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INSTRUCTIONS FOR USE

Reagent kit for the qualitative and quantitative determination of DNA of human herpesvirus type 4 (EBV) by polymerase chain reaction with real-time detection "EBV-test"

TS 21.20.23-057-97638376-2022

Version 3 dated 07.12.2023

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
SVC	sampling volume control
EBV (HHV4)	Epstein-Barr virus (EBV) or human herpesvirus 4 (HHV4)

Introduction

Target analyte: Human herpes virus 4 (EBV) genomic DNA specific region.

The scientific validity of the target analyte lies in the specificity (DNA sequence uniqueness) in relation to Human herpesvirus 4 (EBV) genome.

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

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

Indications for use: a reagent kit is recommended for use in clinical laboratory diagnostics for the examination of 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 herpesvirus 4 to choose an appropriate therapy and evaluate its effectiveness regardless of the disease form and stage in all population groups.

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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^{-} NA cells and contributes to suboptimal control of EBV in immunosuppressed children with post-transplant lymphoproliferative disorder // Front. Immunol. – 2020, №11.

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 kit is not sterile.

1. Intended use

1. Intended use: EBV-test reagent kit is designed for qualitative and quantitative determination of human herpesvirus 4 (EBV) DNA 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 herpesvirus 4 to choose an appropriate 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 appropriate therapy and evaluating its effectiveness in patients with detected human herpesvirus 4. The results are taken into account in the comprehensive diagnosis of the disease.

Potential consumers of a medical device:

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

2. Method principle

Method

Qualitative and quantitative PCR with real-time hybridization-fluorescence detection.

Test sample type

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

Detection principle

DNA amplification process happens in a reaction buffer using specific to the corresponding DNA regions primers and the *Taq* polymerase enzyme and consists of a series of DNA temperature denaturation and primer annealing repeated cycles.

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

The kit contains reagents for highly specific regions of human herpesvirus type 4 DNA, as well as human genomic DNA (the human *ALB* gene for sampling volume control, hereinafter referred to as SVC) detection (Table 1).

Table 1 – Test targets

Channel corresponding to the fluorophore	
FAM/Green	HEX/Yellow
EBV	SVC

SVC allows to evaluate DNA isolation efficiency and 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 stages. A false positive result can be detected with a negative control sample.

A reagent kit with an expired shelf life cannot be used.

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 the temperature regime violation 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 results from other test systems, the therapy used.

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

3. The reagent kit composition

Configuration form

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

Test sample number

Each EBV-test 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 test 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 composition

Table 2 – EBV-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 for a specific region of human herpesvirus 4 genomic DNA (FAM/Green);

2. Primers and a probe for SVC (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 herpesvirus 4 and the *ALB* gene region (hereinafter referred to as SVC).

Channel	**Concentration (copies/ml)**	
	PC-1	**PC-2**
FAM/Green (EBV)	1 000 000 = 10^6	10 000 = 10^4
HEX/Yellow (SVC)		

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 case of qualitative analysis, only PC-1 is used in one repetition. In 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 deionized DNase-free 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 – EBV-test reagent kit

Indicator	Characteristics and standards	Clause in TS
1. Technical characteristics		
1.1. 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
PC-1	Transparent colorless liquid	Section 7, clause 7.6
PC-2	Transparent colorless liquid	Section 7, clause 7.6
NC	Transparent colorless liquid	Section 7, clause 7.6
1.2. Completeness	Clause 1.4 TS 21.20.23-057-97638376-2022	Section 7, clause 7.9
1.3. Labelling	Clause 4 TS 21.20.23-057-97638376-2022	Section 7, clause 7.9
1.4. Packaging	Clause 5 TS 21.20.23-057-97638376-2022	Section 7, clause 7.9
2. Functional characteristics		
Positive result with PC-1	Fluorescence signal growth registered in tubes with PC-1 in the channels FAM, HEX Ct≤28.	Section 7, clause 7.7.2
Positive result with PC-2	Fluorescence signal growth registered in tubes with PC-2 in the channels FAM, HEX Ct≤32.	Section 7, clause 7.7.2
Negative result with NC	Ct is not indicated in tubes with NC in the FAM and HEX channels (i.e. no fluorescence accumulation curve)	Section 7, clause 7.7.2
Reaction in tubes with ESS-SC	In tubes with ESS-SC, Ct is not indicated in the FAM channel (i.e. no fluorescence accumulation curve), and Ct ≤ 32 in the HEX channel.	Section 7, clause 7.7.2
Reaction in tubes with ESS-SenC-4	In tubes with ESS-SenC-4 in the FAM channel Ct ≤ 35 and in the HEX channel Ct is not indicated (i.e. no fluorescence accumulation curve).	Section 7, clause 7.7.2
"Linearity" test	The correlation ratio of PC-1, PC-2 and a standard sample (SS) is not less than 0.98	Section 7, clause 7.7.2

Precision test: coefficient of variation (CV) under repeatability conditions	Ct coefficient of variation of each calibration sample PC-1 and PC-2 repetition (at least 4) under repeatability conditions is not more than 5%.	Section 7, clause 7.7.2
Concentration evaluation accuracy test	The obtained value of human herpesvirus 4 DNA concentration should correspond to the concentration given in a standard sample certificate ESS-1 (6 log10 copies/ml) and ESS-2 (4 log10 copies/ml), with ± 0.5 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 human herpesvirus 4 (EBV) DNA.

The absence of non-specific positive amplification results in the presence of following organisms and viruses in the genomic NA sample was shown: herpes simplex virus type 1 and 2, herpes simplex virus type 5, 6 and 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 international recommendations **CLSI EP-17A2**, the limit of detection (LOD) was established by the method of standard sample dilution analysis:

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain;

According to the study results, human herpesvirus 4 DNA detection limit in 100 µl samples with 95% detection rate when using NA-Extra isolation kits (RC No. RZN 2021/15428 dated June 5, 2023) and Ribo-sorb (RC No. FSR 2008/03993 dated February 22, 2019) for each cycler is:

Test analyte	Cycler used	Concentration, copies/ml (LOD) with 95% confidence probability	
		DNA isolation kit	
		NA-Extra	RIBO-sorb
Human herpesvirus 4 (EBV) 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 95%CI: 331,32 - 438,68 copies/ml	384 95%CI: 330.32 - 437.68 copies/ml

4.2.3 Quantification limit

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

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain;

According to the study results, human herpesvirus 4 DNA 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 June 5, 2023) and Ribo-sorb (RC No. FSR 2008/03993 dated February 22, 2019) for each cycler is:

Test analyte	Cycler used	Concentration, copies/ml (LOD) with 95% confidence probability	
		DNA isolation kit	
		NA-Extra	RIBO-sorb
Human herpesvirus 4 (EBV) 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

EBV-test linearity in accordance with GOST R 51352-2013 was determined by testing a series of dilutions of a standard sample:

- AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), 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 EBV-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 no more than ± 0.22 log10.

4.2.5 Precision under repeatability and reproducibility conditions:

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

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

4.2.6 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 valid 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 sampling procedure – 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 leucocytes, 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 sampling procedure		
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 leucocytes, 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 the analysis: 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 when sampling human venous blood.

Limitations on the test material use:

- blood samples collected in test tubes with heparin or sodium citrate as an anticoagulant cannot be used 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. The analysis of such samples may result in unreliable results;

4.2.7 Metrological traceability of the end–user IVD medical device calibrators - PC-1, PC-2, included in EBV-test reagent kit, and the used calibrators ESS-1, ESS-2 and ESS-SenC-4 was carried out in accordance with the Calibration Hierarchy with the reference measurement method (RMM) and the primary standard sample (SS) (clause 5.2 GOST R ISO 17511-2022).

The common calibration hierarchy, indicating the measurement uncertainty at each stage, is shown in Table 5.

Table 5 – Calibration hierarchy results

Sample type	Sample	Measurement procedure	Measurement uncertainty
Primary standard sample	AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Spain	Flow cytometric measurement, FCM	$u_{ref} = 0{,}5$
Primary calibrator	Rehydrated standard sample AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065)	The primary calibrator is prepared by rehydrating the lyophilized standard sample AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL (MBC065), manufactured by Vircell, Испания	$u_{p.2} = 0{,}1$
Secondary calibrator	Dilution panel of the rehydrated sample AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL	It was prepared by the primary calibrator dilution in accordance with the SS certificate	$u_{p.3} = 0{,}1$

	(MBC065), manufactured by Vircell, Spain		
Used calibrator	ESS-SenC-4, ESS-1, ESS-2	Manufacturer's measurement method - quantitative PCR with real-time hybridization-fluorescence detection	$u_{p.4} = 0{,}11$
IVD medical device calibrator of the end user	PC-1, PC-2	Manufacturer's measurement method - quantitative PCR with real-time hybridization-fluorescence detection	$u_{p.5} = 0{,}13$ $u_{cal} = 0{,}5$
Combined standard uncertainty			$u(y) = 0{,}5$
Expanded combined uncertainty			$U(y) = 1$
Maximum acceptable measurement uncertainty			$Umax(y) = 1$

The attributed concentration of end-user calibrators PC-1 is $1x10^{6}$ copies/ml, PC-2 is $1x10^{4}$ copies/ml with 0.5 log copies/ml uncertainty.

The combined standard measurement uncertainty for the recorded values of EBV DNA determined amount with the end-user EBV-test kit is u(y) = 0.5 log copies/ml.

4.2.8 Biological reference intervals

The biological reference interval of the EBV DNA level among 238 patients aged 0 to 67 years, based on clinical trials results, ranges from 2.71 to 5.9 log10 copies/ml. The EBV DNA concentration median in the sample is 3.89 log10 copies/ml.

4.3. Clinical efficiency characteristics

238 samples of human clinical material (55 - whole blood, 50 - blood leucocytes, 50 - oropharyngeal swabs, 55 - saliva, 14 - biopsies of internal organs, 14 - cerebrospinal fluid) were used for clinical trials. These samples were obtained from patients with a confirmed herpesvirus infection diagnosis caused by human herpesvirus 4 (EBV), regardless of the disease form and stage, from all population groups that were taken from a residual aliquots biological bank.

To evaluate diagnostic specificity and cross-reactivity in clinical trials, **158 samples of human clinical material** (35 whole blood, 31 blood leucocytes, 35 oropharyngeal swabs, 33 saliva, 12 biopsies of internal organs, 12 cerebrospinal fluid), not containing human herpesvirus 4 (EBV) DNA, but with the confirmed positive presence of genomic NA of the following organisms and viruses: human herpesvirus type 6, human herpesvirus type 5 (CMV), varicella zoster virus, parvovirus B19, herpes simplex virus type 1 and 2, herpesvirus type 8, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae were also examined with the tested kit EBV-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 with "Reagent kit for the qualitative and quantitative determination of human herpesvirus type 4 (EBV) DNA by polymerase chain reaction with real-time detection "EBV-test" according to TS 21.20.23-057-97638376-2022", produced by TestGene LLC and a comparison kit: "Reagent kit for the detection and quantitative detection of Epstein-Barr virus (EBV) DNA in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® EBV-screen/monitor-FL" according to TS 9398-086-01897593-2012, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor (RC No. FSR 2010/09503 dated February 27, 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:

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia);
- CFX 96 cycler (Bio-Rad, USA);
- Rotor-Gene Q cycler (Qiagen, Germany);
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA);
- 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	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	55	35	100% (95% CI:96.7%-100%)	100% (95% CI:94.87%-100%)
Blood leucocytes	50	31	100% (95% CI:96.38%-100%)	100% (95% CI:94.22%-100%)
Oropharyn-geal swabs	50	35	100% (95% CI:96.38%-100%)	100% (95% CI:94.87%-100%)
Saliva	55	33	100% (95% CI:96.7%-100%)	100% (95% CI:94.56%-100%)
Biopsies of internal organs	14 samples in three repetitions = 42	12 samples in three repetitions = 36	100% (95% CI:95.70%-100%)	100% (95% CI:95.01%-100%)
Cerebrospinal fluid	14 samples in three repetitions = 42	12 samples in three repetitions = 36	100% (95% CI:95.70%-100%)	100% (95% CI:95.01%-100%)

4.3.2 Method comparison: accuracy

Data obtained from testing **238 samples of human clinical material** (55 - whole blood, 50 – blood leukocytes, 50 - oropharyngeal swabs, 55 - saliva, 14 - biopsies of internal organs, 14 - cerebrospinal fluid) from patients with herpesvirus infection diagnosis caused by human herpesvirus type 4 (EBV), allow to conclude on the reliable conformity of the results of human herpesvirus type 4 (EBV) 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 (EBV) DNA by polymerase chain reaction with real-time detection "EBV-test" according to TS 21.20.23-057-97638376-2022", produced by TestGene LLC and a **comparison kit:**

- Reagent kit for the detection and quantification of Epstein-Barr virus (EBV) DNA in clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection "AmpliSens® EBV-screen/monitor-FL" according to TS 9398-086-01897593-2012, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor (RC No. FSR 2010/09503 dated February 27, 2019)

when performing PCR analysis using **cyclers:**

- DTprime detection 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 cycler (Xian TianLong Science and Technology Co, China).

The systematic error of human herpesvirus 4 (EBV) 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**	**Cycler used**	**Number of samples**	**Coefficient of correlation**	**Intersection**	**Slope**
EBV-test reagent kit, manufactured by TestGene LLC, **compared** with AmpliSens® EBV-screen/monitor-FL reagent kit, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor (RC No. FSR 2010/09503 dated February 27, 2019)	Whole blood	log10 copies /ml	DTprime	55	09982	-0.2235	1.0055
			CFX 96	55	0.9958	0.0138	0.9961
			Rotor-Gene Q	55	0.9961	0.0137	0.9967
			Quant Studio 5	55	0.9966	0.0379	0.9896
			FLUORITE	55	0.9959	-0.0129	1.003
	Blood leucocytes	log10 copies /ml	DTprime	50	0.9954	0.0095	0.998
			CFX 96	50	0.9946	0.0089	0.9984
			Rotor-Gene Q	50	0.9956	-0.0205	1.0038
			Quant Studio 5	50	0.9957	-0.034	1.0058
			FLUORITE	50	0.9957	0.0258	0.9946
	Oropharyn-geal swabs	log10 copies /ml	DTprime	50	0.9979	0.0403	0.9875
			CFX 96	50	0.9973	0.0048	0.9974
			Rotor-Gene Q	50	0.9973	0.032	0.9936

			Quant Studio 5	50	0.9975	0.0071	0.9998
			FLUORITE	50	0.9975	0.0277	1.007
	Saliva	log10 copies /ml	DTprime	55	0.9969	-0.0472	1.0115
			CFX 96	55	0.9957	0.0446	0.9884
			Rotor-Gene Q	55	0.9969	0.0423	0.9905
			Quant Studio 5	55	0.9954	0.0392	0.9917
			FLUORITE	55	0.997	-0.0109	1.0005
	Biopsies of internal organs	log10 copies /ml	DTprime	14	0.9908	0.0529	0.9817
			CFX 96	14	0.9917	-0.0164	1.0047
			Rotor-Gene Q	14	0.9892	0.0846	0.9761
			Quant Studio 5	14	0.991	-0.0466	1.0138
			FLUORITE	14	0.9924	-0.0665	1.0191
	Cerebrospi-nal fluid	log10 copies /ml	DTprime	14	0.9939	0.0503	0.9829
			CFX 96	14	0.995	0.0017	0.9982
			Rotor-Gene Q	14	0.9938	-0.0242	1.0068
			Quant Studio 5	14	0.996	-0.0054	1.0014
			FLUORITE	14	0.9949	0.0264	0.9896

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 - human herpesvirus type 4 (EBV) DNA concentration was drawn using the studied medical device "EBV-test", manufactured by TestGene LLC, **LOT: 202309-301**, and Y - human herpesvirus type 4 (EBV) DNA concentration using the studied medical device "EBV-test", manufactured by TestGene LLC, LOT: **202309-302**.

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

Sample type	Unit	Cycler used	Number of samples	Coefficient of correlation	Intersec-tion	Slope
Whole blood	log10 copies/ml	DTprime	55	0.9924	0.0257	0.9924
		CFX 96	55	0.984	-0.093	1.0204
		Rotor-Gene Q	55	0.9878	0.0954	0.9726
		Quant Studio 5	55	0.9802	0.0473	0.9901
		FLUORITE	55	0.9802	0.0473	0.9901

Blood leucocytes	log10 copies/ml	DTprime	50	0.986	-0.012	1.0049
		CFX 96	50	0.9818	0.1537	0.9621
		Rotor-Gene Q	50	0.9955	0.0175	0.9954
		Quant Studio 5	50	0.9843	-0.0338	1.0107
		FLUORITE	50	0.984	-0.0091	1.0022
Oropharyn-geal swabs	log10 copies/ml	DTprime	50	0.9857	-0.0029	1.001
		CFX 96	50	0.9968	0.022	0.9947
		Rotor-Gene Q	50	0.9899	0.0244	0.9937
		Quant Studio 5	50	0.9829	0.1535	0.9645
		FLUORITE	50	0.9882	-0.0203	1.0046
Saliva	log10 copies/ml	DTprime	55	0.9967	-0.0127	1.0018
		CFX 96	55	0.984	0.0489	0.9885
		Rotor-Gene Q	55	0.987	0.0996	0.9765
		Quant Studio 5	55	0.9858	0.0507	0.9892
		FLUORITE	55	0.9889	-0.0263	1.0022
Biopsies of internal organs	log10 copies/ml	DTprime	42	0.9952	0.0199	0.9929
		CFX 96	42	0.9929	0.0428	0.9856
		Rotor-Gene Q	42	0.9892	0.0722	0.9779
		Quant Studio 5	42	0.9899	-0.037	1.0123
		FLUORITE	42	0.9911	0.0646	0.9791
Cerebrospi-nal fluid	log10 copies/ml	DTprime	42	0.9961	0.0178	0.9922
		CFX 96	42	0.9935	-0.0427	1.0164
		Rotor-Gene Q	42	0.9909	-0.0514	1.0188
		Quant Studio 5	42	0.9849	0.0041	0.9987
		FLUORITE	42	0.9939	-0.0093	1.0015

The obtained data allow to conclude on the reliable conformity of the results of human herpesvirus type 4 (EBV) DNA concentration quantitative detection in clinical samples obtained with different lots of the studied medical device "Reagent kit for the qualitative and quantitative determination of human herpesvirus type 4 (EBV) DNA by polymerase chain reaction with real-time detection" EBV-test" according to TS 21.20.23-057-97638376-2022", produced by TestGene LLC.

5. Risks associated with the reagent kit use

The border risk zone includes the following hazards:

1. Loss of functional properties of the reagents included in the kit due to transportation, storage or use under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding the permissible levels;

3. Contamination of reaction mixtures and test DNA samples with contents from PC-1 and PC-2 tubes or with 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 DNA of the human herpesvirus type 4 (EBV) by polymerase chain reaction with real-time detection "EBV-test" according to TS 21.20.23-057-97638376-2022" 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 non-flammable and non-explosive. The reagents included in EBV-test kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in EBV-test kit 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 (zones). 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 stage of the process 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;

- only specially trained personnel is allowed 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 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

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 [4] 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 consumables with "DNase-free" label 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 tubes with an optically transparent lid for PCR (Axygen, USA, RC No. FSZ 2012/11892 dated August 26, 2014):
   - 0.2 ml PCR tubes,
   - 0.1–0.2 ml PCR tubes in strips,
   - PCR plates with optically transparent film.
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 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 Pharmedopolis RT LLC, Russia (registration certificate No. RZN 2021/13989 dated November 26, 2021);
8. When collecting oropharyngeal swabs – use sterile saline solution or phosphate buffer solution (PBS);

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

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 leucocytes, 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. 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! Heparin and sodium citrate cannot be used as an anticoagulant.

Initial clinical material transportation and storage conditions:

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

ATTENTION! Do freeze or heat the blood tube above 25°C.

Do not use hemolyzed and chylous blood. The analysis of such samples may result in invalid results!

8.2. Leucocytes collection

It is obtained from whole peripheral and/or umbilical blood. Blood can be stored at room temperature for up to 6 hours from the collection moment. To select leucocytes, centrifuge a blood tube for 20 minutes at 3,000 rpm. Using a filter tip, carefully collect 0.2 ml of the leukocyte mass from the surface of the cell sediment 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 collection

Collect at least 1.0 ml of 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 temperatures from 2 to 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/thaw the material only once.

8.4 Oropharyngeal swabbing

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 taking the sample, 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 temperatures from 2 to 8°C – up to 3 days;
- at -20°C – up to 1 week;
- at -70°C – for a long time.

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

8.5 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 2-5 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 temperatures from 2 to 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 lids 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 reagents 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 05, 2023);

- when using blood leucocytes, 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 EBV-test reagent kit.

Tested DNA samples storage conditions:

- at temperatures from 2 to 8°C – up to 1 day;
- at temperatures from -18 to -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 consumables with "DNase-free" label when working with DNA. It is mandatory to use a separate pipette tip with an aerosol barrier for each reaction component.

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

Kit components preparation for testing

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

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

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 the equipment and materials in it, using disinfectants suitable for use in PCR laboratories, and turn on the UV lamp for 20-30 minutes.

10. Testing procedure

The PCR test 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 pre-PCR area – a room for reagent dispensing and preparation for PCR amplification)

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

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 QUANTITATIVE ANALYSIS

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

- Label 0.1-0.2 µl PCR tubes;
- In a separate disposable sterile Eppendorf type 1.5-2.0 ml 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 tested samples. Mix on 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 appropriate tubes for the tested samples. Do not add DNA preparation into tubes with PC-1 and NC;
- Add PC-1 and NC into the appropriate tubes;
- To remove drop from the walls, centrifuge the tubes for 1-3 seconds on a vortex microcentrifuge.

Table – 5 Tubes layout for qualitative analysis

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

FOR QUANTITATIVE 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 Eppendorf type 1.5-2.0 ml 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 tested samples. Mix on 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 appropriate tubes for the tested samples. Do not add DNA preparation into tubes with PC-1, PC-2 and NC;

- Add PC-1, PC-2 and NC into the appropriate tubes;

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

Table 6 – 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 hybridization fluorescence detection of amplification products in real time;

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

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

2. Program the device to perform the corresponding amplification program and fluorescence signal detection, following the Instructions for the used device. Specify the analysis type: quantitative or qualitative with standards. PCR protocol is listed in Table 7;

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	00:20	-	
3	95	00:15	-	40
	64	00:20	FAM, HEX	

3. Specify samples number and identifiers, PC-1 and PC-2 standards and their concentrations (see Table 8), label the tubes location on the thermoblock matrix in accordance with their layout;

Table 8 – Calibration samples concentration

Channel	Concentration (copies/ml)	
	PC-1	PC-2
FAM/Green (EBV)	1 000 000 = 10^6	10 000 = 10^4
HEX/Yellow (*ALB*)		

4. Make sure that the following detection channels are included in optical measurement parameters of the amplification program.

FAM/Green и HEX/Yellow;

5. Start PCR with a fluorescent signal detection.

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

11. Result registration and interpretation

Results registration is carried out automatically during amplification with the used device software.

Recommendations on threshold line setting

For cyclers of any models, a threshold line is set individually for each detection channel at a level corresponding to 5-10% of the maximum fluorescence level obtained for PC-1 in the last amplification cycle.

Result interpretation is performed using Ct channel values shown in Table 1. Only Ct values obtained at a PCR stage with fluorescence detection are taken into account (i.e., corresponding to stage 3 – see Table 7).

First, evaluate reaction and Ct values in the control samples. Test samples results interpretation begins only after correct PC-1, PC-2 and NC reactions.

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

Result interpretation in control samples

The following results should be obtained for negative and positive control samples (Table 9).

Table 9 – Test results for PC and NC

Control sample	Channel corresponding to the fluorophore	
	FAM/Green	HEX/Yellow
NC	Ct not indicated or > 35	

PC-1 and PC-2	Ct ≤ 32

When the obtained values for NC differ from those indicated in Table 9, the results of the entire production series are considered invalid. In this case, it is required to take special measures to eliminate possible contamination.

When obtaining values for PC that differ from those indicated in Table 9, it is required to repeat amplification of the entire samples batch.

When re-obtaining values for PC that differ from those indicated in Table 9, it is required to replace the reagents.

Result interpretation in DNA test samples

Result analysis during the qualitative analysis is shown in Table 10.

Table – 10 Result interpretation principle during qualitative analysis

Ct values		**Result**
FAM/Green (EBV)	**HEX/Yellow (SVC)**	
Ct ≤ 35	Not considered	Human herpesvirus 4 (EBV) DNA **detected**
Ct absent or Ct > 35	Ct ≤ 35	Human herpesvirus 4 DNA **not detected or below the detection limit**
Ct absent or Ct > 35		**Invalid** result

Results analysis during quantitative analysis.

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

It is required to draw a calibration line based on the obtained Ct values for calibration samples and their concentrations. When using a calibration line, the absolute concentrations of the tested samples are calculated. Ct values ≤ 35 in the FAM channel are taken into account for the samples.

PCR efficiency should be in 90-110% range, difference between Ct values of the repetitions of each positive control sample, PC-1 and PC-2, should be less than 1. Otherwise, reperform the test, starting from the DNA isolation stage.

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 "concentration less than 800 copies/ml" or "concentration more than 1×10^7 copies/ml" respectively, are issued without specifying the exact value.

Relative concentration for assessing the viral load per 10^5 human cells is calculated using the following formula:

$$\frac{number\ of\ EBV\ DNA\ copies\ in\ ml}{number\ of\ human\ DNA\ copies\ in\ ml} * 2 * 10^5$$

An invalid result may be obtained due to the presence of inhibitors in DNA obtained from clinical material, test protocol incorrect implementation, non-compliance with the PCR temperature regime, etc. In this case, the conclusion is not issued, it is required to retake the biomaterial from the patient and retest it.

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

Diagnostic value of the obtained assay result:

The obtained assay result can be used by a qualified specialist (doctor) in combination with other data (the common clinical picture and other assay types) for early diagnosis of herpesvirus infection in patients regardless of the disease form and stage in all population groups and for choosing an appropriate therapy and evaluating its effectiveness in patients with detected human herpesvirus 4.

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

12. Storage, transportation and usage conditions

Storage

Store EBV-test reagent kit in the manufacturer's package at -16°C…-24°C during the entire kit shelf life, it is allowed to store at 2°C…8°C up to 14 days.

It is not allowed to freeze/thaw EBV-test reagent kit more than 10 times.

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

Transportation

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

Transport at -16°C…-24°C during the entire kit shelf life. Transportation is allowed at 2°C…8°C up to 14 days. Atmospheric pressure is not subject to control, as it does not affect the device quality.

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

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

Shelf life

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

Shelf life of opened kit components

12 months from the acceptance date of the manufacturer's QCD, if stored at -16°C…-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 production and 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 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 production and 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 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.

Tubes and packaging of EBV-test reagent kit 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 EBV-test reagent kit quality and safety during the shelf life in compliant with the kit transportation and storage requirements, as well as rules of operation.

In case of 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
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, kits, the test-systems, control materials, culture media. Requirements to devices and to supporting documentation.
GOST R ISO 23640-2015	In vitro diagnostic medical devices. Evaluation of stability 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 23640-2015	In vitro diagnostic medical devices. Evaluation of stability of in vitro diagnostic reagents.
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 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

Labeling 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.