INSTRUCTIONS FOR USE

Reagent kit for genotyping of hepatitis C virus by multiplex Real-Time RT-PCR "HEPA-C-GEN-test"

IVD

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Content

List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
RT	reverse transcription
DNA	deoxyrubonucleic acid
cDNA	complementary DNA
RNA	ribonucleic acid
HCV	hepatitis C virus
ICS	internal control sample
NC	negative control sample
PC	positive control sample
SenC	Sensitivity Control
SC	Specificity Control

Introduction

Hepatitis C virus infection is a leading cause of acute and chronic liver disease. 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 glomerulonephritis and B-cell lymphoma. An important characteristic of the hepatitis C virus is that it has several genotypes (currently 8 genotypes are known), the determination of which is necessary for the appropriate antiviral therapy selection, prognostic assessment of the disease course and possible complications.

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

The scientific validity of the target analyte lies in the specificity of the target analyte (RNA sequence uniqueness) in regard to the 1a, 1b, 2, 3, 4, 5a and 6 virus genotypes.

Hepatitis C virus belongs to the flaviviridae viruses family. The genome is a single-stranded linear RNA molecule and consists of the following structural and functional elements: 5 'UTR-*C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3`UTR*[1,2]. High heterogeneity is an important feature of the genome.

To differentiate genotypes in the HEPA-C-GEN-test reagent kit were used following gene fragments: for 1a — *NS5B*, 1b — *NS5B*, 2 — *NS5B*, 3 — *NS3*, 4 — *NS5B*, 5a — *NS5B*, 6 — gene of the capsid C structural protein (core protein). The choice is determined by the relative conservatism of the regions among the same genotype. It should be noted that different test systems can use different regions to differentiate genotypes (for example, 5'UTR[3]), and if recombinant viruses are detected, there may be a difference between results of genotyping test systems from different manufacturers. For

[1] Chevaliez S., Pawlotsky J.-M. Chapter 1. HCV Genome and Life Cycle. In: Tan S.-L. (ed.), Hepatitis C Viruses: Genomes and Molecular Biology. Norfolk (UK): Horizon Bioscience, 2006. P. 5–47.

[2] González-Candelas F., López-Labrador F.X., Bracho M.A. Recombination in Hepatitis C Virus // Viruses. 2011. Vol. 3. P. 2006–2024.

example, a recombinant variant 2k/1b with a recombination in the *NS2*[3,4,5] region is widespread in the Russian Federation. This recombination joint is also characteristic of most other known recombinant genotypes[3]. However, most antiviral therapy drugs used for hepatitis C treatment are *NS3/4A, NS5A, NS5B*[6,7,8,9] inhibitors. It determines the therapeutic feasibility of detecting a genome region located below the most common recombination joint (the NS2 gene region).

Scope of the reagent kit: clinical laboratory diagnostics of infectious diseases.

Indications and contraindications

Indications: hepatitis C virus (genotypes 1a, 1b, 2a, 2, 3, 4, 5a and 6) genotyping in patients with detected hepatitis C virus to select a drug for antiviral therapy in patients with confirmed viremia, in accordance with the Guidelines: Acute Hepatitis C in Adults, B17.1 (Author: Non-Profit Partnership National Infectious Disease Society (NNOI)) and to determine the need for antiviral therapy for patients and regimens in accordance with the Clinical Guidelines: "Chronic viral hepatitis C (HVGS) in adults," B18.2 (Approved by Specialized Commission of the Ministry of Health of the Russian Federation in the field of "Infectious diseases," in coordination

[3] Cai Q., Zhao Z., Liu Y., Shao X., Gao Z. Comparison of three different HCV genotyping methods: Core, NS5B sequence analysis and line probe assay // International Journal of Molecular Medicine. 2012. P. 347–352.

[4] Kalinina O., Norder H., Mukomolov S., Magnius L.O. A Natural Intergenotypic Recombinant of Hepatitis C Virus Identified in St. Petersburg // Journal of Virology. 2002. Vol. 76 (8). P. 4034–4043.

[5] Demetriou V.L., Kyriakou E., Kostrikis L.G. Near-full genome characterization of two natural intergenotypic 2k/1b recombinant hepatitis C virus isolates // Advances in Virology. 2011 (710438). P. 1–7.

[6] Nikolaeva L.I., Sapronov. Hepatitis C virus: targets for therapy and new drugs // Questions of Virology. 2021. Vol. 57. No. 5. P. 10–15.

[7] Keikha M., Eslami M., Yousefi B., Ali-Hassanzadeh M., Kamali A., Yousefi M., Karbalaei M. HCV genotypes and their determinative role in hepatitis C treatment // Virus Disease. 2020. Vol. 31. P. 235–240.

[8] Kumar A., Rajput M.K., Paliwal D., Yadav A., Chhabra R., Singh S. Genotyping & diagnostic methods for hepatitis C virus: A need of low-resource countries // Indian Journal of Medical Research. 2018. Vol. 147 (5). P. 445–455.

[9] Zopf S., Kremer A.E., Neurath M.F., Siebler J. Advances in hepatitis C therapy: What is the current state - what come's next? // World Journal of Hepatology. 2016. Vol. 8 (3). P. 139–147.

with Scientific Council of the Ministry of Health of the Russian Federation).

Population and demographic aspects of the reagent kit usage: no population or demographic usage aspects of the HEPA-C-GEN-test reagent kit were identified.

Sterility: the product is not sterile.

1. Intended Use

Intended Use: HEPA-C-GEN-test reagent kit is designed for hepatitis C virus and its genotypes' specific genomic RNA regions qualitative detection by a single-stage allele-specific multiplex polymerase chain reaction with hybridization fluorescence detection with reverse transcription in real-time in RNA samples isolated from K2-EDTA human plasma in patients with detected hepatitis C virus for genotyping hepatitis C virus and selecting a drug for antiviral therapy in patients with confirmed viremia according to the Guidelines: Acute Hepatitis C in Adults, B17.1 (Author: Non-Profit Partnership National Infectious Disease Society (NNOI)) and to determine the need for antiviral therapy for patients and regimens in accordance with the Clinical Guidelines: "Chronic viral hepatitis C (HVGS) in adults," B18.2 (Approved by Specialized Commission of the Ministry of Health of the Russian Federation in the field of "Infectious diseases," in coordination with Scientific Council of the Ministry of Health of the Russian Federation).

Functional use: the results obtained can be used to select a drug for antiviral therapy in patients with confirmed viremia.

Reagent kit potential consumers:

Kit for research use only.

2. Method Principle

Method

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

Test sample type

The test material is RNA samples isolated from K2-EDTA human plasma.

Detection Principle

Specific genomic RNA regions of hepatitis C virus genotypes qualitative detection by multiplex single-stage allele-specific polymerase chain reaction with hybridization-fluorescence detection with reverse transcription in real-time (RT-PCR-RT) in RNA samples isolated from clinical material includes three stages:

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

Single-stage reverse transcription and specific regions amplification using primers specific to these regions are carried out with RNA samples in a reaction buffer.

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

The oligonucleotide mixtures contain primers and fluorescent-labeled oligonucleotide probes that hybridize with the complementary region of the amplified target DNA and get destroyed by *Taq*-polymerase. The dye and quencher separate, and fluorescence intensity increases in the corresponding range of the optical spectrum. It allows to registrate specific amplification product accumulation by measuring the fluorescent signal intensity in real time.

HEPA-C-GEN-test-A reagent kit configuration form is designed to detect genotypes 1a, 1b, 2 and 3. It contains an oligonucleotide mixture A.

HEPA-C-GEN-test-AB reagent kit configuration form is designed to detect genotypes 1a, 1b, 2, 3, 4, 5a and 6. It includes oligonucleotide mixtures A and B.

Oligonucleotide mixture A contains reagents for detecting genotypes 1a, 1b, 2 and 3, as well as an ICS (Table 1).

Oligonucleotide mixture B contains reagents for detecting genotypes 4, 5a and 6, as well as an ICS (Table 1).

Table 1 – Assay Targets

Oligonucleotide mixture	**Channel corresponding to a fluorophore**				
	FAM / Green	**HEX / Yellow**	**ROX / Orange**	**Cy5 / Red**	**Cy5.5 / Crimson**
A	HCV genotype 1a	ICS	HCV genotype 1b	HCV genotype 2	HCV genotype 3
B	HCV genotype 4	ICS	-	HCV genotype 5a	HCV genotype 6

ICS allows to evaluate the RNA extraction quality and effectiveness and determine the possible presence of reverse-transcriptase inhibitors and amplification in the sample. Their presence can lead to false negative results.

Method limitations

A possible reason for obtaining a false positive result is contamination during DNA extraction or during multiplex PCR reaction stages. A false positive result can be detected with a negative control sample.

Damage to the integrity of the package during transportation.

Use of expired kit or kit storage conditions violation.

Violation of storage conditions during samples transportation.

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

3. Reagent Kit Components

Reagent kit configuration forms:

The HEPA-C-GEN-test reagent kit comes in two configuration forms:

- HEPA-C-GEN-test-A for genotypes 1a, 1b, 2, and 3 detection;
- HEPA-C-GEN-test-AB for genotypes 1a, 1b, 2, 3, 4, 5a, and 6 detection.

Choosing of a configuration form is determined by the population characteristics of the genotype distribution[10,11].

Test samples number

1. The HEPA-C-GEN-test-A configuration form is designed for 96 reactions, that corresponds to 94 test samples (47 in duplicates), negative and positive samples during a single run of the cycler for 96 wells or 32 single test samples with negative and positive control samples in each run.

2. The HEPA-C-GEN-test-AB configuration form is designed for 192 reactions, that corresponds to 92 test samples (46 in duplicate tests), negative and positive control samples in two runs for 96 wells or 32 single test samples with negative and positive control samples in each run.

[10] Clinical guidelines "Chronic viral hepatitis C (CHC) in adults" of the Ministry of Health of the Russian Federation, 2018. 90 p.

[11] Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection. World Health Organization, 2018. 84 p.

Reagent kit components

Table 2 – HEPA-C-GEN-test-A reagent kit configuration form components

No.	Reagent	Description	Quantity, Volume
1.	RT-PCR buffer	Transparent colorless liquid	1 tube, 480 µl
2.	Oligonucleotide mixture A	Transparent colorless liquid, may have a lilac shade	1 tube, 1440 µl
3.	PC	Transparent colorless liquid	1 tube, 160 µl
4.	NC	Transparent colorless liquid	2 tubes, 1600 µl each
5.	ICS	Transparent colorless liquid	1 tube, 950 µl

Note: Operational documentation (instructions for use and quality certificate) is not included in the bill of materials, but is included in the reagent kit delivery scope. To ensure compliance with transportation conditions the reagent kit is placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. The thermal container is put into an individual package with the instructions for use and the quality certificate for every reagent kit batch.

Table 3 – HEPA-C-GEN-test-AB reagent kit configuration form components

No.	Reagent	Description	Quantity, Volume
1.	RT-PCR buffer	Transparent colorless liquid	1 tube, 960 µl
2.	Oligonucleotide mixture A	Transparent colorless liquid, may have a lilac shade	1 tube, 1440 µl
3.	Oligonucleotide mixture B	Transparent colorless liquid, may have a blue shade	1 tube, 1440 µl
4.	PC	Transparent colorless liquid	1 tube, 320 µl
5.	NC	Transparent colorless liquid	4 tubes, 1600 µl each
6.	ICS	Transparent colorless liquid	1 tube, 930 µl

Note: Operational documentation (instructions for use and quality

certificate) is not included in the bill of materials, but is included in the reagent kit delivery scope. To ensure compliance with transportation conditions the reagent kit is placed in a reusable polyurethane foam thermal container filled with ice packs for temporary storage and transportation. The thermal container is put into an individual package with the instructions for use and the quality certificate for every reagent kit batch.

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

Oligonucleotide mixtures are ready for use and contain primers and probes designed to identify specific targets (Table 1).

PC is ready for use and is a plasmid DNA mixture with synthetic inserts of amplified fragments of genomic cDNA of hepatitis C virus detected genotypes and bacteriophage genome fragment in 10% TE buffer (10 mM Tris, 1 mM EDTA).

NC is ready for use and is DNase- and RNase-free deionized water.

ICS is ready for use and is a reinforced RNA.

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 4 – HEPA-C-GEN-test reagent kit

Indicator name	Characteristics and standards	Clause in Technical Specification (TS)
1. Technical characteristics		
1. Appearance		
HEPA-C-GEN-test-A configuration form		
RT-PCR buffer	Transparent colorless liquid	Section 7, clause 7.6
Oligonucleotide mixture A	Transparent colorless liquid, may have a lilac shade	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

HEPA-C-GEN-test-AB configuration form		
RT-PCR buffer	Transparent colorless liquid	Section 7, clause 7.6
Oligonucleotide mixture A	Transparent colorless liquid, may have a lilac shade	Section 7, clause 7.6
Oligonucleotide mixture B	Transparent colorless liquid, may have a blue shade	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
1.2. Completeness	According to clause 1.4 of TS 21.20.23-022-97638376-2020	Section 7, clause 7.12
1.3. Marking	According to clause 4 of TS 21.20.23-022-97638376-2020	Section 7, clause 7.12
1.4. Packaging	According to clause 5 of TS 21.20.23-022-97638376-2020	Section 7, clause 7.12
2. Functional characteristics		
2.1 Positive result with PC	Fluorescence signal growth registered in PC tubes for a reaction mixture A: in the FAM, ROX, Cy5 and Cy5.5 channels Ct≤30, HEX Ct≤32; for a reaction mixture B: in the FAM, Cy5 and Cy5.5 channels Ct≤30, HEX Ct≤32.	Section 7, clause 7.8.2
2.2 Negative result with NC	In tubes with NC in the FAM, ROX, Cy5 and Cy5.5 channels Ct is not indicated (i.e. there is no fluorescence accumulation graph) or Ct> 35, and in the HEX channel Ct≤32.	Section 7, clause 7.8.2
2.3 Reaction in tubes with Specificity Control (SC)	In tubes with SC in the FAM, ROX, Cy5 and Cy5.5 channels Ct is not indicated (i.e. there is no fluorescence accumulation graph), and in the HEX channel Ct≤32.	Section 7, clause 7.8.2
2.4 Reaction in test tubes with Sensitivity Control (SenC)	In tubes with SenC in the FAM, ROX (only for reaction mixture A), Cy5 and Cy5.5 channels in all repetitions (at least 3) of each reaction mixture Ct≤35, and in the HEX channel Ct≤32.	Section 7, clause 7.8.2

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

Standard control samples for sensitivity determination (SenC-1a, SenC-1b, SenC-2, SenC-3, SenC-4, SenC-5a, SenC-6) are plasmid mixtures with synthetic inserts of a hepatitis C virus genomic cDNA fragment of the detected hepatitis

C virus genotypes in 4,000 copies/ml concentration and a bacteriophage genome fragment in 100,000 copies/ml concentration in 10% TE-buffer (1 mm Tris, 0.1 mm EDTA):

A specificity control sample (SC) is human genomic DNA solution extracted from the 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

Specific to hepatitis C virus 1a, 1b, 2, 3, 4, 5a and 6 RNA genotypes.

4.2.1.1 The hepatitis C virus RNA (1a to 6) detection possibility was confirmed via NIBSC standard:

– 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques. NIBSC code: 14/290 that consists of seven vials representing six major genotypes: vial 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 6.

When testing using the 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques. NIBSC code: 14/290 reliable results were obtained; it confirms hepatitis C various genotypes (from 1a to 6) detection possibility by the HEPA-C-GEN-test reagent kit.

4.2.1.2 Analytical specificity: studying the potentially cross-reacting substances effect.

The following non-specific reactions absence and simultaneous NA strains presence was confirmed:

- from the ATCC collection (American Type Culture Collection, USA): *Propionibacterium acnes* (ATCC® 29399 ™), *Staphylococcus aureus* subsp. *aureus*, Strain Seattle 1945 (ATCC® 25923™), *Staphylococcus epidermidis*, FDA Strain PCI 1200 (ATCC® 12228™), *Staphylococcus haemolyticus* ATCC™ 29970™.

- from the "GKPM-OBOLENSK" collection: *Escherichia coli* M-17 (strain number B-2929), *Candida albicans* NCTC 885-653 (strain number B-7940)

in a maximum concentration of 10^6 to 10^7 cells per ml.

4.2.1.3 Analytical specificity: studying the potentially interfering substances effect

The list of studied potentially interfering substances is given in Section 8.3 of the Instructions.

According to the results of the study the following substances were classified as PCR inhibitors:

1) anticoagulants — heparin in 0.15 IU/ml concentration and sodium citrate in 0.1 mM/ml concentration. Heparin and sodium citrate cannot be used as anticoagulants when sampling peripheral blood.

2) heparin in 1 IU/ml concentration is used for anticoagulant therapy. The presence of heparin in the blood of patients undergoing anticoagulant therapy may lead to inaccurate PCR results, so it is recommended to sample blood from such patients before the next administration of the drug.

Other interfering substances in validating concentrations of interferents do not affect the specific regions of the hepatitis C virus and its genotypes genomic RNA qualitative detection results using the HEPA-C-GEN-test reagent kit.

4.2.2 Analytical sensitivity (detection limit, LOD)

According to GOST R 51352-2013 and taking into account the international **CLSI EP-17A2** guidelines, the limit of detection (LOD) was determined by the dissolving analysis method according to the WHO International Standards:

- WHO International Standard 6th WHO International Standard for hepatitis C virus RNA for nucleic acid amplification techniques NIBSC code: 18/184, in 257,000 IU/tube (~ 5.41 log10 IU/tube) concentration

in HCV-negative human K2-EDTA plasma within the range of the expected detection limit for 100 µl and 1000 µl volume samples.

Each of the 7 dissolvings was analyzed using the HEPA-C-GEN-test reagent kit during 3 different days in 30 repetitions to calculate the percentage of positive results. Results were determined according to international **CLSI EP-17A2** guidelines by probit analysis method.

Sample volume	**Used cycler**	**Concentration, IU/ml (LOD) with 95% confidence probability**	**95% Confidence Interval**
100 µl	DTprime	1514.8	95%CI: 1513.37-1516.23
	CFX 96	1520.4	95%CI: 1518.97-1521.83
	Rotor Gene Q	1510.9	95%CI: 1509.47-1512.33
	Quant Studio 5	1510.2	95%CI: 1508.77-1511.63
1000 µl	DTprime	77.3	95%CI: 75.87-78.73
	CFX 96	77.1	95%CI: 75.67-78.53
	Rotor Gene Q	77.1	95%CI: 75.67-78.53
	Quant Studio 5	76.5	95%CI: 75.07-77.93

NIBSC standard panel samples were used to verify **detection limit when testing different genotypes of hepatitis C virus (1a to 6)**:

– 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques. NIBSC code: 14/290 containing seven vials representing six major genotypes: vial 14/276 — HCV genotype 1a, 14/278 — HCV genotype 1b, 14/280 — HCV genotype 2i, 14/282 — HCV genotype 3a, 14/284 — genotype HCV4r, 14/286 — HCV genotype 5a, 14/288 — genotype 6.

The obtained results confirmed the HEPA-C-GEN-test reagent kit ability to detect genotypes 1a, 1b, 2i, 3a, 4r, 5a, 6 in ~ 1500 IU/ml concentration in 100 µl of K2-EDTA plasma samples, ~ 77 IU/ml in 1000 µl of K2-EDTA plasma samples with 95% upper one-sided confidence interval exceeding the expected 95% detection rate.

4.2.3 Precision under repeatability and reproducibility conditions:

1. The variation coefficient under the kit repeatability conditions does not exceed 3%.

2. The variation coefficient under the kit reproducibility conditions does not exceed 5%.

4.3. Clinical efficiency characteristics

41 K2-EDTA human plasma samples, sampled from patients with detected hepatitis C virus aged 12 to 65 years (17 women and 24 men) that had various stages of the disease were tested to conduct clinical trials.

This number of samples was collected in accordance with the International CLSI EP09-A3 guidelines, as well as in accordance with the GOST R 51352-2013.

NIBSC standard samples: 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques were also considered as clinical samples to evaluate the kit performance against various HCV genotypes (1b to 6). NIBSC code: 14/290, consisting of seven vials representing six main genotypes: vial 14/276 — HCV genotype 1a, 14/278 — HCV genotype 1b, 14/280 — HCV genotype 2i, 14/282 — HCV genotype 3a, 14/284 — genotype HCV4r, 14/286 — HCV genotype 5a, 14/288 — genotype 6.

Each sample was tested in two rounds using the HEPA-C-GEN-Test test reagent kit manufactured by TestGene LLC to assess inter-lot repeatability.

Quality, safety and efficacy of the tested medical device was studied in 156 tests.

The tests were carried out by a prospective parallel study of the diagnostic characteristics of the each configuration form of the HEPA-C-GEN-test reagent kit based on comparison of the test results of the same clinical material samples by the HEPA-C-GEN-test reagent kit manufactured by TestGene LLC and by the AmpliSens® HCV-genotype-FL reagent kit produced by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia.

Correct work of the reagent kit is indicated by the concordance of the results.

When testing K2-EDTA blood plasma samples from patients with the hepatitis C virus RNA absence (negative samples), but with confirmed presence of the following microorganisms DNA/RNA: HIV-1 — 2 samples, Adenovirus 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 I — 4 samples, herpes simplex virus II — 3 samples, no cross-reactivity was detected, no non-specific reactions were

detected.

Cyclers used for PCR testing:

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

HEPA-C-GEN-test-A reagent kit configuration form was designed to detect genotypes 1a, 1b, 2 and 3, so the diagnostic characteristics calculation regarding to genotypes 4, 5 and 6 was not carried out.

Diagnostic characteristics confidence intervals (CI) were calculated via the Clopper and Pearson method (Clopper-Pearson Confidence Interval; Clopper C., Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial // Biometrika. 1934. Vol. 26 (4). P. 404–413. doi:10.2307/2331986).

The reagent kit diagnostic characteristics were calculated with 95% confidence probability.

Results reproducibility is 100%.

Table 5 — Clinical efficacy

Analyte to be determined	**Positive samples observations number**	**Negative samples observations number**	**Diagnostic sensitivity with 95% confidence probability**	**Diagnostic specificity with 95% confidence probability**
HEPA-C-GEN-test-A				
HCV genotype 1a	10	292	100% (95% diagnostic interval: 69,15%-100%)	100% (95% diagnostic interval: 98,74%-100%)
HCV genotype 1b	30	252	100% (95% diagnostic interval: 88,43%-100%)	100% (95% diagnostic interval: 98,55%-100%)
HCV genotype 2	8	296	100% (95% diagnostic interval: 63,06%-100%)	100% (95% diagnostic interval: 98,76%-100%)
HCV genotype 3	6	300	100% (95% diagnostic interval: 54,07%-100%)	100% (95% diagnostic interval: 98,78%-100%)
«HEPA-C-GEN-test-AB»				
HCV genotype 1a	14	10	100% (95% diagnostic interval: 69,15%-100%)	100% (95% diagnostic interval: 98,74%-100%)
HCV genotype 1b	20	8	100% (95% diagnostic interval: 88,43%-100%)	100% (95% diagnostic interval: 98,55%-100%)
HCV genotype 2	8	14	100% (95% diagnostic interval: 63,06%-100%)	100% (95% diagnostic interval: 98,76%-100%)

HCV genotype 3	4	4	100% (95% diagnostic interval: 54,07%-100%)	100% (95% diagnostic interval: 98,78%-100%)
HCV genotype 4	2	2	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,82%-100%)
HCV genotype 5a	2	2	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,82%-100%)
HCV genotype 6	2	2	100% (95% diagnostic interval: 15,81%-100%)	100% (95% diagnostic interval: 98,82%-100%)

5. Risks associated with the reagent kit usage

The risk zone includes the following hazards:

1. The kit reagents functional properties loss due to transportation, storage or usage under inappropriate conditions;

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

3. Reaction mixtures and test RNA samples contamination with contents from the PC tube or with 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, analysis and disposal due to unqualified personnel work;

6. Usage of an unusable kit (after the expiration date or in case of damaged package).

No risks have been identified in the risk zone area.

Total residual risk of using the HEPA-C-GEN-test reagent kit for hepatitis C virus genotyping by multiplex RT-PCR-RT method is acceptable, the benefits of its usage exceed the risk.

6. Safety Precautions

All components and reagents included in the HEPA-C-GEN-test reagent kit belong to low-hazard substances. Precautions against any special, unusual environmental risks when using or selling the product are not provided.

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

Reagents included in the HEPA-C-GEN-test kit are not toxic because 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 sanitary and epidemiological requirements for the prevention of infectious diseases, and with the requirements organization of laboratories using nucleic acids amplification methods when working with material containing microorganisms pathogenicity groups I–IV.

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

The work should be carried out in a laboratory performing clinical material molecular-biological (PCR) testing in accordance with sanitary and epidemiological requirements.

The following requirements should always be met when working:

- Remove unused reagents in accordance with sanitary and epidemiological requirements for the management of medical waste.

ATTENTION! It is unacceptable to open the tubes and spray the contents when removing waste after amplification (tubes containing PCR products), 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 this instruction;
- only specially trained personnel are allowed to work with the kit (a specialist with higher medical education who has been trained in licensed qualification courses to work with Pathogenic Biological Agents (PBA) of pathogenicity groups III and IV and to conduct PCR testing, as well as a laboratory assistant with secondary special medical education);
- do not use the kit after the expiration date;
- avoid contact with skin, eyes and mucous membrane. In case of contact, immediately flush the affected area with water and seek medical assistance.

The necessary precautions are not provided for the magnetic fields effects, external electrical influences, electrostatic discharges, pressure or pressure changes, overloads, or sources of thermal ignition.

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

unusual risks during product 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 reactions preparation)

Equipment for multiplex RT-PCR:

1. Class II and III biological safety cabinet;
2. Vortex;
3. Set of electronic or automatic variable volume dispensers;
4. Refrigerator for + 2°C… + 8°C with a freezer not higher than -16 °C;
5. Cycler[12] with real-time fluorescence detection in the channels corresponding to the FAM/Green, HEX/Yellow, ROX/Orange and Cy5/Red, Cy5.5/Crimson fluorophores. For example: CFX96 (BioRad, USA), DTprime (DNA-Technology LLC, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

Materials and reagents not included in the kit:

ATTENTION! It is necessary to use only disposable sterile plastic consumables with "DNase-free" and "RNase-free" labels when working with RNA.

1. Disposable tips with aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);
2. 1.5 or 2.0 ml disposable sterile Eppendorf type tubes;
3. Thin-walled disposable PCR tubes with an optically transparent lid (when using plate type cyclers) or optically transparent walls (when using rotary type cyclers): 0.1 or 0.2 ml[13] PCR tubes, or 0.1 or 0.2 ml PCR strip tubes, or PCR plates with an optically transparent film (e.g. Axygen, US) compatible with the used cycler;
4. Lab coat and disposable gloves without talcum powder;
5. Container with disinfectant;
6. Test tube rack for 0.1 or 0.2 ml tubes or for 0.1 or 0.2 ml stripped tubes (e.g., InterLabService LLC, Russia);
7. A kit for RNA extraction from blood plasma (see section 8.2 of the instructions)

[12] Cyclers should be maintained, calibrated and used according to the manufacturer's recommendations. Use of this kit in an uncalibrated device may affect the performance of the reagent kit.

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

8. Test Samples

Test sample type

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

8.1. Clinical material collection procedure

Clinical material sampling 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. Immediately 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 anticoagulants.

ATTENTION! The presence of heparin in the blood of patients 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.

Transportation and storage conditions of the initial clinical material - blood:

- at temperatures from + 2°C to + 8°C — less than 6 hours;
- at room temperature — less than 2 hours.

Do not freeze blood.

Plasma should be sampled within 2 hours (when stored at room temperature) or 6 hours (when stored at temperatures from + 2°C to + 8°C) after the clinical material collection. For that centrifuge a tube with blood at 800-1600g speed for 20 minutes at room temperature. After centrifugation transfer the upper fraction (plasma) into a separate plastic 1.5 or 2.0 ml tube free of RNases.

K2-EDTA blood plasma transportation and storage conditions:

It is allowed to 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 refreezing and thawing of K2-EDTA plasma samples.

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

Material pre-processing

No preparation required.

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

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

It is recommended to use the following reagent kits for human RNA sample isolation from K2-EDTA blood plasma

- NA-Extra reagent kit for DNA/RNA extraction from clinical material, manufactured by TestGene LLC, Russia (registration certificate: RZN 2021/15428 dated 24.09.2021).

The protocol and the instructions of the reagent kit used must be strictly followed during the RNA extraction procedure.

Add 10 µl of ICS from the HEPA-C-GEN-test reagent kit to plasma intended for RNA isolation.

The NC sample also undergoes the extraction procedure in a 100 µl volume with the addition of 10 µl ICS. If the instruction of the manufacturer of reagent kits for RNA extraction provides for the use of a larger sample volume adjust the NC volume to the required one with saline solution or with TE buffer.

Conditions for possible storage of analyzed RNA samples:

- at + 2°C... + 8 °C — up to 4 hours (recommended),
- at -18°C... -22 °C — up to a week,
- at temperature less than -80 °C — up to 1 year.

8.3 Interfering substances and restrictions on the tested material use

The potentially interfering substances effect on the HEPA-C-GEN-test reagent kit performance has been examined for potentially interfering substances that may occur during normal use of the HEPA-C-GEN-test

reagent kit and that presumably can affect the ability of the reagent kit to produce valid results.

Interfering substances may 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 the DNA isolation procedure;

3) substances encountered during the sampling procedure of clinical material — in this case, anticoagulants.

The tested interfering substances concentrations are shown in Table 6.

Table 6

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 µg/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 viral hepatitis C	
Interferon Alfa	1000 IU/ml
Pegylated interferon alpha	0.036 mg/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

According to the examination 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 sampling peripheral blood.

2) heparin in 1 IU/ml concentration used for anticoagulant therapy. Heparin presence in blood of patients 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.

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

Limitations on test material use:

– test material usage is not allowed under storage and transportation conditions violation (temperature, duration, multiple freezing and thawing):

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

– heparin presence 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.

9. Kit components preparation of for testing

Installation, adjustment, calibration of the medical device for commissioning is not required.

ATTENTION! It is necessary to use only disposable sterile plastic consumables with "DNase-free" and "RNase-free" labels when working with RNA. It is mandatory to use a separate tip with an aerosol barrier for each reaction component.

ATTENTION! The reaction mixture components should be mixed immediately before the assay.

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

1. Thoroughly mix the contents of the tubes with the RNA isolated for the assay, RT-PCR buffer, oligonucleotide mixture, NC and PC, turn each tube 10 times or mix using vortex at low speed for 3-5 seconds, then remove the drops from the tube lids by short centrifugation.

2. Select the required 0.1 or 0.2 ml PCR tubes number (with optically

transparent lids or walls — depending on the type of the used cycler) according to the following calculation: for HEPA-C-GEN-test-AB configuration form — 2 x the number of test samples + 2 x PC + 2 x NC; for HEPA-C-GEN-test-A configuration form — 1 x number of test samples + 1 x PC + 1 x NC (see Table 7).

Table 7 – Test tubes labeling principle

	Test samples			PC	NC
	1	2	n		
Reaction mixture A (all configuration forms)	+	+	+	+	+
Reaction mixture B (HEPA-C-GEN-test-AB configuration form)	+	+	+	+	+

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 amplification products detection;
3. Results interpretation.

A) RT-PCR preparation

(is produced in pre-PCR area — a room for reagent dispensing and preparation for PCR amplification).

Reaction mixture A (for all the configuration forms) preparation requires:

1. RT-PCR buffer — 5 µl,
2. Oligonucleotide mixture A —15 µl,
3. Sample (RNA test sample, PC, NC) — 5 µl.

Total reaction volume is 25 µl.

Reaction mixture B (for HEPA-C-GEN-test-AB configuration form) preparation requires:

1. RT-PCR buffer — 5 µl,
2. Oligonucleotide mixture B — 15 µl,
3. Sample (RNA test sample, PC, NC) — 5 µl.

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume.

Prepare the reaction tubes in the following order:

1. Label 0.1 or 0.2 ml PCR tubes (see Table 7).
2. For all configuration forms: in a separate disposable sterile 1.5 or 2.0 ml Eppendorf type tube prepare the reaction mixture A: (n + 3) x5 µl of

PCR-buffer and (n + 3) x15 µl of oligonucleotide mixture A, where n is the number of test samples.

3. For the HEPA-C-GEN-test-AB configuration form: in a separate disposable sterile 1.5 or 2.0 ml Eppendorf type tube prepare the reaction mixture B: (n + 3) x5 µl of PCR buffer and (n + 3) x15 µl of oligonucleotide mixture B, where n is the number of test samples.

4. Add 20 µl of reaction mixtures A or B into the corresponding PCR tubes (if the mixtures are included in the configuration form) (see Table 7).

5. Add 5 µl of isolated RNA into the corresponding test tubes. Do not add RNA into PC and NC tubes.

6. Add 5 µl of PC into the appropriate tubes.

7. Add 5 µl of the NC that has passed the NA extraction into the appropriate tubes.

8. To discharge drops from the walls, centrifuge the tubes for 1-3 seconds on a microcentrifuge-vortex.

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

(performed in the PCR area – a room for PCR amplification)

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

2. Program the device to perform the corresponding RT-PCR and fluorescent signal detection program according to the instructions of the used device. The RT-PCR protocol compatible with most cycler models (DTprime, Rotor-Gene Q, QuantStudio 5) is shown in Table 8; protocol for CFX96 cycler (BioRad, USA) is shown in Table 9.

3. Specify the number and identifiers of samples, mark the tubes location on the matrix of the thermoblock in accordance with their layout.

4. Make sure that the FAM/Green, HEX/Yellow, ROX/Orange, Cy5/Red, Cy5.5/Crimson detection channels are included in the optical measurement parameters of the amplification program.

5. Start PCR with fluorescent signal detection.

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

Table 8 — RT-PCR protocol (for DTprime, Rotor-Gene Q, QuantStudio 5)

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, ROX/Orange, Cy5/Red, Cy5.5/Crimson	
	67	00:30	–	

Table 9 – RT-PCR protocol (for CFX96)

Stage	Temperature, °C	Time, min.: sec.	Detection Channels	Total Cycles
1	52	40:00	☐	☐
2	95	02:00	☐	☐
3	95	00:15	☐	5
	60	00:15		
	67	00:30		
4	95	00:15	☐	45
	60	00:15	FAM/Green, HEX/Yellow, ROX/Orange, Cy5/Red, Cy5.5/Crimson	
	67	00:30	☐	

ATTENTION! In case of using the QuantStudio 5 cycler, it is necessary to adjust optical filters before starting the amplification protocol (the VIC signal may be registered in the ROX channel if Δ Rn > 100,000; it can lead to obtaining false positive results)! For that, press the "Action" button in the "Method" tab, then select "Optical filter settings" in the pop-up menu, and in the "PCR Filter settings" section choose only the following filter combinations: x1 - m1, x2 - m2, x4 - m4, x5 - m5, x6 - m6.

11. Results registration and interpretation

Results interpretation is carried out automatically upon RT-PCR completion using the software of the used device.

Recommendations for installing the threshold line

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

The results are interpreted according to the Ct values in the FAM/Green, HEX/Yellow, ROX/Orange, Cy5/Red and Cy5.5/Crimson channels (Table 1).

First, the reaction passage and Ct values in control samples are evaluated. The test samples results interpretation starts just after the correct passage of PC and NC.

ATTENTION! In case of using Rotor-Gene 6000, Rotor-Gene 3000, Rotor-Gene Q and similar cyclers, activate the “Dynamic Tube” and “Noise slope correction” functions, set the 10% value in the "Outlier Removal" section for all detection channels except Cy5.5/Crimson, which should be 20% in the "Outlier Removal" section.

Results interpretation in control samples

The following NC and PC results should be obtained for each reaction mixture (Table 10).

Table 10 – NC and PC test results

Control sample	Ct values in detection channels corresponding to fluorophores				
	FAM / Green	HEX / Yellow	ROX / Orange	Cy5/ Red	Cy5.5 / Crimson
Reaction mixture A					
NC	> 35 or absent	≤ 32	> 35 or absent	> 35 or absent	> 35 or absent
PC	≤30	≤32	≤30	≤30	≤30
Reaction mixture B					
NC	> 35 or absent	≤ 32	not considered	> 35 or absent	> 35 or absent
PC	≤30	≤32	not considered	≤30	≤30

If the obtained NC values differ from those shown in Table 10, the entire assay results are considered unreliable. In this case, special measures should be taken to eliminate possible contamination. At the same time if there are no signals in the detection channels (Table 11) the results should be considered reliable; re-testing of such samples is not required.

If obtained PC values differ from those indicated in Table 10, repeated amplification of the entire sample batch is required. If after repeated amplification PC results differ from those indicated in Table 10, the reagents must be replaced.

Results interpretation in control samples

Results interpretation principles are shown in Table 11.

The genotype is determined by comparing RT-PCR results in the corresponding reaction mixtures (Table 11) according to the following conditions:

for all configuration forms:

- if the result corresponds to one genotype, then this genotype is shown in the result;
- if several genotypes are detected in one test sample, and Ct values in the corresponding channels differ for not more than 8 cycles, then several genotypes are indicated; except genotypes 1a and 1b. There may be a slight rise in the graph for 1a genotype if 1b is detected, (in this case, 1a is not taken into account);
- if several genotypes are indicated for one test sample, and Ct values in the corresponding channels differ by more than 8 cycles, then genotypes with higher Ct values are not taken into account;
- if Ct values for genotypes were not obtained for one test sample, while for ICS $Ct \leq 32$, it is indicated that the genotype was not determined;
- In case of an invalid and doubtful result, a conclusion is not issued, it is necessary to retake the biomaterial from the patient and retest it. If a doubtful result is repeated, repeat the test with a reagents kit from another manufacturer or another method. The reasons for obtaining an invalid result may be low DNA concentration, inhibitors' presence in the RNA sample obtained from clinical material, incorrect analysis protocol execution, non-compliance with the PCR temperature regime, et al.

Table 11 – Results interpretation Principle

Ct values in the detection channels					Result
FAM / Green	**ROX / Orange**	**Cy5/ Red**	**Cy5.5 / Crimson**	**HEX / Yellow**	
Reaction mixture A					
Genotype 1a	**Genotype 1b**	**Genotype 2**	**Genotype 3**	**ICS**	
–				≤ 32	genotype, corresponding to the channel is not detected
–	–	–	–	> 32	invalid result
≤ 35				not considered	Viral RNA corresponding to the channel was detected
> 35				not considered	Test result for the target corresponding to the channel is doubtful
Reaction mixture B					
Genotype 4	**–**	**Genotype 5**	**Genotype 6**	**ICS**	
–	not considered	–	–	≤ 32	genotype, corresponding to the channel is not detected
–	not considered	–	–	> 32	invalid result
≤ 35				not considered	Viral RNA corresponding to the channel is detected
> 35				not considered	Test result for the target corresponding to the channel is doubtful

"Not considered" — the result is not taken into account during interpretation; "–" — there is no fluorescence signal.

For HEPA-C-GEN-test-A configuration form:

– if the genotype is not determined, it is necessary to check the HCV RNA concentration in the test sample using a reagent kit for determining the viral load, as at a concentration below 4,000 copies/ml there is no genotyping possibility, or use a reagent kit to detect other genotypes of

hepatitis C (the HEPA-C-GEN-test-AB configuration form).

For HEPA-C-GEN-test-AB configuration form:

– If the genotype is not determined, it is necessary to check the concentration of HCV RNA in the sample under study using a reagent kit for determining the viral load, as at a concentration below 4,000 copies/ml there is no genotyping possibility. The genotype may also not be determined due to the presence of other genotypes of the hepatitis C virus that are not detected by this reagent kit (for example, 1c, 7, 8), as well as due to the presence of molecular genetic polymorphisms located in the hybridization region of primers and probes in RT-PCR.

12. Storage, Transportation and Usage Conditions

Storage

The HEPA-C-GEN-test reagent kit in the manufacturer's packaging should be stored at -18°C…-22 °C for 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-B-test-Q kit more than 10 times.

After opening, store the reagents under the same conditions as before opening.

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

Transportation

The HEPA-C-GEN-test reagent kit should be transported by all types of covered vehicles in accordance with transportation rules applicable to this transport type.

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

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

To ensure compliance with the transportation conditions throughout the entire transportation period, the reagent kit is placed in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. The type, volume and number of ice packs placed in the thermal container with the 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 the HEPA-C-GEN-test reagent kit is 12 months from the date of acceptance by the manufacturer's quality control department (QCD), 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, if stored at -18°C… -22°C.

Shelf life of the kit components prepared for work

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

13. Disposal

Reagent kits that have become unusable including the ones with expired shelf life, are subject to disposal in accordance with sanitary and epidemiological requirements for the management of medical waste.

According to medical waste classification the kits belong to Class A (epidemiologically safe waste, which is similar in composition to solid household waste).

Unused reagents are collected in a single-use labeled packaging of any color (except yellow and red) in accordance with sanitary and epidemiological requirements for the management of medical waste.

Used tubes and materials are disposed of in accordance with the requirements for disinfection, pre-sterilization, cleaning and sterilization of medical devices.

Liquid components (reagents, chemical agents) are disposed by draining into a sewer with a reagent preliminary dilution with tap water 1:100 and removing the packages remains as industrial or household garbage. HEPA-C-GEN-test reagent kit consumer packaging is subject to mechanical destruction with the removal of residues as industrial or household garbage.

Personnel carrying out the reagent kit destruction must comply with the safety rules for carrying out one or another destruction method.

14. Warranty, Contacts

The manufacturer guarantees the MTB-RESIST-II-Test reagent kit quality and safety during the shelf-life period in compliance with the product transportation and storage requirements, as well as in compliance with the usage rules. In case of complaints about the reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company TestGene (TestGene, LLC),
9, 44 Inzhenerny Proezd, office 13, Ulyanovsk, 432072,
Russian Federation
www.testgene.com

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