APPROVED BY
CEO of
TestGene LLC
Toropovskii A.A.
August 17, 2022

INSTRUCTIONS FOR USE

Reagent kit for the qualitative and quantitative determination of human herpes virus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection"HHV6 test"

TS 21.20.23-043-97638376-2021

Version 2 dated August 17, 2022

Content

List of abbreviations

The following abbreviations and designations are used in these instructions:

PCR	polymerase chain reaction
DNA	deoxyribonucleic acid
NC	negative control sample
CS-1	calibration sample 1
CS-2	calibration sample 2
PC	positive control sample
SVC	sampling volume control
HHV-6	human herpesvirus 6

Introduction

Target analyte: a specific region of human herpesvirus 6 (HHV-6) genomic DNA.

The scientific validity of the target analyte lies in its specificity (DNA sequence uniqueness) in relation to the human herpesvirus 6 (HHV6) genome.

Herpes virus type 6 (HHV-6) is a DNA virus, belonging to the subfamily *Betaherpesvirinae*, the genus *Roseolovirus*, has two serological subtypes – 6A and 6B. HHV-6 has only recently been added to the list of known human pathogens. HHV-6 is the etiological agent of several infectious diseases: sudden exanthema, fever with convulsive syndrome, and infectious mononucleosis. In addition, HHV-6 is a co-factor of oncological and lymphoproliferative diseases (nasopharyngeal carcinoma, non-Hodgkin lymphoma, peripheral T-cell lymphoma, B-cell lymphoma, sinusoidal large B-cell lymphoma, pleomorphic T-cell lymphoma, Hodgkin disease)[1].

The main route of the virus transmission in natural condition is airborne. Vertical transmission is also possible: viral antigens have been detected in abortive material from spontaneous abortions. The virus can be transmitted through sexual contact and perinatally (from mother to child during pregnancy or birth). Long-term reproduction during acute infection and HHV-6 persistence in the blood cells of apparently healthy individuals, including donors, are serious risk factors for virus transmission during blood transfusions and organ and tissue transplants[2].

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

Indications for use: HHV6-test reagent kit is recommended for use in clinical laboratory diagnostics to test clinical material (whole blood, blood leukocytes, oropharyngeal swabs, saliva, internal organs biopsy samples, cerebrospinal fluid, urine) in patients with suspected herpesvirus infection, regardless of the disease form and stage in all population groups.

[1] Melekhina E.V., Chugunova O.L., Nikolich A.D., and others. The course of infection associated with human herpesvirus type 6 in children // Children's hospital. – 2013. - №4.

[2] Savenkova M.S., Vashura L.V. Herpes type 6: epidemiology, diagnosis, clinical variants of the course // Effective pharmaceutical therapy. Pediatrics. - No.2 (23).

Contraindications for 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 HHV6-test reagent kit use were identified.

Sterility: the product is not sterile.

1. Intended use

1.1. Intended use: HHV6-test reagent kit is designed for qualitative and quantitative detection of human herpesvirus type 6 (HHV6) DNA by allele-specific polymerase chain reaction with real-time hybridization and fluorescence detection in a DNA sample isolated from clinical material (whole blood, blood leukocytes, oropharyngeal swabs, saliva, internal organs biopsy samples, cerebrospinal fluid, urine), in patients with suspected herpesvirus infection, regardless of the disease form and stage in all population groups.

Functional purpose: the results obtained can be used for early diagnosis of herpesvirus infection in patients, regardless of the disease form and stage in all population groups. The results are taken into account in the comprehensive disease diagnosis.

Potential consumers of a medical device:

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

2. Method principle

Method

Qualitative and quantitative allel-specific polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection.

Test sample type

Material for PCR is DNA samples isolated from whole blood, blood leukocytes, oropharyngeal swabs, saliva, internal organs biopsy samples, cerebrospinal fluid, urine.

Detection principle

PCR-based DNA amplification process, which is the basis of the test system, takes place in a reaction buffer using primers specific to the

corresponding DNA regions and the *Taq* polymerase enzyme. It involves a series of repeated cycles of DNA denaturation and primer annealing.

PCR Mixture contains fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA target and are destroyed by *Taq* polymerase, as a result the fluorescent dye and quencher are separated, and the fluorescence intensity increases. This allows the specific amplification product accumulation to be recorded by measuring the fluorescent signal intensity in real time.

The kit contains reagents for highly specific regions of human herpesvirus 6 DNA, as well as human genomic DNA (sampling volume control, hereinafter SVC) detection (Table 1).

Table 1 – Test targets

Channel corresponding to the fluorophore	
FAM/Green	HEX/Yellow
Human herpesvirus 6 DNA	SVC

SVC allows to evaluate the DNA isolation efficiency and the possible presence of inhibitors in the sample, which can lead to false negative results.

Method limitations

A possible reason for obtaining a false positive result is contamination at DNA isolation or PCR reaction stage. A false positive result can be detected 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 reagent appearance does not match the description.

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

The clinical diagnosis conclusion cannot be based on the assay results with this medical device only. For diagnostic purposes, the results should be used in combination with other data: symptoms, the common clinical picture, the assay results from other test systems, the therapy used.

Total reaction time is 65 minutes (excluding sample preparation).

3. Reagent kit components

Configuration form

HHV6-test reagent kit is designed in 1 configuration form – HHV6-test.

Number of test samples

Each HHV6-test reagent kit contains reagents designed to perform 96 reactions, which equates to:

- detection of 94 test samples, negative and positive control samples, or 32 single test samples detections with negative and positive control samples in each test during qualitative analysis;
- detection of 91 test samples, calibration samples and a negative control sample, or 16 single test samples detections with calibration samples and a negative control sample in each test during quantitative analysis.

Reagent kit components

Table 2 – HHV6-test reagent kit components

No.	Reagent name	Description	Quantity, volume
1	PCR Buffer	Transparent colorless liquid	1 tube, 384 µl
2	Primer Mix	Transparent, colorless liquid, may have a shade of lilac	1 tube, 960 µl
3	CS-1	Transparent colorless liquid	2 tubes, 1600 µl each
4	CS-2	Transparent colorless liquid	2 tubes, 1600 µl each
5	PC	Transparent colorless liquid	1 tube, 192 µl
6	NC	Transparent colorless liquid	2 tubes, 1600 µl each

Note: The operational 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 place a reagent kit in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared ice packs. Place the thermal container, instructions for use and a quality certificate for each batch of products supplied in a cardboard box.

PCR Buffer is ready for use and contains all the basic reagents, including a thermostable hot-start DNA polymerase, deoxynucleotide triphosphates, uracil-DNA glycosylase and an optimized buffer.

Primer Mix is ready for use and contains a multiplex mix of primers and probes:

1. primers and a probe for the human herpesvirus 6 genomic DNA specific region, detection is carried out in the FAM/Green channel;

2. primers and a probe for SVC, detection is carried out in the HEX/Yellow channel.

Reaction in HEX/Yellow Ct ≤ 32 indicates sufficient material sampling quality, nucleic acid efficient isolation, and the absence of PCR inhibitors. If there is no reaction in the HEX/Yellow channel or Ct > 32 and at the same time there is no reaction in the FAM/Green channel, the result should be considered invalid, and the sample should be re-tested starting from DNA isolation. If an invalid result is repeated, the biomaterial should be taken from this patient again.

Positive control sample (PC) is ready for use and is a mixture of plasmid DNA with synthetic insertions of amplified DNA fragments: a specific DNA fragment of human herpesvirus 6 and a COMT gene region. PC is in 10% TE buffer (1 mM Tris, 0.1 mM EDTA).

Negative control sample (**NC)** is ready for use and is deionized DNase-free water.

Calibration sample CS-2 is a mixture of plasmid DNA with synthetic insertions of amplified DNA fragments of human herpesvirus 6 and a human COMT gene region with 1×10^6 copies/ml concentration in a TE buffer (10 mM Tris, 1 mM EDTA).

The calibration sample CS-2 is a mixture of plasmid DNA with synthetic insertions of amplified DNA fragments of human herpesvirus 6 and a human COMT gene region with $1x10^4$ copies/ml concentration in a 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 – HHV6-test reagent kit

Indicator	Characteristics and standards	Clause in TS
1. Technical characteristics		
1) Appearance		
PCR Buffer	Transparent colorless liquid	Section 7, Clause 7.6
Primer Mix	Transparent, colorless liquid, may have a shade of lilac	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
PC	Transparent colorless liquid	Section 7, Clause 7.6
NC	Transparent colorless liquid	Section 7, Clause 7.6
1.2. Completeness	According to clause 1.4 TS 21.20.23-043-97638376-2021	Section 7, Clause 7.9
1.3. Labelling	According to clause 4 TS 21.20.23-043-97638376-2021	Section 7, Clause 7.9
1.4. Packaging	According to clause 5 TS 21.20.23-043-97638376-2021	Section 7, Clause 7.9
2. Functional characteristics		
2.1 Positive result with PC	Fluorescence signal growth recorded in tubes with PC in the channels FAM Ct ≤ 32, HEX Ct ≤ 32.	Section 7, Clause 7.7.2
2.2 Negative result with NC	In tubes with NC in the channels FAM and HEX Ct is not indicated (i.e. there is no fluorescence accumulation curve)	Section 7, Clause 7.7.2
2.3 Reactions in tubes with SC	In tubes with SC, Ct is not indicated in the FAM channel (that is, there is no fluorescence accumulation curve), and in the channel HEX Ct ≤ 32.	Section 7, Clause 7.7.2
2.4 Reactions in tubes with SenC	In tubes with a SenC in the FAM channel, in all repetitions (at least 4) Ct ≤ 35 and with the standard deviation value in SenC repetitions no more than 5%, and in the channel HEX Ct ≤ 32.	Section 7, Clause 7.7.2
2.5 "Linearity" test	The correlation coefficient of CS-1, CS-2 and the standard sample (SS) is at least 0.98	Section 7, Clause 7.7.2

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 7.7.2
2.7 Concentration evaluation accuracy test	The obtained value of human herpesvirus 6 DNA concentration should correspond to the concentration given in a standard sample passport ESS-1 (6 log10 copies/ml) and ESS-2 (4 log10 copies/ml), with ± 0.4 log10 concentration tolerance	Section 7, Clause 7.7.2

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

4.2. Analytical efficiency characteristics

4.2.1. Analytical specificity

It is specific to the human herpesvirus 6 (HHV6) DNA.

The absence of nonspecific positive amplification results was shown in the presence of the following organisms and viruses in the genomic NA sample: herpes simplex virus type 1 and 2, Epstein-Barr virus, herpes simplex virus type 8, Varicella zoster virus, *Parvovirus* B19, human cytomegalovirus, *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus agalactiae*.

4.2.2 Limit of detection

According to GOST R 51352-2013 and taking into account international recommendations **CLSI EP-17A2**, the limit of detection (LOD) is established by the dilution analysis method of the International Standard – 1st WHO International Standard for Human Herpes virus 6B (HHV-6B) DNA (NIBSC code: 15/266).

Based on the study results, the limit of detection of HHV6 DNA in 100 µl samples with 95% detection rate when using NA-Extra isolation kit (RC No. RZN 2021/15428 dated September 24, 2021) for the cycler:

- DTprime – 368 copies/ml (95% CI: 296.4 – 439.5 copies/ml), 206.1 IU/ml (95% CI: 163.1 – 249.0 IU/ml);

- CFX 96 – 380 copies/ml (95% CI: 308.4 – 451.5), 212.8 IU/ml (95% CI: 169.8 – 255.7 IU/ml);

- Rotor-Gene Q – 379 copies/ml (95% CI: 307.4 – 450.5), 212.2 IU/ml (95% CI: 169.2 – 255.1 IU/ml);

- Quant Studio 5 – 370 copies/ml (95% CI: 298.4 – 441.5), 207.2 IU/ml (95% CI: 164.2 – 250.1 IU/ml).

Based on the study results, the limit of detection of HHV6 DNA in 100 µl samples with 95% detection rate when using RIBO-sorb isolation kit (RC No. FSR 2008/03993 dated February 22, 2019) for the cycler:

- DTprime – 373.8 copies/ml (95% CI: 302.2 – 445.3 copies/ml), 209.3 IU/ml (95% CI: 166.3 – 252.2 IU/ml);

- CFX 96 – 374.5 copies/ml (95% CI: 302.9 – 446.1), 209.7 IU/ml (95% CI: 166.7 – 252.6 IU/ml);

- Rotor-Gene Q – 377.5 copies/ml (95% CI: 305.9 – 449.1), 211.4 IU/ml (95% CI: 168.4 – 254.3 IU/ml);

- Quant Studio 5 – 374.4 copies/ml (95% CI: 302.8 – 445.9), 209.7 IU/ml (95% CI: 166.7 – 252.6 IU/ml).

4.2.3 Limit of quantification

According to GOST R 51352-2013 and taking into account international recommendations **CLSI EP-17A2**, the limit of quantification (LOQ) is established by the dilution analysis method of the International Standard – 1st WHO International Standard for Human Herpes virus 6B (HHV-6B) DNA (NIBSC code: 15/266).

Based on the study results, the limit of quantification (LOQ) of HHV6 DNA in 100 µl samples with 95% detection rate using the "NA-Extra" isolation kit (RC No. RZN 2021/15428 dated September 24, 2021) for the cycler:

- DTprime – 401.6 copies/ml (95% CI: 330.0 – 473.1 copies/ml), 224.9 IU/ml (95% CI: 181.9 – 267.8 IU/ml);

- CFX 96 – 403.6 copies/ml (95% CI: 332.0 – 475.1), 226.0 IU/ml (95% CI: 183.0 – 268.9 IU/ml);

- Rotor-Gene Q – 407.2 copies/ml (95% CI: 335.6 – 478.7), 228.0 IU/ml (95% CI: 185.0 – 270.9 IU/ml);

- Quant Studio 5 – 404.2 copies/ml (95% CI: 332.6 – 475.7), 226.4 IU/ml (95% CI: 183.4 – 269.3 IU/ml).

Based on the study results, the limit of quantification (LOQ) of

HHV6 DNA in 100 µl samples with 95% detection rate when using Ribo-sorb isolation kit (RC No. FSR 2008/03993 dated February 22, 2019) for the cycler:

- DTprime – 403.7 copies/ml (95% CI: 332.1 – 475.2 copies/ml), 226.1 IU/ml (95% CI: 183.1 – 269.0 IU/ml);
- CFX 96 – 400.6 copies/ml (95% CI: 329.0 – 472.1), 224.4 IU/ml (95% CI: 181.4 – 267.3 IU/ml);
- Rotor-Gene Q – 403.7 copies/ml (95% CI: 332.1 – 475.2), 226.0 IU/ml (95% CI: 183.0 – 268.9 IU/ml);
- Quant Studio 5 – 401.4 copies/ml (95% CI: 329.8 – 472.9), 224.8 IU/ml (95% CI: 181.8 – 267.7 IU/ml).

4.2.4 Linear measurement range

The linear measurement range was verified using an international standard – 1st WHO International Standard for Human Herpes virus 6B (HHV-6B) DNA (NIBSC code: 15/266).

Based on the results of the linear range study, it can be concluded that for 100 µl samples, the analysis results with HHV6-test reagent kit are linear in the range from 400 copies/ml to 10^7 copies/ml (from 224 IU/ml to $5.6*10^6$ IU/ml).

4.2.5 Precision under repeatability and reproducibility conditions:

1. The coefficient of variation under the kit repeatability conditions does not exceed 3%.
2. The coefficient of variation under the kit reproducibility conditions does not exceed 5%.

4.3. Clinical efficiency characteristics

For clinical trials, 238 samples of human clinical material (45 – whole blood, 40 – blood leukocytes, 40 – oropharyngeal swabs, 45 – saliva, 14 – internal organs biopsy samples, 14 – cerebrospinal fluid, 40 – urine) were used from patients diagnosed with of herpes virus infection caused by human herpesvirus 6 (HHV-6), regardless of the disease form and stage in all population groups.

The biological reference interval of human herpesvirus 6 DNA in the studied population of patients aged 6 months to 45 years ranged from 516 copies/ml to 795507 copies/ml.

To evaluate diagnostic specificity and cross-reactivity in clinical trials by the tested reagent kit "HHV-6", **158 human clinical material samples** (27 – whole blood, 26 – blood leukocytes, 28 – oropharyngeal swabs, 28 – saliva, 12 – internal organs biopsy samples, 12 – cerebrospinal fluid, 25 – urine) that did not contain HHV-6 DNA, but with the confirmed positive presence of genomic NA of the following organisms and viruses: herpes simplex virus type 1 and 2, Epstein-Barr virus, herpes virus type 8, Varicella zoster virus, *Parvovirus* B19, human cytomegalovirus, *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus agalactiae*, were also tested.

This number of samples was selected taking into account the requirements of **GOST R 51352-2013** and the recommendations of the International Guideline **CLSI EP09-A3**.

Each sample was tested in two series using the tested reagent kit "Reagent kit for the qualitative and quantitative determination of human herpes virus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection "HHV6-test" according to TS 21.20.23-043-97638376-2021", produced by TestGene LLC and comparison kits:

- when testing whole blood samples, blood leukocytes, oropharyngeal swabs, saliva, and internal organs biopsy samples, cerebrospinal fluid: Reagent kit for the quantitative detection of herpes virus type 6 (HHV6) DNA in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® HHV6-screen-titer-FL" according to TS 9398-094-01897593-2012, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate no. FSR 2010/09506 dated March 13, 2019);
- when testing urine samples: Reagent kit for the detection and quantification of Epstein-Barr virus (EBV), human cytomegalovirus (CMV), and herpes virus type 6 (HHV6) DNA by real-time polymerase chain reaction "AmpliPrime® EBV / CMV / HHV6" according to TS 21.20.23-090-09286667-2020, manufactured by NextBio LLC (RC No. RZN 2021/15314 dated September 17, 2021).

The results matched, indicating that the medical device was functioning correctly.

DNA was isolated from clinical samples using the following DNA isolation kits:

- to isolate DNA from blood, oropharyngeal swabs, urine: Reagent kit for DNA/RNA isolation from clinical material "NA-Extra" according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (Registration certificate No. RZN 2021/15428 dated September 24, 2021);

- to isolate DNA from blood leukocytes, saliva, internal organs biopsy samples, and cerebrospinal fluid: Reagent kit for RNA/DNA isolation from clinical material "RIBO-sorb" according to TS 9398-004-01897593-2008 produced by the Central Research Institute of Epidemiology of Rospotrebnadzor (registration certificate No. FSR 2008/03993 dated February 22, 2019).

The following cyclers, recommended by the tested reagent kit manufacturer, were used to carry out a PCR test with HHV6-test:

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

4.3.1 The diagnostic characteristics study results based on clinical material samples are shown in Table 7.

Test material type	Number of observations with positive samples	Number of observations with negative samples	Diagnostic sensitivity with 95% confidence probability	Diagnostic specificity with 95% confidence probability
Whole blood	90	54	100% (95% CI: 95.98%-100%)	100% (95% CI: 93.40%-100%)
Blood leukocytes	80	52	100% (95% CI: 98.70%-100%)	100% (95% CI: 93.15%-100%)
Oropharyngeal swabs	80	56	100% (95% CI: 95.49%-100%)	100% (95% CI: 93.62%-100%)
Saliva	90	56	100% (95% CI: 95.98%-100%)	100% (95% CI: 93.62%-100%)
Internal organ biopsy samples	84	72	100% (95% CI: 95.70%-100%)	100% (95% CI: 95.01%-100%)
Cerebrospinal fluid	84	72	100% (95% CI: 95.70%-100%)	100% (95% CI: 95.01%-100%)
Urine	80	50	100% (95% CI: 95.49%-100%)	100% (95% CI: 92.89%-100%)

4.3.2 Method comparison: accuracy

Data obtained from testing **238 human clinical material samples** (45 – whole blood, 40 – blood leukocytes, 40 – oropharyngeal swabs, 45 – saliva, 14 – internal organs biopsy samples, 14 – cerebrospinal fluid, 40 – urine) from patients diagnosed with human herpesvirus infection type 6 (HHV-6), allow to conclude on the results reliable conformity of quantitative detection of human herpesvirus type 6 (HHV6) DNA concentration in clinical samples obtained using the **tested medical device** "Reagent kit for the qualitative and quantitative determination of human herpesvirus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection "HHV6-test" according to TS 21.20.23-043-97638376-2021", manufactured by TestGene LLC and a **comparison kit:**

- when testing samples of whole blood, blood leukocytes, oropharyngeal swabs, saliva, internal organs biopsy samples, cerebrospinal fluid: Reagent kit for the detection and quantification of herpes virus type 6 (HHV6) DNA in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® HHV6-screen titer-FL" according to TS 9398-094-01897593-2012, produced by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (registration certificate no. FSR 2010/09506 dated March 13, 2019);
- when testing urine samples: Reagent kit for the detection and quantification of Epstein-Barr virus (EBV), human cytomegalovirus (CMV), and herpes virus type 6 (HHV6) DNA by real-time polymerase chain reaction "AmpliPrime® EBV / CMV / HHV6" according to TS 21.20.23-090-09286667-2020, manufactured by NextBio LLC (RC No. RZN 2021/15314 dated September 17, 2021).

When conducting a PCR analysis using **cyclers:**

- DTprime detecting cycler (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 systematic error of HHV6 DNA concentration logarithm measurement does not exceed 3%.

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	Used cycler	Number of samples	Correlation coefficient	Intersection	Slope
Reagent kit "HHV6-test", manufactured by TestGene LLC **in comparison** with a reagent kit "AmpliSens® HHV6-screen-titer-FL", manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia, (RC No. FSR 2008/02552 dated November 22, 2019)	Whole blood	log10 copies/ml	DTprime	45	0.9936	0.0098	0.9961
			CFX 96	45	0.9938	0.0225	0.9942
			Rotor-Gene Q	45	0.9933	0.0361	0.9885
			Quant Studio 5	45	0.9955	0.016	0.9951
	Blood leukocytes	log10 copies/ml	DTprime	40	0.9912	0.0246	0.9947
			CFX 96	40	0.992	0.0034	1.0008
			Rotor-Gene Q	40	0.9978	0.0832	0.9799
			Quant Studio 5	40	0.9936	-0.0048	1.0006
	Oropharyngeal swabs	log10 copies/ml	DTprime	40	0.9958	0.0804	0.9823
			CFX 96	40	0.9961	0.9907	0.0349
			Rotor-Gene Q	40	0.9964	0.0513	0.9868
			Quant Studio 5	40	0.9959	0.0425	0.9881
	Saliva	log10 copies/ml	DTprime	45	0.9928	0.1015	0.9928
			CFX 96	45	0.9942	0.0479	0.99
			Rotor-Gene Q	45	0.9942	0.0664	0.9823
			Quant Studio 5	45	0.998	-0.0048	1.0006
	Internal organ biopsy samples	log10 copies/ml	DTprime	42	0.9907	-0.0867	1.031
			CFX 96	42	0.9908	-0.0369	1.0153
			Rotor-Gene Q	42	0.9938	-0.0417	1.0179
			Quant Studio 5	42	0.9925	-0.1029	1.0354
	Cerebrospinal liquid	log10 copies/	DTprime	42	0.9949	0.0417	0.9876
			CFX 96	42	0.9927	0.0301	0.9905
			Rotor-Gene Q	42	0.9932	0.0615	0.9816

		ml	Quant Studio 5	42	0.9912	0.0814	0.9765
Reagent kit HHV6-test, produced by TestGene LLC **in comparison with** a reagent kit "AmpliPrime® EBV/ CMV/ HHV6", produced by FBIS Center of Epidemiology of Rospotrebnadzor, Russia, (RC RZN 2021/15314 dated September 17, 2021)	Urine	log10 copies/ ml	DTprime	40	0.9909	0.0056	0.9942
			CFX 96	40	0.9932	0.0436	0.9901
			Rotor-Gene Q	40	0.9938	0.0211	0.9968
			Quant Studio 5	40	0.9909	-0.0096	1.0036

4.3.3 Interlot correlation determination results

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* - HHV6 DNA concentration was constructed using the tested medical device "HHV6-test", manufactured by TestGene LLC, LOT: **202206-201**, and *Y* - HHV6 DNA concentration using the tested medical device "HHV6-test", manufactured by TestGene LLC, LOT: **202206-202**.

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**	**Used cycler**	**Number of samples**	**Correla-tion coefficient**	**Intersec-tion**	**Slope**
Whole blood	log10 copies/ml	DTprime	45	0.9932	0.016	0.9977
		CFX 96	45	0.9927	-0.004	1.0031
		Rotor-Gene Q	45	0.9921	-0.0172	1.0074
		Quant Studio 5	45	0.9915	0.0089	0.9996
Blood leukocytes	log10 copies/ml	DTprime	40	0.9912	- 0,0459	1.0162
		CFX 96	40	0.9901	- 0,0431	1.0117
		Rotor-Gene Q	40	0.9911	0.1147	0.9695
		Quant Studio 5	40	0.9922	0.1308	0.9665

Oropharyn-geal swabs	log10 copies/ml	DTprime	40	0.9948	- 0,047	1.0097
		CFX 96	40	0.9935	- 0,0109	1.0017
		Rotor-Gene Q	40	0.9947	- 0,0046	1.0036
		Quant Studio 5	40	0.993	- 0,0621	1.0148
Saliva	log10 copies/ml	DTprime	45	0.9921	0.0035	1.0008
		CFX 96	45	0.9918	0.1349	0.964
		Rotor-Gene Q	45	0.994	- 0,0081	1.0003
		Quant Studio 5	45	0.9924	0.0164	0.9943
Urine	log10 copies/ml	DTprime	40	0.9868	- 0,0007	1.0041
		CFX 96	40	0.9864	0.1461	0.9619
		Rotor-Gene Q	40	0.9843	0.066	0.9798
		Quant Studio 5	40	0.9912	- 0,1202	1.0299
Internal organ biopsy samples	log10 copies/ml	DTprime	42	0.9953	0.0579	0.9848
		CFX 96	42	0.9979	0.0519	0.983
		Rotor-Gene Q	42	0.9932	0.0577	0.9795
		Quant Studio 5	42	0.9962	0.022	0.9946
Cerebrospinal liquid	log10 copies/ml	DTprime	42	0.9923	0.0912	0.9728
		CFX 96	42	0.9927	0.0584	0.9856
		Rotor-Gene Q	42	0.9907	- 0,0718	1.0237
		Quant Studio 5	42	0.9916	- 0,0278	1.0084

The obtained data allow to conclude on the reliable conformity of the results of human herpesvirus type 6 DNA concentration quantitative detection in clinical samples obtained with different lots of the tested medical device "Reagent kit for the qualitative and quantitative determination of human herpes virus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection "HHV6-test" according to TS 21.20.23-043-97638376-2021", produced by TestGene LLC.

5. Risks associated with the reagent

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 levels;
3. Contamination of reaction mixtures and DNA test samples with contents from a PC tube or amplification products;
4. Testing using a poor quality DNA sample (low concentration and/or poor purification);
5. Failure to comply with the sample preparation, testing and disposal requirements due to the unqualified personnel work;

6. Use of an unusable kit (use after the expiration date or in case of damaged packaging).

No risks identified in the unacceptable risk zone.

The cumulative residual risk of using a medical device "Reagent kit for the qualitative and quantitative determination of human herpesvirus type 6 (HHV6) DNA by polymerase chain reaction with real-time detection "HHV6-test" is acceptable, the benefits of its use exceed the risk.

6. Safety precautions

The class, depending on the potential risk of use – 2b – in accordance with the medical devices nomenclature classification approved by the Order of the Ministry of Health of the Russian Federation dated 06.06.2012 N 4n.

The reagents in the kit are non-flammable. The outer packaging is neither self-igniting nor explosive. The reagents included in HHV6-test kit have low vapor elasticity and exclude the possibility of inhalation poisoning.

The reagents included in HHV6-test are non-toxic, as 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:

- dispose of unused reagents in accordance with applicable rules and regulations;

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

- the laboratory process should be unidirectional. The testing is carried out in separate rooms (areas). Work should begin in the Isolation Area and continue in the Amplification and Detection Area. Do not return samples, equipment and reagents to the area where the previous process stage was carried out;

- use and change disposable filter tips for automatic dispensers during each operation. Disposable plastic items must be disposed of in a special container with a disinfectant that can be used to disinfect medical waste;

- table surfaces, as well as rooms in which PCR is performed, must be exposed to ultraviolet radiation for 30 minutes before and after work completion;

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

- a reagent kit cannot be used after the expiration date;

- do not use a reagent kit if the inner packaging is damaged, or the reagent appearance does not match the description;

- allow only specially trained personnel to work with the kit (a specialist with higher medical education who has completed training in licensed courses specializing in PCR diagnostics, as well as a laboratory assistant with secondary specialized medical education);

- use disposable gloves, lab coats, eye protection while handling

samples and reagents. Wash your hands thoroughly after finishing work;

- all kit components are non-toxic to humans in the used concentrations. In case of kit components contact with the skin or mucous membranes, rinse the affected area with plenty of water.

The necessary precautions regarding the effects of magnetic fields, external electrical influences, electrostatic discharges, pressure or pressure changes, overload or sources of thermal ignition 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 the product use or sale are not provided.

7. Required equipment and materials

Equipment:

1. Class II and III biological safety box;
2. Vortex;
3. A set of electronic or automatic variable volume dispensers;
4. Refrigerator from +2°C to +8°C with freezer below - 16°C;
5. Cycler[3] with real-time fluorescence detection in channels corresponding to FAM/Green, HEX/Yellow fluorophores: CFX96 (BioRad, USA, RC No. FSZ 2008/03399 dated June 21, 2016), DTprime (LLC NPO DNA-Technology, Russia, RC No. FSR 2011/10229 dated March 3, 2011), Rotor-Gene Q (Qiagen, Germany, No. FSZ 2010/07595 dated August 10, 2010), QuantStudio 5 (Thermo Fisher Scientific, USA, RC No. RZN 2019/8446 dated June 6, 2019).

Materials and reagents not included in the product:

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

1. Disposable tips with aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl;
2. Disposable 1.5 ml Eppendorf type tubes;
3. Thin-walled disposable PCR tubes with optically transparent lid:

- 0.2 ml PCR tubes,
- 0.1-0.2 ml PCR tubes in strips,
- PCR plates with optically transparent film.

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

4. Separate lab coat and disposable talc-free gloves;

5. Container with disinfectant solution;

6. Test tube racks for 0.2 ml tubes or 0.2 ml tubes in strips;

7. To take a swab from the oropharynx, it is recommended to use "Medical disposable sterile probe according to TS 32.50.13-002-28731857-2020", manufactured by PharMedPolis RT LLC, Russia (registration certificate No. RZN 2021/13989 dated November 26, 2021);

8. When taking oropharyngeal swabs, use a sterile saline solution or phosphate buffer (PBS) solution;

9. DNA extraction kit (see Section 8.8 of the Instructions).

8. Test samples

Test sample type

Material for PCR is DNA samples isolated from whole blood, blood leukocytes, oropharyngeal swabs, saliva, internal organs biopsy samples, cerebrospinal fluid, urine.

8.1 Human whole peripheral venous blood sampling

To obtain plasma, collect peripheral venous blood (at least 4-5 ml) into a test tube with EDTA-K2 added as an anticoagulant. To mix the blood with the anticoagulant after the material sampling, turn the tube 2-3 times.

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

Initial clinical material transportation and storage conditions:

- at 2°C... +8°C – up to 6 hours;
- at room temperature – up to 2 hours.

ATTENTION! It is important to avoid freezing and heating the blood tube above +25°C.

Do not use hemolyzed and chylous blood. Unreliable results may be obtained when analyzing such samples!

8.2 Blood leukocytes collection

They are obtained from whole peripheral and/or umbilical cord blood. Blood can be stored at room temperature for 6 hours after collection. To select leukocytes, centrifuge a blood tube for 20 minutes at 3,000 rpm. Using a filter tip, collect carefully 0.2 ml of the leukocyte mass from the cell sediment surface and transfer into a sterile 1.5–2.0 ml

tube.

Storage conditions:

- at a temperature below -68°C - for a long time.

8.3 Urine sample collection and preparation.

For analysis, collect 15-25 ml of the first-void urine in a special dry sterile bottle or 50-60 ml container. To collect a urine sample, first wash the genital area to prevent contamination by discharge.

Sample preparation

Shake the bottle with urine. Transfer 1.0 ml of urine using a filter tip into a sterile disposable 1.5 ml tube. Centrifuge for 5 minutes at 12,000 rpm. If there is a large amount of salt, resuspend only 1.0 ml of the salt sediment top layer and then concentrate again. Using a vacuum aspirator with a trap flask (or a variable volume dispenser), remove completely the supernatant using a separate tip without a filter for each sample, without collecting the sediment. Add transport medium to the sediment to increase a final volume up to 0.2 ml and mix thoroughly on a vortex.

Material and pre-processed samples storage and transportation conditions:

- at +2... +8°C – up to 1 day;
- at a temperature -20°C – up to 1 week;
- at a temperature of -70°C – for a long time.

8.4 Cerebrospinal fluid sampling

Collect at least 1.0 ml cerebrospinal fluid using disposable needles in disposable plastic 1.5 or 2.0 ml tubes.

ATTENTION! Sample pre-processing is not required.

Material storage and transportation conditions:

- at +2... +8°C – up to 1 day;
- at a temperature -20°C – up to 1 week;
- at a temperature -70°C – for a long time.

It is allowed to freeze and thaw the material only once.

8.5 Oropharyngeal sample collection

Take swabs with dry cotton swabs on a plastic base with rotational movements from the surface of the tonsils, palatine arches and the

posterior wall of the oropharynx.

After sampling collection, place the swab (the applied part of the probe with a cotton swab) in a sterile disposable Eppendorf type tube with 500 µl of sterile saline solution or phosphate buffered saline (PBS) solution, and brake off carefully the plastic rod at a distance up to 0.5 cm from the applied part, leaving the applied part of the probe with the material inside. Close the tube tightly with a lid.

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

- at room temperature – up to 6 hours;
- at a temperature +2... +8°C – up to 3 days;
- at a temperature below -20°C – up to 1 week;
- at a temperature of -70°C – for a long time.

It is allowed to freeze and thaw the material only once.

8.6 Saliva sample collection

Before saliva collection, rinse the mouth three times with saline solution. Collect at least 1.0 ml of saliva in disposable sterile 2 ml plastic tubes. Close the tube tightly with a lid.

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

- at room temperature – up to 6 hours;
- at a temperature +2... +8°C – up to 1 day;
- at a temperature below -20°C – up to 1 week;
- at a temperature -70°C – for a long time.

It is allowed to freeze and thaw the material only once.

8.7 Biopsy samples collection and preparation

Place puncture samples (micro-biopsy samples) in microtubes with screw lids or 1.5 ml Eppendorf type tubes containing 0.1 ml of transport medium.

ATTENTION! Sample pre-processing is not required.

Place macro-biopsy samples – 0.1-1.0 g tissue pieces in a cooled porcelain mortar and add 0.5-1.0 ml of cooled isotonic sodium chloride solution, cut into small pieces with sterile scissors and grind with a pestle. Take the supernatant liquid (0.1-0.2 ml) through a cotton swab using a sterile filter tip into sterile microtubes.

8.8 DNA isolation from biological material

To isolate a human genomic DNA sample from biological material, it is recommended to use the following reagent kits:

- when using blood, oropharyngeal swabs and urine as clinical material: Reagent kit for DNA/RNA isolation from clinical material "NA-Extra" according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated September 24, 2021);

- when using blood leukocytes, saliva, internal organs biopsy samples, and cerebrospinal fluid as clinical material: Reagent kit for RNA/DNA isolation from clinical material "RIBO-sorb" according to TS 9398-004-01897593-2008 produced by the Central Research Institute of Epidemiology of Rospotrebnadzor (registration certificate No. FSR 2008/03993 dated February 22, 2019).

ATTENTION! Simultaneously with DNA isolation from the tested clinical samples, it is required to perform all the steps of sample preparation with a negative control sample (NC) and calibration samples CS-1 and CS-2 with 100 µl volume, which are included in HHV6-test reagent kit.

DNA test samples storage conditions:

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

8.9 Interfering substances and limitations on the test material use

The effect of potentially interfering substances on HHV6-test reagent kit performance has been evaluated for potentially interfering substances that may occur during HHV6-test reagent kit normal use and may affect the kit's ability to provide accurate results.

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

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

2) substances found in specific samples types (for example, blood

hemoglobin);

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

The concentrations of interfering substances studied are listed in Table 4.

Table 4

Interfering substances	Maximum concentration
Endogenous interfering substances and anticoagulants	
Hemoglobin	260 µg/ml
Cholesterol	150 mg/dL
Triglycerides	250 mg/dL
Exogenous interfering substances	
Substances found during the clinical material sampling	
Heparin (anticoagulant)	0.15 IU/ml
Sodium citrate (anticoagulant)	0.1 mM/ml
EDTA-K2 (anticoagulant)	0.5 mM/ml
During anticoagulant therapy	
Heparin	1 IU/ml
Medications prescribed for herpesvirus infection	
Aciclovir	2.37 µg/100 µl

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

1) anticoagulants – heparin at 0.15 copies/ml concentration and sodium citrate at 0.1 mM/ml concentration. It is not allowed to use heparin and sodium citrate as an anticoagulant for peripheral blood sampling;

2) heparin at 1 copies/ml concentration, used in anticoagulant therapy. Heparin presence in the blood of patients undergoing anticoagulant therapy can lead to unreliable PCR results, therefore, it is recommended to take blood from such patients before the next administration of the drug.

Limitations on the test material use:

- test material cannot be used in case of storage and transportation conditions violation (temperature, duration, repeated freezing and thawing);
- it is not allowed to use samples contaminated with extraneous biological material;
- do not use hemolyzed and chylous blood. Unreliable results may be obtained when analyzing such samples;
- blood samples collected in test tubes with heparin or sodium citrate as an anticoagulant are not suitable for testing;
- heparin presence in the blood of patients undergoing anticoagulant therapy can lead to unreliable PCR results, therefore, it is recommended to take blood from such patients before the next administration of the drug.

9. Components preparation for testing

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

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

ATTENTION! Mix the reaction mixture components according to Table 5 in PCR tubes before analysis.

Components preparation for testing

1. Mix thoroughly the contents of the tubes with DNA isolated for analysis, NC, CS-1, CS-2, which have passed the DNA isolation stage, Primer Mix, PCR Buffer, PC, turning each tube 10 times or mixing on a vortex at low speed for 3-5 seconds, and then remove drops from the tube lids by short centrifugation;
2. Select the required number of strips or tubes for DNA test samples and DNA control samples amplification.

Before performing PCR, wet clean the PCR-box, as well as equipment and materials used in it with disinfectants suitable for use in PCR laboratories, turn on the UV lamp for 20-30 minutes.

10. Testing procedure

PCR assay consists of the following stages:

1. PCR preparation;
2. DNA amplification with hybridization-fluorescence detection of amplification products in real time;
3. Results interpretation (described in detail in Section 11).

A) PCR preparation

(carried out in the pre-PRC area - a room for reagents dispensing and preparing for PCR-amplification)

Total reaction volume – 20 µl.

ATTENTION! It is forbidden to change the reaction volume. When the volume changes, the method sensitivity decreases dramatically!

To carry out one reaction, you need:

1. PCR Buffer – 4 µl;
2. Primer Mix – 10 µl;
3. Sample (test sample, PC or CS-1 and CS-2; NC that has passed the DNA isolation stage) – 6 µl.

FOR QUALITATIVE ANALYSIS

Prepare reaction tubes according to Table 5 in the following order:

1. Label 0.1–0.2 µl PCR tubes;
2. In a separate disposable sterile 1.5–2.0 ml Eppendorf type tube, prepare the reaction mixture: (N+3)x4 µl of PCR Buffer + (N+3)x10 µl of Primer Mix, where N – the number of tested samples. Mix on a vortex by short centrifugation;
3. Add 14 µl of the prepared reaction mixture into each PCR tube;
4. Add 6 µl of isolated DNA into the appropriate tubes for tested samples. Do not add DNA preparation into the tubes with PC and NC;
5. Add PC and NC into the appropriate tubes;
6. To remove drops from the walls, centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge.

Table 5 – Layout of test tubes for qualitative analysis

	Sample 1	Sample N	PC	NC
Primer Mix	○	○	○	○

FOR QUANTITATIVE ANALYSIS

Prepare reaction tubes according to Table 6 in the following order:

1. Label 0.1–0.2 µl PCR tubes;

2. In a separate disposable sterile 1.5 – 2.0 ml Eppendorf type tube, prepare the reaction mixture: (N+6)x4 µl of PCR Buffer + (N+6)x10 µl of Primer Mix, where N – the number of tested samples. Mix on a vortex by short centrifugation;

3. Add 14 µl of the prepared reaction mixture into each PCR tube;

4. Add 6 µl of isolated DNA into the appropriate tubes for tested samples. Do not add DNA preparation into tubes with CS-1 and CS-2, NC;

5. Add NC, CS-1 and CS-2 into the appropriate tubes;

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

Table 6 – Layout of the tubes for quantitative analysis

	Sample 1	Sample N	CS-1	CS-1	CS-2	CS-2	NC
Primer Mix	○	○	○	○	○	○	○

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

(carried out in the PCR area, a room for PCR amplification)

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

2. Program the device to perform the appropriate program of amplification and fluorescent signal detection, following the instructions for the used device. Specify the analysis type – qualitative or quantitative with standards. PCR protocol is listed in Table 7;

3. Specify the samples number and identifiers, CS-1 and CS-2

standards with their concentrations, mark the tubes location on the thermal block matrix in accordance with their layout;

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

5. Start PCR with a fluorescent signal detection;

6. Upon the program completion start analyzing the results.

Table 7 – PCR protocol

Stage	Temperature, °C	Time, min:sec	Detection channels	Total cycles
1	95	02:00	-	1
2	95	00:15	-	5
	64	00:15		
3	95	00:05		40
	64	00:15	FAM, HEX	

11. Results registration and interpretation

The results are recorded automatically during amplification with the software of the used device.

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 10-20% of the maximum fluorescence level obtained for a positive control sample in the last amplification cycle.

Interpret the results using the Ct values of the FAM/Green and HEX/Yellow channels (Table 1). Only the Ct values obtained at the PCR with fluorescence detection stage are taken into account (that is, corresponding to stages 3 – see Table 7).

First, evaluate the reaction and Ct values in the control samples. Begin results interpretation in the tested samples only if the PC and NC reactions are correct.

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

Results interpretation in control samples

The following results should be obtained for negative and positive control samples (Table 8).

Table 8 – Study results for PC and NC

Added material	Selected fluorophore	
	FAM (human herpesvirus 6, HHV6)	HEX (SVC)
NC	Ct not indicated or > 35	Ct not indicated
PC	Ct ≤ 32	Ct ≤ 32

When obtained values for a negative control sample differ from those indicated in Table 8, the results of the entire series are considered unreliable. In this case, it is required to take special measures to eliminate possible contamination.

When obtained values for a positive control sample differ from those indicated in Table 8, repeat amplification of the entire samples batch.

When re-obtaining values for PC that differ from those indicated in Table 8, replace the reagents.

Results interpretation in DNA test samples

The results analysis during the qualitative analysis is shown in Table 9.

Table – 9 Results interpretation principle during qualitative analysis

Ct values		Result
FAM/Green (HHV6)	HEX/Yellow (SVC)	
Ct ≤ 35	Not considered	Human herpesvirus 6 (HHV6) DNA **detected**
Ct absent	Ct ≤ 35	Human herpesvirus 6 (HHV6) DNA **not detected**

Ct > 35	Not considered	Human herpesvirus 6 DNA test result **doubtful**
Ct > 35 or absent		Invalid result

Results interpretation during quantitative analysis.

Results interpretation is carried out automatically using the software supplied with the used detection cycler, or manually. Based on the obtained Ct values for calibration samples and their concentrations, it is necessary to draw a calibration line. When using a calibration line, the tested samples concentrations are calculated. For the samples Ct values ≤ 35 in the FAM channel are taken into account. When Ct > 35 for samples (when Ct ≤ 35 for SVC), the result is considered doubtful.

PCR efficiency should be in 90-110% range, and the difference between the Ct values of the repetitions of each calibration sample, CS-1 and CS-2, should not exceed 1.5. Otherwise, it is required to repeat analysis, starting from the DNA isolation stage. If one of the two duplicates CS-1 or CS-2 has a Ct value that deviates greatly from the others, it can be ignored when drawing the calibration line.

The reason for obtaining an invalid result may be the presence of inhibitors in the DNA preparation obtained from clinical material, incorrect testing protocol implementation, 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.

In case of an invalid and doubtful result, the conclusion is not issued, it is required to re-take the biomaterial from the patient and re-test it.

If a doubtful result is repeated, it is required to repeat the study with a reagent kit from another manufacturer or by another method.

Diagnostic value of the obtained study result:

The obtained positive or negative study result can be used by a qualified specialist (doctor), taking into account the data of the clinical picture and other test types in combination, for the early diagnosis of herpesvirus infection in patients regardless of the disease form and stage of all population groups.

12. Reagent kit storage, transportation and operation conditions

Storage

Store HHV6-test reagent kit in the manufacturer's packaging at -18... -20°C during the entire kit shelf life, it can be stored at +2... +8°C for up to 90 days.

It is allowed to freeze/thaw HHV6-test reagent kit up to 10 times.

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

Transportation

Transport HHV6-test reagent kit in all types of covered vehicles in accordance with the transportation rules applicable to this transport type.

Transport at -18... -20°C during the entire kit shelf life. Transportation is allowed at +2... +8°C for up to 90 days, or at +15... +25 °C for 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, place a reagent kit 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 the thermal container with the transported reagent kits, as well as the thermal container volume 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 HHV6-test reagent kit – 12 months from the acceptance date of the manufacturer's QCD, if all transportation, storage and operation conditions are met. A reagent kit cannot be used after the expiration date.

Shelf life of the opened kit components

12 months from the acceptance date of the manufacturer's QCD, if stored at -18... -20°C.

Shelf life of the kit components prepared for work

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

13. Disposal

Reagent kits that have become unusable, including due to expiration dates, must be disposed of in accordance with the requirements 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 and 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 of 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 packaging residues as industrial or household waste.

HHV6-test reagent kit consumer packaging and tubes are 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

The manufacturer guarantees quality and safety of HHV6-test reagent kit during the shelf life if compliant with transportation and storage requirements as well as rules of operation.

If you have any complaints about the kit quality, undesirable events or incidents, submit information to:

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
E-mail: help@testgen.ru

Annex A

Designation	Document name
GOST ISO 14971-2011	Medical devices. Application of risk management to medical devices.
GOST R 51088-2013	In vitro diagnostic medical devices. Reagents, reagent kits, the test systems, control materials, culture medium. Requirements to devices and to supporting documentation.
GOST R ISO 23640-2015	In vitro medical devices. Evaluation of stability of in vitro diagnostic reagents.
GOST R 51352-2013	In vitro diagnostic medical devices. Test methods.
GOST R EN 13612-2010	Performance evaluation of in vitro diagnostic medical devices.
GOST R ISO 18113-1-2015	In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Part 1. Terms, definitions and general requirements.
GOST R ISO 18113-2-2015	In vitro diagnostic medical devices. Information provided by the manufacturer (labelling). Part 2. In vitro diagnostic reagents for professional use.
GOST R ISO 15223-1-2020	Medical devices. Symbols to be used with medical device labels, labelling, and information to be supplied. Part 1. General requirements.
GOST ISO 13485-2017	Medical products. Quality management systems. Requirements for regulatory purposes.