# INSTRUCTIONS FOR USE

## Reagent kit for hepatitis D virus RNA qualitative and quantitative detection by RT-PCR-RT "HEPA-D-test-Q"

IVD

Version 4 dated 15.08.2022

## Content

## List of abbreviations

The following abbreviations and designations are used in this instruction:

| | |
|---|---|
| PCR | polymerase chain reaction |
| RT | reverse transcription |
| DNA | deoxyribonucleic acid |
| cDNA | complementary DNA |
| RNA | ribonucleic acid |
| HDV | hepatitis D virus |
| ICS | internal control sample |
| NCS | negative control sample |
| PCS | positive control sample |
| CS1 | calibration sample No. 1 |
| CS2 | calibration sample No. 2 |
| SenC | sensitivity control sample |
| SC | specificity control sample |

## Introduction

Hepatitis D virus is a potentially life-threatening infection that can lead to chronic liver disease and crease a high risk of death. Hepatitis D virus, when coinfected or superinfected with hepatitis B, significantly complicates the disease course. Hepatitis D viral load assessment makes it possible to adjust therapy and evaluate its effectiveness.

**Target analyte:** specific regions of hepatitis D virus genomic RNA (hepatitis D, hepatitis delta, HDV).

**The scientific validity of the target analyte** lies in its specificity (RNA sequence uniqueness) in relation to hepatitis D virus genomes.

Hepatitis D virus is a member of the genus Deltavirus (family undefined). The genome is represented by a circular single-stranded RNA molecule containing approximately 1700 nucleotides. Hepatitis B virus presence is required for viral replication[1].

HDV infection can occur in two forms: coinfection and superinfection. Coinfection is an infectious process when simultaneous infection with hepatitis B and hepatitis B viruses occurs. Superinfection is an infectious process that develops when a patient with chronic hepatitis B virus infection or a hepatitis B virus carrier is infected with hepatitis D virus. Co-infection is in nature an acute severe disease with a high risk of developing fulminant hepatitis (up to 20%). When superinfection, when the disease overlaps with already altered liver tissue, in 70-80% of cases the infection leads to rapid development of cirrhosis.

Hepatitis D virus replication suppresses hepatitis B virus replication in liver cells (viral interference phenomenon). As a result, many serological markers may not be detected in the blood of the patient or their concentration may be reduced.

HDV infection recognition in addition to hepatitis B is very important, because the disease course, therapeutic approaches and prognosis in hepatitis D differ from those for hepatitis B without hepatitis D. Hepatitis D virus RNA quantitative detection in blood plasma should

[1]Hepatitis D. Worldwide health organization (WHO). Newsletter, 2020.

be carried out during therapy. Viral load detection allows to determine the stage of the disease, identify the activity of the process, and monitor the effectiveness of antiviral therapy.

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

**Indications and contraindications for use:**

Indications for use: suspicion of hepatitis D virus infection and viral load detection in patients with detected hepatitis D virus to choose an appropriate therapy and evaluate its effectiveness.

Contraindications for use: when used by specially trained personnel and taking into account the intended use, no contraindications have been identified.

**Population and demographic aspects of the kit use:** no population, demographic aspects of HEPA-D-test-Q reagent kit use have been detected.

**Sterility:** the product is not sterile.

## 1. Intended use

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

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

**Potential consumers of a kit:**

The kit is intended for research use only.

## 2. Method principle

**Method**

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

**Test sample type**

The material for the study is RNA samples isolated from human K2-EDTA blood plasma.

**Detection principle**

Hepatitis D virus RNA quantitative detection by multiplex allele-specific polymerase chain reaction with hybridization-fluorescent detection in a 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. Result interpretation.

RNA samples are subject to one-step reverse transcription reactions and amplification of specific regions using primers specific to them in the reaction buffer.

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

Oligonucleotide mixtures contain fluorescently labeled oligonucleotide probes, which hybridize with a complementary region of the amplified DNA target and are hydrolyzed (destroyed) by Taq polymerase, as a result the fluorescent dye and quencher are separated, and the fluorescence intensity increases over the corresponding range of the optical spectrum. This allows register the accumulation of a specific amplification product by measuring the fluorescent signal intensity in real time.

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

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

To draw a calibration line, necessary to determine the concentrations of hepatitis D virus genomic RNA in the test sample, calibration samples CS1 and CS2 are used.

Table 1 – Test targets

| **The channel corresponding to fluorophore** | |
|---|---|
| **FAM/Green** | **HEX/Yellow** |
| Hepatitis D virus RNA | ICS |

**Method limitations**

A possible reason for obtaining a false positive result is contamination at the stage of RNA isolation or RT-PCR reaction. A false positive result can be detected by using a negative control sample.

A reagent kit cannot be used after the expiration date.

Do not use the reagent kit if the inner packaging is damaged or the appearance of the reagent does not correspond to the description.

A reagent kit transported or stored in violation of the temperature regime cannot be used.

The clinical diagnosis conclusion cannot be based only on the assay results using this kit. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical the picture, the assay results from other test systems (for example, concentration determination of anti-HDV with ELISA or chemiluminescence immunoassay), the therapy used.

If viral load is very low (less than 40 IU/ml), which may be due to the antiviral therapy used or peculiarities of the disease course, false negative results may be obtained. In these cases, it is recommended to use a larger volume of clinical material for nuclear acid isolation to lower the test system sensitivity threshold.

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

## 3. Reagent kit components

HEPA-D-test-Q reagent kit is available in one configuration form– HEPA-D-test-Q.

### Number of test samples

HEPA-D-test-Q reagent kit is designed for 96 reactions, it equates to quantitative detection of 88 test samples, calibration samples, negative and positive control samples in a single run of a 96-well cycler or 10 single runs of test samples with calibration, negative and positive control samples in each run. In qualitative analysis, it corresponds to the detection of 94 test samples, negative and positive control samples with a single run of a 96-well cycler or 32 single runs of test samples with calibration, negative and positive control samples in each run.

### Reagent kit components

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

| No. | Reagent name | Description | Quantity, volume |
|---|---|---|---|
| 1. | RT-PCR buffer | Transparent colorless liquid | 1 test tube, 480 µl |
| 2. | Oligonucleotide mixture | Transparent colorless liquid, may have a shade of lilac | 1 test tube, 1,440 µl |
| 3. | PC | Transparent colorless liquid | 1 test tube, 50 µl |
| 4. | NC | Transparent colorless liquid | 1 test tube, 1,000 µl |
| 5. | ICS | Transparent colorless liquid | 1 test tube, 950 µl |
| 6. | CS1 | Transparent colorless liquid | 2 test tubes, 1,500 µl |
| 7 | CS2 | Transparent colorless liquid | 2 test tubes, 1,500 µl |

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

RT-PCR buffer contains all the basic reagents, including a warm-start reversease, 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 water solution of TE (1 mM Tris, 0.1 mM EDTA).

PC is ready for use, it is a plasmid DNA mixture with synthetic insertions of hepatitis D virus genomic cDNA amplified fragment at a concentration of 1.37x106 IU/ml (107 copies/ml) and cDNA fragment of the bacteriophage genome in 10% TE buffer (10 mM Tris, 1 mM EDTA).

NC is ready for use, it is RNase-free deionized water.

ICS is ready for use, it is an armored RNA preparation.

CS1 is a specific fragment of hepatitis D virus genome detected by a reagent kit at a concentration of 1.37x105 IU/ml (106 copies/ml) in TE buffer (10 mM Tris, 1 mM EDTA).

CS2 is a specific fragment of hepatitis D virus genome detected by a reagent kit at a concentration of $4.11x10^2$ IU/ml ($3x10^3$ copies/ml) in TE buffer (10 mM Tris, 1 mM EDTA).

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-D-test-Q reagent kit

| **Indicator name** | **Characteristics and standards** | **Clause in TS** |
|---|---|---|
| **1. Technical characteristics** | | |
| 1. Appearance | | |
| RT-PCR buffer | Transparent colorless liquid | Section 7, clause 7.6 |
| Oligonucleotide mixture | Transparent colorless liquid, may have a shade of lilac | 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 |
| CS1 | Transparent colorless liquid | Section 7, clause 7.6 |
| CS2 | Transparent colorless liquid | Section 7, clause 7.6 |
| 1.2. Completeness | According to clause 1.4 TS 21.20.23-020-97638376-2020 | Section 7, clause 7.9 |
| 1.3. Labelling | According to clause 4 TS 1.20.23-020-97638376-2020 | Section 7, clause 7.9 |
| 1.4. Packaging | According to clause 5 TS 21.20.23-020-97638376-2020 | Section 7, clause 7.9 |
| **2. Functional characteristics** | | |
| 2.1Positive result with PC | Fluorescence signal growth registration in test tubes with PC in channels FAM Ct≤30, HEX Ct≤30. | Section 7, clause 7.7.2 |
| 2.2 Negative result with NC | In test tubes with NC channels FAM Ct> 35 or not indicated (i.e. there is no fluorescence accumulation diagram), and in the HEX channel Ct≤32. | Section 7, clause 7.7.2 |
| 2.3 Reaction in tubes with SC | In test tubes with SC, Ct is not indicated in the FAM channel (i.e. there is no fluorescence accumulation diagram), and in the HEX channel Ct≤32. | Section 7, clause 7.7.2 |
| 2.4 Reaction in tubes with SenC | In test tubes with SenC in the FAM channel in all replicates (at least 4) Ct≤35 and with a standard deviation value in the SenC replicates not exceeding 5%, and in HEX channel Ct≤32. | Section 7, clause 7.7.2 |
| 2.5 “Linearity” test | Correlation ratio of CS1, CS2 and standard sample (SS) is not less than 0.98 | Section 7, clause 7.7.2 |

| | | |
|---|---|---|
| 2.6 Precision test: coefficient of variation (CV) under repeatability Conditions | Coefficient of variation Ct for replicates of each calibration sample CS1 and CS2 under repeatability conditions is no more than 5%. | Section 7, clause 7.7.2 |
| 2.7 Accuracy concentration detection test | The obtained value of hepatitis D virus RNA concentration should correspond to the concentration given in the standard sample passport with a tolerance of ±0.4 lg concentration. | Section 7, clause 7.7.2 |

Note: when carrying out the control PCR, as SenC and SC are used:

- a sensitivity control sample (SenC), which is a specific fragment of hepatitis D virus genome detected by a reagent kit in 10% TE buffer (10 mM Tris, 1 mM EDTA) at a concentration of 40 IU in 1 ml.

- specificity control sample (SC), which is a mixture solution of human genomic DNA isolated from the Jurkat cell line at a concentration of 1,000 copies per 5 µl (200,000 copies/ml).

In case of the kit malfunction, deviations in its functioning that may affect safety, or changes in the analytical characteristics of the product, immediately stop using the kit inform the manufacturer (see section 14 of the Instructions).

**4.1. Analytical efficiency characteristics**

**4.1.1. Analytical specificity**

HEPA-D-test-Q reagent kit specific to the hepatitis D (hepatitis D, hepatitis delta, HDV) genomic RNA.

For HEPA-D-test-Q reagent kit the manufacturer selected regions of a non-coding RNA fragment close to the hepatitis D virus RNA ribozyme sequence as regions for primers and probes hybridization.

**4.1.1.1. Confirmation of in vitro specificity using the WHO international standard:** 1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays, PEI code 7657 /12, at a concentration of 575,000 IU/ml.

To carry out the assay, QuantStudio 5 cycler (Thermo Fisher Scientific, USA), Registration Certificate No. RZN 2019/8446 dated June 6, 2019, was used.

The correlation coefficient was 0.9911, which corresponds to a high correlation strength of the HDV RNA concentration in standard samples obtained using the tested kit “Reagent kit for hepatitis D virus RNA qualitative and quantitative detection in human blood plasma samples using real-time polymerase chain reaction with hybridization-fluorescent detection with reverse transcription (RT-PCR) “HEPA-D-test-Q” according to TS 21.20.23-020-97638376-2020”, produced by TestGene LLC and the expected HDV RNA concentration in standard panel samples, prepared using the 1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays), PEI code 7657/12, at a concentration of 575,000 IU/mL.

The maximum deviation of the average concentration (log10 IU/ml) obtained by HEPA-D-test-Q reagent kit in duplicate from the expected concentration for standard samples was -0.18 log10 from the log10 concentration.

#### 4.1.1.2. Analytical specificity: cross-reactivity evaluation

Based on the results of cross-reactivity evaluation carried out during the assay of NA strains of the following microorganisms and viruses at a concentration of no more than 1x105 copies/ml and no less than 1x103 copies/ml:

– standard samples from the NIBSC collection: hepatitis A virus (NIBSC code: 00/562), hepatitis B virus (NIBSC code: 10/264), hepatitis C virus (NIBSC code: 14/150), human immunodeficiency virus type 1 (NIBSC code : 16/194), cytomegalovirus (NIBSC code: 09/162), Epstein-Barr virus (NIBSC code: 09/260), varicella zoster virus (NIBSC code: W1044), human herpes virus type 6 (NIBSC code: 15/ 266), parvovirus B19 ((NIBSC code: 99/800), herpes simplex virus type 1 (NIBSC code: 16/368), herpes simplex virus type 2 (NIBSC code: 17/122);

– strains of microorganisms from the ATCC collection (American Type Culture Collection, USA): Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Streptococcus pyogenes Group A ATCC 19615, Streptococcus agalactiae Group B ATCC 13813;

using the test HEPA-D-test-Q reagent kit in accordance with the interpretation of the results set out in the instructions for use, negative results were obtained for all tested samples.

Obtaining negative results confirms the absence of nonspecific positive results in relation to NA of the following organisms and viruses: hepatitis viruses A, B, C, human immunodeficiency virus type 1, cytomegalovirus, Epstein-Barr virus, herpes simplex virus 1 and 2 types, herpes virus type 6, varicella zoster virus, parvovirus B19, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, S. agalactiae.

**4.1.1.3. Analytical specificity: testing the effect of potentially interfering substances**

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

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

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

2) heparin at a concentration of 1 IU/ml, 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 indicated interferent concentrations do not affect the results of hepatitis D virus RNA qualitative and quantitative determination using the HEPA-D- test-Q test kit.

**4.1.2. Limit of Detection (LOD)**

According to the test results, HDV RNA detection limit in K2-EDTA blood plasma samples is:

- 100 µl volume with a detection rate of 95% for the DTprime cycler - 39.4 IU/ml (95% CI: 34.0–44.7), CFX 96 – 39.9 IU/ml (95%CI: 34.5–45.2), Rotor-Gene Q – 39.7 IU/ml (95%CI: 34.3–45.0), Quant Studio 5 – 39.9 IU/ml (95%CI: 34.5–45.2),
- 1000 µl volume with a detection rate of 95% for the DTprime cycler - 8.20 IU/ml (95%CI: 6.77–9.63), CFX 96 – 8.79 IU/ml (95%CI: 7.36–10.22), Rotor-Gene Q – 8.93 IU/ml (95%CI: 7.50–10.36), Quant Studio 5 – 9.13 IU/ml (95%CI: 7.70–10.56).

**4.1.3. Limit quantitative definitions (LOQ)** in K2-EDTA blood plasma samples: According to the test results limit of quantitation (LOQ) for HDV RNA in K2-EDTA plasma samples:

- 100 µl volume with a detection rate of 95% for the DTprime cycler – 112.7 IU/ml (95%CI: 102.3–123.8), CFX 96 – 112.5 IU/ml (95%CI: 102.7 – 124.2), Rotor-Gene Q – 112.5 IU/ml (95%CI: 95%CI: 101.7–123.2), Quant Studio 5 – 112.9 IU/ml (95%CI: 101 ,3–122.8);
- 1000 µl volume with a detection rate of 95% for the DTprime cycler - 12.9 IU/ml (95% CI: 10.0–15.7), CFX 96 – 13.1 IU/ml (95%CI: 10.2 – 15.9), Rotor-Gene Q – 14.0 IU/ml (95%CI: 11.1–16.8), Quant Studio 5 – 12.9 IU/ml (95%CI: 10.0–15.7).

**4.1.4. Linear measuring range** of the tested HEPA-D-test-Q reagent kit:

- in 100 µl K2-EDTA blood plasma samples linear range from 103 IU/ml to 1.36 * 107 IU/ml and demonstrate a maximum deviation from the regression line not more than ± 0.23 log10.
- in 1000 µl K2-EDTA blood plasma samples linear range from 10.3 IU/ml to 1.36 * 106 IU/ml and demonstrate a maximum deviation from the regression line not more than ± 0.21 log10.

**4.1.5. Metrological traceability** of control samples – PC, CS1, CS2, included in HEPA-D-test-Q reagent kit, as well as SOP "Control sample of hepatitis D virus RNA", SenC, used for quality control of the finished HEPA-D-test-Q kit, was carried out using the 1st World Health Organization International Standard for the assay of hepatitis D virus RNA for nucleic acid amplification methods (NAT) (1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays), PEI code 7657/12. The assigned concentration of CS1 is 1.37x105 IU/ml, CS2 – 411 IU/ml, LOQ – 1.37x106 IU/ml, SOP – 1612 IU/ml, SenC – 40 IU/ml.

Based on the obtained results of the calibration and standardization process, it can be concluded that HEPA-D-test-Q reagent set ensures quantitative values for the 1st International Standard of the World Organization health care for the assay of hepatitis D virus RNA for nucleic acid amplification methods (NAT) (1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid

Amplification Techniques (NAT)-Based Assays), PEI code 7657/12, which are similar to the expected values with a deviation of not more than ±0.13 log10 IU/ml (uncertainty).

#### 4.1.6 Precision under repeatability and reproducibility conditions:

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

### 4.2. Clinical efficiency characteristics

In the Russian Federation, there is no duly registered kit for hepatitis D virus RNA quantitative detection in human blood plasma samples. Therefore, human blood plasma samples negative for hepatitis D virus with the addition of the WHO international standard in various concentrations were considered as positive clinical samples: 1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays), PEI code 7657/12.

40 recombinant positive clinical samples prepared using the WHO international standard (PEI code 7657/12,) at different concentrations from the linear range were used for clinical trials (in the form of clinical and laboratory tests of a kit).

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

Each sample was tested in two series using the tested HEPA-D-test-Q reagent kit.

To conduct a PCR assay using the tested HEPA-D-test-Q reagent kit, cyclers, recommended by the tested reagent kit manufacturer, were used:

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

RNA isolation from clinical samples was carried out using a RNA isolation kit:

- A reagent kit for DNA/RNA isolation from clinical material NA-

Extra according to TS 21.20.23-013-97638376-2019 produced by TestGene LLC, Russia (registration certificate: RZN 2021/15428 dated 09.24.2021).

**4.3.1 Results of diagnostic characteristics study on clinical material samples** are shown in Table 4.

Table 4

| Test material type | Number of observations with positive samples | Number of observations with negative samples | Diagnostic sensitivity with confidence level 95 % | Diagnostic specifi city with confidence level 95 % |
|---|---|---|---|---|
| Blood plasma | 80 | 74 | 100% (95% CI:95.49%-100%) | 100% (95% CI:95.14%-100%) |

**4.3.2 Comparison of methods: accuracy.**

Data, obtained by testing 40 human plasma samples (containing EDTA- K2 as an anticoagulant) supplemented in various concentrations with the WHO international standard: 1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)- Based Assays), PEI code 7657/12, allow to conclude on reliable compliance of the results of hepatitis D virus RNA concentration quantitative detection in clinical samples obtained using the test kit HEPA-D-test-Q with expected test samples concentration results during PCR assay using cyclers:

- Detecting cycler DTprime (NPO DNA-Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 3, 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. РЗН 2019/8446 dated June 6, 2019.

**The systematic error** in measuring the logarithm of hepatitis D RNA concentration does not exceed 3%.

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

Table 5

| | **Sample type** | **Uni t** | **Cycler used** | **Number of samples** | **Correlation ratio** | **Intersection** | **Slope** |
|---|---|---|---|---|---|---|---|
| HEPA-D-test-Q reagent kit, produced by TestGene LLC in comparison with expected sample dilution concentrations WHO: 1st World Health Organization Internat ional Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT) – Based Assays), P.E.I. code 7657/12 | Human blood plasma with EDTA-K2 as an anticoagulant | log10 IU/m l | DTprime | 40 | 0.9992 | 0.0143 | 0.9956 |
| | | | CFX 96 | 40 | 0.9994 | -0.0196 | 1.0032 |
| | | | Rotor-Gene Q | 40 | 0.9989 | 0.002 | 0.9991 |
| | | | Quant Studio 5 | 40 | 0.999 | -0.0234 | 1.0054 |

### 4.3.3 Detection results of the interlot correlation (K2- EDTA human blood plasma).

To determine the interlot correlation of measurement results in clinical samples in accordance with the international guideline CLSI EP09-A3, a scatter diagram of the dependent variable X – hepatitis D virus RNA concentration was drawn using the test kit HEPA- D-test-Q, produced by TestGene LLC, LOT: 202207-356, and U - hepatitis D virus RNA concentration using a test kit HEPA-D-test-Q, produced by TestGen LLC, LOT: 202207-357.

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

Table 6

| | **Sample type** | **Unit** | **Cycler used** | **Number of samples** | **Correlation ratio** | **Intersection** | **Slope** |
|---|---|---|---|---|---|---|---|
| HEPA-D-test-Q reagent kit produced by TestGene LLC **LOT: 202207-356** compared with **LOT: 202207-357** | Human Blood plasma EDTA-K2 | log10 IU/ml | DTprime | 40 | 0.9953 | 0.0057 | 1.0003 |
| | | | CFX 96 | 40 | 0.9958 | -0.0425 | 1.0107 |
| | | | Rotor-Gene Q | 40 | 0.997 | 0.0612 | 0.9834 |
| | | | Quant Studio 5 | 40 | 0.9956 | -0.0285 | 1.0043 |

Correlation ratio $R^2$ during the test on each of the cyclers used was more than 0.99. In accordance with the document CLSI EP09-A3 recommendations, using the regression and correlation method, it can be concluded that correlation strength of hepatitis D virus RNA concentration is high in clinical samples obtained with two lots of the test kit “Reagent kit for hepatitis D virus RNA qualitative and quantitative detection by RT-PCR-RT HEPA-D-test-Q according to TS 21.20.23-020-97638376-2020", produced by TestGene LLC.

## 5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

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

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

3. Contamination of reaction mixtures and test RNA samples with contents from a 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. Use of an unsuitable kit (use after the expiration date or in case of damaged packaging).

The cumulative residual risk of using a “Reagent kit for hepatitis D virus RNA qualitative and quantitative detection by RT-RT-PCR “HEPA-D-test-Q" according to TS 21.20.23-020-97638376-2020 is acceptable, the benefits of its use exceed the risk.

## 6. Safety precautions

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

The reagents included in HEPA-D-test-Q kit are non-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 of sanitary and epidemiological requirements for the prevention of infectious diseases, requirements of organization of the work of laboratories using nucleic acid amplification methods when working with material containing microorganisms I– IV pathogenicity group.

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

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!** When removing waste after amplification (tubes containing PCR products), it is unacceptable to open the tubes and splash the contents , as this can 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;
- allow only specially trained personnel to work with the kit (a specialist with a higher medical education who has been trained in licensed specialization courses for working with PBA of pathogenicity group I– II and PCR diagnostics, as well as a laboratory assistant with a secondary specialized medical education);
- do not use the kit after the expiration date;
- do not use the reagent kit if the internal packaging is damaged or the appearance of the reagent does not correspond to the description;
- avoid contact with skin and mucous membranes; in case of contact, immediately rinse the affected area with water and seek medical assistance.

There are no necessary precautions regarding the influence of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload, or thermal inflammation sources.

The kit does not contain substances of human or animal origin with

a potential infectious nature, therefore precautions against any special, unusual risks during the product use or sele are not provided.

## 7. Required equipment and materials

### Equipment for multiplex PCR:

1. Protection class II and III biological safety box;
2. Vortex;
3. Variable volume dispensers, allowing to take liquid volumes of 20–200 µl, 200–1000 µl
4. Refrigerator from 2°C to 8°C with freezer less than -16°C;
5. Cycler[2] with real-time fluorescent detection via channels corresponding to FAM/Green and HEX/Yellow fluorophores: 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 product:

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

1. Disposable pipette tips with aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (for example, Axygen, USA);
2. Disposable sterile1.5 or 2.0 ml tubes of the Eppendorf type;
3. Thin-walled disposable tubes with an optically transparent lid (when using plate type cyclers) or optically transparent walls (when using rotary type cyclers) for PCR: 0.1 or 0.2 ml[3] PCR tubes, or 0.1 or 0.2 ml PCR tubes in strips, or PCR plates with optically transparent film (for example, Axygen, USA), compatible with the cycler used;
4. Disposable gown and disposable gloves without talcum powder;
5. Container with disinfectant solution;
6. Test tube racks for 0.1 or 0.2 ml tubes or for stripped 0.1 or 0.2 ml tubes;

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

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

7.Kit for isolating RNA from blood plasma K2-EDTA (see paragraph. 8.2. Instructions).

## 8.Test samples

### Test sample type

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

### 8.1. The procedure for clinical material collection

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 pathogenicity group II.

### Material sampling for assay

4 or 6 ml peripheral blood is collected in the morning on an empty stomach into a test tube (vacuum tube) containing a K2-EDTA solution as an anticoagulant. Immediately after blood sampling, turn the tube upside down 3-4 times to mix the blood with the K2-EDTA 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, so it is recommended to take blood from such patients before the next administration of the drug.

**Conditions for transportation and storage of the initial clinical material - blood:**

– at temperatures from +2°C to +8°C – up to 6 hours;

– at room temperature – up to 2 hours.

Do not freeze blood.

Plasma should be isolated within 2 hours (when stored at room temperature) or 6 hours (when stored at temperatures from +2°C to +8°C) after material sampling, for this purpose a test tube with blood is centrifuged 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 RNase-free plastic tube.

**Conditions K2-EDTA blood plasma for transportation and storage:**

Plasma can be stored at temperatures from +2 °C to +8°C for up to 5 days, at temperatures from -18°C to -22°C up to 3 months, at a temperature -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 analytical sensitivity of the kit is possible due to the use of a larger plasma volume, if this is provided by the RNA isolation kit used.

**Material pre-processing**

No preparation required.

Accounting, storage, transfer 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 (hazard) groups I–II, current sanitary rules on the procedure for accounting, storage, transfer and transportation of microorganisms of pathogenicity groups I–IV.

**8.2. Procedure for collection of a human RNA sample isolated from K2-EDTA blood plasma**

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 produced by TestGene LLC, Russia

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

Add 10 µl of ICS from HEPA-D-test-Q reagent kit to plasma intended for RNA isolation. NC sample, CS1 and CS2 in a volume of 100 µl also undergo the isolation procedure with the addition of 10 µl of ICS. If the reagent kit manufacturer's instructions for RNA isolation allow to use of a larger sample volume, increase the volume of NC, CS1 and CS2 to the required volume with saline solution or TE buffer.

**RNA test samples storage conditions:**

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

### 8.3. Interfering substances and restrictions on the test material use

The effect of potentially interfering substances on HEPA-D-Test-Q reagent kit performance has been tested for potentially interfering substances that may occur during normal use of HEPA-D-Test-Q reagent kit, and presumably affect the reagent kit ability to produce valid results.

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

1) substances used in a patient's treatment (for example, medicines);

2) substances found in specific sample types of - in this case, contamination of the clinical sample with blood components (hemoglobin, triglycerides, bilirubin) can inhibit PCR if insufficient purification during the RNA isolation procedure;

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

The tested concentrations of interfering substances are shown in Table 7.

Table 7

| Interfering substances | Maximum concentration |
|---|---|
| **Endogenous interfering substances** | |
| Hemoglobin | 260 µg/ml |
| Triglycerides | 37 mmol/L |
| Bilirubin | 210 µmol/L |
| Heparin (anticoagulant) | 0.15 IU/mL |
| Sodium citrate (anticoagulant) | 0.1 mM/ml |
| EDTA-K2 (anticoagulant) | 0.5 mM/ml |
| **Exogenous interfering substances** | |
| With anticoagulant therapy | |
| Heparin | 1 IU/ml |
| Drugs prescribed for hepatitis D virus | |
| Interferon alpha | 1000 IU/ml |
| Pegylated interferon alfa | 0.036 µg/ml |
| Bulevirtide | 0.4*10-3 mg/ml |
| Drugs prescribed for hepatitis B virus | |
| Lamivudine | 0.02 mg/ml |
| Entecavir | 0.1*10-3 mg/ml |
| Telbivudin | 0.12 mg/ml |

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

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

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

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

**Limitations on the test material use:**

- the test material cannot be used in case of storage and transportation conditions violation (temperature, duration, repeated 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, so it is recommended to take blood from such patients before the next administration of the drug.

## 9. Kit components preparation for testing

Installation, assembling, adjustment, calibration of the kit for commissioning is not required.

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

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

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 use in PCR laboratories, and turn on the UV lamp for 20–30 minutes. Before the test, it is necessary to defrost the kit components at room temperature.

For qualitative assay:

1. Mix thoroughly the tubes contents with RNA isolated for the test, RT-PCR buffer, a mixture of oligonucleotides, NC and PC, turning upside down each tube 10 times or mixing on a vortex at low speed for 3-5 seconds, then discharge drops from the tube lids by short centrifugation.

2. Select the required number of tubes (with optically transparent lids or walls - depending on the type of a detecting cycler used) with a volume of 0.1 or 0.2 ml for PCR based on the following calculation: the test samples number + 1 x PC + 1 x NC + 3 x CS1 + 3 x CS2.

For quantitative assay:

1. Mix thoroughly the tubes contents with RNA isolated for the test, RT-PCR buffer, a mixture of oligonucleotides, CS1, CS2, NC and PC, turning upside down each tube 10 times or mixing on a vortex at low speed for 3–5 seconds, then discharge the drops from the tube lids by short centrifugation.

2. Select the required number of tubes (with optically transparent lids or walls - depending on the type of a detecting cycler used) with a volume of 0.1 or 0.2 ml for PCR based on the following calculation: the test samples number + 1 x PC + 1 x NC + 3 x CS1 + 3 x CS2.

## 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-fluorescent real-time detection of amplification products;
3. Result interpretation.

### A) RT-PCR preparation

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

**The total reaction volume is 25 µl.**

**ATTENTION!** It is forbidden to change the reaction volume.

To prepare reaction mixtures for 1 reaction, you need:

1. RT-PCR buffer – 5 µl,
2. An oligonucleotide mixture – 15 µl,
3. A sample (RNA, PC, NC test sample) – 5 µl.

It is necessary prepare reaction tubes as follows:

1. Label 0.1 or 0.2 ml PCR tubes.

2.In a separate 1.5 or 2.0 ml disposable sterile tube of the Eppendorf type prepare a reaction mixture: (n+9)x5 µl of PCR buffer and (n+9)x15 µl of an oligonucleotide mixture, where n is the number of test samples.

3.Add 20 µl of the prepared reaction mixture into each PCR tube.

4.Add 5 µl of isolated RNA into the appropriate tubes for the test samples. Do not add RNA preparation into PC and NC tubes.

5.For quantitative assay: add 5 µl of calibration samples that have passed through the RNA isolation stage into the appropriate tubes for CS1 and CS2 (see Section 8.2).

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

7.Add 5 µl of NC that has passed through the RNA isolation stage into the appropriate tube (see Section 8.2).

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

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

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

1. Place the tubes in the reaction module of the real-time PCR device. It is recommended to place 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 PCR program and detect the fluorescent signal, following the instructions for the device used. Test type: quantitative with standards. The PCR protocol is listed in Table 8.

3. Specify the number and identifiers of samples, CS1 and CS2 calibration samples with an indication of their concentrations, mark the tubes location on the thermoblock matrix in accordance with their layout.

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 8 - PCR protocol

| Stage | Temperature, °C | Time, min.:sec. | Detection channels | Total number of 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. Result 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 model, 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 result interpretation is performed using the Ct values of the FAM/Green and HEX/Yellow channels (Table 1). Only Ct values obtained at the PCR stage with fluorescent detection are taken into account (i.e., corresponding to stage 4 - see Table 8).

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

**ATTENTION!** If Rotor-Gene Q cycler is used, activate the functions Dynamic Tube, Noise slope correction, set 10% value in the Outlier Removal section.

**Result interpretation in control samples**

For NC and PC, the following results should be obtained (Table 9).

Table 9 – 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 9, the results of the entire tested series are considered unreliable. In this case, special measures must be taken to eliminate possible contamination.

When obtaining values for PC that differ from those indicated in Table 9, repeated amplification of the entire sample batch is required. When reobtaining values for PC that differ from those indicated in Table 9, it is necessary to replace the reagents.

**Result interpretation in the tested clinical samples**

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

Based on the obtained Ct values for calibration samples and their concentrations, it is necessary to draw a calibration line. When using a calibration line, the concentrations of the test samples are calculated. For samples, Ct ≤ 35 values are taken into account from the FAM channel. When a Ct value > 35 is obtained for the samples (with a Ct value ≤ 32 for ICS), the result is considered doubtful.

For quantitative analysis: PCR efficiency should be more than 90%, the difference between the Ct values for replicates of each calibration sample, CS1 and CS2 should be no more than 1. Otherwise, it is necessary to reperform the test, starting from the RNA isolation step. If one of three replicates CS1 or CS2 has a Ct value that deviates sharply from the rest, it is allowed to ignore it when drawing a calibration line. If

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

Qualitative assay results interpretation principles are shown in Table 10.

Results interpretation principles of quantitative assays in 100 µl and 1000 µl K2-EDTA blood plasma samples are shown in Tables 10 and 11.

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 the clinical sample.

Table 10 – The results interpretation principle of qualitative assay in the K2-EDTA blood plasma test clinical samples

| Channels corresponding to Fluorophores | | Result interpretation |
|---|---|---|
| FAM/Green(H DV), IU/ml | HEX/Yellow (ICS), Ct | |
| ≤ 35 | not considered | Hepatitis D virus RNA detected |
| – | ≤ 32 | No hepatitis D virus RNA detected |
| – | > 32 | invalid result |
| > 35 | not considered | doubtful result |

Designations: “not considered” – result is not taken into account; “ ” – there is no fluorescence signal, the concentration is not indicated.

Table 11 – The result interpretation principle of quantitative assays in 100 μl K2-EDTA blood plasma test clinical samples

| Channels corresponding to Fluorophores | | Result interpretation |
|---|---|---|
| FAM/Green(H DV), IU/ml | HEX/Yellow (ICS), Ct | |
| 103– 1.37 x 107 | not considered | positive result indicating a specific concentration in IU/ml |
| < 1.03 x102 | not considered | positive result indicating "less than 103 IU/ml" |
| > 1.37 x 107 | not considered | positive result indicating "more than 1.37 x 107 IU/ml" |
| – | ≤ 32 | negative result (concentration is not specified) |
| – | – | invalid result |

Designations: "not considered" – the result is not taken into account during interpretation; "-" – there is no fluorescence signal, the concentration is not indicated.

Table 12 – The result interpretation principle of quantitative assay in 1000 μ K2-EDTA blood plasma test clinical samples

| Channels corresponding to Fluorophores | | Result interpretation |
|---|---|---|
| FAM/Green(H DV), IU/ml | HEX/Yellow (ICS), Ct | |
| 20 – 1.37 x 106 | not considered | positive result indicating a specific concentration in copies/ml |
| < 20 | not considered | positive result indicating "less than 20 IU/ml" |
| > 1.37 x 106 | not considered | positive result indicating "more than 1.37 x 106 IU/ml" |
| – | ≤ 32 | negative result (concentration is not specified) |
| – | – | invalid result |

Designations: "not considered" – the result is not taken into account during interpretation; "-" – there is no fluorescence signal, the concentration is not indicated.

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

If a doubtful result is repeated, repeat the test with reagent kit from another manufacturer or another method.

**Diagnostic value of the obtained assay result:**

The obtained assay results should be used in combination with other data: the clinical picture, the results of other assay types, for hepatitis D virus diagnosis as well as to choose an appropriate therapy and evaluate its effectiveness.

## 12. Storage, transportation and usage conditions

**Storage**

HEPA-D-test-Q reagent kit in the manufacturer's packaging should be stored at temperatures from -18 to -22°C during the entire kit shelf life; at temperatures from 2 to 8°C up to than 30 days.

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

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

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

**Transportation**

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

Transport at temperatures from -18 to -22°C during the entire it shelf life. Transportation is allowed at temperatures from 2 to 8°C for up to 30 days, or at temperatures from 15 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 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 number of iced packs placed in a thermal container with transported reagent kits, as well as the volume of the thermal container are selected depending on the

transportation duration and conditions.

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

**Shelf life**

The shelf life of HEPA-D-test-Q reagent kit is 12 months from the acceptance date by the manufacturer's quality control department (QCD) if all transportation, storage and usage 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 acceptance date by the manufacturer's QCD, if stored at temperatures from -18 to -22°C.

**Shelf life of kit components prepared for use**

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-D-test-Q reagent kit consumer packaging is subject to mechanical destruction with 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 obligations, contacts

Manufacturer guarantees quality and safety of HEPA-D-test-Q reagent kit during shelf life if compliant 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, Inzhenerny 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
Email: help@testgen.ru