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INSTRUCTIONS FOR USE

Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT "HEPA-C-test-Q"

TS 21.20.23-019-97638376-2020

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List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
RT	reverse transcription
RNA	ribonucleic acid
HCV	hepatitis C virus
ICS	internal control sample
NC	negative control sample
PC	positive control sample
CS-1	calibration sample No. 1
CS-2	calibration sample No. 2
SenC	sensitivity control
SC	specificity control

Introduction

Hepatitis C virus infection is a leading cause of acute and chronic liver diseases. Chronic infection can lead to cirrhosis and liver cancer. The course of chronic hepatitis C can be characterized by extrahepatic manifestations, among which the most acute are cryoglobulinemic vasculitis, cryoglobulinemic nephritis and B–cell lymphoma. Hepatitis C viral load assessment makes it possible to adjust therapy and evaluate its effectiveness.

Target analyte: specific regions of hepatitis C genomic RNA (hepatitis C, HCV)

Scientific validity of the target analyte lies in its specificity (RNA sequence uniqueness) in relation to hepatitis C virus genome.

Hepatitis C virus belongs to the flavivirus family (Flaviviridae). The genome is represented by a single-stranded linear RNA molecule. High heterogeneity is an important genome feature. HCV, due to its high mutational activity, is able to avoid the effects of the immune system protective mechanisms. There are 6 virus genotypes and many subtypes, which have different significance for the disease prognosis and the antiviral therapy effectiveness.[1]

HCV RNA detection indicates the virus reproduction in the body and it is a method of disease diagnostics. The virus genetic material can be detected by PCR in 10-12 days after infection – during this period specific antibodies, that are formed a few months after infection, are absent and biochemical indicators of liver function are within the reference values.

Viral load quantitative detection, taking into account the HCV genotype, makes it possible to control the therapy and predict the disease course.

Treatment effectiveness is evaluated by the RNA amount before and during therapy. The blood viral load usually decreases several times in the first three months of successful treatment. The therapy is effective, vireamia decreases twice in the first 4-12 weeks of the therapy course and after the end of the course, the virus genetic material is not detected in the blood. Not decreasing vireamia after 12 weeks from the course start indicates its ineffectiveness.

[1] Hepatitis C. World Health Organization (WHO). The newsletter. 2020.

It is recommended to conduct a test before the therapy start, in the 4th, 12th and 24th treatment weeks and 24 weeks after the end of the course. The therapy duration and the frequency of hepatitis C virus RNA quantitative detection depends on the virus genotype and the liver damage stage. [2]

The scope of the reagent kit: clinical laboratory testing of infectious diseases.

Indications and contraindications for use

Indications for use: suspected hepatitis C virus infection and viral load detection in patients with detected hepatitis C virus to select an appropriate therapy and evaluate its effectiveness.

Contraindications to use: when used by specially trained personnel and taking into account the intended use, were not identified.

Population, demographic aspects of the medical device use: no population, demographic aspects of HEPA-C-test-Q reagent kit use were identified.

Sterility: the device is not sterile.

1. Intended use

Intended use: HEPA-C-test-Q reagent kit is designed for qualitative and quantitative detection of hepatitis C virus (hepatitis C, HCV) RNA by one-step allele-specific polymerase chain reaction with hybridization-fluorescence detection with real-time reverse transcription (RT-PCR-RT) in a RNA sample isolated from human K2-EDTA blood plasma in patients with suspected hepatitis C virus infection and patients with detected hepatitis C virus to select an appropriate therapy and evaluate its effectiveness.

Purpose of use: the results obtained can be used to diagnose hepatitis C virus, select appropriate therapy and evaluate its effectiveness. The results are taken into account in the comprehensive disease diagnosis.

Potential consumers of the medical device:

The kit is intended for professional use in medical centers and clinical diagnostic laboratories. The professional level of potential users is a clinical laboratory diagnostics doctor, a medical laboratory technician, a

[2] Recommendations on diagnosis and treatment of adult patients with hepatitis C. Ministry of Health of the Russian Federation. - M. 2014.

laboratory technologist.

2. Method principle

Method

One-step allele-specific polymerase chain reaction (PCR) in real time with hybridization-fluorescence detection with reverse transcription (RT-PCR-RT).

Test sample type

Test material is RNA samples isolated from K2-EDTA human blood plasma.

Detection principle

Hepatitis C virus RNA quantitative detection by multiplex allele-specific polymerase chain reaction with hybridization-fluorescence detection in an RNA sample isolated from clinical material includes three stages:

1. RT-PCR preparation;
2. RNA reverse transcription and DNA PCR amplification with hybridization-fluorescent real-time detection of amplification products;
3. Results interpretation.

One-step reverse transcription reactions and specific regions amplification using primers specific to them are carried out with RNA samples in a reaction buffer.

RT-PCR Buffer contains all the main reagents, including a warm-start revertase, a thermostable hot start DNA polymerase, deoxynucleotide triphosphates and an optimized buffer.

Oligonucleotides mixtures contain fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified RNA target and get hydrolyzed (destroyed) by *Taq*-Polymerase. The dye and quencher separate, and fluorescence intensity increases in the corresponding range of the optical spectrum. It allows to register specific amplification product accumulation by measuring the fluorescent signal intensity in real time.

The kit contains reagents for detection of hepatitis C virus genomic RNA highly specific regions (targets), as well as an internal control sample (ICS) (Table 1).

ICS allows to evaluate quality and effectiveness of RNA isolation and possible presence of amplification inhibitors in a sample, the presence of which can lead to false negative results.

CS-1 and CS-2 calibration samples are used to make the calibration line, which is required to determine concentrations of hepatitis C virus genomic RNA in a test sample.

Table 1 – Test targets

Channel corresponding to a fluorophore	
FAM / Green	**HEX / Yellow**
Hepatitis C virus RNA	ICS

Method limitations

A possible reason for obtaining a false positive result is contamination at RNA isolation or RT-PCR reaction stages. A false positive result can be detected with a negative control sample.

Damage to packaging integrity during transportation.

Use of an expired kit or kit storage conditions violation.

Violation of storage conditions during sample transportation.

Clinical diagnosis assessment cannot be based only on the assay results obtained with this kit. For diagnostic purposes, the results should be used in combination with other data: symptoms, common clinical picture, results from other test systems (e.g., anti-HCV concentration detection with enzyme immunoassay or chemiluminescence assay) of the applied therapy.

False negative results may be obtained if viral load is very low (less than 48 IU/ml), which may occur due to the used antiviral therapy or the disease course peculiarities. In these cases, it is recommended to use a larger volume of clinical material to isolate nucleic acids in order to lower the test system sensitivity threshold.

RT-PCR time ranges from 120 to 145 minutes (excluding sample preparation), depending on the cycler used.

3. Reagent kit components

HEPA-C-test-Q reagent kit is designed in one configuration form – HEPA-C-test-Q.

Number of test samples

HEPA-C-test-Q reagent kit is designed for 96 reactions, it equates to detection of 88 (44 samples if carried out in duplicates) test samples, calibration samples, negative and positive control samples during a single run of a 96 well cycler or 10 single test sample detections with calibration, negative and positive control samples in each test.

Reagent kit components

Table 2 – HEPA-C-test-Q reagent kit components

No.	Reagent	Description	Quantity, Volume
1.	RT-PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2.	Oligonucleotide Mixture	Transparent colorless liquid, may have a lilac shade	1 tube, 1440 µl
3.	PC	Transparent colorless liquid	1 tube, 50 µl
4.	NC	Transparent colorless liquid	1 tube, 1000 µl
5.	ICS	Transparent colorless liquid	1 tube, 950 µl
6.	CS-1	Transparent colorless liquid	2 tubes, 1500 µl each
7	CS-2	Transparent colorless liquid	2 tubes, 1500 µl each

Note: Operational documentation (instructions for use and quality certificate) is not included in the device, but is included in the device delivery set. To ensure compliance with transportation conditions a reagent kit must be placed in a reusable polyurethane foam thermal container with prepared ice packs for temporary storage and transportation. The thermal container, instructions for use and the quality certificate for each batch of devices supplied are placed into an individual package.

The RT-PCR Buffer contains all main reagents, including a warm-start revertase, a thermostable hot start DNA polymerase, deoxynucleotide triphosphates and an optimized buffer.

Oligonucleotide Mixture is ready for use and contains primers and probes designed to identify specific targets – see Table 1. The Oligonucleotide Mixture is in a 10% nuclease-free TE water solution (1 mM Tris, 0.1 mM EDTA).

PC is a ready for use plasmid DNA mixture with synthetic insertions of an amplified fragment of hepatitis C virus genomic cDNA in 3 703 700 IU/ml concentration and a fragment of a bacteriophage genome in a 10% TE buffer (10 mM Tris, 1 mM EDTA).

NC is ready for use RNase-free deionized water.

ICS is a ready for use reinforced RNA.

CS-1 is a mixture of plasmid RNA with synthetic insertions of hepatitis C virus RNA amplified fragments in 370 370 IU/ml concentration in a TE buffer (10 mM Tris, 1 mM EDTA) (1 000 000 copies/ml).

CS-2 is a mixture of plasmid RNA with synthetic insertions of hepatitis C virus RNA amplified fragments in concentration 1.111 IU/ml in a TE buffer (10 mM Tris, 1 mM EDTA) (3 000 copies/ml).

The kit contains no products for medical use, materials of human or animal origin.

4. Reagent kit characteristics

4.1. Technical and functional characteristics

Table 3 - HEPA-C-test-Q reagent kit

Indicator	Characteristics and standards	Clause in Technical Specification (TS)
1. Technical characteristics		
1. Appearance		
RT-PCR Buffer	Transparent colorless liquid	Section 7, clause 7.6
Oligonucleotides Mixture	Transparent colorless liquid, may have a shade of lilac color	Section 7, clause 7.6
PC	Transparent colorless liquid	Section 7, clause 7.6

NC	Transparent colorless liquid	Section 7, clause 7.6
ICS	Transparent colorless liquid	Section 7, clause 7.6
CS-1	Transparent colorless liquid	Section 7, clause 7.6
CS-2	Transparent colorless liquid	Section 7, clause 7.6
1.2. Completeness	In accordance with clause 1.4 TS 21.20.23-019-97638376-2020	Section 7, clause 7.12
1.3. Marking	In accordance with clause 4 TS 21.20.23-019-97638376-2020	Section 7, clause 7.12
1.4. Package	In accordance with clause 5 TS 21.20.23-019-97638376-2020	Section 7, clause 7.12
2. Functional characteristics		
2.1 Positive result with PC	Fluorescence signal growth registration in tubes with PC in the FAM Ct≤30, HEX Ct≤30.	Section 7, clause 7.8.2
2.2 Negative result with NC	In tubes with NC in the FAM and HEX channels Ct > 35 or not indicated (i.e. there is no fluorescence accumulation curve)	Section 7, clause 7.8.2
2.3 Reaction in tubes with Specificity Control (SC)	In tubes with SC Ct is not indicated in the FAM channel (i.e. there is no fluorescence accumulation curve), and in the HEX channel Ct ≤32.	Section 7, clause 7.8.2
2.4 Reaction in tubes with Sensitivity Control (SenC)	In tubes with SenC in the FAM channel in all repetitions (at least 4) Ct≤35 and with a standard deviation value of SenC repetitions not more than 5%, and in the HEX channel Ct ≤32.	Section 7, clause 7.8.2
2.5 "Linearity" test	The correlation ratio of CS-1, CS-2 and a reference material (RM) is not less than 0.98	Section 7, clause 7.8.2
2.6 Accuracy test: coefficient of variation (CV) under repeatability conditions	The coefficient of variation Ct for repetitions of each calibration sample CS-1 and CS-2 under repeatability conditions is not more than 5%.	Section 7, clause 7.8.2
2.6 Concentration determination accuracy test	The obtained value of hepatitis C virus RNA concentration should correspond to the concentration given in the Reference Material Certificate, with a tolerance of ± 0.4 lg concentration.	Section 7, clause 7.8.2

Note: During the control PCR, as SenC and SC are used:

- a control sample for sensitivity detection (SenC), i.e. a mixture of plasmids with synthetic insertions of hepatitis C virus genomic cDNA fragment in 92 IU per 1 ml concentration and a fragment of a bacteriophage genome in a 10 % TE buffer (10 mM Tris, 1 mM EDTA).

- a specificity control sample (SC), i.e., a mixture solution of human genomic DNA extracted from Jurkat cell line in 1 000 copies per 5 µl (200 000 copies/ml) concentration.

4.2 Analytical efficiency characteristics

4.2.1 Analytical specificity

HEPA-C-test-Q reagent kit is specific to the hepatitis C (hepatitis C, HCV) genomic RNA.

For HEPA-C-test-Q reagent kit, 5`-UTR regions, conservative for genotypes 1-8, were selected as regions for primers and probes hybridization.

The possibility to detect and quantify equally different HCV genotypes were confirmed. When testing using 4th HCV RNA Genotype Panel samples for Nucleic Acid Amplification Techniques. NIBSC code: 14/290 in all obtained values, the correlation ratio R2 of the expected HCV concentration and the obtained concentrations ≥ 0.98, which confirms the possibility of detection and quantitative determination by the test HEPA-C-test-Q reagent kit of equally different hepatitis C genotypes (from 1a to 6). Maximum deviation of the average concentration (log10 IU/ml) obtained by HEPA-C-test-Q reagent kit in two repetitions for reference materials was 0.05 log10 from the log10 concentration established using AmpliSens® HCV- Monitor-FL kit according to TS 9398-035-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2007/00577 dated 27.12.2019).

4.2.2 Limit of detection (LOD)

According to the assay results, RNA HCV detection limit in blood plasma samples K2-EDTA:

- **100 µl** volume with a detection rate of 95% for the DTprime cycler – 92.8 IU/ml (95% CI: 91.57-94.43), CFX 96 – 98.8 IU/ml (95% CI: 97.37–

100.23), Rotor-Gene Q – 97.6 IU/ml (95% CI: 96.17-99.03), Quant Studio 5 – 97.8 IU/ml (95% CI: 96.37–99.23)[3].

- **1000 µl** volume with detection rate of 95% for DTprime cycler – 9.21 IU/ml (95% CI: 7.78-10.64), CFX 96 – 10.20 IU/ml (95% CI: 8.77–11.63), Rotor-Gene Q – 9.58 IU/ml (95% CI: 8.15–11.01), Quant Studio 5 – 8.91 IU/ml (95% CI: 7.48–10.34)[4].

4.2.3 Detection limit when testing different genotypes (from 1a to 6) HCV.

The test results using the NIBSC standard - 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques, NIBSC code: 14/290, consisting of seven bottles representing six main genotypes: bottle 14/276 – HCV genotype 1a, 14/278 – HCV genotype 1b, 14/280 – HCV genotype 2i, 14/282 – HCV genotype 3a, 14/284 – HCV genotype 4R, 14/286 – HCV genotype 5a, 14/288 – genotype 6l, confirmed the HEPA-C-test-Q reagent kit ability to detect genotypes 1a, 1b, 2i, 3a, 4r, 5a, 6l in ~92 IU/ml concentration per 100 µl K2-EDTA blood plasma samples, ~9.2 IU/ml per 1000 µl K2-EDTA blood plasma samples with an upper one-sided confidence interval of 95%, exceeding the expected 95% detection rate.

4.2.4. Limit of quantitation (LOQ) in K2-EDTA blood plasma samples:

- 100 µl volume with a 95% confidence probability for the DTprime cycler – 272.8 IU/ml (95% CI: 271.3 – 274.2), CFX 96 – 273.3 IU/ml (95% CI: 271.8-274.7), Rotor-Gene Q – 275.5 IU/ml (95% CI: 274.0-276.9), QuantStudio 5 – 276.1 IU/ml (95%CI: 274.6– 277.5).

- 1000 µl volume with a 95% confidence probability for DTprime cycler – 29.2 IU/ml (95% CI: 27.7-30.6), CFX 96 – 30.4 IU/ml (95% CI: 28.9–31.8), Rotor-Gene Q – 28.5 IU/ml (95% CI: 27.0-29.9), QuantStudio 5 – 29.5 IU/ml (95%CI: 28.1–30.9).

4.2.5 Limit of quantitation (LOQ) verification when testing various HCV genotypes (from 1a to 6).

[3] converting to copies / ml: when isolated from 100 µl of plasma and elution with a volume 25 µl – at least 92 IU / ml (250 copies / ml).

[4] converting to copies / ml: when isolated from 1000 µl of plasma and elution with a volume 25 µl – at least 9.2 IU / ml (25 copies / ml).

The test results using the NIBSC Standard - 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques. NIBSC code: 14/290, consisting of seven bottles representing six basic genotypes: bottle 14/276 – HCV genotype 1a, 14/278 – HCV genotype 1b, 14/280 – HCV genotype 2i, 14/282 – HCV genotype 3a, 14/284 – HCV4r genotype, 14/286 – HCV genotype 5a, 14/288 – genotype 6l, confirmed HEPA-C-test-Q reagent kit limit of quantitation (LOQ) in relation to genotypes 1a to 6 in concentration ~ 275 IU/ml in 100 µ K2-EDTA blood plasma samples, ~30 IU/ml in 1000 µl K2-EDTA blood plasma samples with an upper one-sided confidence interval of 95% exceeding the expected 95% detection rate.

4.2.6 Linear measurement range of the test HEPA-C-test-Q reagent kit:

- in 100 µl K2-EDTA blood plasma samples: linear range from 275 IU/ml to $3.7*10^7$ IU/ml, maximum deviation from the regression line is not more than ± 0.4 log10.[5]
- in 1000 µl K2-EDTA blood plasma samples: linear range from 28 IU/mL to $3.7*10^6$ IU/mL, maximum deviation from the regression line is not higher than ± 0.4 log10.

4.2.7 Linear measurement range verification when testing various genotypes (from 1a to 6) of HCV.

HEPA-C-test-Q reagent kit linear measuring range during testing is from 1a to 6:

- in 100 µl K2-EDTA blood plasma samples: linear range from 275 IU/ml to $3.7*10^7$ IU/ml, maximum deviation from the regression line is not more than ± 0.4 log10.
- in 1000 µl K2-EDTA blood plasma samples: linear range from 28 IU/ml to $3.7*10^6$ IU/ml, maximum deviation from the regression line is not more than ± 0.4 log10.

4.2.8 Metrological traceability of control samples – PC, CS-1, CS-2, which are included in the HEPA-C-test-Q reagent kit, was conducted using the 6th WHO International Standard for hepatitis C virus RNA for

[5] converting to copies / ml: in 100 µl K2-EDTA blood plasma samples: linear range from 750 copies/ml to 10^8 copies / ml; in 1000 µl K2-EDTA blood plasma samples: linear range from 80 copies / ml to 10^8 copies / ml

nucleic acid amplification techniques, NIBSC code: 18/184. The assigned CS-1 concentration is 370 370 IU/ml, CS-2 – 1 111 IU/ml, PC – 3 703 704 IU/ml.

Based on the results of the calibration and standardization process, it can be concluded that HEPA-C-test-Q reagent kit provides quantitative values for the 4th WHO International Standard for HBV DNA for NAT, NIBSC code: 10/266, which are similar to the expected values with a deviation of not more than ±0.05 log10 IU/ml (uncertainty).

4.2.9 Accuracy in repeatability and reproducibility conditions:

1. The kit coefficient of variation under repeatability conditions is not more than 3%.
2. The kit coefficient of variation under reproducibility conditions is not more than 5%.

4.3. Characteristics of clinical efficiency

4.3.1 Specificity

The specificity of the "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q", manufactured by TestGene LLC, was determined by testing negative RNA HCV samples from individual donors and pregnant women. The status in regards to RNA hepatitis C virus in the samples was established by a reagent kit for the hepatitis C virus (HCV) RNA detection in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection AmpliSens® HCV-FL according to TS 9398-019-01897593-2012, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate no. FSR 2011/10235 dated November 04, 2019).

In total 54 K2-EDTA blood plasma samples were tested with two lots of HEPA-C-test-Q reagent kits. 54 K2-EDTA blood plasma samples did not contain HCV RNA. When testing this sample panel, HEPA-C-test-Q reagent kit specificity was 100.0% (with a one-sided 95% confidence interval of 99.5%).

The lower limit of the specificity confidence interval with 95% confidence probability was determined by the Clopper and Pearson method (Clopper-Pearson Confidence Interval; Clopper, C., & Pearson, E. (1934).

The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika, 26(4), 404-413. doi:10.2307/2331986).

Used cyclers, recommended by the reagent kit manufacturer to carry out a PCR test with HEPA-C-test-Q reagent kit:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany)
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA).

4.3.2 Analytical specificity: potentially interfering substances effect checking

The list of tested potentially interfering substances is given in Section 8.3 of the Instructions for Use.

Based on the assay results, the following substances were classified as PCR inhibitors:

1) anticoagulants - heparin at 0.15 IU/ml concentration and sodium citrate at 0.1 mM/ml concentration. It is not allowed to use heparin and sodium citrate as an anticoagulant when taking peripheral blood.

2) heparin at 1 IU/ml concentration, used in anticoagulant therapy. The presence of heparin in patients' blood undergoing anticoagulant therapy can lead to inaccurate PCR results, therefore, it is recommended to collect blood from such patients before the next administration of the drug.

Other interfering substances at validated interferent concentrations have no effect on the test results. A negative result was obtained for all HCV RNA-negative samples, a positive result was obtained for all HCV RNA-positive samples. Besides, the average log10 titer of each HCV-positive sample containing potentially interfering substances was between -0.03 log10 and 0.02 log10 of an average log10 titer of a corresponding positive sample.

4.3.3 Analytical specificity: potentially cross-reactive agents effect checking

The results showed that during testing of 30 blood plasma K2-EDTA samples from patients which showed the hepatitis C virus RNA absence (negative samples), but with the confirmed DNA/RNA presence of the following microorganisms: HIV-1 – 2 samples, adenovirus type 5 – 3 samples, varicella zoster virus – 1 sample, cytomegalovirus – 2 samples,

Staphylococcus aureus – 2 samples, Epstein-Barr virus – 2 samples, hepatitis A virus – 1 sample, hepatitis B virus – 3 samples, human T-cell lymphotropic virus type 2 – 2 samples, human herpes virus type 6 – 2 samples, human papillomavirus – 3 samples, herpes simplex virus type 1 – 4 samples, herpes simplex virus, type 2 – 3 samples, no cross-reactivity was observed, no nonspecific reactions were detected.

A positive result was obtained for all HCV RNA-positive samples. Besides, the average log10 titer of each HCV-positive sample containing potentially interfering substances was between -0.09 log10 and 0.07 log10 of the average log10 titer of a corresponding positive sample.

4.3.4 Analytical sensitivity: limit of detection (LOD) for genotypes from 1a to 6

Clinical samples and NIBSC panel samples: 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques. NIBSC code: 14/290, consisting of seven bottles representing six basic genotypes: bottle 14/276 - HCV genotype 1a, 14/278 – HCV genotype 1b, 14/280 – HCV genotype 2i, 14/282 - HCV genotype 3a, 14/284 – HCV4r genotype, 14/286 – HCV genotype 5a, 14/288 – genotype 6l, was diluted in K2-EDTA blood plasma to the LOD concentration established by the manufacturer using the WHO International Standard 6th for hepatitis C virus RNA for nucleic acid amplification techniques, NIBSC code: 18/184 (genotype 1b), determined based on probit analysis of the 95% detection rate of LOD (92 IU/ml in 100 µl K2-EDTA blood plasma samples, 9.2 IU/ml in 1000 µl K2-EDTA blood plasma samples).

The obtained results confirmed HEPA-C-test-Q reagent kit ability to detect genotypes from 1a to 6 at a concentration of 92 IU/ml in 100 µl K2-EDTA blood plasma samples, 9.2 IU/ml in 1000 µl K2-EDTA blood plasma samples with an upper one-sided confidence interval of 95%, exceeding the expected detection rate of 95%.

4.3.5 Detection results of the interlot correlation (human blood plasma K2-EDTA)

To determine the interlot correlation of measurement results in clinical samples in accordance with the international guidelines CLSI EP09–A3, a scatter diagram of the dependent variable X - HCV RNA concentration was drawn using the test kit "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT "HEPA-C-test-Q",

manufactured by TestGeneLLC, LOT: 202111-425, and U – concentration of HCV RNA using the test kit "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT "HEPA-C-test-Q", manufactured by TestGene LLC, LOT: 202111-426.

The statistical processing results of the obtained data on the interlot correlation detection in accordance with recommendations of the CLSI EP09-A3 document using the regression and correlation method.

	Sample	Unit	Cycler used	Number of samples	Correlation ratio	Intersection	Slope
HEPA-C-test-Q reagent kit manufactured by TestGene LLC **LOT: 202111-425 in comparison** with a HEPA-C-test-Q reagent kit manufactured by LLC TestGene **LOT: 202111-426**	Human blood plasma K2-EDTA	log10 IU/ml	Dtprime	48	0.991	0.0593	0.9869
			CFX 96	48	0.9937	-0.021	1.0028
			Rotor-Gene Q	48	0.9924	0.1216	0.9622
			Quant Studio 5	48	0.9915	0.0281	0.9922

Correlation ratio R^2 during testing on each of the used cyclers was more than **0.99.** In accordance with the CLSI EP09-A3 document recommendations, using the regression and correlation method, it can be concluded that correlation strength of HCV RNA concentration is high in clinical samples obtained with **two lots of the studied medical device** "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q", manufactured by TestGene LLC.

4.3.6 Method comparison: accuracy

HCV RNA concentration was determined in 48 clinical samples, using the studied medical device "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q" in two series with cyclers recommended by the manufacturer of the studied reagent kit:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia); registration certificate no. FSR 2011/10228 dated March 03, 2011;

- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;

- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated August 10, 2010;

- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019.

The obtained results were compared with the results obtained using the comparison kit AmpliSens® HCV-Monitor-FL, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00577 dated December 27, 2019).

The results of the obtained data statistical processing compared with methods (accuracy) in accordance with the recommendations of the CLSI EP09-A3 document using the regression and correlation method.

	Sample	**Unit**	**Cycler used**	**Number of samples**	**Correlation ratio**	**Intersection**	**Slope**
HEPA-C-test-Q reagent kit manufactured by TestGene LLC **in comparison** with AmpliSens® HCV-Monitor-FL reagent kit, produced by FBIS Central Research Institute of Epidemiology Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00577 dated December 27, 2019)	Human blood plasma K2- EDTA	log10 IU/ ml	DTprime	48	0.9959	-0.061	1.0165
			CFX 96	48	0.9956	-0.0055	1.0003
			Rotor-Gene Q	48	0.9952	0.0322	0.9878
			Quant Studio 5	48	0.9941	-0.0292	1.0071

The obtained data allow to conclude on the reliable conformity of the HCV RNA concentration quantitative detection results in clinical samples obtained with the **studied medical device** "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C- test-Q, produced by TestGene LLC and a **comparison kit** AmpliSens® HCV-Monitor-FL, manufactured by FBI S Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00577 dated December 27, 2019) during PCR analysis with **cyclers:**

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 03, 2011;

- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;

- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 August 10,2010;

- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019.

The systematic error in measuring the logarithm of HCV RNA concentration does not exceed: for DTprime cycler: ±0.09 log10 IU/ml, for the CFX 96 cycler: ±0.08 log10 IU/ml, for Rotor-Gene Q cycler: ±0.07 log10 IU/ml, for QuantStudio 5 cycler: ±0.09 log10 IU/ml.

4.3.7 Precision detection (clinical samples)

Precision (within-batch reproducibility) of the test kit "Reagent kit for hepatitis C virus RNA quantitative detection by the RT-PCR-RT "HEPA-C-test-Q" was evaluated by 10-fold measurement of two human blood plasma K2-EDTA samples with an established HCV RNA concentration from the linear measurement range, with a registered reagent kit AmpliSens HCV- Monitor-FL, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00577 dated 27.12.2019) using the Rotor-Gene Q (Qiagen, Germany, No. FSZ 2010/07595 dated August 10, 2010).

The concentration values were considered as having a log-normal distribution and analyzed expressed in log10.

The average value, standard deviation and variation coefficient were estimated. Precision was considered acceptable if the coefficient of variation did not contradict the data in the technical documentation.

The coefficient of variation (CV) during the results precision determination of HCV RNA in human blood plasma K2-EDTA samples, with the studied medical device "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q", does not exceed the value stated by the manufacturer and is not more than 3%.

4.3.8 Precision detection (calibration samples CS-1 and CS-2)

Precision (within-batch reproducibility) of calibration samples as included in the studied medical device "Reagent kit for hepatitis C virus

RNA quantitative detection by the RT-PCR-RT "HEPA-C-test-Q" was evaluated by 10-fold evaluation of each level of calibration samples CS-1 and CS-2 in one analytical series.

The obtained results indicate adequate operation of the analytical system. Based on the obtained results, it can be concluded that the coefficient of variation (CV) in the results precision determination of calibration samples of two levels CS-1 and CS-2 does not exceed the one stated by the manufacturer and is not more than 3%.

4.3.9 Reproducibility detection (calibration samples CS-1 and CS-2)

Reproducibility of calibration samples CS-1 and CS-2 included in the studied medical device "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q" was evaluated by evaluating each level of CS-1 and CS-2 calibration samples in 10 additional analytical series.

Reproducibility data were obtained by testing different reagent kit batches, reactions were performed in different laboratories, by different operators, on different days, on different PCR cyclers.

The obtained results indicate adequate operation of the analytical system under reproducibility conditions: when testing different batches of reagent kits, in different laboratories, by different operators, on different days, on different PCR cyclers.

Based on the obtained results, it can be concluded that the coefficient of variation (CV) during the result reproducibility detection of calibration samples of two levels — CS-1 and CS-2 — did not exceed the one stated by the manufacturer and was not more than 3%.

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. Loss of functional properties of reagents included in the kit due to transportation, storage or use under inappropriate conditions;

2. Clinical material contamination with inhibitory substances in concentrations exceeding the permissible ones;

3. Contamination of reaction mixtures and studied RNA samples with contents from the PC tube or amplification products;

4. Testing with a poor quality RNA sample (low concentration

and/or poor purification);

5. Failure to comply with the requirements for sample preparation, testing and disposal due to work with unqualified personnel;

6. The use of an unsuitable kit (use after the expiry or in case of damaged package).

The cumulative residual risk of using a medical device "Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q" is acceptable, the benefits of its use exceed the risk.

6. Safety precautions

The class, depending on the potential risk of use, is 3 in accordance with the medical devices nomenclature classification approved by the order of the Ministry of Health of the Russian Federation dated 06.06.2012 N 4n.

All components and reagents included in HEPA-C-test-Q reagent kit belong to hazard class 4 (low-hazard substances) in accordance with GOST 12.1.007-76 "Occupational safety standards system. Harmful substances. Classification and general safety requirements".

The reagents included in HEPA-C-test-Q kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in HEPA-C-test-Q kit are non-toxic, since they are prepared by mixing individual non-toxic components.

Work with material infected or suspected of infection is carried out in accordance with the requirements of sanitary and epidemiological rules for the safe work with microorganisms of pathogenicity groups I-II (SP 1.3.3118–13), MU "Work organization of laboratories using nucleic acids amplification methods when working with material containing microorganisms pathogenicity groups I–IV " (MU 1.3.2569-09).

It is required to simultaneously ensure and comply with the biological safety rules and requirements for the organization and conduct of these works by personnel in order to prevent premises and equipment contamination with nucleic acids and (or) amplicons of the tested samples.

The work should be carried out in a laboratory performing PCR essays of clinical material in compliance with sanitary and epidemiological rules SanPiN SanPiN 2.1.3684-21" Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public

premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow methodological recommendations "Guidelines for disinfection, presterilization cleaning and sterilization of medical devices" (MU 287-113), MU "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I-IV" (MU 1.3.2569-09).

The following requirements should always be met when working:

- remove unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures";

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and spray the contents, as this may lead to contamination of the laboratory area, equipment and reagents with PCR products;

- use the kit strictly for its intended purpose, according to these Instructions for Use;

- allow only specially trained personnel to work with the kit (a specialist with higher medical education who has been trained in licensed specialization courses for working with pathogenic biological agents of pathogenicity groups I–II and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

- do not use the kit after the expiry date;

- avoid contact with skin, eyes and mucous membranes; in case of contact, immediately rinse the affected area with water and seek medical assistance.

The necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, sources of thermal inflammation are not provided.

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special,

unusual risks during device use or sale are not provided.

7. Required equipment and materials

Work with a reagent kit is carried out in the working area 3 (for preparation of reactions) (MU 1.3.2569-09).

Equipment for multiplex PCR:

1. Class II and III biosafety cabinet
2. Vortex;
3. Variable volume dispensers, allowing to take liquid volumes of 20-200 µl, 200-1000 µl
4. Refrigerator for 2°C to 8°C with a freezer for less than -16°C;
5. Cycler[6] with real-time fluorescence detection via channels corresponding to FAM/Green and HEX/Yellow fluorophores: CFX96 (BioRad, USA), DTprime, (NPO DNA Technology LLC, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the device:

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic consumables with "RNase-free" label.

1. Disposable tips with aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. Disposable sterile 1.5 or 2.0 ml Eppendorf type tubes;
3. Thin-walled disposable PCR tubes with an optically transparent lid (when detecting through a lid) or optically transparent walls (when detecting through a tube wall): 0.1 or 0.2 ml[7] PCR tubes, or 0.1 or 0.2 ml PCR tubes in strips, or PCR plates with an optically transparent film (for example, Axygen, USA), compatible with the used cycler;
4. Disposable lab coat and disposable talc-free gloves;
5. Container with disinfectant solution;
6. Test tube rack for 0.1 or 0.2 ml tubes or for stripped 0.1 or 0.2 ml tubes;
7. Kit for the nucleic acid isolation from blood plasma (see Section 8.2 of the Instructions for Use).

[6] The cyclers must be maintained, calibrated and used in accordance with the manufacturer's recommendations. The use of this kit in an uncalibrated device may affect the performance of the reagent kit.

[7] Make sure that PCR tubes are compatible with the cycler used.

8. Test samples

Test sample type

Test material is RNA samples isolated from human blood plasma K2-EDTA.

8.1. Clinical material collection procedure

ATTENTION! Before starting work, study the methodological recommendations "Taking, transporting, storing clinical material for PCR diagnostics" developed by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Sampling of clinical material and its packaging is carried out by an employee of a medical organization trained in the requirements and rules of biological safety when working and collecting material suspected of infection with microorganisms of the pathogenicity group II.

Material sampling for assay

4 or 6 ml peripheral blood is taken in the morning on an empty stomach in a test tube (vacuum tube) containing EDTA-K2 solution as an anticoagulant. Right after blood sampling, turn the tube upside down 3-4 times to mix the blood with the EDTA-K2 solution.

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

ATTENTION! The heparin presence in the blood of patients undergoing anticoagulant therapy can lead to inaccurate PCR results, therefore, it is recommended to take blood from such patients before the next administration of the drug.

Initial clinical material transportation and storage conditions

– at +2°C…+8 ° C – up to 6 hours;

– at room temperature – up to 2 hours.

Do not freeze blood.

Plasma K2-EDTA should be isolated within 2 hours (if stored at room temperature) or 6 hours (if stored at +2°C…+8°C) after material sampling, for that centrifugate the blood tube at 800-1600 g for 20 minutes at room temperature. After centrifugation, transfer the upper fraction (plasma) into a separate 1.5 or 2.0 ml plastic tube, free of RNases.

Blood plasma transportation and storage conditions:

Store plasma at +2°C…+8°C up to 5 days, at -18°C…-22°C up to 3 months, at -70°C – for a long time.

ATTENTION! Avoid repeated freezing and thawing of plasma

samples.

To isolate RNA, use at least 100 µl of plasma. An increase in the kit analytical sensitivity is possible due to use of a larger plasma volume, if this is provided by the RNA isolation kit used.

Material pre-processing

No preparation required.

Accounting, storage, handing and transportation of clinical material suspected of hepatitis virus presence should be carried out in accordance with the current sanitary and epidemiological rules for the work safety with microorganisms of pathogenicity groups I-II (SP 1.3.3118–13), current sanitary rules on the procedure for accounting, storage, transfer and transportation of microorganisms of pathogenicity groups I– IV.

Clinical material (Class B) disposal, as extremely epidemiologically hazardous waste, is carried out in accordance with SanPiN 2.1.3684-21.

8.2. Collection of human RNA sample isolated from blood plasma K2-EDTA

To isolate a human RNA sample from blood plasma, it is recommended to use the following reagent kits:

A reagent kit for DNA/RNA isolation from clinical material NA-Extra according to TS 21.20.23-013-97638376-2019 manufactured by TestGene LLC, Russia (registration certificate: RZN 2021/15428 dated 24.09.2021).

During the RNA isolation procedure, the protocol and the instructions of the used reagent kit must be strictly followed.

Add 10 µl of ICS from HEPA-C-test-Q reagent kit to plasma intended for the RNA isolation. 100 µl of NC sample, CS-1 and CS-2 also undergo the isolation procedure with addition of 10 µl of ICS (it is not required to use CS-1 and CS-2 when conducting qualitative analysis).

If the reagent kit manufacturer's Instructions for RNA isolation allow to use a larger sample volume, increase the volume of NC, CS-1 and CS-2 to the required volume with saline solution or TE buffer.

Test RNA samples storage conditions:

- at +2°C …+8°C up to 4 hours (recommended),
- at -18°C …-22°C up to one week,
- at below -80°C up to one year.

8.3. Interfering substances and restrictions on the test material use

The potentially interfering substances effect on HEPA-C-test-Q reagent kit performance was tested for potentially interfering substances that may occur during normal use of HEPA-C-test-Q reagent kit, and presumably affect the reagent kit ability to give valid results.

Interfering substances can originate from the following external and internal sources:

1) substances used in a patient' treatment (e.g., medicines);
2) substances found in specific sample types - in this case, contamination of a clinical sample with blood hemoglobin can inhibit PCR with insufficient purification during DNA isolation;
3) substances found during the procedure of clinical material sampling – in this case, anticoagulants.

The concentrations of interfering substances tested are shown in Table 6.

Table 6

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 μl/ml
Heparin (anticoagulant)	0.15 IU/ml
Sodium Citrate (anticoagulant)	0.1 mM/ml
EDTA-K2 (anticoagulant)	0.5 mM/ml
Cholesterol	150 mg/dl
Triglycerides	250 mg/dl
Exogenous interfering substances	
With anticoagulant therapy	
Heparin	1 IU/ml
Drugs prescribed for hepatitis C virus	
Interferon alpha	1000 IU/ml
Pegylated interferon alpha	0,036 μl/ml
Ribavirin	0.04 mg/ml
Narlaprevir	0.02 mg/ml
Paritaprevir	0.015 mg/ml
Dasabuvir	0.05 mg/ml
Sofosbuvir	0.08 mg/ml
Daclatasvir	0.012 mg/ml
Ledipasvir	0.018 mg/ml
Ombitasvir	0.0025 mg/ml

Based on the assay results, the following substances were classified as PCR inhibitors:

1) anticoagulants - heparin at 0.15 IU/ml concentration and sodium citrate at 0.1 mM /ml concentration. It is not allowed to use heparin and sodium citrate as anticoagulants when taking peripheral blood.

2) heparin at 1 IU/ml concentration, used for anticoagulant therapy. The heparin presence in blood of patients undergoing anticoagulant therapy can lead to inaccurate PCR results, therefore it

is recommended to take blood from such patients before the next administration of the drug.

To reduce the PCR inhibitor amount, it is required to follow the rules for clinical material sampling.

Limitations on test material use:

- the test material cannot be used in case of storage and transportation conditions violation (temperature, duration, multiple freezing-thawing);

- it is not allowed to use samples contaminated with extraneous biological material.

- heparin presence in blood of patients undergoing anticoagulant therapy can lead to inaccurate PCR results, therefore, it is recommended to take blood from such patients before the next administration of the drug.

9. Kit components preparation for testing

It is not required to install, assemble, adjust, calibrate the medical device for commissioning.

ATTENTION! When working with RNA, it is required to use only disposable sterile plastic consumables with "RNase-free" label. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

ATTENTION! The reaction mixture components should be mixed right before testing.

Before preparing the reaction mixtures, it is required to wet clean the PCR box, as well as the equipment and materials contained in it, using disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes. Before the test, it is required to defrost the kit components at room temperature.

1. Mix thoroughly the tubes contents with the RNA isolated for test, PCR Buffer, Oligonucleotide Mixture, CS-1, CS-2, NC and PC, turning upside down each tube 10 times or mixing using vortex at low speed for 3-5 seconds, then discharge drops from the tube lids by short centrifugation (it is not required to use CS-1 and CS-2 when conducting qualitative analysis).

2. Take the required number of 0.1 or 0.2 ml PCR tubes (with optically transparent lids or walls, depending on the type of detecting

cycler used) based on the calculation: the test samples number[8] + 1 x PC + 1 x NC + 3 x CS-1 + 3 x CS-2 (it is not required to use CS-1 and CS-2 when conducting qualitative analysis).

10. Testing procedure

The PCR test consists of the following stages:

1. RT-PCR preparation;
2. RNA reverse transcription and DNA PCR amplification with hybridization-fluorescence real-time detection of amplification products;
3. Result interpretation

A) RT-PCR preparation;

(carried out in a pre-PCR area – a room for reagent dispensing and preparation for PCR amplification)

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume.

Reaction mixture preparation for 1 reaction requires:

1. PCR Buffer – 5 µl,
2. Oligonucleotide Mixture – 15 µl,
3. Sample (RNA, PC, NC test samples) – 5 µl.

Prepare reaction tubes as follows:

1. Label the 0.1 or 0.2 ml PCR tubes.
2. In a separate disposable sterile 1.5 or 2.0 ml Eppendorf type tube prepare a reaction mixture: (n+9)x5 µl of PCR Buffer and (n+9)x15 µl of Oligonucleotide Mixture, where n is a number of test samples.
3. Add 20 µl of the prepared reaction mixture into each PCR tube.
4. Add 5 µl of isolated RNA to appropriate tubes for the test samples. Do not add RNA to PC and NC tubes.
5. Add 5 µl of calibration samples that have passed through RNA isolation stage into the corresponding tubes for CS-1 and CS-2 (see Section 8.2) (it is not required to use CS-1 and CS-2 when conducting qualitative analysis).
6. Add 5 µl of PC into the corresponding tube.
7. Add 5 µl of NC to the corresponding tube, which has passed through RNA isolation stage (see Section 8.2).

[8] To improve accuracy, it is recommended to analyze each sample twice.

8. To discharge drops from the walls, centrifugate the tubes for 1-3 seconds using vortex.

B) RNA reverse transcription and DNA PCR amplification with hybridization-fluorescence real-time detection of amplification products;

(carried out in a PCR area – a room for PCR amplification)

1. Install the tubes in a reaction module of a real-time PCR device. It is recommended to install the test tubes in the center of the thermal block to evenly press the tubes with a heating lid.

2. Program the device to perform the corresponding PCR program and fluorescence signal detection, following the Instructions for the device used. Test type: quantitative with standards. The PCR protocol is listed in Table 7.

3. For qualitative analysis: specify the number and identifiers of samples, CS-1 and CS-2 standards with indication of their concentrations, mark the tubes location on the matrix thermal block in accordance with their installation.

4. Make sure that the FAM/Green and HEX/Yellow detection channels are involved in the optical measurement parameters of the amplification program.

5. Start PCR with a fluorescent signal detection.

6. At the end of the program, start analyzing the results.

Table 7 – RT-PCR protocol

Stage	Temperature, °C	Time, min.:sec.	Detection channels	Total cycles
1	52	40:00	-	-
2	95	02:00	-	-
3	95	00:05	-	5
	60	00:15	-	
	67	00:30	-	
4	95	00:05	-	45
	60	00:15	FAM/Green, HEX/Yellow	
	67	00:30	-	

11. Results registration and interpretation

Results registration is carried out automatically upon PCR completion with the used device software.

Recommendations on setting the threshold line

For cyclers of any models, the threshold line is set individually for each detection channel at a level corresponding to 5-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

The results interpretation is carried out using the Ct values of the FAM/Green and HEX/Yellow channels (Table 1). Only Ct values obtained at the PCR stage with fluorescence detection are taken into account (i.e., corresponding to stage 4 – see Table 7).

First, the reaction outcome and Ct values in the control samples are evaluated. Test samples results interpretation starts only after the correct PC and NC passage.

ATTENTION! If Rotor-Gene 6000, Rotor-Gene 3000, Rotor-Gene Q and similar cyclers are used, activate the "Dynamic Tube", "Noise slope correction" functions, set 10% value in the "Outlier Removal" section.

Result interpretation in control samples

The following results should be obtained for NC and PC (Table 8).

Table 8 – Test results for NC and PC

Control sample	Ct values for detection channels corresponding to fluorophores	
	FAM/Green	HEX/Yellow
NC	> 35 or absent	≤ 32
PC	≤ 30	≤ 30

When obtaining values for NC that differ from those indicated in Table 8, the results of the entire staged series are considered unreliable. In this case, special measures should be taken to eliminate possible contamination.

When obtaining values for the PC that differ from those indicated in Table 8, repeat amplification of the entire sample batch.

When reobtaining PC values that differ from those indicated in Table 8, it is required to replace the reagents.

Result interpretation in the tested clinical samples

Result interpretation is carried out automatically using the software supplied with the used detection cycler, or manually.

Based on the obtained Ct values for calibration samples and their concentrations, it is required to make a calibration line. When using a calibration line, the concentrations of the test samples are calculated.

Hepatitis C RNA is detected if Ct ≤ 35 in the FAM channel. When Ct > 35 in the FAM channel and Ct ≤ 32 in the HEX channel, the result is considered doubtful. Hepatitis C RNA is not detected if there is no Ct in the FAM channel, and Ct ≤ 32 in the HEX channel. The result is considered invalid if Ct > 35 or absent in the FAM channel, and Ct > 32 or absent in the HEX channel. Quantitative analysis is possible if hepatitis C RNA is detected in a sample.

PCR efficiency should be more than 90% and less than 110%, the difference between Ct values in each calibration sample repetitions, CS-1 and CS-2, should be not more than 1. Otherwise, it is required to reperform the test, starting from RNA isolation stage. If one of the three CS-1 or CS-2 duplicates has a Ct value that deviates sharply from the rest, it is allowed to ignore it when making a calibration line.

ATTENTION! If a plasma volume exceeding 100 µl was used for RNA extraction (while maintaining the same volume of calibration samples used for RNA extraction), recalculate the obtained hepatitis C RNA concentration by multiplying the obtained concentration value by the 100/V ratio, where V is plasma volume used for RNA isolation. Measurement accuracy: ±0.4 lg of concentration.

ATTENTION! The Reference Card for results interpretation, supplied with the reagent kit, may specify a K coefficient needed to correct the obtained concentration value (if the coefficient is missing, assume its value as 1). To obtain the exact concentration, multiply the obtained value by the coefficient.

The general formula for concentration correction is:

$C = C_{obtained} \times 100 \times K / V$, where V is the plasma volume used

for RNA extraction, and K is the coefficient specified in the reagent kit Reference Card.

Further result interpretation principles are shown in Table 9.

The reason for obtaining an invalid result may be the presence of inhibitors in the RNA preparation obtained from clinical material, incorrect implementation of the test protocol, non-compliance with the PCR temperature regime etc.

The reason for obtaining a doubtful result may be an insufficient virus concentration in a clinical sample.

Table 9 – The result interpretation principle in the tested clinical samples

Channels corresponding to fluorophores		**Result interpretation**
FAM/Green (HCV), IU/ml	HEX/Yellow (ICS), Ct	
275 – $3.7*10^7$ IU/ml	not considered	positive result with a specific concentration in IU/ml indication
< 275 IU/ml	not considered	positive result with "less than 275 IU/ml" indication (less than $3*10^3$ copies/ml)
> $3.7*10^7$ IU/ml	not considered	positive result with "more than $3.7*10^7$ IU/ml" indication (more than 10^8 copies/ml)
-	≤ 32	negative result (concentration is not specified)
-	-	invalid result

Note: "not considered" – the result is not taken into account during interpretation;

"-" – there is no fluorescence signal, the concentration is not specified.

Note: the Table shows the values for RNA isolation from a 100 µl volume; when isolated from 1000 µl, the concentration values will be 10 times less than those indicated.

To recalculate the results in copies/ml, it is recommended to use the coefficient: 1 IU/ml = 2.7 copies/ml[9].

[9] McCullough J., Alter H.J., Ness P.M. Interpretation of pathogen load in relationship to infectivity and pathogen reduction efficacy // Transfusion. 2019. Vol. 59. P. 1132–1146.

In case of an invalid and doubtful result, a conclusion is not issued, it is necessary to retake biomaterial from a patient and retest it. At the same time, for doubtful results, it is recommended to isolate RNA from a larger plasma volume.

If a doubtful result repeats, retest with a reagent kit from another manufacturer or another method.

12. Storage, transportation and usage conditions

Storage

Store HEPA-C-test-Q reagent kit in the manufacturer's packaging at -18°C…-22°C during the entire kit shelf life, it is allowed to store at +2°C…+8°C up to 30 days.

It is not allowed to freeze/thaw HEPA-C-test-Q kit more than 10 times.

After opening, store at the same conditions as before opening.

A reagent kit stored in violation of the regulated regime cannot be used.

Transportation

Transport HEPA-C-test-Q reagent kit by all types of covered vehicles in accordance with transportation rules applicable to this transport type.

Transport at temperatures from -18°C…-22°C during the entire kit shelf life. Transportation is allowed at temperatures from +2°C…+8°C up to 30 days, or at temperatures from +15°C…+25°C up to 5 days.

Atmospheric pressure is not subject to control, as it does not affect the device quality.

To ensure compliance with transportation conditions throughout the entire transportation period, a reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. The type, volume and quantity of ice packs placed in a thermal container with transported reagent kits, as well as the volume of the thermal container are selected depending on the duration and conditions of transportation.

Reagent kits transported in violation of the temperature regime cannot be used.

Shelf life

The shelf life of HEPA-C-test-Q reagent kit is 12 months from the acceptance date of the manufacturer's QCD (Quality Control Dept), if all transportation, storage and operation conditions are met. A reagent kit with an expired shelf life cannot be used.

Shelf life of the opened kit components

12 months from the date of acceptance of the manufacturer's QCD (Quality Control Dept), if stored at temperatures from -18°C… -22°C.

Shelf life of prepared kit components

One hour under conditions that prevent the components from drying out, as well as extraneous biological material contamination.

13. Disposal

Reagent kits that got out of order, including due to expiration date, are subject to disposal in accordance with the requirements of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of the territories of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures".

According to the classification of medical waste, the kits belong to class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with paragraph 170 of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" are collected in reusable containers or disposable bags of any color (except yellow and red).

The remaining tubes and materials after the work are disposed in accordance with the methodological recommendations "Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices" (MU 287-113).

Liquid components (reagents) are destroyed by draining into

the sewer with preliminary reagent dilution with tap water 1:100 and removal of package residues as industrial or household garbage.

HEPA-C-test-Q reagent kit consumer package is subject to mechanical destruction with the removal of residues as industrial or household waste.

Personnel destroying the reagent kit must comply with the safety rules of a particular destruction method.

14. Warranty, contacts

Manufacturer guarantees quality and safety of HEPA-C-test-Q reagent kit during shelf life if compliance with transportation and storage requirements as well as rules of operation. If you have any complaints about the quality of the kits, please contact:

Limited Liability Company TestGene (TestGene LLC)
9, 44th Ingenerny Proezd, office 13, Ulyanovsk, 432072, Russia
Phone number: +7 (499) 705-03-75
www.testgene.com

Technical Support Service:
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

The instructions for use comply with the requirements of the order of the Ministry of Health of the Russian Federation dated 09.01.2014 No. 2n, order of the Ministry of Health of the Russian Federation dated 19.01.2017 No. 11n, GOST 51088-2013.

Annex A

Reagent kit for hepatitis C virus RNA quantitative detection by RT-PCR-RT HEPA-C-test-Q complies with the following interstate product standards:

Designation	Document name
GOST ISO 14971-2011	Medical products. Application of risk management to medical devices
GOST R 51088-2013	Medical devices for in vitro diagnostics. Reagents, reagent kits, test systems, control materials, culture medium. Requirements to devices and supporting documentation.
GOST R ISO 23640-2015	Medical devices for in vitro diagnostics. Reagent stability testing for in vitro diagnostics
GOST R 51352-2013	Medical devices for in vitro diagnostics. Test methods.
GOST R EN 13612-2010	Evaluation of functional characteristics of medical devices for in vitro diagnostics
GOST R 56894-2016	Summary technical documentation for demonstrating conformity to the essential principles of safety and performance of in vitro diagnostic medical devices.
GOST R ISO 18113-1-2015	Medical devices for in vitro diagnostics Information provided by the manufacturer (marking). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-1-2015	Medical devices for in vitro diagnostics Information provided by the manufacturer (marking). Part 2. In vitro diagnostic reagents for professional use.

GOST R ISO 23640-2015	Medical devices for in vitro diagnostics Reagent stability testing for in vitro diagnostics.
GOST R ISO 15223-1-2020	Medical products. Symbols to be used with medical device labels, labelling, and information to be supplied. Part 1. Basic requirements
GOST ISO 13485-2017	Medical products. Quality management systems. Requirements for regulatory purposes
GOST 2.114-2016	A unified system of design documentation. Technical specifications
GOST 2.104-2006	Unified System for design documentation. Basic records
GOST R 1.3-2018	Standardization in the Russian Federation. Technical specifications for the products. General requirements for content, design, designation and updating

Note – The above standards were in force at the time of the instructions for use approval. In the future, when using the document, it is advisable to check the validity of the reference normative documents at the current moment. If the reference document is replaced or modified, then the replaced (modified) document should be used when applying this document.