APPROVED BY
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INSCTRUCTIONS FOR USE

Reagent kit for the qualitative and quantitative determination of DNA of human herpes viruses types 4, 5 and 6 (EBV, CMV, HHV6) by the polymerase chain reaction method with real-time detection "Herpes-test"

TS 21.20.23-055-97638376-2022

Version 3 dated December 7, 2023

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
PC-1	positive control sample 1
PC-2	positive control sample 2
SAC	sampling volume control
EBV, HHV4	Epstein-Barr virus, human herpesvirus 4
CMV, HHV5	cytomegalovirus, human herpesvirus 5
HHV6	human herpesvirus 6
SC	specificity control
SenC	sensitivity control
RM	reference material
IRM	in-house reference material

Introduction

Target analyte: specific regions of human herpes viruses types 4, 5 and 6 (HHV4, HHV5, HHV6) genomic DNA.

The scientific validity of the target analytes lies in the specificity (DNA sequence uniqueness) in relation to the genomes of human herpes viruses types 4, 5 and 6 (HHV4, HHV5, HHV6).

Herpesviruses (Herpesviridae) are a large family of DNA viruses, in total more than 100 types are known. 8 of these types can infect humans, and after acute infection, these viruses remain for life in various body cells and can reactivate[1].

Herpesvirus 4 or Epstein-Barr virus (EBV) is characterized by the ability to transform B cells, causing diseases such as infectious mononucleosis (IM), post-transplant lymphoproliferative disease (PTLD) and Burkitt lymphoma[2]. It is known that saliva is the primary route of EBV transmission, whereas alternative EBV transmission routes can be parenchymal organs or stem cells transplantation and, in rare cases, blood transfusion. Primary EBV infection is usually asymptomatic and occurs in early childhood, but delayed infection can lead to IM[3].

Herpes virus type 5 (cytomegalovirus, CMV) is transmitted through blood or other biological fluids, as well as with organs transplanted during transplantation. The infection can be transmitted transplacentally or during birth. Primary infection in adults can be asymptomatic or manifest as various syndromes, including mononucleosis, hepatitis, or pneumonia.

Herpes virus type 6 (HHV-6) has two serological subtypes – 6A and 6B. HHV-6 is the etiological agent of several infectious diseases: sudden exanthema, fever with seizures, and infectious mononucleosis. In addition,

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[1] Shikova E., Reshkova V., Kumanova A. et al. Cytomegalovirus, Epstein-Barr virus, and human herpesvirus-6 infections in patients with myalgic encephalomyelitis /chronic fatigue syndrome // J. Med. Virol. – 2020, №9 (12).

[2] Ok C.Y., Li L., Young K.H. EBV-driven B-cell lymphoproliferative disorders: from biology, classification and differential diagnosis to clinical management // Exp. Mol. Med. – 2015, №47.

[3] Lam J.K.P, Azzi T., Hui K.F. et al. Co-infection of cytomegalovirus and Epstein-Barr virus diminishes the frequency of $CD56^{dim}$ $NKG2A^{+}$ KIR^{-} NK cells and contributes to suboptimal control of EBV in immunosuppressed children with post-transplant lymphoproliferative disorder // Front. Immunol. – 2020, №11.

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)[4]. 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[5].

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

Indications for use: Herpes-test reagent kit is recommended for use in clinical laboratory diagnostics to test clinical material (whole blood, blood leukocytes, oropharyngeal smears, saliva, biopsies of internal organs, cerebrospinal fluid), in patients with suspected herpes virus infection and patients with detected human herpes viruses types 4, 5 or 6 to choose an adequate therapy and evaluate its effectiveness, regardless of the disease form and stage in all population groups.

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 a reagent kit use were identified.

Sterility: the product is not sterile.

[4] 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.

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

1. Intended use

Intended use: Herpes-test reagent kit is designed for qualitative and quantitative detection of human herpes viruses types 4, 5 and 6 (HHV4, 5, 6) by 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, biopsies of internal organs, cerebrospinal fluid) in patients with suspected herpesvirus infection and patients with detected human herpes viruses types 4, 5 or 6 to select adequate therapy and evaluate its effectiveness 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, and for choosing an adequate therapy and evaluating its effectiveness in patients with detected human herpesviruses types 4, 5, 6. 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, medical technologist, a medical laboratory technician.

2. Method principle

Method

Qualitative and quantitative 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, biopsies of internal organs, and cerebrospinal fluid.

Detection principle

DNA amplification process 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 temperature 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 herpes viruses of type 4, 5 and 6 DNA, as well as human genomic DNA (human *ALB* gene for sample adequacy control, hereinafter - SAC) detection (Table 1).

Table 1 – Test targets

Channel corresponding to the fluorophore			
FAM/Green	HEX/Yellow	ROX/Orange	Cy5/Red
HHV6	SAC	HHV5	HHV4

SAV 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.

The 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

Herpes-test reagent kit is designed in one configuration form.

Test sample number

Herpes-test reagent kit contains reagents designed to perform 96 reactions, which corresponds to:

When conducting qualitative analysis

- detection of 94 test samples, negative and positive (PC-1) control samples
- 32 single samples detections with negative and positive control samples in each test;

When conducting quantitative analysis

- detection of 91 test samples, calibration samples (PC-1 and PC-2) and a negative control sample;
- 16 single test samples detections with calibration samples and a negative control sample in each test.

Reagent kit components

Table 2 – Herpes-test reagent kit components

No.	Reagent name	Description	Quantity, volume
1	PCR Buffer	Transparent colorless liquid	1 tube, 480 µl
2	Primer Mix	Transparent, colorless liquid, may have a shade of lilac	1 tube, 480 µl
3	PC-1	Transparent colorless liquid	1 tube, 480 µl
4	PC-2	Transparent colorless liquid	1 tube, 480 µl
5	NC	Transparent colorless liquid	2 tubes, 1600 µl each

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

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

1. Primers and a probe to a genomic DNA specific region of human herpes virus type 6 (FAM/Green), human herpes virus type 5 (ROX/Orange), human herpes virus type 4 (Cy5/Red);

2. Primers and a probe for SAV (HEX/Yellow).

Positive control samples (PC-1 and PC-2) are ready for use and are a mixture of plasmid DNA with synthetic insertions of amplified DNA fragments: DNA specific fragments of human herpes viruses types 4, 5, 6 and the *ALB* gene region (hereinafter - SAV).

Channel	**Concentration (copies/ml)**	
	PC-1	**PC-2**
FAM/Green (HHV6)	1 000 000 = 10^6	10 000 = 10^4
HEX/Yellow (SAC)		
ROX/Orange (HHV5)		
Cy5/Red (HHV4)		

PC-1 and PC-2 are in 10% TE buffer (1 mM Tris, 0.1 mM EDTA) and contain DNA sodium salt from salmon testes 20 ng/µl and sodium azide 0.05% as a preservative.

PC-1 is both a positive control and a calibration sample. In the case qualitative analysis only PC-1 is used in one repetition. In the case of quantitative analysis PC-1 and PC-2 are used, each in two repetitions.

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

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 – Herpes-test reagent kit

Indicator	Characteristics and standards
1. Technical characteristics	
1.1. Appearance	
PCR Buffer	Transparent colorless liquid
Primer Mix	Transparent, colorless liquid, may have a shade of lilac
PC-1	Transparent colorless liquid
PC-2	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to clause 1.4 TS 21.20.23-055-97638376-2022
1.3. Labelling	According to clause 4 TS 21.20.23-055-97638376-2022
1.4. Packaging	According to clause 5 TS 21.20.23-055-97638376-2022
2. Functional characteristics	
Positive result with PC-1	Fluorescence signal growth recorded in tubes with PC-1 in FAM, ROX, Cy5, HEX channels Ct ≤ 28
Positive result with PC-2	Fluorescence signal growth recorded in tubes with PC-2 in FAM, ROX, Cy5, HEX channels Ct ≤ 32
Negative result with NC	Ct is not indicated in tubes with NC in the FAM, ROX, Cy5, and HEX channels (i.e., there is no fluorescence accumulation curve)
Reaction in tubes with IRM-SC	Ct is not indicated in tubes with IRM-SC in the FAM, ROX, Cy5 channels (that is, there is no fluorescence accumulation curve), and Ct ≤ 32 in the HEX channel.
Reaction in tubes with IRM-SenC-4	Ct is not indicated in tubes with IRM-SenC-4 in the FAM, HEX, and ROX channels, and Ct ≤ 35 in the Cy5 channel.
Reaction in tubes with IRM-SenC-5	Ct is not indicated in tubes with IRM-SenC-5 in the FAM, NEX, Cy5 channels, and Ct ≤ 35 in the ROX channel.
Reaction in tubes with IRM-SenC-6	Ct is not indicated in tubes with IRM-SenC-6 in the ROX, NEX, Cy5 channels, and Ct ≤ 35 in the FAM channel.
"Linearity" test	The correlation coefficient of PC-1, PC-2 and reference material (RM) is at least 0.98
Precision test: coefficient of variation (CV) under repeatability conditions	Ct coefficient of variation of each calibration sample PC-1 and PC-2 repetitions (at least 4) under repeatability conditions does not exceed 5%.

Concentration evaluation accuracy test	The obtained value of human herpes viruses types 4, 5, 6 DNA concentration should correspond to the concentration given in the reference material certificate IRM-1 (6 log10 copies/ml) and IRM-2 (4 log10 copies/ml), with ± 0.5 log10 copies/ml concentration tolerance

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 human herpes viruses types 4, 5, and 6 DNA.

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

4.2.2 Limit of detection

In accordance with GOST R 51352-2013 and taking into account the international recommendations of CLSI EP-17A2, the limit of detection was established by the method of reference material dilution analysis:

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain;
- AMPLIRUN® CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain;
- AMPLIRUN® HERPES DNA CONTROL (MBC025-R), manufactured by Vircell, Spain.

According to the study results, human herpes viruses types 4, 5, and 6 DNA the limit of detection (LOD) in 100 µl samples with 95% detection rate when using NA-Extra isolation kits (RC No. RZN 2021/15428 dated September 24, 2021) and RIBO-sorb (RC No. FSR 2008/03993 dated February 22, 2019) for each cycler is:

Test analyte	Used cycler	Concentration, copies/ml (LOD) with 95% confidence probability	
		DNA isolation Kit	
		NA-Extra	RIBO-sorb
Human herpes virus type 6 (HHV6) DNA	DTprime	380 95%CI: 326.32 - 433.68 copies/ml	371 95%CI: 317.32 -424.68 copies/ml
	CFX 96	372 95%CI: 318.32 -425.68 copies/ml	370 95%CI: 316.32 - 423.68 copies/ml
	Rotor-Gene Q	376 95%CI: 322.32 - 429.68 copies/ml	366 95%CI: 312.32 -419.68 copies/ml
	Quant Studio 5	389 95%CI: 335.32 - 442.68 copies/ml	374 95%CI: 320.32 -427.68 copies/ml
	FLUORITE	390 95%CI: 336.32 - 443.68 copies/ml	385 95%CI: 331.32 - 438.68 copies/ml
Human herpes virus type 5 (HHV5) DNA	DTprime	389 95%CI: 335.32 - 442.68 copies/ml	375 95%CI: 321.32 - 428.68 copies/ml
	CFX 96	379 95%CI: 325.32 - 432.68 copies/ml	376 95%CI: 322.32 -429.68 copies/ml
	Rotor-Gene Q	381 95%CI: 327.32 -434.68 copies/ml	370 95%CI: 316.32 - 423.68 copies/ml
	Quant Studio 5	374 95%CI: 320.32 - 427.68 copies/ml	387 95%CI: 333.32 - 440.68 copies/ml
	FLUORITE	375 95%CI: 321.32 - 428.68 copies/ml	382 95%CI: 328.32 - 435.68 copies/ml
Human herpes virus type 4 (HHV4) DNA	DTprime	374 95%CI: 320.32 - 427.68 copies/ml	374 95%CI: 320.32 - 427.68 copies/ml
	CFX 96	377 95%CI: 323.32 - 430.68 copies/ml	382 95%CI: 328.32 - 435.68 copies/ml
	Rotor-Gene Q	376 95%CI: 322.32 - 429.68 copies/ml	386 95%CI: 332.32 - 439.68 copies/ml
	Quant Studio 5	380 95%CI: 326.32 - 433.68 copies/ml	378 95%CI: 324.32 - 431.68 copies/ml
	FLUORITE	385	384

		95%CI: 331.32 - 438.68 copies/ml	95%CI: 330.32 - 437.68 copies/ml

4.2.3 Limit of quantitative detection

In accordance with GOST R 51352-2013 and taking into account the international recommendations of **CLSI EP-17A2**, the limit of quantitative determination (LOQ) was established by the method of reference material dilution analysis:

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain;
- AMPLIRUN® CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain;
- AMPLIRUN® HERPES DNA CONTROL (MBC025-R), manufactured by Vircell, Spain.

According to the study results, human herpes viruses types 4, 5, and 6 DNA the limit of quantitative detection (LOQ) in 100 µl samples with 95% detection rate when using NA-Extra isolation kits (RC No. RZN 2021/15428 dated September 24, 2021) and RIBO-sorb (RC No. FSR 2008/03993 dated February 22, 2019) for each cycler is:

Test analyte	**Used cycler**	**Concentration, copies/ml (LOD) with 95% confidence probability**	
		DNA isolation Kit	
		NA-Extra	**RIBO-sorb**
Human herpes virus type 6 (HHV6) DNA	DTprime	783 95%CI: 729.32 - 836.68 copies/ml	767 95%CI: 713.32 - 820.68 copies/ml
	CFX 96	779 95%CI: 725.32 - 832.68 copies/ml	783 95%CI: 729.32 - 836.68 copies/ml
	Rotor-Gene Q	775 95%CI: 721.32 - 828.68 copies/ml	778 95%CI: 724.32 - 831.68 copies/ml
	Quant Studio 5	775 95%CI: 721.32 - 828.68 copies/ml	777 95%CI: 723.32 - 830.68 copies/ml
	FLUORITE	768 95%CI: 714.32 - 821.68 copies/ml	770 95%CI: 716.32 - 823.68 copies/ml
Human herpes virus type 5 (HHV5) DNA	DTprime	782 95%CI: 728.32 - 835.68 copies/ml	773 95%CI: 719.32 - 826.68 copies/ml

	CFX 96	774 95%CI: 720.32 - 827.68 copies/ml	773 95%CI: 719.32 - 826.68 copies/ml
	Rotor-Gene Q	785 95%CI: 731.32 - 838.68 copies/ml	778 95%CI: 724.32 - 831.68 copies/ml
	Quant Studio 5	776 95%CI: 722.32 - 829.68 copies/ml	769 95%CI: 715.32 - 822.68 copies/ml
	FLUORITE	772 95%CI: 718.32 - 825.68 copies/ml	766 95%CI: 712.32-819.68 copies/ml
Human herpes virus type 4 (HHV4) DNA	DTprime	786 95%CI: 732.32 - 839.68 copies/ml	769 95%CI: 715.32 - 822.68 copies/ml
	CFX 96	784 95%CI: 730.32 -837.68 copies/ml	777 95%CI: 723.32 - 830.68 copies/ml
	Rotor-Gene Q	778 95%CI: 724.32 - 831.68 copies/ml	766 95%CI: 712.32 - 819.68 copies/ml
	Quant Studio 5	774 95%CI: 720.32 - 827.68 copies/ml	781 95%CI: 727.32 - 834.68 copies/ml
	FLUORITE	766 95%CI: 712.32 - 819.68 copies/ml	767 95%CI: 713.32 - 820.68 copies/ml

4.2.4 Linear measurement range

Herpes-test linearity in accordance with GOST R 51352-2013 was determined by testing a series of dilutions of reference materials:

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain;
- AMPLIRUN® CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain;
- AMPLIRUN® HERPES DNA CONTROL (MBC025-R), manufactured by Vircell, Spain.

and positive controls.

Based on the linear range study results, it can be concluded that for 100 µl samples, the analysis results with Herpes-test reagent kit are linear in the range from 800 copies/ml to 10^7 copies/ml and show a maximum deviation from the regression line not higher than ± 0.22 log10.

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%.

4.2.6 Interfering substances and limitations on the test material use

The effect of potentially interfering substances on a reagent kit performance was studied regarding potentially interfering substances that may occur during a reagent kit normal use, and presumably affect the reagent kit ability to produce reliable results.

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

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

2) substances found in specific sample types (e.g., blood hemoglobin);

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

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

Table 4

Clinical material type	Interfering substances	Maximum concentration
Endogenous interfering substances and anticoagulants		
whole blood, blood leukocytes, oropharyngeal swabs, saliva, biopsies of internal organs, cerebrospinal fluid	Hemoglobin	0.20 mmol/100 µl
whole blood	Triglycerides	0.0037 mmol/100 µl
oropharyngeal swabs, saliva,	Mucin	0.23 mg/100 µl
Exogenous interfering substances		
Substances found during the clinical material collection		
whole blood	Heparin (anticoagulant)	0.015 IU/100 µl
whole blood	Sodium citrate (anticoagulant)	0.01 mM/100 µl

whole blood	EDTA-K2 (anticoagulant)	0.05 mM/ 100 µl
Drugs prescribed for herpesvirus infection		
whole blood, blood leukocytes, oropharyngeal swabs, saliva, biopsies of internal organs, cerebrospinal fluid	Acyclovir	2.37 µg/100 µl
	Lactoferrin	0.1 µg/100 µl

Based on the study results, the following substances were classified as PCR inhibitors during testing: anticoagulants – heparin at 0.015 IU/100 µl concentration and sodium citrate at 0.01 mM/100 µl concentration. It is not allowed to use heparin and sodium citrate as an anticoagulant for human venous blood collection.

Limitations on the test material use:

- blood samples collected in test tubes with heparin or sodium citrate as an anticoagulant are not suitable for testing.
- the 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;

4.2.7 Metrological traceability of the end–user IVD medical device calibrators - PC-1, PC-2, included in Herpes-test reagent kit, and the used calibrators IRM-1, IRM-2 and IRM-SenC-4, IRM-SenC-5, IRM-SenC-6 was carried out in accordance with the Calibration Hierarchy with the reference measurement method and the primary reference material (RM) (clause 5.2 GOST R ISO 17511-2022).

The common calibration hierarchy with indicated measurement uncertainty at each stage is shown in Table 5.

Table 5 – Calibration hierarchy results

Sample type	Analyte	Sample	Measurement method	Measurement uncertainty
	EBV DNA	AMPLIRUN® EPSTEIN-BARR	Flow cytometry, FCM	$u_{ref} = 0.5$

Primary reference material		VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain		
	CMV DNA	AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain		$u_{ref} = 0.5$
	HHV6 DNA	AMPLIRUN HHV-6 DNA CONTROL (MBC025-R), manufactured by Vircell, Spain		$u_{ref} = 0.5$
Primary calibrator	EBV DNA	Reconstituted reference material AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065)	The primary calibrator is prepared by the lyophilized primary RM reconstitution	$u_{p.2} = 0.1$
	CMV DNA	Reconstituted reference material AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016)		$u_{p.2} = 0.1$
	HHV6 DNA	Reconstituted reference material AMPLIRUN HHV-6 DNA CONTROL (MBC025-R)		$u_{p.2} = 0.1$
Secondary calibrator	EBV DNA	Dilution panel of the reconstituted sample AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065)	The dilution panel is prepared by the primary calibrator dilution in accordance with the RM certificate.	$u_{p.3} = 0.1$
	CMV DNA	Dilution panel of the reconstituted material AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016)		$u_{p.3} = 0.1$
	HHV6 DNA	Dilution panel of the reconstituted material AMPLIRUN HHV-6 DNA CONTROL (MBC025-R)		$u_{p.3} = 0.1$

Used calibrator	EBV DNA	IRM-SenC-4, IRM-1, IRM-2	Manufacturer's measurement method - quantitative PCR with real-time hybridization-fluorescence detection	$u_{p.4} = 0.1$
	CMV DNA	IRM-SenC-5, IRM-1, IRM-2		$u_{p.4} = 0.13$
	HHV6 DNA	IRM-SenC-6, IRM-1, IRM-2		$u_{p.4} = 0.12$
IVD medical device calibrator of the end user	EBV DNA	PC-1, PC-2	Manufacturer's measurement method - quantitative PCR with real-time hybridization-fluorescence detection	$u_{p.5} = 0.12$ $u_{cal} = 0.5$
	CMV DNA	PC-1, PC-2		$u_{p.5} = 0.12$ $u_{cal} = 0.5$
	HHV6 DNA	PC-1, PC-2		$u_{p.5} = 0.11$ $u_{cal} = 0.5$
Combined standard uncertainty				$u(y) = 0.5$
Combined expanded uncertainty				$U(y) = 1$
Maximum acceptable measurement uncertainty				*Umax(y)* = 1

The assigned concentration **in relation to human herpes virus type 4 (EBV) DNA** of IRM-1 is 1×10^6 copies/ml, IRM-2 is 1×10^4 copies/ml, IRM-SenC-4 - 800 copies/ml.

The assigned concentration **in relation to human herpes virus type 5 (CMV) DNA** of IRM-1 is 1×10^6 copies/ml, IRM-2 is 1×10^4 copies/ml, IRM-SenC-5 - 800 copies/ml.

The assigned concentration **in relation to human herpes virus type 6 (HHV6) DNA** of IRM-1 is 1×10^6 copies/ml, IRM-2 is 1×10^4 copies/ml, IRM-SenC-6 - 800 copies/ml.

The assigned concentration **in relation to human herpes virus type 4 (EBV) DNA** of PC-1 is $1x10^6$ copies/ml, PC-2 is $1x10^4$ copies/ml.

The assigned concentration **in relation to human herpes virus type 5 (CMV) DNA** of PC-1 is $1x10^6$ copies/ml, PC-2 is $1x10^4$ copies/ml.

The assigned concentration **in relation to human herpes virus type 6 (HHV6) DNA** of PC-1 is $1x10^6$ copies/ml, PC-2 is $1x10^4$ copies/ml.

The combined standard measurement uncertainty for the recorded values of the detectable amount of CMV DNA using the end-user Herpes-test kit is **u(y) = 0.5 log copies/ml.**

The combined standard measurement uncertainty of the value assigned to the end-user IVD medical device calibrators (PC-1, PC-2) u_{cal}

does not exceed the permissible ratio *Umax(y)* of specification for the IVD medical device, taking into account the coverage factor *k* (*k* = 2, for an approximate 95% confidence probability):

$$u_{cal} = 0{,}5 \leq \frac{1}{2} Umax(y) = 0{,}5$$

The estimated combined expanded measurement uncertainty U(y) does not exceed the maximum acceptable measurement uncertainty Umax(y):

$$U(y)=1 \leq Umax(y)=1$$

4.2.8 Biological reference intervals

The biological reference interval for human herpes virus type 4 (EBV) DNA level among 238 patients aged 0 to 67 years, based on the clinical trials results, ranges from 2.44 to 5.84 log10 copies/ml. The EBV DNA concentration median in the sample is 3.91 log10 copies/ml.

The biological reference interval for human herpesvirus type 5 (CMV) DNA level among 211 patients aged 12 to 45 years, based on the clinical trials results, ranges from 2.71 to 5.44 log10 copies/ml. The CMV DNA concentration median in the sample is 4.11 log10 copies/ml.

The biological reference range for human herpes virus type 6 (HHV6) DNA levels among 198 patients aged 0 to 45 years, based on the clinical trials results, ranges from 2.34 to 5.9 log10 copies/ml. The HHV6 DNA concentration median in the sample is 3.90 log10 copies/ml.

4.3. Clinical efficiency characteristics

For clinical trials, 648 samples of human clinical material were used (147 - whole blood, 135 - blood leukocytes, 135 - oropharyngeal smears, 145 - saliva, 43 - biopsies of internal organs, 43 - cerebrospinal fluid) from patients diagnosed with herpes virus infection caused by human herpes viruses types 4, 5 or type 6, regardless of the disease form and stage of all population groups.

Test sample type	Human herpes virus type 4 (EBV)	Human herpes virus type 5 (CMV)	Human herpes virus type 6 (HHV6)	**Total**
Whole blood	55	47	45	147
Blood leukocytes	50	45	40	135
Oropharyngeal swabs	50	45	40	135
Saliva	55	45	45	145
Biopsy of internal organs	14	15	14	43
Cerebrospinal fluid	14	15	14	43
Total number of samples containing tested analyte	238	212	198	648

To evaluate diagnostic specificity and cross-reactivity in clinical trials, **158 samples of human clinical material** (35 - whole blood, 31 - blood leukocytes, 35 - oropharyngeal swabs, 33 - saliva, 12 - biopsies of internal organs, 12 - cerebrospinal fluid), not containing human herpes viruses types 4, 5, 6 DNA, but with the confirmed positive presence of genomic NA of the following organisms and viruses: human simplex type 1 and 2, herpes simplex virus type 8, Varicella-Zoster virus, Parvovirus B19, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus agalactiae* were also examined with the tested kit Herpes-test.

In accordance with international guideline CLSI EP09-A3 recommendations, it is recommended to perform clinical studies using at least 40 clinical samples. **In order to conduct studies using biopsies of internal organs and cerebrospinal fluid of a larger sample volume, according to CLSI EP09-A3 recommendations, each sample was tested in 3 repetitions,** starting from the DNA isolation procedure.

Each sample was tested in two series using reagent kit for the qualitative and quantitative determination of DNA of human herpes viruses type 4, 5 and 6 (EBV, CMV, HHV6) by polymerase chain reaction

with real-time detection "Herpes-test" according to TS 21.20.23-055-97638376-2022", produced by TestGene LLC and a comparison kit:

- Reagent kit for the detection and quantification of Epstein-Barr virus (EBV), cytomegalovirus (CMV) and herpes virus type 6 (HHV6) DNA in clinical material by polymerase chain reaction with real-time hybridization-fluorescence detection "AmpliSens® EBV/CMV/HHV6-screen-FL" according to TS 9398-095-01897593-2012, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor (RC No. FSR 2010/09502 dated October 18, 2019).

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

Cyclers used to carry out a PCR test, recommended by the reagent kit manufacturer:

- 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);
- FLUORITE cycler (Xian TianLong Science and Technology Co, China)

Confidence intervals (CI) of diagnostic characteristics will be calculated using the Clopper and 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). The diagnostic characteristics of the tested kit were calculated with 95% confidence probability.

4.3.1 Diagnostic characteristics test results based on clinical material samples are shown in Table 7.

Test material type	Tested analyte	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	EBV	110	70	100% (95% CI:96.7%-100%)	100% (95% CI:94.87%-100%)
	CMV	94	70	100% (95% CI:96.15%-100%)	100% (95% CI:94.87%-100%)
	HHV6	90	70	100% (95% CI:95.98%-100%)	100% (95% CI:94.87%-100%)
Blood leukocytes	EBV	100	62	100% (95% CI:96.38%-100%)	100% (95% CI:94.87%-100%)
	CMV	90	62	100% (95%	100% (95%

				CI:95.98%-100%)	CI:94.87%-100%)
	HHV6	80	62	100% (95% CI:95.49%-100%)	100% (95% CI:94.87%-100%)
Oropharyn-geal swabs	EBV	100	70	100% (95% CI:96.38%-100%)	100% (95% CI:94.87%-100%)
	CMV	90	70	100% (95% CI:95.98%-100%)	100% (95% CI:94.87%-100%)
	HHV6	80	70	100% (95% CI:95.49%-100%)	100% (95% CI:94.87%-100%)
Saliva	EBV	110	66	100% (95% CI:96.7%-100%)	100% (95% CI:94.56%-100%)
	CMV	90	66	100% (95% CI:95.98%-100%)	100% (95% CI:94.56%-100%)
	HHV6	90	66	100% (95% CI:95.98%-100%)	100% (95% CI:94.56%-100%)
Internal organs biopsy samples	EBV	84	24	100% (95% CI:95.7%-100%)	100% (95% CI:85.75%-100%)
	CMV	90	24	100% (95% CI:95.98%-100%)	100% (95% CI:85.75%-100%)
	HHV6	84	24	100% (95% CI:95.7%-100%)	100% (95% CI:85.75%-100%)
Cerebro-spinal fluid	EBV	84	24	100% (95% CI:95.7%-100%)	100% (95% CI:85.75%-100%)
	CMV	90	24	100% (95% CI:95.98%-100%)	100% (95% CI:85.75%-100%)
	HHV6	84	24	100% (95% CI:95.7%-100%)	100% (95% CI:85.75%-100%)

4.3.2 Methods comparison: accuracy

Data obtained from testing 648 samples of human clinical material (147 - whole blood, 135 – blood leukocytes, 135 - oropharyngeal swabs, 145 - saliva, 43 - biopsies of internal organs, 43 - cerebrospinal fluid) from patients diagnosed with herpesvirus infection caused by human herpesvirus types 4, 5 and 6, allow to conclude on the reliable conformity of the results of human herpes viruses types 4 (EBV), 5 (CMV) and 6 (HHP6) DNA concentration quantitative detection in clinical samples obtained using **the studied medical device** reagent kit for the qualitative and quantitative determination of human herpesvirus type 4, 5 and 6 (EBV, CMV, HHV6) DNA by polymerase chain reaction with real-time detection "Herpes-test" according to TS 21.20.23-055-97638376- 2022, produced by TestGene LLC and **a comparison kit:**

- Reagent kit for the detection and quantification of Epstein-Barr virus (EBV), cytomegalovirus (CMV) and herpes virus type 6 (HHV6) DNA in clinical material by polymerase chain reaction with real-time

hybridization-fluorescence detection "AmpliSens® EBV/CMV/HHV6-screen-FL" according to TS 9398-095-01897593-2012, produced by FBIS Central Research Institute of Epidemiology of Rospotrebnadzor (RU No. FSR 2010/09502 dated October 18, 2019),

when performing PCR analysis with **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;

- FLUORITE (Xian TianLong Science and Technology Co, China, RC No. RZN 2022/16415 dated January 24, 2022).

The systematic error of human herpes viruses type 4, 5 and 6 (CMV, EBV, 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.

Tested analyte	**Sample type**	**Unit**	**Used cycler**	**Number of samples**	**Coefficient of correlation**	**Intersection**	**Slope**
Human Herpes virus type 4 (CMV)	Whole blood	log10 copies /ml	DTprime	55	0.0137	0.9937	0.9967
			CFX 96	55	0.0169	0.9963	0.9963
			Rotor-Gene Q	55	0.0154	0.9948	0.9968
			Quant Studio 5	55	0.0394	0.9867	0.9949
			FLUORITE	55	0.0242	0.993	0.995
	Blood leukocytes	log10 copies /ml	DTprime	50	- 0,0143	1.0045	0.9961
			CFX 96	50	0.0298	0.9912	0.9955
			Rotor-Gene Q	50	- 0,0158	1.0035	0.997
			Quant Studio 5	50	- 0,0311	1.0086	0.9961
			FLUORITE	50	0.0749	0.9824	0.9948
	Oropharyn-geal swabs		DTprime	50	0.018	0.9939	0.9984
			CFX 96	50	0.0491	0.9914	0.9981

		log10 copies /ml	Rotor-Gene Q	50	- 0,0341	1.0064	0.9972
			Quant Studio 5	50	- 0,0115	1.0028	0.9977
			FLUORITE	50	- 0,0305	1.0097	0.9985
	Saliva	log10 copies /ml	DTprime	55	0.9961	0.9974	0.9961
			CFX 96	55	- 0,0043	1.0033	0.997
			Rotor-Gene Q	55	- 0,0483	0.9876	0.9938
			Quant Studio 5	55	- 0,0296	1.0073	0.9963
			FLUORITE	55	0.0011	1.0011	0.997
	Internal organs biopsy samples	log10 copies /ml	DTprime	14	- 0,0173	1.0056	0.9909
			CFX 96	14	- 0,0268	1.0085	0.9898
			Rotor-Gene Q	14	- 0,0218	1.0074	0.99
			Quant Studio 5	14	- 0,0287	1.0106	0.9913
			FLUORITE	14	0.0449	0.9846	0.9922
	Cerebro-spinal fluid	log10 copies /ml	DTprime	14	- 0,0466	1.0162	0.9959
			CFX 96	14	0.0558	0.9843	0.9952
			Rotor-Gene Q	14	- 0,0119	1.0004	0.9948
			Quant Studio 5	14	0.0563	0.9849	0.9959
			FLUORITE	14	- 0,0296	1.0085	0.9952
Human herpes virus type 5 (EBV)	Whole blood	log10 copies /ml	DTprime	47	-0.0351	1.0065	0.9975
			CFX 96	47	0.012	0.9956	0.9983
			Rotor-Gene Q	47	- 0,008	1.0028	0.9982
			Quant Studio 5	47	- 0,0085	1.0028	0.9982
			FLUORITE	47	0.0214	0.9932	0.9975
	Blood leukocytes	log10 copies /ml	DTprime	45	0.0043	0.9997	0.9954
			CFX 96	45	- 0,0079	1.0021	0.996
			Rotor-Gene Q	45	- 0,0603	1.0121	0.9947
			Quant Studio 5	45	- 0,0061	1.002	0.9954
			FLUORITE	45	0.0347	0.9904	0.9951
	Oropharyn-geal swabs	log10 copies /ml	DTprime	45	0.0129	1.9987	0.9976
			CFX 96	45	0.0281	0.9972	0.9972
			Rotor-Gene Q	45	-0.0085	1.0025	0.9978
			Quant Studio 5	45	0.0162	0.9963	0.9974
			FLUORITE	45	-0.0256	1.0065	0.9977
	Saliva		DTprime	45	0.0251	0.9933	0.9971

		log10 copies /ml	CFX 96	45	0.0644	0.9848	0.998
			Rotor-Gene Q	45	0.0325	0.9918	0.9977
			Quant Studio 5	45	0.0021	0.9977	0.9975
			FLUORITE	45	- 0,0005	1.0006	0.997
	Internal organs biopsy samples	log10 copies /ml	DTprime	15	- 0,0954	1.0214	0.997
			CFX 96	15	0.1045	0.9778	0.9956
			Rotor-Gene Q	15	0.0092	0.9977	0.9965
			Quant Studio 5	15	- 0,0505	1.0136	0.9961
			FLUORITE	15	0.069	0.9849	0.9956
	Cerebro-spinal fluid	log10 copies /ml	DTprime	15	- 0,0132	1.0044	0.998
			CFX 96	15	- 0,0195	1.0039	0.9964
			Rotor-Gene Q	15	0.0201	0.9959	0.9983
			Quant Studio 5	15	0.0426	0.989	0.9987
			FLUORITE	15	0.0254	0.993	0.9975
Human herpes virus type 6 (HHV6)	Whole blood	log10 copies /ml	DTprime	45	0.022	0.9954	0.9969
			CFX 96	45	0.0425	0.9916	0.9966
			Rotor-Gene Q	45	0.0076	0.9964	0.9963
			Quant Studio 5	45	0.0712	0.9829	0.9968
			FLUORITE	45	- 0,0127	1.0047	0.9971
	Blood leukocytes	log10 copies /ml	DTprime	40	0.0527	0.9862	0.9971
			CFX 96	40	0.0231	0.995	0.9967
			Rotor-Gene Q	40	0.1033	0.978	0.9966
			Quant Studio 5	40	0.0107	0.9918	0.9959
			FLUORITE	40	0.0122	0.9974	0.996
	Oropharyn-geal swabs	log10 copies /ml	DTprime	40	- 0,0087	1.0005	0.9981
			CFX 96	40	0.0643	0.9841	0.998
			Rotor-Gene Q	40	- 0,0151	1.0026	0.9984
			Quant Studio 5	40	0.0229	0.9901	0.9984
			FLUORITE	40	0.0064	0.9986	0.9979
	Saliva	log10 copies /ml	DTprime	45	- 0,0203	1.0037	0.9982
			CFX 96	45	0.0228	0.9956	0.998
			Rotor-Gene Q	45	0.0416	0.9877	0.9975
			Quant Studio 5	45	- 0,0175	1.0036	0.9975
			FLUORITE	45	0.0419	0.9884	0.9976

	Internal organs biopsy samples	log10 copies /ml	DTprime	14	0.015	0.9943	0.9977
			CFX 96	14	- 0,0287	1.0082	0.9981
			Rotor-Gene Q	14	- 0,006	0.9986	0.9975
			Quant Studio 5	14	0.0309	0.9937	0.9983
			FLUORITE	14	- 0,0226	1.0049	0.9977
	Cerebro-spinal fluid	log10 copies /ml	DTprime	14	0.0019	0.9978	0.9976
			CFX 96	14	- 0,0273	1.0043	0.9985
			Rotor-Gene Q	14	- 0,0282	1.0048	0.9977
			Quant Studio 5	14	0.0219	0.9972	0.9982
			FLUORITE	14	- 0,0203	1.0058	0.9973

4.3.3 Interlot correlation determination results

To **determine the interlot correlation** of measurement results in clinical samples in accordance with the international guidelines CLSI EP09–A3, a scattering diagram of the dependent variable X - DNA concentration of the tested analytes was drawn using the studied medical device "Herpes-test", manufactured by TestGene LLC, **LOT: 202309-305**, and Y - DNA concentration of the tested analytes using the studied medical device "Herpes-test", manufactured by TestGene LLC, **LOT: 202309-306**.

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.

Tested analyte	**Sample type**	**Unit**	**Used cycler**	**Number of samples**	**Coeffi-cient of correla-tion**	**Intersec-tion**	**Slope**
Human Herpes virus type 4 (EBV)	Whole blood	log10 copies /ml	DTprime	55	0.9861	-0.0632	1.019
			CFX 96		0.9857	0.0567	0.9872
			Rotor-Gene Q		0.9817	-0.0342	1.0118
			Quant Studio 5		0.9851	0,0347	0.9869
			FLUORITE		0.9851	0.0347	0.9869
	Blood leukocytes	log10 copies /ml	DTprime	50	0.9816	0.0536	0.9846
			CFX 96		0.9844	-0.1485	1.0334
			Rotor-Gene Q		0.9818	-0.0182	1.0065
			Quant Studio 5		0.9831	-0.0396	1.011
			FLUORITE		0.9831	-0.0396	1.011
	Oropharyn-geal swabs	log10 copies /ml	DTprime	50	0.9918	0.0622	0.9865
			CFX 96		0.9933	-0.018	1.0041
			Rotor-Gene Q		0.9926	-0.0323	1.0104

			Quant Studio 5		0.9815	0.1026	0.9736
			FLUORITE		0.9918	0.0622	0.9865
	Saliva	log10 copies /ml	DTprime	55	0.9873	-0.0089	1.0028
			CFX 96		0.9807	1.1555	0.9631
			Rotor-Gene Q		0.9892	-0.0519	1.0145
			Quant Studio 5		0.9842	0.0616	0.988
			FLUORITE		0.9846	0.0566	0.9878
	Internal organs biopsy samples	log10 copies /ml	DTprime	14	0.9811	0.0553	0.9836
			CFX 96		0.986	-0.0954	1.0297
			Rotor-Gene Q		0.9869	0.125	0.9604
			Quant Studio 5		0.9843	-0.0754	1.0258
			FLUORITE		0.9905	-0.0681	1.0226
	Cerebro-spinal fluid	log10 copies /ml	DTprime	14	0.9954	-0.0136	1.0047
			CFX 96		0.993	0.0311	0.9878
			Rotor-Gene Q		0.991	0.0267	0.9946
			Quant Studio 5		0.9939	-0.0445	1.0129
			FLUORITE		0.9905	-0.0681	1.0226
Human herpes virus type 5 (CMV)	Whole blood	log10 copies /ml	DTprime	47	0.9911	-0.0086	0.998
			CFX 96		0.9898	0.018	0.994
			Rotor-Gene Q		0.9937	0.0547	0.9864
			Quant Studio 5		0.9921	0.0108	0.9952
			FLUORITE		0.9932	-0.0475	1.012
	Blood leukocytes	log10 copies /ml	DTprime	45	0.9862	0.1499	0.9661
			CFX 96		0.9824	0.1936	0.9554
			Rotor-Gene Q		0.9887	-0.0315	1.0086
			Quant Studio 5		0.9862	-0.017	1.0014
			FLUORITE		0.9873	0.0439	0.9876
	Oropharyn-geal swabs	log10 copies /ml	DTprime	45	0.991	0.0797	0.9807
			CFX 96		0.9922	0.0904	0.9757
			Rotor-Gene Q		0.9909	0.063	0.9864
			Quant Studio 5		0.9886	0.0024	1.0042
			FLUORITE		0.9896	-0.1468	1.0312
	Saliva	log10 copies /ml	DTprime	45	0.9879	0.1678	0.9607
			CFX 96		0.9879	-0.0058	1.0019
			Rotor-Gene Q		0.9884	-0.0505	1.0125
			Quant Studio 5		0.9892	-0.0784	1.0195
			FLUORITE		0.997	-0.0005	1.0006
	Internal organs biopsy samples	log10 copies /ml	DTprime	15	0.9932	0.0228	0.9966
			CFX 96		0.9884	0.0372	0.9894
			Rotor-Gene Q		0.9822	0.0051	1
			Quant Studio 5		0.9903	-0.0703	1.016
			FLUORITE		0.9852	-0.0078	1.0029
	Cerebro-spinal fluid	log10 copies /ml	DTprime	15	0.9914	0.018	0.998
			CFX 96		0.9901	0.0307	0.9914
			Rotor-Gene Q		0.9862	0.0199	0.9946
			Quant Studio 5		0.9874	0.181	0.9573
			FLUORITE		0.9877	0.0649	0.9879

Human herpes virus type 6 (HHV6)	Whole blood	log10 copies /ml	DTprime	45	0.9827	-0.0712	1.0205
			CFX 96		0.9861	0.0178	0.9991
			Rotor-Gene Q		0.9848	0.0059	0.9979
			Quant Studio 5		0.9845	0.1182	0.9713
			FLUORITE		0.9824	-0.0598	1.0138
	Blood leukocytes	log10 copies /ml	DTprime	40	0.991	-0.0305	1.006
			CFX 96		0.9873	-0.0665	1.0135
			Rotor-Gene Q		0.9834	0.0096	0.9962
			Quant Studio 5		0.9873	-0.0492	1.0155
			FLUORITE		0.9835	-0.1423	1.0333
	Oropharyn-geal swabs	log10 copies /ml	DTprime	40	0.989	0.1001	0.9784
			CFX 96		0.9888	-0.0209	1.0039
			Rotor-Gene Q		0.9911	-0.0085	1.005
			Quant Studio 5		0.9899	0.0094	0.996
			FLUORITE		0.9911	-0.0329	1.0091
	Saliva	log10 copies /ml	DTprime	45	0.992	0.1195	0.9687
			CFX 96		0.9913	0.089	0.9788
			Rotor-Gene Q		0.9911	-0.058	1.0156
			Quant Studio 5		0.9909	-0.0305	1.0063
			FLUORITE		0.9909	-0.0319	1.0076
	Internal organs biopsy samples	log10 copies /ml	DTprime	14	0.9905	0.0917	0.9757
			CFX 96		0.9922	0.0716	0.9801
			Rotor-Gene Q		0.992	-0.0518	1.017
			Quant Studio 5		0.9919	0.0947	0.9759
			FLUORITE		0.9909	-0.0459	1.0165
	Cerebro-spinal fluid	log10 copies /ml	DTprime	14	0.9894	-0.0589	1.016
			CFX 96		0.9885	0.1312	0.9672
			Rotor-Gene Q		0.9892	0.0342	0.9854
			Quant Studio 5		0.9893	0.0751	0.9824
			FLUORITE		0.9901	-0.0059	0.9975

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 operation under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding the permissible levels;
3. Contamination of reaction mixtures and DNA test samples with contents from PC-1 or PC-2 tubes or 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 unqualified personnel work;
6. Use of an unsuitable kit (use after the expiry 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 DNA of human herpes viruses types 4, 5 and 6 (EBV, CMV, HHV6) by the polymerase chain reaction method with real-time detection "Herpes-test" according to TS 21.20.23-055-97638376-2022" is acceptable, the benefit of its use exceeds 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.

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

The reagents included in Herpes-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".

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 dated January 28, 2021 "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 of MU 287-113, MU 1.3.2569-09.

The following requirements should always be met when working:

- dispose of unused reagents in accordance with SanPiN 2.1.3684-21 dated January 28, 2021 "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";

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 the 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, and eye protection while handling samples and reagents. Wash your hands thoroughly after finishing work;

- all kit components are non-toxic to humans in the stated 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

The work with Herpes-test reagents kit is carried out in the working area 3 (for reaction preparation) (MU 1.3.2569-09).

Equipment:

1. Class II and III biosafety cabinet (e.g., microbiological safety boxes BMB-II-Laminar-C according to TS 32.50.50-010-51495026-2020, manufactured by Lamsystems, RC No. FSR 2012/13259 dated July 29, 2021 or Cabinet for sterile work DNA/RNA UV-Cleaner UVC/T-M-AR, Biosan, Latvia, RC No. RZN 2023/19369 dated January 18, 2023);

2. Vortex (e.g., Microspin 12 high-speed mini-centrifuge, BIOSAN SIA, Latvia, RC No. FSZ 2011/10116 dated July 11, 2011 or CM-70M centrifuge-mixer, manufactured by SIA ELMI, Latvia, RC No. RZN 2016/4616 dated May 31, 2023);

3. Variable volume dispensers that allow to take liquid volumes of 0.5–10 µl, 10-100 µl or 20-200 µl, 100-1000 µl (e.g., Eppendorf Research Plus, Germany, RC No. FSZ 2011/11028 dated November 15, 2011 or Biohit, Finland, RC No. FSZ 2012/12201 dated May 18, 2012);

4. Refrigerator from +2°C to +8°C with a freezer below -16°C (e.g., combined laboratory refrigerator XL-250 POZIS, XL-250-1 POZIS according to TS 9452-203-07503307-2012, manufactured by POZIS, RC No. RZN 2016/4043 dated May 8, 2019);

5. Cycler[6] with real-time fluorescence detection in channels corresponding to the FAM/Green, HEX/Yellow fluorophores:

- CFX96 (BioRad, USA, RC No. FSZ 2008/03399 dated June 21, 2016),
- DTprime (NPO DNA Technology LLC, Russia, RC No. FSR 2011/10229 dated March 3, 2011),
- Rotor-Gene Q (Qiagen, Germany, RC No. FSZ 2010/07595 dated August 10, 2010),
- QuantStudio 5 (Thermo Fisher Scientific, USA, RC No. RZN 2019/8446 dated June 6, 2019),
- FLUORITE (Xian TianLong Science and Technology Co, China, RC No. RZN 2022/16415 dated January 24, 2022).

Materials and reagents not included in the kit:

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

1. Disposable tips with an aerosol barrier up to 1000 µl, 200 µl, 20 µl and 10 µl (Axygen, USA, RC No. FSZ 2012/12077 dated February 27, 2014);

2. Disposable Eppendorf type 1.5–2.0 ml tubes (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014);

3. Thin-walled disposable PCR tubes with an optically transparent cap (Axygen, USA, RC No. FSZ 2012/11892 August 26, 2014):

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

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

4. Separate lab coat disposable talc-free gloves;

5. Container with disinfectant solution;

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

7. To take a swab from the oropharynx, it is recommended to use a "disposable sterile medical probe according to TS 32.50.13-002-28731857-2020", manufactured by Pharmmedpolis RT LLC, Russia (registration certificate No. RZN 2021/13989 dated December 9, 2022);

8. When collecting oropharyngeal swabs – use sterile saline solution or phosphate buffer solution (PBS);

9. DNA isolation kit (see Section 8.7 of the Instructions).

8. Test samples

Test sample type

PCR material is DNA samples isolated from whole blood, blood leukocytes, oropharyngeal swabs, saliva, biopsies of internal organs, and cerebrospinal fluid.

Material sampling for assay

ATTENTION! Before starting work, review the guidelines "Sampling, transportation and storage of clinical material for PCR diagnostics" developed by 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 III.

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 upside down 2-3 times.

ATTENTION! Heparin and sodium citrate cannot be used 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! Do not freeze or heat 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 from the collection moment. 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 1.5–2.0 ml sterile tube.

Storage conditions:

- at a temperature below -70°C - for a long time.

8.3 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 -20°C – up to 1 week;
- at -70° C – for a long time.

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

8.4 Oropharyngeal swabbing

Collect samples 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 cap.

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

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

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

8.5 Saliva collection

Before saliva collection, rinse the mouth three times with saline solution. Collect at least 1.0 ml of saliva in disposable sterile 2-5 ml plastic tubes. Close the tube tightly with a cap.

ATTENTION! Sample pre-processing is not required.

Material storage conditions:

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

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

8.6 Biopsies samples collection and preparation

Place puncture samples (microbioptates) in microtubes with screw caps or 1.5 ml Eppendorf type tubes containing 0.1 ml of transport medium.

ATTENTION! Sample pre-processing is not required.

Place macrobioptates – 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.

8.7 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 and oropharyngeal swabs as clinical material: a reagent kit for DNA/RNA isolation from the clinical samples "NA-Extra" according to TS 21.20.23-013-97638376-2019, manufactured by

TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated June 5, 2023);

- when using blood leukocytes, saliva, biopsy samples of internal organs, and cerebrospinal fluid as clinical material: a reagent kit for RNA/DNA isolation from clinical material "RIBO-sorb" according to TS 9398-004-01897593-2008 produced by FBIS 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 carry out all sample preparation stages for 100 µl negative control sample (NC), included in Herpes-test reagent kit.

DNA test samples storage conditions:

- at 2... 8°C – up to 1 day;
- at -18... -22°C – up to 1 month;
- at -70° C – for a long time.

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 DNase-free consumables when working with DNA. 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 6 in PCR tubes before testing.

Kit components preparation for testing

1. Mix thoroughly the contents of the tubes with DNA isolated for testing, NC, which have passed the DNA isolation stage, PC-1 (for qualitative and quantitative analysis), PC-2 (for quantitative analysis only), Primer Mix, PCR Buffer, turning each tube upside down 10 times or mixing using a vortex at low speed for 3-5 seconds, and then remove drops from the tube caps by short centrifugation;

2. Take the required number of strips or tubes for DNA test samples and DNA control samples amplification.

For qualitative analysis:

Number of samples + NC + PC-1.

For quantitative analysis:

Number of samples + NC + 2x PC-1 + 2x PC-2.

Before performing PCR, wet clean the PCR-box, as well as equipment and materials 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 amplification products hybridization-fluorescence detection in real time;
3. Results interpretation (described in detail in Section 11).

A) PCR preparation

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

To carry out one reaction, you need:

1. PCR Buffer – 5 µl;
2. Primer Mix – 5 µl;
3. Sample (test sample, PC-1 and PC-2, NC, which passed the DNA isolation stage) – 15 µl.

Total reaction volume – 25 µl.

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

FOR QUALITATIVE ANALYSIS

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

- Label 0.1-0.2 µl PCR tubes;
- In a separate disposable sterile 1.5-2.0 ml Eppendorf type tube, prepare the reaction mixture: (N+3) x5 µl of PCR Buffer + (N+3)x5 µl of Primer Mix, where N is the number of test samples. Mix using a vortex at low speed for 3-5 seconds, and then remove drops by short centrifugation;
- Add 10 µl of the prepared reaction mixture into each PCR tube;
- Add 15 µl of isolated DNA into the corresponding tubes for the test samples. Do not add DNA preparation into tubes with PC-1 and NC;
- Add PC-1 and NC into the corresponding tubes;
- To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a vortex microcentrifuge.

Table 7 – Tubes layout for qualitative analysis

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

FOR QUANTITATIVE ANALYSIS

Prepare reaction tubes according to Table 7 in the following order:

- Label 0.1-0.2 µl PCR tubes;
- In a separate disposable sterile 1.5-2.0 ml Eppendorf type tube, prepare the reaction mixture: (N+6) x5 µl of PCR Buffer + (N+6)x5 µl of Primer Mix, where N is the number of test samples. Mix using a vortex at low speed for 3-5 seconds, and then remove drops by short centrifugation;
- Add 10 µl of the prepared reaction mixture into each PCR tube;
- Add 15 µl of isolated DNA into the corresponding tubes for the test samples. Do not add DNA preparation into tubes with PC-1, PC-2 and NC;
- Add PC-1, PC-2 and NC into the corresponding tubes;
- To remove drops from the walls, centrifuge the tubes for 1-3 seconds using a vortex microcentrifuge.

Table 7 – Tubes layout for quantitative analysis

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

B) DNA PCR amplification with amplification products hybridization-fluorescence detection 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 heated lid;

2. Program the device to perform the corresponding program of amplification and fluorescent signal detection, following the instructions for the device used. Specify the analysis type – qualitative or quantitative with standards. PCR protocol is listed in Table 8;

Table 8 – PCR protocol for Herpes-test

Stage	Temperature, ˚C	Time, min.:sec.	Detection channels	Total cycles
1	95	02:00	-	1
2	95	00:15	-	5
	64	00:20	-	
3	95	00:15	-	40
	64	00:20	FAM, HEX, ROX, Cy5	

3. Specify number and identifiers of the samples, standards PC-1 and PC-2 with their concentrations (see Table 9), mark the tubes location on the thermal block matrix in accordance with their layout;

Table 9 – Calibration samples concentrations

Channel	Concentration (copies/ml)	
	PC-1	PC-2
FAM/Green (HHV6)	1 000 000 = 10^6	10 000 = 10^4
HEX/Yellow (*ALB*)		
ROX/Orange (HHV5)		
Cy5/Red (HHV4)		

4. Make sure that the following detection channels are involved in the optical measurement parameters of the amplification program: FAM/Green, HEX/Yellow, ROX/Orange and Cy5/Red;

5. Start PCR with a fluorescent signal detection;

6. Upon the program completion start analyzing the results.

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 ~5-10% of the maximum fluorescence level obtained for PC-1 in the last amplification cycle.

Interpret the results based on the Ct channel values shown in Table 1. Only the Ct values obtained at the PCR with fluorescence detection

stage are taken into account (that is, the corresponding stage 3 – see Table 8).

First, evaluate the reaction and Ct values in the control samples. Begin results interpretation in the tested samples only in case of PC-1, PC-2 and NC correct outcome.

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

Results interpretation in control samples

The following results should be obtained for negative and positive control samples (Table 10).

Table 10 – Study results for PC and NC

Control sample	Channel corresponding to the fluorophore			
	FAM/Green	HEX/Yellow	ROX/Orange	Cy5/Red
NC	Ct not indicated or > 35			
PC-1 and PC-2	Ct ≤ 32			

When obtained values NC differ from those indicated in Table 10, the results of the entire series are considered unreliable. In this case, it is required to take special measures to eliminate possible contamination.

When obtaining values for PC-1 and PC-2 that differ from those indicated in Table 10, repeat amplification of the entire samples batch.

When re-obtaining values for PC that differ from those indicated in Table 10, replace the reagents.

Results interpretation in DNA test samples

The results analysis during the qualitative assay is shown in Table 11.

Results analysis during quantitative analysis.

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

Based on the obtained Ct values for the calibration samples and their concentrations, it is necessary to draw a calibration line. When using a calibration line, the absolute concentrations of the tested samples are

calculated. For samples Ct ≤ 35 in the FAM, ROX, and Cy5 channels are considered.

PCR efficiency should be in 90-110% range, and the difference between Ct values of the repetitions of each positive control sample, PC-1 and PC-2, should not exceed 1.5. Otherwise, the analysis must be repeated from the DNA isolation stage.

Table – 11 The results interpretation principle during qualitative analysis

Ct values				Result
FAM/Green (HHV6)	HEX/Yellow (SAC)	ROX/Orange (HHV5)	Cy5/Red (HHV4)	
Ct ≤ 35	Not considered	Ct absent	Ct absent	Human herpes virus type 6 (HHV6) DNA **detected**
Ct absent	Not considered	Ct ≤ 35	Ct absent	Human herpes virus type 5 (HHV5) DNA **detected**
Ct absent	Not considered	Ct absent	Ct ≤ 35	Human herpes virus type 4 (HHV4) DNA **detected**
Ct ≤ 35	Not considered	Ct ≤ 35	Ct absent	Human herpes virus types 5 and 6 DNA **detected**
Ct absent	Not considered	Ct ≤ 35	Ct ≤ 35	Human herpes virus types 4 and 5 DNA **detected**
Ct ≤ 35	Not considered	Ct absent	Ct ≤ 35	Human herpes virus types 4 and 6 DNA **detected**
Ct ≤ 35	Not considered	Ct ≤ 35	Ct ≤ 35	Human herpes virus types 4, 5 and 6 DNA **detected**
Ct absent or Ct > 35	Ct ≤ 35	Ct absent or Ct > 35	Ct absent or Ct > 35	DNA of the corresponding herpes virus channels **not detected or below the detection limit**
Ct absent or Ct > 35	Ct > 35	Ct absent or Ct > 35	Ct absent or Ct > 35	**Invalid result for the corresponding herpes type**

If the target analyte concentration is in the range of $8x10^{2} – 1x10^{7}$ copies/ml, the exact concentration in copies/ml is indicated. If the concentration is less or greater than the specified range, the results are "concentration less than 800 copies/ml" or "concentration more than 1×10^{7} copies/ml", respectively, without specifying the exact value.

The relative concentration to estimate the viral load per 10^{5} human cells is calculated using the following formula:

$$\frac{\text{EBV/CMV/HHV6 DNA copy number per ml}}{\text{DNA copy number per ml}} * 2 * 10^{5}$$

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; noncompliance with the PCR temperature regime, etc. In case of an invalid 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 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 early herpesvirus infection diagnosis in patients regardless of the disease form and stage of all population groups and for choosing adequate therapy and evaluating its effectiveness in patients with the detected human herpes virus type 4, 5 or 6.

The results obtained with the kit should be used in combination with other data: symptoms, the general clinical picture, results from other test type, and the therapy used.

12. Reagent kit storage, transportation and operation conditions

Storage

Store Herpes-test reagent kit in the manufacturer's packaging at -16... -24°C during the entire kit shelf life, it can be stored at +2... +8°C for up to 14 days.

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

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

Transportation

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

Transport at -16... -24°C during the entire kit shelf life. Transportation is allowed at +2... +8°C for up to 14 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 Herpes-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 -16... -24°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, public premises, organization and conduct of sanitary and anti-epidemic (preventive) measures".

According to the medical waste classification, the kits belong to class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with clause 170 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 labelled 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 MU 287-113 (Guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices).

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

Herpes-test reagent kit tubes and packaging 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

The manufacturer guarantees Herpes-test reagent kit quality and safety during 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, please contact:

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

Technical Support Service:

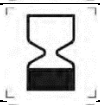
Phone number: +7 927 981 58 81
E-mail: help@testgen.ru

Annex A

Designation	Document name
GOST ISO 14971-2021	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 supporting documentation.
GOST R ISO 23640-2015	In vitro diagnostic medical devices. Stability evaluation of in vitro diagnostic reagents.
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 supplied 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 information to be supplied by the manufacturer. Part 1. General requirements.
GOST R ISO 17511-2022	In vitro diagnostic medical devices. Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human biological samples.
GOST ISO 13485-2017	Medical devices. Quality management systems. Requirements for regulatory purposes.

Annex B

Labelling symbols

	Consult instructions for use
	In vitro diagnostic medical device
	Temperature limitation
	Batch code or Lot number
	Use by…
	Date of manufacture
	Fragile, handle with care
	This icon shows the correct position of the load in space. This side up. Do not turn over or tip on its side a transport packaging with this symbol. Store and transport it vertically only.