR Mix	Internal Control	1x25µL	1 x 50 µL	2 x 50 µL
Natural	RAS R-IC EX Mix		1x250µL	1 x 500 µL	2 x 500 µL
White	MBGW	Molecular Biology Grade water	1 x 500 µL	1 x 500 µL	2 x 500 µL

Materials required but not supplied

The materials which are required but not supplied are listed below:

1. Viral RNA Extraction kit
2. Biosafety Cabinet
3. PCR Hood

4. Calibrated variable micropipettes
5. Sterile pipette filter tips (aerosol free)
6. Vortex mixer
7. Dry Bath
8. Benchtop centrifuge with rotor for 1.5 mL reaction tubes
9. Real Time PCR machine
10. Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate
11. Cooling block (96 x 0.2 mL tubes)
12. 1.5 mL centrifuge tubes
13. 1.5 mL centrifuge tube stand
14. Cooling block (2 mL tubes)
15. Sterile Nitrile gloves
16. Facemask
17. Head cap
18. Lab coats

Quality Systems

In accordance with ISO-certified Quality Management System (9001:2008 and 13485: 2003) of RAS Lifesciences, each lot of Amplisure™ HCV quantitative kit is tested against predetermined specifications to ensure consistent product quality.

Sample Type/Collection/Storage/Transport

Sample Type

Plasma (K_2EDTA-Blood)
Heparinized Blood must not be used as they inhibit the PCR reaction

Sample Collection, Storage and Transport

3-5 mL of blood has to be drawn into a K_2EDTA vaccutainer. Cap and swirl the tubes for uniform mixing of blood and K_2EDTA. When K_2EDTA is used, whole blood can be collected in tubes with or without a gel separator. The K_2EDTA blood

samples are centrifuged (20 minutes at 1000–1500 x *g)* to separate plasma from cellular material and in the case of a non gel separator tube, the plasma should be removed to a secondary sterile tube within 4 hours of phlebotomy.

Collection and storage of unstabilized whole blood is not recommended for PCR analysis, because RNA degradation occurs in blood stored *ex vivo*. The sensitivity of the assay can be reduced if whole blood samples are frozen or stored for a longer period of time.

Plasma separated in a gel separator tube may be transported to the laboratory *in situ.* Plasma should be shipped at 2 to 8 °C and stored at -20 °C as it is stable for up to five days at 2 to 8 °C and longer if frozen at -20 °C or -70 °C or lower. Do not store plasma samples in a "frost -free" freezer as the temperature is cycled several times per day on this type of freezer, causing degradation of nucleic acid targets.

Sample material should be transported in a leak proof, unbreakable transport container to avoid leakage of sample. The samples should be transported following the local and national instructions for the transport of pathogen material.

Assay Procedure

RNA Extraction

Amplisure™ HCV quantitative kit has been validated using the following Viral RNA extraction kits:

1- Roche High Pure Viral RNA kit (Cat. No. 11858882001)
2- QIAamp Viral RNA Mini Kit (Cat. No. 52904)

Follow the manufacturer's instructions mentioned in the manual for Viral RNA extraction. Different pack sizes of the above mentioned kits can be used. However

the customer can also validate their own extraction process using other Viral RNA extraction Kits.
Recommended sample volume for extraction and elution are as follows:

Name of the RNA Isolation Kit	**Recommended Sample volume *(to be taken for RNA Extraction)***	**Recommended Final Elution volume**
Roche High Pure Viral RNA kit (Cat. No.11858882001)	200 µL	50 µL
QIAamp Viral RNA Mini Kit (Cat. No. 52904	140 µL	50 µL

The analytical sensitivity of the assay in consideration of the purification was determined using the above defined volumes.

Use of Internal Control (IC)

Internal controls are supplied (RAS R-IC Ex Mix and RAS IC PCR Mix) along with Amplisure™ HCV quantitative kit. This allows the user to control the Viral RNA isolation procedure as well as to check for possible PCR inhibition.

Internal control should only be used once, either at the Extraction step or at the PCR step

Usage of Internal Control at the RNA extraction step

If internal control (IC) is required to be added at the time of RNA extraction, add 10 µL of RAS *R-IC Ex mix* per isolation to the lysis buffer along with other components of kit used for lysis (as per kit instructions) and vortex for 5 seconds prior to usage.

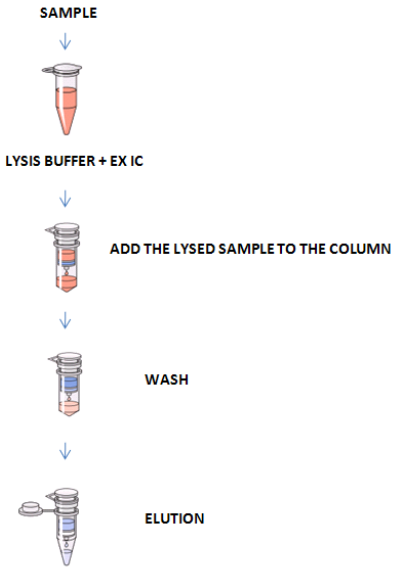


Fig. 1- Viral RNA Extraction Overview

Usage of Internal Control at the Real time PCR step

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the PCR master mix as described below. Addition of Internal control should be done only once, either at the time of sample extraction or during PCR setup.

qPCR Protocol

Preparation of Reaction Mastermix

Quantitation procedure with Amplisure™ HCV quantitative kit involves *1 step RT qPCR*. It is recommended that a minimum of three standards and a negative control (MBGW should be used as negative control) are required to be included in a single run for acquiring proper results.

Set up a real time single step RTPCR reaction as follows:

1- qPCR reaction mix composition without Internal Control (When R-IC Ex Mix is added during RNA extraction)

Components	Volume per reaction (µL) (for final vol. of 30 µL)
RAS qRNA PCR Mix	15.0
RAS RT Mix	7.0
RAS HCV PPM	2.0
RNA/HCQS/ MBGW	5.0
MBGW	1.0

2- qPCR reaction mix composition with Internal Control (When R-IC Ex Mix is not added during RNA extraction)

Components	Volume per reaction (µL) (for final vol. of 30 µL)
RAS qRNA PCR Mix	15.0
RAS RT Mix	7.0
RAS HCV PPM	2.0
RNA/HCQS/ MBGW	5.0
RAS IC PCR Mix	1.0

1. **Addition of IC PCR mix (if required) should be done at the time of preparation of master mix.**
2. **The results may be inconsistent, if the IC PCR mix is added individually.**

Place the PCR plate/tubes/strips in real time thermocycler.

PCR Programming

The Amplisure™ HCV quantitative kit is validated on the following instruments:

- Rotor-Gene™ 6000
- Rotor-Gene™ Q 5plex
- ABI 7500 DX Real-Time PCR System
- ABI 7300 Real-Time PCR System
- Eppendorf Realplex 4
- Bio-Rad™ CFX 96

Plate Setup

1. Program the plate setup by labeling the slots as per tube/strip/plate labels. The sequence of labeling of slots should be the same way as the tube/strip/plate is kept in the machine.
2. Select the type of sample (Unknown/Standard/NTC) for each slot.
3. When Standard is selected as sample type, mention the quantitation value in IU/µL (2E4, 2E3 etc)
4. Select the channel for acquisition (FAM/Yakima Yellow)

S. No.	Name of channel	Source wavelength (nm)	Detection wavelength (nm)
1.	FAM (Pathogen target)	470	510
2.	Yakima Yellow (Internal Control)	530	555

5. For background calibration in different instruments, follow the procedure described below:

   Rotor-Gene™ 6000 - Perform 'Gain optimization'
   Rotor-Gene™ Q 5plex - Perform 'Gain optimization'
   ABI 7500 DX Real-Time PCR System - Select Passive Reference dye 'ROX'
   ABI 7300 Real-Time PCR System - Select Passive Reference dye 'ROX'
   Eppendorf Realplex 4 - Select 'ROX' for background calibration
   Bio-Rad™ CFX 96 - Select 'ROX' for background calibration

Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com

Cycling conditions

1. Configure the following program in the machine.

Steps	No. of cycles	Temperature (°C)	Time
1 (Reverse Transcription)	1	42	15 min.
2 (Initial denaturation)	1	95	10 min.
3 (PCR cycling)	40	95	15 sec.
		60	30 sec
		72*	30 sec
* **Plate Read/Data Acquisition in FAM and Yakima Yellow channel**			

2. Set the reaction volume as 30 µL.
3. Plate read/Data Acquisition for FAM and Yakima Yellow channel should be incorporated in the third stage of step 3 (72◦C/30 sec).
4. The ideal run time for the assay is 120 minutes. Note: *In case of Eppendorf Realplex 4, select RAMP rate as 35%.*

Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com

Data Analysis

Analyze the data after completion of the run. Check the R_n/*Cycle* amplification plot and R_n/*Cycle* amplification plot to observe the amplification signal generated by different samples in the run. Compare both the plots for data analysis. Also look for noisy signals, if observed as it might not give you a proper result.

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to R_n/*Cycle* amplification plot. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals.

Result

The values for unknown samples would appear in the result column in *IU/µL* in FAM Channel. Samples showing no amplification in FAM channel should show amplification in Yakima Yellow channel, and then only results should be considered. The negative control should not show any value in the result column.

Interpretation

Interpret the values for unknown samples, only if the Slope of Standards is between -3.1 to – 3.6 and PCR efficiency is between 90%-110% (0.9 - 1.1) and there should be no amplification in negative control.

Observation	Interpretation	Conclusion
Amplification signal detected in HCV channel (FAM) and in Internal control channel (Yakima Yellow)	Quantify HCV RNA	Proceed for further Analysis
Amplification signal detected in HCV channel (FAM) but no signal in Internal Control channel (Yakima Yellow)	HCV RNA detected. For accurate quantitation repeat the quantitation with diluted(1:100) And undiluted sample	
Amplification signal not detected in HCV channel (FAM) but detected in Internal control channel (Yakima Yellow)	HCV RNA below detection limit	
No Amplification signal detected in HCV channel (FAM) as well as Internal control channel (Yakima Yellow)	Reaction can't be interpreted as Inhibition of PCR or incorrect extraction	Repeat the extraction and PCR

To convert the results from *IU/µL* to IU/mL use the following formula:

$$\text{Result (IU/mL)} = \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

*** For calculating the result of diluted sample (1:100); multiply the observed IU/mL value by dilution factor, 100**

$$\text{Result of 1:100 diluted sample (IU/mL)} = \text{Dilution Factor x (100)} \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Troubleshoot

Observation	Possible cause	Solution
No amplification signal for Standards in FAM channel	1. One of the components may not have been added. 2. Incorrect channel selection 3. Incorrect programming of the real time machine. 4. Instrument is not working properly	1. Repeat the assay by following the correct protocol and addition of reagents 2&3. Please recheck the PCR program 4. Contact manufacturer of thermocycler for technical support.
Weak amplification signal for standards (Signal below threshold) in FAM Channel	1. Improper PCR programming. 2. Inaccurate dispensing of reagents 3. Possible deterioration of kit components due to improper storage	1. Repeat the assay by following the correct protocol 2. Minimize Pipetting errors/Check for calibration status of pipettes
Amplification observed in FAM channel in negative control	1. Aerosols generated 2. Environmental Pipettes, /Work area. 3. Possible contamination of Kit reagents /	1. Use fresh aliquots of Standards/Kit Reagents (if available) 2. Clean the PCR rack/Pipettes thoroughly as per GLP 3. Clean and Fumigate the work area overnight prior to use

For any other technical query; please contact amplisure@raslifesciences.com

Assay Characteristics

Analytical Sensitivity

The analytical sensitivity of PCR assay as well as the analytical sensitivity in consideration of the purification (RNA Extraction) was assessed for the Amplisure™ HCV quantitative kit.

The analytical sensitivity is determined using WHO standards/HCV positive clinical samples in combination with a particular extraction method.

Analytical Sensitivity of qPCR assay

The analytical sensitivity is determined independent from the selected extraction method, using an international standard of known concentration ie. 4th WHO International Standard for Hepatitis C Virus for Nucleic acid amplification techniques (NIBSC code: 06/102).

To determine the analytical sensitivity of the Amplisure™ HCV quantitative kit, a standard dilution series was set up from 0.10 IU/µL to 41.6 IU/µL and analyzed with Amplisure™ HCV quantitative kit.

Testing was carried out on 5 different days on 3 replicates. The results were analyzed by statistical analysis.

The analytical sensitivity of the Amplisure™ HCV quantitative kit is 0.20 IU/µL.

Analytical Sensitivity in consideration of Extraction Method

The analytical sensitivity in consideration of the purification (Roche High Pure Viral RNA kit; Cat. No.11858882001 and QIAamp Viral RNA Mini Kit; Cat. No. 52904) of the Amplisure™ HCV quantitative kit was also determined using a dilution series of the International Standard for Hepatitis C Virus for Nucleic acid amplification techniques (NIBSC code: 06/102) from 2.6 to 2600 IU/mL spiked in healthy plasma specimens (Negative for HCV by Real time PCR).

These were subjected to RNA extraction using the Roche High Pure Viral RNA kit (Roche, extraction volume: 0.2 mL, elution volume: 50 µL)

Each of the dilutions was analyzed with the Amplisure™ HCV Quantitative Kit on 5 different days on 3 replicates. The results were determined by statistical analysis.

The analytical sensitivity in consideration of the purification of the Amplisure™ HCV quantitative kit, is 50 IU/mL (with Roche High Pure Viral RNA kit)

Linear Range

The linear range (analytical measurement) of the Amplisure™ HCV quantitative kit was determined by analyzing a dilution series of HCV quantitation standards from 0.2IU/µL to $2x10^7$ IU/µL.

Each dilution was tested in replicates (n= 3) using the Amplisure™ HCV quantitative kit

The linear range of the Amplisure™ HCV quantitative kit has been determined to cover concentrations from ***0.4 IU/µL to 2 x 10^7 IU/µL***.

Conversion Factor (IU/µL to Copies/µL)

The 4th WHO International Standard for Hepatitis C Virus for Nucleic acid amplification techniques (NIBSC code: 06/102) has been assigned an International Unit (IU) by the WHO as the number of genome equivalents.

The conversion factor value for Amplisure™ HCV quantitative kit has been assigned after calibration of the HCQS (HCV quantitation standards) with the 4th WHO International Standard for Hepatitis C Virus for Nucleic acid amplification techniques (NIBSC code: 06/102).The Conversion Factor (IU/µL to Copies/µL) is entirely dependent on the current PCR assay kit. A Correlation and Regression

analysis was performed to compare the two standard curves of HCV RNA values obtained by Amplisure™ HCV quantitative kit (copies/µL) and HCV NIBSC standards (IU/µL).

The correlation coefficient between the expected and estimated values was very good

r = 0.9912.

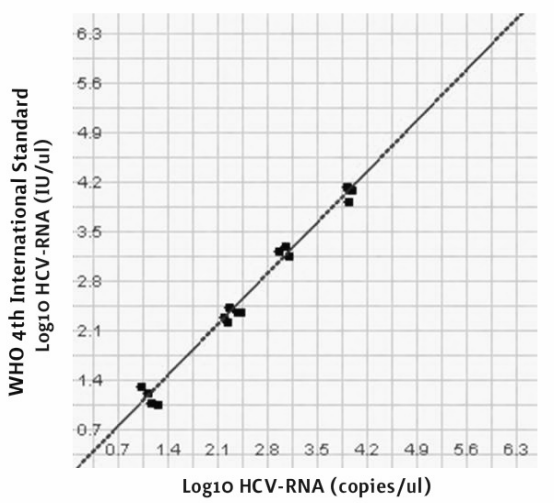


Fig 2. Correlation and Regression plot of HCV NIBSC standards (IU/µL) and HCV RNA (copies/µL)

The conversion factor calculated for Amplisure™ HCV quantitative kit from IU/µL to Copies/ µL is 2.18, i.e. **1 IU/µL = 2.18 copies/ µL**

To convert into copies/mL use the following formula:

$$\textbf{Result (copies/mL)} = \frac{\textbf{Calculated Value (copies/µL) x Elution Volume (µL)}}{\textbf{Sample Volume (mL)}}$$

Specificity

The specificity of the Amplisure™ HCV quantitative kit is ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all published sequences (Genbank) by BLAST analysis.

Detection of Different HCV genotypes (1-6) using International Standard

The detectability of all relevant genotypes (1-6) was ensured by a Database alignment and by a qPCR run with the following genotypes, using International standard (2nd HCV RNA genotype panel for Nucleic Acid Amplification Techniques –NIBSC code: 08/264) and using ≥ 300 positive HCV samples covering all genotypes.

International HCV Genotype Controls	Source of International controls	Result obtained with Amplisure™ HCV quantitative kit
Geno 1	NIBSC	Detected
Geno 2	NIBSC	Detected
Geno 3	NIBSC	Detected
Geno 4	NIBSC	Detected
Geno 5	NIBSC	Detected
Geno 6	NIBSC	Detected

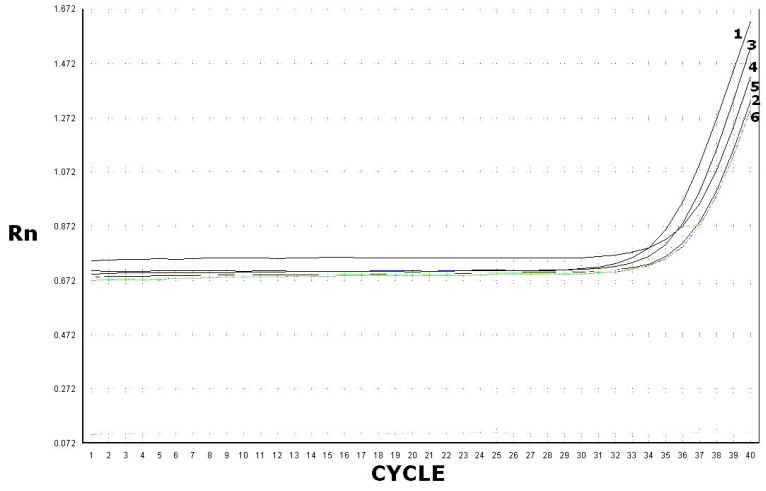


Fig3: Detection of HCV RNA Genotype panel (NIBSC) using Amplisure® HCV Quantitative Kit

Cross Reactivity Data

A potential cross-reactivity of the Amplisure™ HCV quantitative kit was tested using the control group listed below. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

The specificity was also validated with 50 different healthy plasma specimens/other various sample types. These did not generate any amplification signals with the HCV specific primers and probes, which are included in the Amplisure™ HCV quantitative kit.

Pathogen Tested	Cross Reactivity with the HCV Primers/Probes
Adenovirus	-ve
Herpes Simplex Virus-1	-ve
Herpes Simplex Virus-2	-ve
Epstein Barr virus	-ve
Human Immunodeficiency Virus	-ve
Cytomegalo virus	-ve
Hepatitis B virus	-ve
Enterovirus	-ve
BK virus	-ve
MTB complex	-ve
Plasmodium sps.	-ve

Precision

The precision data of the Amplisure™ HCV quantitative kit have been generated for HCV positive clinical specimens and HCV quantitation standards.

Intra-assay variability

The precision data consists of the *Intra-assay variability* which is variability of multiple results of samples of the same concentration within one experiment.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HCV quantitative kit have been collected using all the quantitation

standards (HCQS1- HCQS4). Also included were three different samples with different viral loads.

Inter-assay variability

The precision data consists of the *Inter-assay variability* which is variability of multiple results of samples of the same concentration within different experiments.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HCV quantitative kit have been collected using all the quantitation standards (HCQS1- HCQS4). Also included were three different samples with different viral loads. Testing was performed with 3 replicates for 10 days.

The precision data was calculated on basis of the CT values obtained.

Sample Type	Mean [Log Value (IU/µL)]	Variability Testing	Standard Deviation	Coefficient of Variation (%)
HCQS1	Log 4.3	Intra-assay variability	0.012	0.001
		Inter-assay variability	0.090	0.004
HCQS2	Log 3.3	Intra-assay variability	0.009	0.001
		Inter-assay variability	0.080	0.003
HCQS3	Log 2.3	Intra-assay variability	0.009	0.001
		Inter-assay variability	0.250	0.009
HCQS4	Log 1.3	Intra-assay variability	0.020	0.001
		Inter-assay variability	0.050	0.001
HCV Control 1	Log 5.2	Intra-assay variability	0.190	0.008
		Inter-assay variability	0.290	0.012
HCV Control 2	Log 3.7	Intra-assay variability	0.100	0.003
		Inter-assay variability	0.220	0.007
HCV Control 3	Log 2.2	Intra-assay variability	0.140	0.004
		Inter-assay variability	0.340	0.010

Performance study on samples

Biological performances of the Amplisure™ HCV quantitative kit on plasma samples have been evaluated in an Indian Microbiology Laboratory, using samples collected during the laboratory's routine activity.

The laboratory's routine technique is a commercial real-time quantitative PCR kit which was used to amplify a fragment of 102 bp of the Hepatitis C genome. The results were expressed directly in IU/mL.

The samples of plasma were extracted with the Roche High Pure Viral RNA kit (Cat. No.11858882001) using the protocol recommanded by the manufacturer on 200 µL with an elution volume of 50 µL. The extracted samples were amplified on Rotor-Gene Q using both kit reagents following manufacturer's instructions.

A total of 99 plasma samples was tested.

Results of the concordance obtained on the plasma samples:

		COMMERCIAL PCR KIT		Total
		+	-	
Amplisure™ HCV quantitative kit	+	79	2*	81
	-	0	18	18
	Total	79	20	99

Global concordance: 97/99 = 98.0% [92.9; 99.8](95%exact CI)

Of 99 samples tested, 2 gave discordant results.

* Of these 2 samples negative for the commercial HCV RG PCR kit and positive with Amplisure® HCV and two were found to be positive by sequencing and identified as HCV genotype 3a and 3b.

Quantitative analysis of positive plasma samples in the two kits

The quantitative analysis of results for plasma between the two techniques was carried out on confirmed positive samples.

It showed good correlation between the quantifications of the two techniques for plasma. Indeed, the mean difference in quantification between the two PCR techniques was around +0.34 $\log_{10}$ IU/mL.

Pearson coefficient correlation: R=0.857

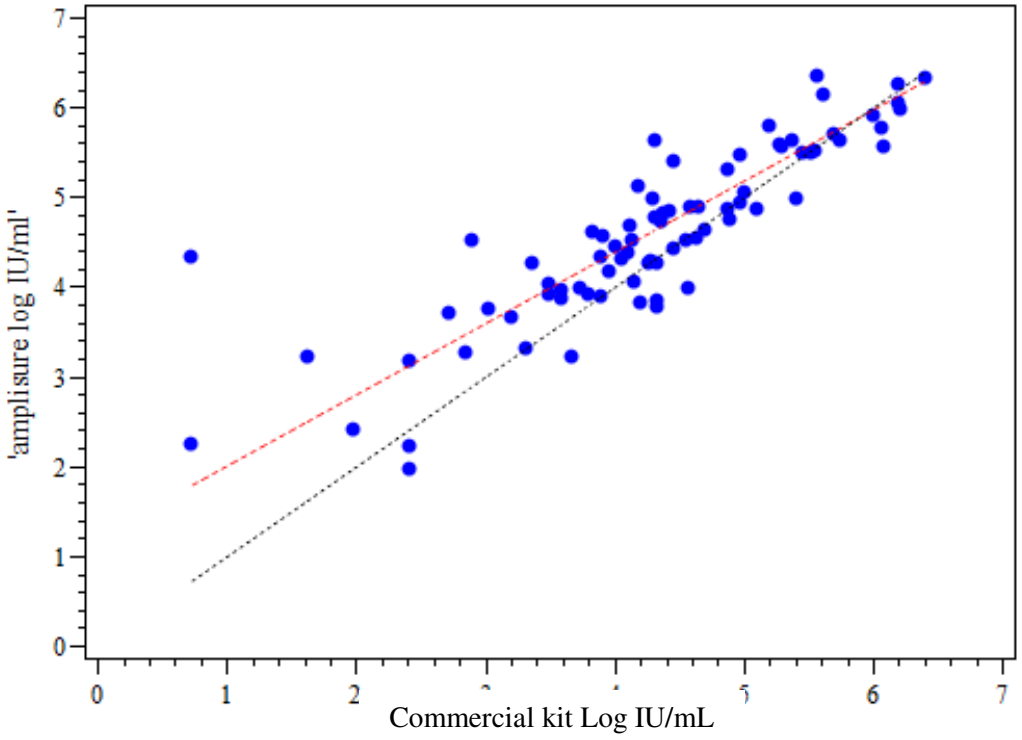


Fig. 4 : The quantification performance of the Amplisure HCV kit on blood plasma was therefore demonstrated.

Abbreviations

Abbreviation	*Expansion*
HCV	Hepatitis C Virus
RNA	Ribonucleic Acid
IC	Internal Control
RNases	Ribonucleases
PCR	Polymerase Chain Reaction
BSL2	Bio Safety Level 2
BSL3	Bio Safety Level 3
mL	Milli Liters
µL	Micro Liters
K_2EDTA	Potassium Ethylene Diamine Tetra Acetate
G	Relative Centrifugal Force
qPCR Protocol	Quantitative PCR protocol
MBGW	Molecular Biology Grade Water
RT PCR	Reverse Transcriptase PCR
NTC	No Template Control
FAM	Carboxyfluorescein
ROX	Carboxy-X-rhodamine
NIBSC	National Institute for Biological Standards and Control
IU	International Units
WHO	World Health Organization
BLAST	Basic Local Alignment Search Tool
Rxn	Reaction

References

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3. Narahari S, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort Infect Genet Evol 2009;(4):643-5

Ordering Information

QT-HCV-25 : 25 rxns
QT-HCV-50 : 50 rxns
QT-HCV-100 : 100 rxns