DNA-TECHNOLOGY

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For research use only

HCV Quantitative REAL-TIME PCR Kit

INSTRUCTION FOR USE

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Q3-P612-23/9EU

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1. INTENDED USE

The **HCV QUANTITATIVE REAL-TIME PCR Kit** is intended for research and diagnostic applications. The **HCV QUANTITATIVE REAL-TIME PCR Kit** is an *in vitro N*ucleic Acid Test (NAT) – pathogen-detection-based product. The **HCV QUANTITATIVE REAL-TIME PCR Kit** is intended for quantitative determination of hepatitis C virus RNA in human biological material (blood plasma) by real-time RT-PCR method.

Indications for the analysis:

- the need for quantitative determination of hepatitis C virus RNA;
- monitoring of viral load during therapy.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HCV QUANTITATIVE REAL-TIME PCR Kit.**

The **HCV QUANTITATIVE REAL-TIME PCR Kit** can be used in research practice.

Potential users: personnel qualified in molecular diagnostics methods and in working with pathogenic microorganisms and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

Method: reverse transcription of RNA followed by amplification of synthesized cDNA fragments by real-time polymerase chain reaction (RT-PCR); quantitative analysis.

Method principle: based on the use of reverse transcription of RNA and subsequent amplification of cDNA, which consists of repeated cycles of temperature denaturation of DNA, annealing primers with complementary sequences and subsequent completion of polynucleotide chains from these primers by Taq-polymerase.

The RNA reverse transcription stage and PCR amplification of cDNA stage are performed in one test tube.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA during the initial heating of the tube.

The **HCV QUANTITATIVE REAL-TIME PCR Kit** includes the Internal control RNA-IC “A”, which is intended to assess the quality of the RNA extraction and polymerase chain reaction.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into PCR-mix. When a specific product is formed, the DNA probe is destroyed and the quenching agent stops affecting the fluorescent label, which leads to an increase in the fluorescence level. The number of destroyed probes (hence the fluorescence level) increases in proportion to the number of specific amplicons formed, and the fluorescence level is measured at each amplification cycle.

The DNA probes used to detect the amplification product of the desired HCV cDNA include the Fam fluorescent tag.

The DNA probes used to detect the product of the internal control sample include the Hex fluorescent tag.

Table 1 shows the detection channels of amplification products.

To quantify HCV RNA, the reagent kit includes calibration samples in two concentrations: HCV-ST1 – 5.0×10^7 IU/mL and HCV-ST2 – 5.0×10^4 IU/mL.

Two standards (HCV-ST1 and HCV-ST2) are used to develop a standard curve to quantification HCV concentration in blood plasma.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
RNA HCV	IC*	-	-	-

*- Internal control RNA-IC “A”

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **HCV QUANTITATIVE REAL-TIME PCR Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

The **HCV QUANTITATIVE REAL-TIME PCR Kit** is also approved for use with CFX96 (Bio-Rad) real-time thermal cycler.

3. CONTENT

The **HCV QUANTITATIVE REAL-TIME PCR Kit** content is represented in Table 2.

Table 2. The **HCV QUANTITATIVE REAL-TIME PCR Kit** content, package S (standard) for Q3-P612-S3/9EU and Q3-P612-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or slightly pink transparent liquid under white wax layer	1440 µL (15 µL in each tube)	96 tubes or 12 8-tube strips
Enzyme Taq/RT	Colorless transparent viscous liquid	55 µL	1 tube
RT-PCR-buffer “V”	Colorless transparent liquid	1620 µL (810 µL in each tube)	2 tubes
Internal control RNA-IC “A”	Colorless transparent liquid	1.0 mL	1 tube
Positive control*	Colorless transparent liquid	130 µL	1 tube
Standard HCV-ST1 ($5x10^7$ IU/mL)	Colorless transparent liquid	360 µL	1 tube
Standard HCV-ST2 ($5x10^4$ IU/mL)	Colorless transparent liquid	360 µL	1 tube
Strip’s caps**	12 8-caps		

*- marking as C+ is allowed

**- for detection kit packaged in strips REF Q3-P612-S3/9EU

All components are ready to use and do not require additional preparation for operation.

The **HCV QUANTITATIVE REAL-TIME PCR Kit** is intended for single use and designed for 96 tests (94 defined samples, one positive control and one negative control) (6 independent assay procedures for 8 clinical samples are recommended).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- For blood collection: 4.0 - 9.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. RNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II-III;
- Refrigerator;
- High speed centrifuge (RCF(g) no less than 1600) for Vacuette tubes;
- High speed centrifuge (RCF(g) no less than 14000) for 1.5-2.0 mL tubes;
- Vortex mixer;
- Solid-state thermostat (temperature range 24-65 °C);
- Tube rack for 1.5 mL tubes;
- Tube rack for 2.0 mL tubes;
- RNase and DNase free 1.5 mL microcentrifuge tubes with caps;
- RNase and DNase free 2.0 mL microcentrifuge tubes with caps;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free filtered pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips for semi-automatic pipettes (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** REF P-017-N/1EU is recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Freezing chamber;
- Vortex mixer;
- Rotor for strips (if package in strips is used);
- PCR tube rack for 0.2 mL tubes or strips;
- Tube rack for 1.5 mL tubes;
- Tube rack for 2.0 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Pipette stand;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of **HCV QUANTITATIVE REAL-TIME PCR Kit**, except Enzyme Taq/RT, must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix for amplification must be stored out of light at temperatures from 2 °C to 8 °C during the storage period. The excessive temperature and light can be detrimental to product performance. The Enzyme Taq/RT must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the Enzyme Taq/RT, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the Enzyme Taq/RT in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Enzyme Taq/RT should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kits stored under undue conditions should not be used.

An expired **HCV QUANTITATIVE REAL-TIME PCR Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **HCV QUANTITATIVE REAL-TIME PCR Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with

aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;

- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

Blood plasma obtained from human peripheral whole blood is used for the assay.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control RNA-IC "A": triglycerides – up to 40 mmol/L of plasma sample, hemoglobin – up to 2.0 g/L, bilirubin – up to 340 µmol/L, total protein - 60 g/L.

Sample collection

Peripheral blood

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 4.0 - 9.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. The use of sodium citrate anticoagulant is also applicable. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

ATTENTION! It is not allowed to use heparin as an anticoagulant.

Transportation and storage of the samples

ATTENTION! The overall storage of the sample should not exceed 6 hours.

The transportation and storage temperature from collecting the sample till analysis should be from 2 °C to 8 °C.

ATTENTION! Whole blood cannot be frozen!

Sample preparation (obtaining blood plasma)

1 Centrifuge the tube with blood at RCF(g) 800-1600 for 20 minutes at room temperatures (from 18 °C to 25 °C).

2 Take the upper fraction (plasma) with a dispenser and put it into the new 1.5 mL tube.

The blood plasma can be stored at the temperature from minus 18 °C to minus 22 °C for no more than 3 months, at a temperature of minus 68 °C to minus 72 °C for no more than one year.

ATTENTION! Avoid repeated freezing and thawing of samples.

8. PROCEDURE

General requirements

To prevent cross-contamination of the biological material in the laboratory, the following rules are recommended:

- it is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;
- it is necessary to use negative control samples, starting from the stage of RNA extraction in each protocol;
- use tips with aerosol filters at all stages of the assay;

- observe the assay procedure, open Eppendorf-type tubes without touching the inner part of the tube cap with gloved hand;
- do not touch the edge of the tube with the tip when adding reagents.

8.1 RNA extraction

To isolate HCV RNA from blood plasma, a set of reagents for extraction of nucleic acids from blood plasma with preconcentration is used ("DNA-Technology" made **PREP-NA-ULTRA viral DNA/RNA Extraction Kit)**.

RNA extraction is carried out according to the **PREP-NA-ULTRA** kit instructions.

It is recommended to analyze each clinical specimen in duplicate.

To increase the reliability of the results at the nucleic acid isolation stage, it is recommended to duplicate the test samples (two separate sample preparations should be performed for one test sample).

ATTENTION! The obtained RNA preparation should be used within two hours for the reverse transcription reaction and the polymerase chain reaction.

8.2 The use of control samples at the stage of nucleic acid extraction

8.2.1 Internal control sample

To exclude false negative results during the sample preparation should be used an internal control RNA-IC "A" from the **HCV QUANTITATIVE REAL-TIME PCR Kit.**

Should be introduced internal control RNA-IC "A" in the amount of 10 µL per sample at the stage of nucleic acid extraction.

8.2.2 Negative control sample

To exclude false positive results and to control the quality of the detection use a negative control sample from the nucleic acid extraction stage.

At the stage of nucleic acid extraction, it is obligatory to prepare a negative control sample (from the **PREP-NA-ULTRA** kit) and run it through all extraction stages simultaneously with RNA isolation from clinical samples in accordance with the **PREP-NA-ULTRA** kit instructions for use.

8.3 Standards HCV-ST1 and HCV-ST2.

Standards HCV-ST1 and HCV-ST2 must be run through the nucleic acid extraction step.

Before testing it is necessary to dilute the calibration samples standards HCV-ST1 and HCV-ST2 with the solution of the negative control sample (from the **PREP-NA-ULTRA** kit).

- When separating HCV RNA from 1000 µL of the test sample, 20 µL of the appropriate calibration sample and 980 µL of the negative control should be added to tubes marked "HCV-ST1" and "HCV-ST2".
- When extracting HCV RNA from 250 µL of the test sample, 20 µL of the appropriate calibration sample and 230 µL of the negative control should be added to tubes labeled "HCV-ST1" and "HCV-ST2".

In case of mixed run (including simultaneous analysis of samples isolated from 1000 µL and 250 µL), extract the calibration samples and the negative control from 1000 µL.

Each group of allocated samples should include one negative control (C-), three standards HCV-ST1 (ST1), three standards HCV-ST2 (ST2).

Table 3. Example of marking test tubes for the RNA extraction procedure

Plasma sample	“C–“	HCV-ST1	HCV-ST2
Tube No.1	Tube “C–“	Tube “ST1-1”	Tube “ST2-1”
Tube No.2		Tube “ST1-2”	Tube “ST2-2”
		Tube “ST1-3”	Tube “ST2-3”

8.4 PCR with Reverse Transcription (RT-PCR)

ATTENTION! The reagents and tubes should be kept away from direct sun light.

ATTENTION! When using package S (Q3-P612-S3/9EU), strips, strictly observe the completeness of the strips and caps to them. Do not use the cap strips from the other kits!

8.4.1 Mark the required number of the tubes with paraffin sealed PCR-mix:

- two tubes for each blood plasma test sample (if is necessary analyze each sample in duplicate);
- three tubes for the standard HCV-ST1 “ST1”;
- three tubes for the standard HCV-ST2 “ST2”;
- one tube for a positive control sample “C+";
- one tube for a negative control sample “C-";.

Example: It is necessary to analyze 6 samples. We need to label 12 test tubes for the samples tested; 3 test tubes for “ST1", 3 test tubes for “ST2", one test tube for “C+" and one test tube for “C-". Total number of test tubes is 20.

8.4.2 Vortex the RT-PCR-buffer ”V” and Enzyme Taq/RT thoroughly for 1-3 seconds, then spin briefly for 3-5 seconds.

ATTENTION! Enzyme Taq/RT should be got out from the freezer immediately prior to use.

8.4.3 Prepare the mixture of RT-PCR-buffer “V” and Enzyme Taq/RT. Add to the one tube:

- 15.0 x (N+1) µL of RT-PCR-buffer “V”;
- 0.5 x (N+1) µL of Enzyme Taq/RT,

N is a quantity of the samples to be tested taking to account “ST1”, “ST2” “C-” and “C+”.

Mixture can be stored at temperature from 2 °C to 8 °C for no more than one hour.

Example: to test 6 samples, mark 20 tubes. Prepare the mixture of RT-PCR-buffer “V” and Enzyme Taq/RT for 21 (20+1) tubes. Mix 315 µL of RT-PCR-buffer “V” and 10.5 µL of Enzyme Taq/RT.

ATTENTION! Taking the Enzyme Taq/RT, it is necessary to dip the tip no more than 1.0 mm and observe the rules for dosing viscous liquids. Thoroughly flush the remaining Enzyme Taq/RT from the tip by pipetting at least 5 times.

8.4.4 Vortex the tube with the mixture of RT-PCR-buffer “V” and Enzyme Taq/RT thoroughly. Then spin briefly for 1-3 seconds.

8.4.5 Add 15 µL of the RT-PCR-buffer “V” and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break.

8.4.6 Vortex the tubes with samples and “C-” and “C+” for 3-5 seconds and down the drops for 1-3 seconds.

ATTENTION! Open the cap of the tube/strip, add RNA sample (or control sample), then close the tube/strip before proceeding to the next tube/strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.4.7 Add 20 µL of the RNA sample into corresponding tubes (2 tubes for each sample). Do not add RNA into the “C-”, “C+” tubes. Avoid paraffin layer break.

8.4.8 Add 20 µL of the corresponding standard HCV that passed the RNA extraction step to the tubes marked "ST1" and "ST2" (3 tubes for each standard HCV). Avoid paraffin layer break.

8.4.9 Add 20 µL of negative control sample (C-), which passed whole RNA extraction procedures into corresponding tube. Avoid paraffin layer break.

8.4.10 Add 20 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.4.11 Spin down the tubes for 3–5 seconds to collect drops.

8.4.12 Set the tubes/strips into the Real-time Thermal cycler.

8.4.13 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.4.12) and run PCR. See Table 4.

For use with CFX96 (Bio-Rad) real-time thermal cycler consult user manual for devices. See Table 5.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	47	15	0	1		Cycle
2	95	5	0	1		Cycle
3	95	0	10	50		Cycle
	62	0	20		√	
5	10[1]	…	…	Holding		Holding
√ - optical measurement [1] – holding at 25°C is allowed						

Table 5. The PCR program for CFX96 (Bio-Rad)

Step	Temperature, °C	Time, min:sec	Cycle repeats
1	47	15:00	1
2	95	5:00	1
3	95	0:10	50
4	59 √	0:20	
√ - optical measurement (Plate Read), set the fluorescence measurement on the Fam and Hex channels at 59 °C			

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

9. CONTROLS

The **HCV QUANTITATIVE REAL-TIME PCR Kit** contains positive control sample. Positive control is a cloned part of the virus genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The kit includes the Internal control (RNA-IC "A"). RNA-IC "A" is intended to assess the quality of the RNA extraction and polymerase chain reaction. To reveal possible contamination a negative control is required.

ATTENTION! A negative control sample should go through all stages of RNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

The test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

Recording and analysis of the reaction results is done automatically by the software provided with the detection thermal cycler.

Calculation of HCV RNA concentration in plasma samples (C1) is performed according to the formula:

$$C1 = C2 * k \qquad (1),$$

C1 – RNA concentration in the original plasma sample;

C2 – RNA concentration measured by the calibration curve given by standards CT1 and CT2;

k – conversion coefficient calculated by the formula:

$$k = \frac{V1}{V2} \qquad (2),$$

V1 – volume of standard ST introduced into the negative control sample during the RNA extraction procedure (20 µL);

V2 – volume of plasma from which RNA was extracted (1000 µL or 250 µL).

ATTENTION! **The value of the coefficient *k* for calculating the concentration:**

- When extracting HCV RNA from 1000 µL plasma**, k = 0.02**.
- When extracting HCV RNA from 250 µL plasma, **k = 0.08**.
- When calculating the concentration of a positive control sample **k = 0.02**.

In case of mixed runs (including simultaneous analysis of samples extracted from 1000 µLand 250 µL), it is necessary to select samples that differ from the standard volume of extraction (the standard is extraction of HCV RNA from 1000 µL of plasma).

The Real-time PCR Thermal Cyclers detects results automatically. Analysis will be performed by Real-Time PCR application. The interpretation should be performed in accordance with Table 6.

Table 6. The interpretation of assay results

Detection channel		**Interpretation**
Fam range, calculated concentration IU/mL	**Hex** Cp or Cq	
Analyzed samples		
$1.0x10^2$ – $5.0x10^8$	Is not considered	HCV RNA detected and quantified (IU/mL)
<**100**	Is not considered	HCV RNA detected but not quantified. Calculated titer is below the Lower Limit of Quantitation of the assay. Report results as **"less than 100 IU/mL"**
>**$5.0x10^8$**	Is not considered	HCV RNA detected but not quantified. Calculated titer is above the Upper Limit of Quantitation of the assay. Report results as **"more than $5.0x10^8$ IU/mL"**
Is not specified	≤35	Negative result RNA HCV is not detected
Is not specified	>35 or is not specified	Unreliable result
Positive control sample		
$2.0x10^4$-$2.0x10^5$*	Is not considered	Positive result with RNA concentration in the sample indicated (IU/mL) The results are valid
Negative control sample		
Is not specified	≤35	Negative result (concentration not indicated) The results are valid

When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression) and exclude the first five cycles from the analysis (Analyze Date from Cycle 5 to 50).

Unreliable results may be caused by the presence of inhibitors in the nucleic acid preparation obtained from the clinical material, errors in the pre-analytical stage, incorrect implementation of the analysis Protocol, non-compliance with the temperature mode of amplification, etc. In this case, either re-staging of reverse transcription and polymerase chain reaction, or re-extracting of the nucleic acid preparation, or re-collect of clinical material (performed sequentially) is required.

If a false result is obtained for a positive control, the results of the whole production series are considered to be invalid. In this case the whole batch of samples must be amplified again.

When a positive result for HCV RNA is obtained in a negative control, the results of the entire run are considered unreliable. In this case, special measures are required to detect and eliminate possible contamination.

* – if in the positive control the determined concentration is out of the range $2.0×10^4$ – $2.0×10^5$ IU/mL, it is necessary to repeat the assay.

11. SPECIFICATIONS

a. The analytical **specificity** of the **HCV QUANTITATIVE REAL-TIME PCR Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

In samples of human biological material containing hepatitis C virus RNA, the amplification software of the detection thermal cycler records a positive amplification result for the specific product on the Fam detection channel.

The reagent kit detects the following HCV genotypes: 1a, 1b, 2, 3, 4, 5, 6.

In samples of biological material not containing HCV RNA, the amplification software of the detection thermal cycler records a negative result of specific product amplification by the Fam detection channel and a positive result of internal control (IC) amplification by the Hex detection channel.

The analytical specificity of the reagent kit was evaluated on a panel of nucleic acids from the following organisms: RNA from HAV, HDV, HGV, HIV, DNA from HBV, EBV, CMV, HSV I, HSV II, influenza A virus, influenza B virus, and human DNA at up to 10^8 copies/mL of the sample. No cross-reactions for these organisms and viruses were recorded.

b. Analytical **sensitivity** of the **HCV QUANTITATIVE REAL-TIME PCR Kit** is

- 15 IU/mL for RNA isolation from 1000 µL of plasma sample;
- 50 IU/mL for RNA isolation from 250 µL of plasma sample.

Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

c. Metrological traceability

The reagent kit is subject to the metrological traceability requirements for control samples.

The internationally recognized calibrator used is the 6th WHO International Standard for hepatitis C virus (HCV) RNA for nucleic acid amplification techniques (NAT), NIBSC code 18/184.

The plasma HCV RNA concentration (routine assay) expressed in IU/mL is quantified against HCV-ST1 (5×10^7 IU/mL) and HCV-ST2 (5×10^4 IU/mL), characterized to the 6th WHO International Standard for hepatitis C virus (HCV) RNA for nucleic acid amplification techniques (NAT) code 18/184.

The measurement uncertainty (U) of the plasma HCV RNA concentration (for log10 HCV RNA concentration IU/mL) is 0.4.

d. Linear range: $1.0\times10^2 – 5.0\times10^8$ IU/mL.

e. Coefficient of variation of results (%CV) – no more than 10% (for log10 HCV RNA concentration IU/mL).

f. Diagnostic characteristics

Number of samples (n) - 86;

Diagnostic sensitivity (95% CI) - 100% (91.59-100%);

Diagnostic specificity (95% CI) – 100% (91.96-100%).

ATTENTION! The claimed specifications are guaranteed when RNA extraction is performed with **PREP-NA-ULTRA viral DNA/RNA Extraction Kit** REF P-017-N/1EU.

12. TROUBLESHOOTING

Table 7. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	-	PCR inhibition RNA extraction violation	Repeat RNA extraction Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for In vitro Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of the **HCV QUANTITATIVE REAL-TIME PCR Kit**:

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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14. KEY TO SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
NON STERILE	Non-sterile		Caution

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