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January 24, 2022

INSTRUCTIONS FOR USE

Reagent kit for hepatitis B virus DNA quantitative detection of by RT-PCR "HEPA-B-test-Q"

TS 21.20.23-017-97638376-2019

Version 4 dated 24.01.2022

Content

List of abbreviations

The following abbreviations and designations are used in this instruction:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
HBV	hepatitis B virus
ICS	internal control sample
NC	negative control sample
PC	positive control sample
CS-1	calibration sample No.1
CS-2	calibration sample No.2
SenC	sensitivity control
SC	specificity control

Introduction

Hepatitis B virus is a potentially life–threatening infection that can lead to the development of chronic liver disease and create a high risk of death. Hepatitis B viral load assessment makes it possible to adjust therapy and evaluate its effectiveness.

Target analyte: a specific region of hepatitis B virus genomic DNA (hepatitis B, HBV,) – gene *P* (encoding DNA polymerase) and gene *S* (encoding the surface antigen HBsAg), which is conservative in all known subtypes (A, B, C, D, E, F, G, H, I and J).

Target analyte scientific validity lies in its specificity (DNA sequence uniqueness) in relation to hepatitis B virus genome.

Hepatitis B virus belongs to the hepadnavirus family (Hepadnaviridae). The genome is a circular DNA molecule. Blood is the main pathogenic factor for virus transmission. Specific DNA fragments detection indicates hepatitis B virus presence in a test sample and allows to evaluate viral load, which is required for selecting antiviral therapy and monitoring its effectiveness.[1]

Reagent kit scope: clinical laboratory testing of infectious diseases.

Indications and contraindications for use:

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

Contraindications to use: none were identified if used by specially trained personnel and taking into account the intended use.

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

Sterility: the device is not sterile.

[1] Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. World Health Organization, 2015. 134 p.

1. Intended use

Intended use: HEPA-B-test-Q reagent kit is designed for the *P* gene (encoding DNA polymerase) and the *S* gene (encoding the HBsAg surface antigen) DNA of hepatitis B virus (hepatitis B, HBV) qualitative and quantitative detection by allele-specific polymerase chain reaction with real-time hybridization-fluorescence detection in a DNA sample isolated from human K2-EDTA blood plasma in patients with suspected hepatitis B virus infection and patients with detected hepatitis B virus to select an appropriate therapy and evaluate its effectiveness.

Functional use: obtained results can be used to diagnose hepatitis B virus, select an appropriate therapy and evaluate its effectiveness. The results are taken into account for a comprehensive disease diagnosis.

Potential consumers of the medical device:

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

2. Method principle

Method

Allele-specific polymerase chain reaction (PCR) in real time with hybridization fluorescence detection.

Test sample type

PCR test material is DNA samples isolated from human K2-EDTA blood plasma.

Detection principle

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

1. PCR preparation;
2. DNA PCR amplification with hybridization fluorescence real time detection of amplification products;
3. Results interpretation.

Amplification reactions of specific regions with primers specific to them in a reaction buffer are carried out with DNA samples.

PCR Buffer contains all the main reagents, including a hot start thermostable DNA polymerase with deoxynucleotide triphosphates, uracil

DNA glycosidase and an optimized buffer. Presence of uracil-DNA glycosidase enzyme prevents obtaining false positive results when contaminated with amplification products. The enzyme gets completely inactivated during the first DNA denaturation cycle and does not prevent amplification of the current reaction products.

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

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

ICS allows to evaluate the quality and effectiveness of DNA isolation and amplification inhibitors possible presence in the sample, which may lead to obtaining false negative results.

To construct the calibration line, it is required to determine the concentrations of hepatitis B virus genomic DNA in the test sample, CS-1 and CS-2 calibration samples are used.

Table 1 – Test targets

Channel corresponding to a fluorophore	
FAM / Green	**HEX / Yellow**
Hepatitis B virus DNA	ICS

Method limitations

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

Damage to the integrity of the packaging during transportation.

Use of an expired kit or kit storage conditions violation.

Violation of storage conditions during sample transportation.

The clinical diagnosis assessment cannot be based only on the assay results obtained with this kit. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical

picture, results from other test systems (e.g., HBsAg, anti-HBs concentration evaluation with enzyme immunoassay or immunochemiluminescence assay) of the applied therapy.

False negative results may be obtained if viral load is very low (less than 47 IU/ml), which may occur due to the used antiviral therapy or peculiarities of a disease course. In these cases, it is recommended to use a larger volume of clinical material to isolate nuclear acids in order to lower the test system sensitivity threshold.

PCR reaction time ranges from 80 to 100 minutes (excluding sample preparation), depending on the cycler used.

3. Reagent kit components

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

Number of test samples

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

Reagent kit components

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

No	Reagent	Description	Quantity, volume
1.	PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2.	Oligonucleotide Mixture	Transparent colorless liquid, may have a lilac shade	1 tube, 1 440 µl
3.	PC	Transparent colorless liquid	1 tube, 50 µl
4.	NC	Transparent colorless liquid	1 tube, 1 000 µl
5.	ICS	Transparent colorless liquid	1 tube, 950 µl
6.	CS-1	Transparent colorless liquid	2 tubes, 1 500 ml each
7.	CS-2	Transparent colorless liquid	2 tubes, 1 500 ml each

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

The PCR Buffer contains all the main reagents, including a thermally stable hot start DNA polymerase with, deoxynucleotide triphosphates, uracil DNA glycosidase and an optimized buffer.

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

PC is a ready for use plasmid DNA mixture with synthetic insertions of amplified fragments of hepatitis B virus DNA at 1886792 IU/ml concentration and a bacteriophage genome fragment. PC is in a 10% TE Buffer (1 mM Tris, 0.1 mM EDTA).

NC is a ready for use DNase-free deionized water.

ICS is a plasmid DNA with a synthetic insertion of an amplified DNA fragment of the bacteriophage genome in a TE Buffer (10 mM Tris, 1 mM EDTA).

CS-1 is a plasmid DNA mixture with synthetic insertions of amplified hepatitis B virus DNA fragments at 188 679 IU/ml concentration in a TE Buffer (10 mM Tris, 1 mM EDTA) (1 000 000 copies/ml).

CS-2 is a plasmid DNA mixture with synthetic insertions of amplified hepatitis B virus DNA fragments at 566 IU/ml concentration in a TE Buffer (10 mM Tris, 1 mM EDTA) (3 000 copies/ml).

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

4. Reagent kit characteristics
4.1. Technical and functional characteristics

Table 3 – Technical and functional characteristics of HEPA-B-test-Q reagent kit

Indicator	Characteristics and standards	Clause in Technical Specification (TS)
1. Technical characteristics		
1.1. Appearance		
PCR Buffer	Transparent colorless liquid	Section 7, clause 7.6
Oligonucleotide Mixture	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
CS-1	Transparent colorless liquid	Section 7, clause 7.6
CS-2	Transparent colorless liquid	Section 7, clause 7.6
1.2. Completeness	In accordance with clause 1.4 TS 21.20.23-017- 97638376-2019	Section 7, clause 7.12
1.3. Labeling	In accordance with clause 4 TS 21.20.23-017- 97638376-2019	Section 7, clause 7.12
1.4. Packaging	In accordance with clause 5 TS 21.20.23-017- 97638376-2019	Section 7, clause 7.12
2. Functional characteristics		
2.1 Positive result with PC	Fluorescence signal growth registered in tubes with PC in the FAM Ct≤30, HEX Ct≤30.	Section 7, clause 7.8.2
2.2 Negative result with NC	In tubes with NC in the FAM and HEX channels Ct>35 or not indicated (i.e. no fluorescence accumulation curve)	Section 7, clause 7.8.2
2.3 Reaction in tubes with specificity control (SC)	In tubes with SC Ct is not indicated in the FAM channel (i.e. no fluorescence accumulation curve) and in the HEX channel Ct≤32.	Section 7, clause 7.8.2

2.4 Reaction in tubes with SenC	In tubes with SenC in the FAM channel in all repetitions (at least 4) Ct≤35, a standard deviation value during SenC repetitions not more than 5%, and in the HEX channel Ct≤32.	Section 7, clause 7.8.2
2.5 "Linearity" test	The correlation ratio of CS-1, CS-2 and a reference sample (RS) is not less than 0.98	Section 7, clause 7.8.2
2.6 Precision test: coefficient of variation (CV) under repeatability conditions	Ct coefficient of variation of each calibration sample CS-1 and CS-2 repetition under repeatability conditions is not more than 5%.	Section 7, clause 7.8.2
2.7 Concentration evaluation accuracy test	The obtained value of hepatitis B virus DNA concentration should correspond to the concentration given in a reference sample certificate, with ± 0.4 lg concentration tolerance	Section 7, clause 7.8.2

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

- a control sample for sensitivity (SenC) detection, which is a plasmid mixture with synthetic of hepatitis B virus genomic DNA fragment insertion and a bacteriophage genome fragment insertion in a 10% TE Buffer (10 mM Tris, 1 mM EDTA) at 250 copies per 1 ml concentration each (~47 IU/ml).
- a specificity control sample (SC), which is a mixture of human genomic DNA solution isolated from the Jurkat cell line at 1 000 copies per 5 µl (200 000 copies/ml) concentration.

4.2 Analytical efficiency characteristics

4.2.1 Analytical specificity

HEPA-B-test-Q reagent kit is specific to hepatitis B (hepatitis B, HBV) genomic DNA – gene *P* (encoding DNA polymerase) and gene *S* (encoding the surface antigen HBsAg).

An ability to detect and quantify equally different HBV genotypes was confirmed. When testing with 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques - Based Assays PEI code 5086/08 in all obtained values the correlation coefficient R^2 of the expected HBV concentration and the obtained concentration is ≥ 0.98, which confirms an ability to detect and quantify equally different hepatitis B genotypes (A, B, C, D, E, F, G) by

the studied HEPA-B-test-Q reagent kit. The maximum deviation of the average concentration (log10 IU/ml) obtained by HEPA-B-test-Q reagent kit in two repetitions for the reference samples was 0.05 log10 of log10 concentration, mentioned in the Instructions for Use for the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques - Based Assays PEI code 5086/08.

4.2.2 Limit of detection (LOD)

According to the study results, HBV DNA detection limit in blood plasma samples K2-EDTA[2]:

- **100 µl** volume with a detection rate of 95% for DTprime cycler – 47.8 IU/ml (95% CI: 46.37–49.23), CFX 96 – 47.5 IU/ml (95% CI: 46.07–48.93), Rotor-Gene Q – 47.6 IU/ml (95% CI: 46.17–49.03), Quant Studio 5 – 47.8 IU/ml (95%CI: 46.37– 49.23),
- **1000 µl** volume with a detection rate of 95% for the DTprime cycler – 4.79 IU/ml (95% CI: 7.36–10.22), CFX 96– 4.20 IU/ml (95% CI: 6.77–9.63), Rotor-Gene Q – 5.13 IU/ml (95% CI: 7.70–10.56), Quant Studio 5 – 4.93 IU/ml (95% CI: 7.50–10.36).

4.2.3 Detection limit when testing various HBV genotypes (A, B, C, D, E, F, G).

Study results using the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques - Based Assays PEI code 5086/08, consisting of 15 samples of lyophilized HBV-positive plasma and covering the most common HBV genotypes: Samples 1-3 (genotype A), Samples 4-6 (genotype B), Samples 7-9 (genotype C), Samples 10-12 (genotype D), Sample 13 (genotype E), Sample 14 (genotype F), and Sample 15 (genotype G), confirmed HEPA-B-test-Q reagent kit ability to detect genotypes A, B, C, D, E, F, G at ~47 IU/ml concentration per 100 µl of K2-EDTA blood plasma samples, ~4.7 IU/ml per 1000 µl of K2-EDTA blood plasma samples with 95% upper one-sided confidence interval exceeding the expected 95% detection rate.

[2] To convert the results in copies/ml, it is recommended to use the coefficient: 1 IU/ml = 5.3 copies/ml.

4.2.4 Limit of quantitation (LOQ). Based on the study results, the limit of quantitation (LOQ) in K2-EDTA blood plasma samples[3]:

- 100 µl volume with a 95% confidence probability for the DTprime cycler – 142.8 IU/ml (95% CI: 141.3–144.2), CFX 96 – 142.5 IU/ml (95% CI: 141.0–143.9), Rotor-Gene Q – 142.5 IU/ml (95% CI: 141.0–143.9), Quant Studio 5 – 142.8 IU/ml (95% CI: 141.3– 144.2).

- 1000 µl volume with a 95% confidence probability for the DTprime cycler – 14.2 IU/ml (95% CI: 12.7–15.6), CFX 96 – 14.4 IU/ml (95% CI: 12.9–15.8), Rotor-Gene Q – 14.5 IU/ml (95% CI: 13.0–15.9), Quant Studio 5 – 14.4 IU/ml (95% CI: 12.9–15.8).

4.2.5 Limit of quantitation (LOQ) verification when testing various HBV genotypes (A, B, C, D, E, F, G).

The study results using the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Technique - Based Assays PEI code 5086/08, consisting of 15 lyophilized HBV-positive plasma samples and covering the most common HBV genotypes: Samples 1-3 (genotype A), Samples 4-6 (genotype B), Samples 7-9 (genotype C), Samples 10-12 (genotype D), Sample 13 (genotype E), Sample 14 (genotype F) and Sample 15 (genotype G), confirmed HEPA-B-test-Q reagent kit limit of quantification (LOQ) in relation to A, B, C, D, E, F, G genotypes at ~142 IU/ml concentration per 100 µl of K2-EDTA blood plasma samples, ~14.2 IU/ml per 1000 µl of K2-EDTA blood plasma samples with 95%, upper one-sided confidence interval exceeding the expected 95% detection rate.

[3] To convert the results in copies/ml, it is recommended to use the coefficient: 1 IU/ml = 5.3 copies/ml.

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

- in **100 µl** K2-EDTA blood plasma samples: linear range is from 142 IU/ml to $1.89*10^7$ IU/ml, maximum deviation from the regression line is ± 0.4 log10 or less.
- in **1000 µl** K2-EDTA blood plasma samples: linear range is from 14.2 IU/ml to $1.89*10^6$ IU/ml, maximum deviation from the regression line is ± 0.4 log10 or less.

4.2.7 Linear measurement range verification when testing various HBV genotypes (A, B, C, D, E, F, G).

HEPA-B-test-Q reagent kit linear measurement range during testing A, B, C, D, E, F, G genotypes:

- in **100 µl** K2-EDTA blood plasma samples: linear range is from 142 IU/ml to $1.89*10^7$ IU/ml, maximum deviation from the regression line is ± 0.4 log10 or less.
- in **1000 µl** K2-EDTA blood plasma samples: linear range is from 14.2 IU/ml to $1.89*10^6$ IU/ml, maximum deviation from the regression line is ± 0.4 log10 or less.

4.2.8 Metrological traceability of control samples - PC, CS-1, CS-2, included in HEPA-B-test-Q reagent kit was carried out using 4th WHO International Standard for HBV DNA for NAT NIBSC code: 10/266. The assigned CS-1 concentration is 188679 IU/ml, CS-2 is 566 IU/ml, and PC is 1886792 IU/ml.

Based on the obtained results of the calibration and standardization process it can be concluded that HEPA-B-test-Q reagent kit provides quantitative values for the 4th WHO International Standard for HBV DNA for NAT NIBSC code: 10/266, which are similar to the expected values with a deviation of ±0.4 log10 IU/ml or less (uncertainty).

4.2.9 Accuracy in repeatability and reproducibility conditions:

1. The coefficient of variation under the kit repeatability conditions is 3% or less.
2. The coefficient of variation under the kit reproducibility conditions is 5% or less.

4.3. Characteristics of clinical efficiency

4.3.1 Specificity

Specificity of the "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q manufactured by TestGene LLC was determined by testing HBV DNA-negative samples from individual donors. The status in relation to hepatitis B virus DNA in the samples was established by a reagents kit for hepatitis B virus (HBV) DNA detection in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection AmpliSense® HBV-FL according to TS 9398-030-01897593-2012, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate No. FSR 2007/00585 dated February 27, 2019).

In total 65 K2- EDTA blood plasma samples were tested with two lots of HEPA-B-test-Q reagent kit. 65 K2-EDTA blood plasma samples were negative for HBV DNA presence. During testing this sample panel, HEPA-B-test-Q test specificity was 100.0% (with a one-sided 95% confidence interval of 99.5%).

The lower limit of the specificity confidence interval with 95% confidence probability was determined by the Clopper and Pearson method (Clopper-Pearson Confidence Interval; Clopper, C., & Pearson, E. (1934). The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika, 26(4), 404-413. doi:10.2307/2331986).

Cyclers used to carry out a PCR test with HEPA-B-test-Q reagent kit, recommended by the reagent kit manufacturer:

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

4.3.2 Analytical specificity: potentially interfering substances effect study

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

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

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

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

Other interfering substances at validated interferent concentrations do not affect the test results. A negative result of the HEPA-B-test-Q test was obtained for all HBV DNA negative samples, a positive result was obtained for all HBV DNA positive samples. Besides, the average log10 titer of each HBV-positive sample containing potentially interfering substances was between -0.02 log10 and 0.04 log10 of the average log10 titer of the corresponding positive sample.

4.3.3 Analytical specificity: potentially cross-reagents effect study

The results showed that when testing 31 K2-EDTA blood plasma samples from patients that had no hepatitis B virus DNA (negative samples), but had confirmed presence of the following microorganisms DNA/RNA: HIV-1 – 2 samples, adenovirus type 5 – 3 samples, varicella zoster virus – 1 sample, cytomegalovirus – 2 samples, *Staphylococcus aureus* – 2 samples, Epstein-Barr virus – 2 samples, hepatitis A virus – 1 sample, hepatitis C virus – 4 samples, human T-cell lymphotropic virus type 2 – 2 samples, human herpes virus type 6 – 2 samples, human papillomavirus – 3 samples, herpes simplex virus type 1 – 4 samples, herpes simplex virus, type 2 – 3 samples, no cross-reactivity was observed, no nonspecific reactions were detected.

A positive result was obtained for all HBV DNA-positive samples. Besides, the average log10 titer of each HBV-positive sample containing potentially interfering substances was between -0.06 log10 and 0.05 log10 of the average log10 titer of the corresponding positive sample.

4.3.4 Analytical sensitivity: Limit of detection (LOD) for genotypes A to G

Clinical samples and panel samples of the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques - Based Assays PEI code 5086/08, containing HBV DNA of seven various genotypes (A, B, C, D, E, F, G), were diluted in K2-EDTA blood plasma to the LOD concentration for genotype A, established by the manufacturer using the 4th WHO International Standard for HBV DNA for NAT NIBSC code: 10/266 (HBV A genotype), determined based on a probit analysis of the 95% detection rate of LoD (47 IU/ml in 100 µl K2-EDTA blood plasma samples, 4.7 IU/ml in 1000 ml

K2-EDTA blood plasma samples).

The obtained results confirmed HEPA-B-test-Q reagent kit ability to detect genotypes A, B, C, D, E, F, G at 47 IU/ml concentration per 100 µl of K2-EDTA blood plasma samples, 4.7 IU/ml per 1000 µl of K2-EDTA blood plasma samples with 95% upper one-sided confidence interval, exceeding the expected 95% detection rate.

4.3.5 Interlot correlation determination results (K2-EDTA human blood plasma).

To determine the interlot correlation of measurement results in clinical samples in accordance with the CLSI EP09–A3 international guidelines, a scattering diagram of the dependent variable X - HBV DNA concentration was constructed using the studied medical device "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q", manufactured by TestGene LLC, LOT: 202111-427, and HBV DNA Y - concentration using the studied medical device "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT "HEPA-B-test-Q", manufactured by TestGene LLC, LOT: 202111-428.

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

	Sample type	Unit	Cycler used	Number of samples	Correlation ratio	Intersection	Slope
HEPA-B-test-Q reagent kit, manufactured by TestGene LLC **LOT: 202111-427 in comparison** with HEPA-B-test-Q reagent kit, manufactured by TestGene LLC **LOT: 202111-428**	K2-EDTA human blood plasma	log10 IU/ml	DTprime	55	0.9987	-0.0079	1.0013
			CFX 96	55	0.999	0.0426	0.9911
			Rotor-Gene Q	55	0.999	-0.0013	0.9995
			Quant Studio 5	55	0.9989	0.0382	0.9919

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

4.3.6 Method comparison: accuracy

In 55 clinical samples, the HBV DNA concentration was determined with the studied medical device "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q" in two series using cyclers recommended by the studied reagent kit manufacturer:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 03, 2011;
- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;
- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated August 10, 2010;
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019.

The obtained results were compared with the results obtained using a comparison kit AmpliSens® HBV-Monitor-FL, manufactured by the FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00584 dated August 27, 2019).

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

	Sample type	Unit	Cycler used	Number of samples	Correlation ratio	Intersection	Slope
HEPA-B-test-Q reagent kit, manufactured by TestGene LLC **in comparison** with reagent kit AmpliSens® *HBV* Monitor-FL, manufactured by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00584 dated August 27, 2019)	K2-EDTA human blood plasma	log10 IU/ml	DTprime	55	0.9998	0.0489	0.998
			CFX 96	55	0.9997	0.0383	1.0012
			Rotor-Gene Q	55	0.9997	0.0489	0.9974
			Quant Studio 5	55	0.9998	0.0469	0.9988

The obtained data allow to conclude on the reliable conformity of the results of HBV DNA concentration quantitative detection in clinical samples obtained with the **studied medical device** "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q", manufactured by TestGene LLC and a **comparison kit** AmpliSens® *HBV*-Monitor-FL, manufactured by the FBIS Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (registration certificate No. FSR 2007/00584 dated August 27, 2019).

The systematic error of HBV DNA concentration logarithm measurement does not exceed 3%.

4.3.7 Precision detection (clinical samples)

Precision (within-batch reproducibility) of the studied medical device "Reagent kit for hepatitis B virus DNA quantitative detection by the PCR-RT HEPA-B-test-Q" was evaluated by 10-fold measurement of two K2-EDTA human blood plasma samples with an established HBV DNA concentration from the linear measurement range, using a registered reagent kit AmpliSens HBV- Monitor-FL, manufactured by the FBIS Central Research Institute of Epidemiology of Rospotrebnadzor,

Russia, (registration certificate No. FSR 2007/00584 dated August 27, 2019) using a Rotor-Gene Q (Qiagen, Germany, No. FSZ 2010/07595 dated August 10, 2010).

The concentration values were considered as having a log-normal distribution and studied with log10 expression.

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

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

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

Precision (within-batch reproducibility)) of calibration samples as included in the test studied medical device "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q" was evaluated by 10-fold measurement of each level of calibration samples CS-1 and CS-2 in one analytical series.

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

4.3.9 Reproducibility determination (calibration samples CS1 and CS2)

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

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

The results obtained indicate the adequate operation of the test system under reproducibility conditions: when testing different batches of

reagent kits, in different laboratories, by different operators, on different days, using different PCR cyclers.

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

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 use under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding the permissible ones;
3. Contamination of reaction mixtures and test DNA samples with contents from a PC tube or with amplification products;
4. Testing using a poor quality DNA 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 expiry or in case of damaged packaging).

The cumulative residual risk of using a medical device "Reagent kit for hepatitis B virus DNA quantitative detection by PCR-RT HEPA-B-test-Q" is acceptable, the benefit of its use exceeds the risk.

6. Safety precautions

The class, depending on the potential risk of use, is 3, in accordance with the medical devices nomenclature classification approved by the order of the Ministry of Health of the Russian Federation dated 06.06.2012 No. 4n.

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

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

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

Work with material infected or suspected of being infected is carried

out in accordance with the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases", methodological instructions (MU) "Work organization of laboratories using methods of amplification of nucleic acids when working with material containing microorganisms of pathogenicity groups I–IV" (MU 1.3.2569-09).

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

The work should be carried out in a laboratory performing molecular biological (PCR) essays of clinical material in compliance with sanitary and epidemiological rules SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and implementation of sanitary and anti-epidemic (preventive) measures". Follow methodological recommendations "Guidelines for disinfection, presterilization cleaning and sterilization of medical devices" (MU 287-113), MU "Organization of work of laboratories using nucleic acid amplification methods when working with material containing microorganisms of pathogenicity groups I-IV" (MU 1.3.2569-09).

The following requirements should always be met when working:

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

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

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

- only specially trained personnel is allowed to work with the kit (a

specialist with higher medical education who has been trained in licensed specialization courses for working with PBA of pathogenicity groups I–II and PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

- do not use the kit after the expiry date;

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

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

The kit contains no substances of human or animal origin with a potential infectious nature, therefore, precautions against any special, unusual risks during device use or sale are not provided.

7. Required equipment and materials

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

Equipment for multiplex PCR:

1. Class II and III biosafety cabinet;
2. Vortex;
3. A set of electronic or automatic variable volume dispensers;
4. Refrigerator for +2°C…+8°C with a freezer for below -16°C;
5. Cycler[4] with real-time fluorescence detection in the channels corresponding to FAM/Green and HEX/Yellow fluorophores: CFX96 (BioRad, USA), DTprime (NPO DNA Technology LLC, Russia), Rotor-Gene Q (Qiagen, Germany), QuantStudio 5 (Thermo Fisher Scientific, USA).

[4] 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.

Materials and reagents not included in the device:

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

1. Disposable pipette tips with an aerosol barrier of up to 1000 µl, 200 µl, 20 µl and 10 µl (e.g., Axygen, USA);

2. 1.5 ml or 2.0 ml disposable sterile Eppendorf type tubes;

3. Thin-walled disposable PCR tubes with an optically transparent lid (if detecting through a tube wall) or optically transparent walls (if detecting through a tube wall): 0.1 ml or 0.2 ml[5] PCR tubes, or 0.1 ml or 0.2 ml PCR tubes in strips, or PCR plates with an optically transparent film (for example, Axygen, USA), compatible with the used cycler;

4. Disposable lab coat and disposable gloves without talcum powder;

5. Container with disinfectant solution;

6. Test tube rack for 0.1 ml or 0.2 ml tubes or for stripped 0.1 ml or 0.2 ml tubes;

7. Kit for DNA isolation from K2-EDTA blood plasma (see Section 8.2 of the Instructions for Use)

8. Test samples

Test sample type

PCR test material is DNA samples isolated from K2-EDTA human blood plasma.

8.1. Clinical material collection

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

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

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

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

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

Initial clinical material transportation and storage conditions – blood:

– at +2°C…+8°C – up to 6 hours;

– at room temperature – up to 2 hours.

Do not freeze the blood.

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

K2- EDTA blood plasma transportation and storage conditions:

Store K2-EDTA plasma at +2°C…+8°C up to 5 days, at -18°C…-22°C – up to 3 months, at -70°C – for a long time.

ATTENTION! Avoid repeated freezing and thawing of K2-EDTA plasma samples.

Use at least 100 µl of plasma to isolate DNA. An increase in the kit analytical sensitivity is possible due to the use of a larger K2-EDTA plasma volume, if this is provided by the used DNA isolation kit.

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 current sanitary regulations and epidemiological rules on the work safety with microorganisms of pathogenicity groups I–II (SP 1.3.3118-13), current sanitary rules on the procedure for accounting, storage, transfer and transportation of microorganisms of pathogenicity groups I– IV.

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

8.2 Human DNA sample collection isolated from K2-EDTA blood plasma

To isolate a human DNA sample from K2-EDTA blood plasma, it is recommended to use the following reagent kits:

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

Strictly follow the protocol and the Instructions for Use of the used reagent kit during DNA isolation.

Add 10 µl of ICS of HEPA-B-test-Q reagent kit to K2-EDTA plasma intended for DNA isolation.

NC, CS-1 and CS-2 also undergo isolation procedure in 100 µl volume with 10 µl ICS addition (it is not required to use CS-1 and CS-2 when conducting qualitative analysis). If the reagent kit manufacturer's Instructions for Use for DNA isolation allows to use a larger sample volume, increase NC, CS-1 and CS-2 volume to the required one with saline solution or TE Buffer.

Test DNA samples storage conditions:

- at +2°C ...+8°C – up to 1 day (24 hours),
- at -18°C ...-22°C – up to a month,
- at -80°C – for a long time.

8.3. Interfering substances and test material use restrictions

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

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

1) substances used for a patient' treatment (e.g., medicines);

2) substances found in specific sample types - in this case, clinical sample contamination with blood hemoglobin can inhibit PCR if purified insufficiently during DNA isolation;

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

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

Table 6

Interfering substances	Maximum concentration
Endogenous interfering substances	
Hemoglobin	260 μl/ml
Heparin (anticoagulant)	0.15 IU/ml
Sodium Citrate (anticoagulant)	0.1 mM/ml
EDTA-K2 (anticoagulant)	0.5 mM/ml
Cholesterol	150 mg/dl
Triglycerides	250 mg/dl
Exogenous interfering substances	
With anticoagulant therapy	
Heparin	1 IU/ml
Drugs prescribed for hepatitis B virus	
Interferon alpha	1000 IU/ml
Pegylated interferon alpha	0.036 μl/ml
Lamivudine	0.02 mg/ml
Entecavir	$0.1*10^{-3}$ mg/ml
Telbivudine	0.12 mg/ml

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

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

2) heparin at 1 IU/ml concentration, used for anticoagulant therapy. 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.

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

Limitations on test material use:

- do not use test material if storage and transportation conditions are violated (temperature, duration, multiple freezing-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 for testing

The medical device does not require installation, assembling, adjustment, calibration for commissioning.

ATTENTION! It is required to use only disposable sterile plastic consumables with "DNase-free" label when working with DNA. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

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

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

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

2. Take the required number of 0.1 ml or 0.2 ml PCR tubes (with optically transparent lids or walls, depending on the used detecting cycler type) according to the calculation: the test samples number[6] + 1 x PC + 1 x NC + 3 x CS-1 + 3 x CS-2 (it is not required to use CS-1 and CS-2 when conducting qualitative analysis).

10. Testing procedure

The PCR test consists of the following stages:

1. PCR preparation;
2. DNA PCR amplification and amplification products

[6] It is recommended to analyze each sample twice to improve accuracy.

hybridization fluorescence detection in real time;

3. Results interpretation (described in Section 11).

A) PCR preparation

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

Total reaction volume is 25 µl.

ATTENTION! It is forbidden to change the reaction volume.

To prepare a reaction mixture for 1 reaction, it is required:

1. PCR Buffer – 5 µl;
2. Oligonucleotide Mixture – 15 µl;
3. Sample (DNA, PC, NC test sample) – 5 µl.

Prepare reaction tubes as follows:

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

B) DNA PCR amplification with hybridization fluorescence amplification products detection in real time;

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

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

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

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

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

4. Start PCR with a fluorescent signal detection.

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

Table 7 – PCR protocol

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

If it is required to perform tests simultaneously with HEPA-BCD-test, HEPA-C-test-Q, HEPA-C-GENE-test and HEPA-D-test-Q reagent kits, it is possible to add a preliminary stage at +52°C – 40 minutes at the beginning of the protocol, which corresponds to the reverse transcription stage.

11. Result registration and interpretation

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

Recommendations on threshold line setting

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

The results interpretation is performed using Ct values in the FAM/Green and HEX/Yellow channels (Table 1). Only Ct values obtained at a PCR stage with fluorescence detection are taken into account (i.e.,

corresponding to stage 3 – see Table 7).

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

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

Result interpretation in control samples

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

Table 8 – Test results for NC and PC

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

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

When obtaining values for PC that differ from those indicated in Table 8, repeat amplification of the entire sample batch. When reobtaining values for PC that differ from those indicated in Table 8, it is required to replace the reagents.

Tested clinical samples results interpretation

Result interpretation is carried out automatically via the used detection cycler software, or manually. It is required to make a calibration line based on the obtained Ct values for calibration samples and their concentrations. Calculate the test samples concentrations if using the calibration line.

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

PCR efficiency should be from 90% to 110%, difference between the Ct values of the repetitions of each calibration sample, CS-1 and CS-2, should be no more than 1. Otherwise, it is required to repeat testing starting from a DNA isolation stage. If one of the three CS-1 or CS-2 duplicates Ct value deviates sharply from the others, it is allowed to ignore it when making a calibration line.

If K2-EDTA plasma volume exceeding 100 µl was used for DNA isolation (while maintaining the volume of calibration samples taken for DNA isolation), recalculated the obtained hepatitis B DNA concentration: multiply the obtained concentration value by the ratio 100/V, where V is K2-EDTA plasma volume used for DNA isolation. Measurement accuracy: ±0.4 lg concentration.

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

The general formula for concentration correction is:

$C = C_{obtained} \times 100 \times K / V$, where V is the plasma volume used for RNA extraction, and K is the coefficient specified in the reagent kit Reference Card.

Further results interpretation principles are shown in Table 9.

An invalid result may be obtained due to the presence of inhibitors in the DNA preparation obtained from clinical material, test protocol incorrect implementation, non-compliance with the PCR temperature regime, etc.

A doubtful result may be obtained due to insufficient virus concentration in a clinical sample.

Table 9 – Tested clinical samples results interpretation principle

Channels corresponding to fluorophores		Result interpretation
FAM/Green (HBV), IU/ml	HEX/Yellow (ICS) Ct	
$142 – 1.89*10^7$ IU/ml	not considered	positive result specific concentration indication in IU/ml

< 142 IU/ml	not considered	positive result with "less than 142 IU/ml" (less than 750 copies/ml) indication
> $1.89*10^7$ IU/ml	not considered	positive result with "more than $1.89*10^7$ IU/ml" (more than 100 000 000 copies/ml) indication
-	≤ 32	negative result (concentration is not specified)
-	-	invalid result

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

Note: the table shows values for DNA isolation from 100 µl; if isolated from 1000 µl, the concentration values will be 10 times less than the indicated ones.

In case of an invalid and doubtful result, a conclusion is not issued, it is required to retake the biomaterial from the patient and retest it. At the same time, for doubtful results, it is recommended to isolate DNA from a larger K2-EDTA plasma volume.

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

To recalculate the results in copies/ml, it is recommended to use the coefficient: 1 IU/ml = 5.3 copies/ml [7].

12. Storage, transportation and usage conditions

Storage

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

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

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

Transportation

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

[7] Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. World Health Organization, 2015. 134 p.

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…+25°C up to 5 days.

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

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

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

Shelf life

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

Shelf life of 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 prepared kit components

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

13. Disposal

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

According to the classification of medical waste, the kits belong to class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with paragraph 170 of SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the

maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial, public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures" are collected in reusable containers or disposable bags of any color (except yellow and red).

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

Liquid components (reagents) are destroyed by draining into the sewer with preliminary reagent dilution with tap water 1:100 and removal of package residues as industrial or household waste.

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

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

14. Warranty, contacts

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

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

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

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

Annex A

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

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

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

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