HCV Real-Time PCR Kit

Handbook

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RUO

Quantitative detection of Hepatitis C virus RNA

For research use only

REF 09061025 – 25 tests
09061100 – 100 tests

Kit Contents

	CapColor	Reagents	25 Tests	100 Tests
1	BROWN	HCV Reaction Mix	250 µl	1000 µl
2	YELLOW	HCV Enzyme Mix	20 µl	70 µl
3	BLUE	HCV Internal Control	75 µl	250 µl
4	RED	HCV Quantification Standart 1 (10^7 IU/ml)	100 µl	100 µl
5	RED	HCV Quantification Standart 2 (10^6 IU/ml)	100 µl	100 µl
6	RED	HCV Quantification Standart 3 (10^5 IU/ml)	100 µl	100 µl
7	RED	HCV Quantification Standart 4 (10^4 IU/ml	100 µl	100 µl
8	WHITE	PCR Grade Water	100 µl	100 µl

Storage

All reagents of RTA HCV Real-Time PCR Kit should be stored at -20°C. Storage at higher temperatures should be avoided (e.g. +4°C). Under these conditions, kit contents should be stable through the expiration date printed on the label. The reagents should not be freeze-thawed more than 2 times; otherwise the shelf of the kit will reduce. During the working steps all reagents should be kept on ice.

Intended Use

RTA HCV Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for quantification of Hepatitis C virus (HCV) RNA in human serum or plasma (EDTA) using RTA Viral RNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit and Stratagene Mx3000p/Mx3005p instrument or LightCycler 2.0 INCEPTRA Cycler 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD or Bioneer Exicycler 96 Real-Time PCR Detection System for amplification, detection and analysis. RTA HCV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HCV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings. RTA HCV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HCV RNA or confirmation of the diagnosis of infection with HCV.

Product Use Limitations

- All reagents of the kit is for *in vitro* diagnostic use only.
- RTA HCV Real-Time PCR Kit is not intended for screening of blood and blood products for the presence of HCV RNA or confirmation of the diagnosis of infection with HCV.
- This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. Test with other sample types may result in inaccurate results.
- This kit has been validated for use with RTA Viral RNA Isolation Kit or RTA Viral Nucleic Acid Isolation Kit. Using other isolation kits may adversely affect the performance characteristics of the kit.
- This kit has been validated for use with Stratagene Mx3000p/Mx3005p instrument or LightCycler 2.0 or INCEPTRA Cycler 4840/9620/9640/9660/9680 or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or BIO-RAD CFX96-IVD Real-Time PCR Detection System. Using other instruments may adversely affect the performance characteristics of the kit.
- This kit has been optimized for use with specific PCR plastic consumables listed under Additional Materials Required section of the Handbook. Using other PCR plastic consumables may adversely affect the performance characteristics of the kit.
- Trustworthy results depends on proper sample collection, transport, storage and processing methods.
- It is intended for professional use by properly trained personnel.
- RTA HCV Real-Time PCR Kit is intended for use as an aid in the management of patients with chronic HCV infection undergoing anti-viral therapy to assess response to treatment in conjunction with all relevant clinical and laboratory findings.
- The instructions in user manual should be followed strictly for optimum PCR results.
- The expired kits should not be used. Kit components from different lots should not be mixed.

Product Description

RTA HCV Real-Time PCR assay is a one-step real time reverse transcription PCR assay in which RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by RNA polymerase-mediated cDNA amplification. During cDNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the Thermus aquaticus (Taq) RNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if cDNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. A plot of the log of initial target copy number for a set of standards versus Ct is a straight line. Quantification of the amount of target in unknown samples is accomplished by measuring Ct and using the standard curve to determine starting copy number. RTA HCV real-time PCR assay utilizes external standards to gather quantitative results and includes an internal control, which controls for target isolation and amplification. The target region is situated in 5'-untranslated region (UTR) of HCV genome and is 76-bases long. HCV RNA concentration is noted in International Units/ml (IU/ml). If it is needed to convert to copies/ml, our conversion factor for RTA HCV Real-Time PCR Kit is 4.96 copies/IU. In other words, **1 IU/ml = 4.96 copies/ml**

Pathogen Information

Nearly 170 million people worldwide have been infected with Hepatitis-C Virus (HCV). About 85% of these develop persistent infection and are at risk of long term complications like liver cirrhosis and hepatocellular carcinoma-HCC (1). HCV has been classified into 1-6 genotypes on the basis of the identification of their different genomic sequence (2). The main role of HCV genotyping is to predict the likelihood to maintenance of long-term response to therapy, as the genotypes are associated with susceptibility to antiviral therapy (3).

The genome of hepatitis C virus (HCV) is identified as a separate entity by using a recombinant complimentary RNA (cDNA) approach. Hepatitis-C virus (HCV) belongs to the family Flaviviridae. It is a spherical, 30- 60nm in diameter, enveloped, single stranded RNA virus. With the elimination of HBsAg positive blood, HCV became the commonest cause of post transfusion hepatitis (4).

The clinical course of untreated HCV infection is highly variable with the majority of patients experiencing a slow fluctuating disease that may take 20 years or more for full expression. The incubation period is about 7 weeks with a range of 2 to 26 weeks. After initial exposure HCV-RNA can be detected in serum by PCR within a few weeks. Most patients develop liver cell injury within a few months as indicated by elevated alanine aminotransferase. A large percentage of patients are physically asymptomatic and anicteric. A "silent period" may ensue for weeks to month after initial infection, during which viral titres are low and antibody responses are not detectable. Seroconversion to HCV antibody positive status occurs within 3 months in the majority of the exposed patients, but in some it may take upto 6 months. Fulminant hepatitis C is extremely rare. Approximately half of HCV patients develop chronic active hepatitis and this may progress to liver cirrhosis and hepatocellular carcinoma-HCC (1).

Warnings and Precautions

- All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.
- Before and after work all surfaces should be disinfected with a freshly prepared solution of 10% bleach or antiviral agents.
- Dispose of unused reagents, waste and specimens in accordance with country or local regulations.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wear protective disposable gloves, laboratory coats and eye-wear when handling clinical specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water.
- The procedures should preferably be performed in four separated areas (i.e. for RNA extraction, PCR setup, sample addition, amplification) to aid in preventing contamination.. All supplies for a particular procedure should be stored in the area where that procedure is performed and should not be moved between areas Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to an area and never worn outside of that area. The work should flow in one direction, beginning in the extraction area, moving to the PCR setup area in which PCR Master Mix is prepared, then moving to the third area in which samples, negative control and quantification standards are added, finally moving to amplification area in which real-time PCR equipment is run.
- Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control; to prevent sample contamination, use new, sterile aerosol barrier or positive displacement Rnase/DNase-free pipette tips and sterile pipettes.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
- Store the kit away from any source of contaminating RNA or RNA, especially amplified nucleic acid.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- A single type of HCV RNA assay should be used for monitoring a patient. If RTA HCV Real-Time PCR Kit substitutes another HCV RNA assay, both tests should be used in parallel for at least two subsequent samples.
- Do not use a kit after its expiration date.

Performance Characteristics

Analytical Sensitivity Analytical sensitivity was analyzed by use of a dilution series of WHO standard, and the cutoff value of the kit was determined by probit analysis. A dilution series of a WHO International HCV standard was prepared to give the final concentrations of 160, 80, 40, 20, 10 and 5 IU/ml. Dilutions were extracted by RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029) according to RTA Viral Nucleic Acid Isolation Kit Handbook. Each dilution was tested in 24 replicates for each instrument. Lower limit was calculated by probit analysis done by PASW Statistics 18 program. The 95 % cutoff concentration of RTA HCV Real-Time PCR Kit is 10.4 IU/ml for STRATAGENE, 23.6 IU/ml for LightCycler, 9.5 IU/ml for INCEPTRA Cycler, 16 IU/ml for Rotor-Gene, and 12.6 IU/ml for Applied Biosystems 7500.

	INCEPTRACycler	STRATAGENE	LightCycler	BIO-RAD CFX96-IVD*	Rotor-Gene	ABI 7500	Bioneer Exicycler 96
RTA Viral Nucleic Acid Isolation Kit	9.5 IU/ml	10.4 IU/ml	23.6 IU/ml	15 IU/ml	16 IU/ml	12.6 IU/ml	16 IU/ml

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

Linear Range To determine the upper limit, a dilution series of RTA HCV Reference Material (Genotype 1b) ranging from 1 x 10^3 IU/ml to 1 x 10^6 IU/ml were prepared. High concentration samples (1 x 10^7, 1 x 10^8 and 1 x 10^9 IU/ml) were prepared by using calibrated in vitro transcribed RNA bearing HCV quantification standard. Dilutions and spikes were extracted by RTA Viral Nucleic Acid Isolation Kit according to Handbook. Within this range, the relationship between log of target RNA and Ct values is linear. Linear regression analyses comparing the Ct values-versus- log of target RNA were as follows:

For STRATAGENE, Ct value = -3.452(log of target RNA) + 46.74; with a correlation coefficient (R^2) of 0.998.

For INCEPTRA Cycler, Ct value = -3.29(log of target RNA) + 43.07; with a correlation coefficient (R^2) of 0.995.

For Rotor-Gene, Ct value = -3.26(log of target RNA) + 45.11; with a correlation coefficient (R^2) of 0.996.

For ABI 7500, Ct value = -3.343(log of target RNA) + 42.178; with a correlation coefficient (R^2) of 0.999.

Upper limit is at least 1 x 10^9 IU/ml for STRATAGENE, INCEPTRA Cycler, Rotor-Gene, ABI 7500 and Bioneer Exicycler 96.

Lower limit was calculated by probit analysis done by PASW Statistics 18 program according to the quantification results of HCV Analytical Sensitivity Studies. 95 % lower confidence limit is 10.4 IU/ml for STRATAGENE, 9.5 IU/ml for INCEPTRA Cycler, 21 IU/ml for Rotor-Gene, 21.7 IU/ml for ABI 7500.

Dynamic ranges of RTA HCV Real-Time PCR Kit:
For STRATAGENE 10.4 - 1 x 10^9 IU/ml
For INCEPTRA Cycler 9.5 - 1 x 10^9 IU/ml
For Rotor-Gene 21 - 1 x 10^9 IU/ml
For ABI 7500 21.7 - 1 x 10^9 IU/ml
For BIO-RAD CFX96-IVD 14 - 1 x 10^9 IU/ml*
For Bioneer Exicycler 96 16 - 1 x 10^9 IU/ml*

*: Data taken from RTA Automated DNA/RNA Preparation and PCR Setup System

Precision For each experiment, 24 replicates of 10^5 IU/ml 3rd Hepatitis C Virus (HCV) RNA WHO International Standard were used. The results on basis of Ct values are shown in the following table:

Descriptive Statistics					
	N	Mean	Std.Deviation	Variance	Coefficient of variation (%)
Intra_assay	24	29.63	0.60	0.368	2
Inter_assay	24	29.23	0.50	0.255	1.72
Stratagene_3005p	24	28.43	0.98	0.969	3.46
Inter_batch	24	29.48	0.24	0.059	0.82
LightCycler	24	28.86	0.06	0.005	0.23
Inceptra	24	29.14	0.48	0.239	1.67
BIO-RAD CFX96	24	29.73	0.19	0.039	0.67
Rotor-Gene	24	29.58	0.66	0.444	2.25
ABI 7500 Intra-assay	24	29.49	0.19	0.039	0.67
ABI 7500 Inter-assay	24	29.44	0.43	0.190	1.48
TOTAL	240	29.30	0.62	0.394	2.14

Genotype Detectability The performance of RTA HCV Real-Time PCR Kit was evaluated with SeraCare Life Sciences HCV RNA Genotype Performance Panel. According to the results, the data gathered by RTA HCV Real-Time PCR Kit is compatible with the results of other devices. RTA HCV Real-Time PCR Kit can detect and quantify all of 7 HCV genotypes and subtypes (1a, 1b, 2, 3, 4, 5 and 6).

Diagnostic Specificity HCV negative clinical specimens were analyzed to determine the diagnostic specificity of RTA HCV Real-Time PCR Kit. 56 Hepatitis C virus RNA negative clinical **serum** specimens and 52 Hepatitis C virus RNA negative clinical **EDTA plasma** specimens were used. Viral RNA was extracted from HBV negative clinical specimens by RTA Viral RNA Isolation Kit according to RTA Viral RNA Isolation Kit Handbook. None of the HCV negative clinical specimens gave positive test result for Hepatitis C RNA. Diagnostic specificity of RTA HCV Real-Time PCR Kit is 100 %. All of the Internal Controls of tests gave positive result.

Cross Reactivity To examine the specificity of an assay, cross-reactivity studies should be performed for potential cross-reactive markers. In this study, the specificity of the assay was evaluated by testing. 7 reference organism and 12 clinical specimens which were positive.
RTA HCV Real-Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table below:

Organism	Source	Test Result
Cytomegalovirus (CMV)	Acrometrix (Cat. No: 94-2014)	Negative
Human Herpes Simplex virus type 1 (HSV-1)	NIBSC (Cat. No: 08/224)	Negative
Human Herpes Simplex virus type 2 (HSV-2)	NIBSC (Cat. No: 08/226)	Negative
Epstein-Barr Virus (EBV)	NIBSC (Cat. No: 08/316)	Negative
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 97/650)	Negative
Hepatitis B virus (HBV)	NIBSC (Cat. No: 97/750)	Negative
Mycobacterium tuberculosis	ATCC (25177)	Negative
West Nile virus (3 samples)	Clinical specimens	Negative
Hepatitis G virus (2 samples)	Clinical specimens	Negative
Parvovirus B19	Clinical specimens	Negative
Epstein-Barr Virus (EBV)	Clinical specimens	Negative
Human Immunodeficiency Virus 1 (HIV-1) (3 samples)	Clinical specimens	Negative
Cytomegalovirus (CMV) (2 samples)	Clinical specimens	Negative

Cross-Contamination In this study, cross-contamination between samples was evaluated. To do this, five different runs were performed. In every run, 4 high positive HCV sample and 4 HCV negative samples were used. Then, the kit was evaluated accordingly whether or not any cross-contamination was observed. No cross-contamination was observed during the whole process, and none of the human serum samples exhibited evidence of containing PCR inhibitors as indicated by the amplification of internal control.

Whole System Failure 58 Hepatitis C virus RNA negative clinical serum specimens and 57 Hepatitis C virus RNA negative clinical EDTA plasma specimens were spiked with 4th WHO International HCV standard (NIBSC code: 06/102) to give a final concentrations of 45 IU/ml in the elution volume which is 3 times the 95% positive cutoff value determined by analytical sensitivity study. Spikes were extracted by RTA Automated DNA/RNA Preparation and PCR Setup System according to the instructions written in the handbook. Whole system failure rate of RTA HCV Real-Time PCR Kit is ≤1 %.

Clinical Comparative Study Total 104 clinical samples were tested. According to the results, the data gathered by RTA HCV Real-Time PCR Kit is compatible with the results of other CE-marked devices. Log concentrations of 103 of 104 positive clinical samples are between ±1 log concentrations of the result of comparative device.

Additional Materials Required
- RTA Viral RNA Isolation Kit (Cat No: 09010; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey),
- Real-Time PCR system,
- Disposable powder-free gloves
- Micropipettes (0.5 µl – 1000 µl),
- Sterile micropipette tips with filters,
- Microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for 2.0 ml tubes and for PCR strip tubes,
- PCR Workstation,

Real-Time PCR reaction tubes/plates/capillaries
For BIO-RAD CFX96-IVD:
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates with Bar Codes (BIO-RAD, Cat#: HSP-9955)
- Hard-Shell Thin-Wall 96-Well Skirted PCR Plates (BIO-RAD, Cat#: HSP-9655)
- Microseal 'B' Adhesive Seals, optically clear (BIO-RAD, Cat#: MSB-1001),
- Individual PCR Tubes, Low Tube Strips, 8-tubes strip, 0.2 ml Low Profile, White (BIO-RAD, Cat#: TLS0851)
- Flat Cap Strips, Optically Clear, 8-cap strip, 0.2 ml (BIO-RAD, Cat#: TCS0803)

For STRATAGENE MX 3000p/MX 3005p:
- Optical tube strips (8x Strip) (Agilent Technologies, Cat#: 401428),
- Strip caps for PCR and qPCR applications (Agilent Technologies, Cat#: 401425)

For LightCycler 2.0:
- LightCycler Capilleries (20 µl) (ROCHE, Cat#: 4929292001)

For Rotor-Gene 3000/6000:
- Strip Tubes and Caps, 0.1 ml (Qiagen, Cat#: 981103)

For INCEPTRA Cyclers:
- 96x0,2 ml plate (Bioplastics, Cat#: B70501). EU flat cap plate (Bioplastics, Cat#: B57601),EU 0.1ml 8-tube strip attached Optical wide area cap (Bioplastics, Cat#: K72810B)

For Applied Biosystems 7500:
- MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher, Cat#: 4306737)
- MicroAmp® Optical Adhesive Film (Thermo Fisher, Cat#: 4311971)
- MicroAmp® Optical 8-Tube Strip, 0.2 mL (Thermo Fisher, Cat#: 4316567)
- MicroAmp® Optical 8-Cap Strips (Thermo Fisher, Cat#: 4323032)

Sample Preparation
This kit has been validated for use with human serum or human plasma collected in EDTA anti-coagulant. Aseptic techniques must be employed during collection to prevent the introduction of micro-organisms into the patient's anatomical space, and to prevent the sample from being contaminated during the process of collection. All samples should be regarded as potentially infectious and standard precautions guidelines should be followed by all healthcare workers during sample collection and handling. Samples must be collected into appropriate containers before despatch to the laboratory. Be careful to check for cracks in the containers and to ensure that the lids of containers are properly tightened to prevent leakage of samples during handling and transportation. This can pose infection hazards to transport and laboratory staff. Ensure that the outer surfaces of the containers are not contaminated by the patients' samples.
Store whole blood at room temperature for no longer than 4 hours. Centrifuge blood within 4 hours of collection. Transfer serum or plasma to a screw cap cryovial tube.
Transportation of whole blood, serum or plasma must conform to country or local regulations for the transport of etiologic agents. Serum or plasma samples may be stored at 2-8°C for up to 3 days or frozen at -70°C or colder for long-term storage. Avoid multiple freeze/thaw cycles of specimens.

Protocol
Viral RNA Isolation RTA Viral RNA Isolation Kit (Cat No: 09010; RTA Laboratories, Turkey) or RTA Viral Nucleic Acid Isolation Kit (Cat No: 09029; RTA Laboratories, Turkey) should be used for viral RNA extraction from clinical samples. Please follow the manufacturer's instructions as stated in the kit manual.
Internal Control During RNA isolation, addition of the supplied internal control (IC) is necessary. IC allows the user to monitor RNA extraction step as well as to determine any PCR inhibition. For each sample, add 2.5 µl IC together with Solution RL of the isolation kit for a 50 µl elution. Depending on your final elution volume, the volume of IC to be added can be calculated (0.05 µl IC/1 µl Elution Buffer). There will be no amplification of internal control in the tests where high positive HCV samples are amplified because there is a competition between internal control template and HCV RNA template for using PCR primers and other components. The Ct value of internal control of a *negative sample* should be equal to 33 ± 5, otherwise, it denotes a problem during purification.
Quantification Standards For generating a standard curve to obtain accurate quantification data on the Real-Time system, four quantification standards should be used. For each standard the corresponding concentration should be defined properly to the Real-Time PCR system before each run and the standard curve will be generated accordingly at the end of the reaction. Work with HCV Quantification Standards after preparation of clinical samples and negative control in a separate area. Caps of the tubes or capillaries of Clinical Samples SHOULD be closed in that area.

PCR Protocol

1.Thaw all components, except HCV Enzyme Mix, at room temperature. Thaw HCV Reaction Mix at 37°C for 5 min if there is a precipitate. Put HCV Enzyme Mix on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

2.The final volume of Master Mix is calculated by multiplying single reaction volumes of Reaction Mix and Enzyme Mix by the total sample size. The number of negative controls, quantification standards and the clinical samples should be included when calculating total sample size. Against possible pipetting errors, addition of an extra sample to the total sample size is recommended. PCR Grade Water should be used as the negative control.

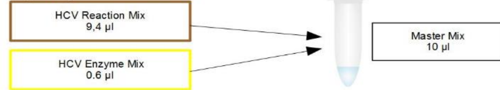
3.To prepare master mix, add 9.4 µl of HCV Reaction Mix (brown tube) and 0.6 µl of HCV Enzyme Mix (yellow cap) for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 10µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 10 µl RNA of each sample, negative control and quantification standards into the tubes. Spin down briefly.

4.Perform the thermal program given in the table below.

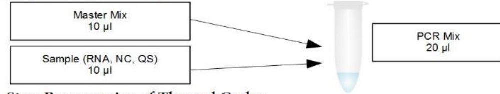
5.Fluorescence is measured at 72°C; FAM and HEX channels should be chosen (See the schema in the next page).

6.Refer to the Operator's Manual of the related instruments to program and analyze the results.

1. Step: Preparation of Master Mix



2. Step: Addition of Samples



3. Step: Programming of Thermal Cycler

Program Name	Cycles	Program for Bioneer Exicycler 96	Cycles	Program for LightCycler	Program for Stratagene and INCEPTRA Cycler	Program for BIO-RAD CFX96-IVD	Program for Rotor-Gene 3000/6000	Program for ABI 7500
cDNA Synth.	1	45ºC, 30 min	1	45ºC, 30 min	45ºC, 30 min	45ºC, 30 min	45ºC, 30 min	45ºC, 30 min
Hot Start	1	95ºC, 10 min	1	95ºC, 10 min	95ºC, 10 min	95ºC, 10 min	95ºC, 10 min	95ºC, 10 min
Amp.*	50	95ºC, 30 sec	45	95ºC, 30 sec	95ºC, 30 sec	95ºC, 30 sec	95ºC, 30 sec	95ºC, 30 sec
		58ºC, 60 sec		60ºC, 60 sec	55ºC, 60 sec	58ºC, 60 sec	57ºC, 60 sec	59ºC, 60 sec
		72ºC, 30 sec		72ºC, 30 sec	72ºC, 30 sec	72ºC, 30 sec	72ºC, 30 sec	72ºC, 30 sec
Cooling	-	-	1	40ºC, 30 sec	-	-		-

* Fluorescence is measured at 72°C; FAM and HEX/VIC/JOE channels should be chosen

Data Analysis

To be able to evaluate the experiment, PCR efficiency of the Standard Curve must be between 90%-110% and R^2 value must be more than 0.98. Otherwise, the experiment should be repeated.
During analysis on STRATAGENE software, adjust threshold fluorescence value manually by entering 1000 for FAM and 500 for HEX.
During analysis on BIO-RAD CFX96-IVD software, adjust threshold fluorescence value manually by entering 500 for FAM and 500 for HEX.
During analysis on INCEPTRA Cycler software, the threshold should be set empirically. At the beginning, the threshold can be set at 50 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Rotor-Gene software, the threshold should be set empirically. At the beginning, the threshold can be set at 0,04 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Applied Biosystems 7500 software, the threshold should be set empirically. At the beginning, the threshold can be set at 10,000 for the analysis, but this value should be fine-tuned depending on the overall amplification peaks.
During analysis on Bioneer Exicycler 96 software, the threshold should be set automatically.
Concentration of each positive sample will be calculated by the software according to the standard curve as International Unit per milliliter (IU/ml) . If it is needed to convert the quantitative results from IU/ml to copies/ml, the conversion factor for RTA HCV Real-Time PCR Kit is 4.96 copies/IU.
In other words, 1 IU/ml = 4.96 copies/ml.
Due to different starting sample volumes and elution volumes during viral RNA isolation, the following formula **SHOULD** be used to calculate the concentration of the original clinical sample:

$$\text{Concentration of the Original Sample (IU/ml)} = \frac{\text{Concentration from Software (IU/ml)} \times \text{Elution Volume (µl)}}{\text{Original Sample Volume (µl)}}$$

The interpretation on the calculated results can be done as follows:

Signal detected in FAM channel	Signal detected in HEX channel	Calculated concentration of the original clinical sample	Conclusion
+	+	<15 IU/ml for BIO-RAD <10.4 IU/ml for STRATAGENE <9.5 IU/ml for INCEPTRA <23.6 IU/ml for LightCycler <16 IU/ml for Rotor-Gene <12.6 IU/ml for ABI 7500 <16 IU/ml for Bioneer Exicycler 96	The result is valid. Quantitation is not possible since the quantitative result is below the analytical sensitivity value of the assay. Reproducibility of the positive result is not guaranteed.
+	+/-	≥15 IU/ml for BIO-RAD ≥10.4 IU/ml for STRATAGENE ≥9.5 IU/ml for INCEPTRA ≥23.6 IU/ml for LightCycler ≥16 IU/ml for Rotor-Gene ≥12.6 IU/ml for ABI 7500 and ≥16 IU/ml for Bioneer Exicycler 96 and ≤1 x 10^9 IU/ml	The result is valid. HCV RNA is detected at the concentration calculated by the software since the quantitative result is within the linear range of the assay.
+	+/-	>1 x 10^9 IU/ml	The result is valid. HCV RNA is detected at a concentration >1 x 10^9 IU/ml. Quantitation is not possible since the quantitative result is above the linear range of the assay.
-	+	N/A	The result is valid. Target (HCV RNA) is not detected.
-	-	N/A	The result is invalid. No diagnostic interpretation can bedone.

References

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