INSTRUCTIONS FOR USE

Reagent kit for the qualitative and quantitative determination of DNA of the human herpesvirus type 5 (CMV) by polymerase chain reaction with real-time detection "CMV-test"

TS 21.20.23-058-97638376-2022

Version 3 dated 07.12.2023

Content

List of abbreviations

The following abbreviations and designations are used in this instruction:

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
CMV, HHV5	cytomegalovirus, human herpesvirus 5
SC	specificity control
SenC	sensitivity control

Introduction

Target analyte: a specific region of the human herpesvirus type 5 (CMV) genomic DNA.

The scientific validity of the target analyte lies in the specificity (DNA sequence uniqueness) in relation to the human herpesvirus type 5 (CMV) genome.

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

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

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

Indications for use: clinical material testing (whole blood, leukocytes, oropharyngeal swabs, saliva, biopsies samples of internal organs, cerebrospinal fluid) in patients with suspected herpesvirus infection and patients with detected human herpesvirus type 5 to choose an appropriate therapy and evaluate its effectiveness regardless of the disease form and stage in all population groups.

Contraindications for use: when used by specially trained personnel and taking into account the intended use have not been identified.

Population, demographic aspects of the medical device use: no population, demographic aspects of the reagent kit use were identified.

Sterility: the device is not sterile.

[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).

1. Intended use

Intended use: CMV-test reagent kit is designed for qualitative and quantitative determination of human herpesvirus type 5 (CMV) DNA by polymerase chain reaction with real-time hybridization and fluorescence detection in a DNA sample isolated from clinical material (whole blood, leukocytes, oropharyngeal swabs, saliva, biopsies samples of internal organs, cerebrospinal fluid), in patients with suspected herpesvirus infection and patients with detected human herpesvirus type 5 to choose an appropriate therapy and evaluate its effectiveness regardless of the disease form and stage in all population groups.

Functional use: the results obtained can be used for herpesvirus infection early diagnosis 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 type 5. The results are taken into account in the complex disease diagnostics.

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 clinical laboratory diagnostics doctor, a 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, leukocytes, oropharyngeal swabs, saliva, biopsies samples of internal organs and cerebrospinal fluid.

Detection principle

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

PCR mixture contains fluorescently labeled oligonucleotide probes that hybridize with a complementary region of the amplified DNA target and are destroyed by *Taq* polymerase, resulting in the fluorescent dye

and quencher separation, and an increase in fluorescence intensity. This allows to register the specific amplification product accumulation by measuring the fluorescent signal intensity in real time.

The kit contains reagents for determination of human herpes virus type 5 highly specific DNA regions, as well as human genomic DNA (human *ALB* gene for sampling volume control, hereinafter SVC) (Table 1)

Table 1 – Test targets

A channel corresponding to a fluorophore	
FAM/Green	HEX/Yellow
CMV	SVC

SVC allowes to evaluate the DNA isolation effectiveness and the inhibitors possible presence in the sample, the presence of 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.

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 regulated regime cannot be used.

A clinical diagnosis cannot be based on the testing results using this kit only. For diagnostic purposes, the results should be combined with other data: symptoms, the general clinical picture, the testing results by other test systems and the therapy used.

The total testing time is 65 minutes (excluding sample preparation).

3. Reagent kit components

Configuration form

A reagent kit is available in one configuration form.

Number of test samples

Each kit contains reagents designed for 96 reactions, which equates to:

When conducting a qualitative analysis

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

When conducting quantitative analysis

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

Reagent kit components

Table 2 – 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 lilac shade	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 a ready for use buffer containing all the basic reagents, including a thermostable hot start DNA polymerase, deoxynucleotide triphosphates (dNTP), uracil DNA glycosylase and an optimized for PCR buffer.

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

1. Primers and a probe for a specific region of human herpesvirus type 5 (CMV) genomic DNA, detection is carried out in the FAM/Green channel;
2. Primers and a probe for SVC, detection is carried out in the HEX/Yellow channel.

Positive control samples (PC-1 and PC-2) are ready for use and are a mixture of plasmid DNA with amplified DNA fragments synthetic insertions: the human herpesvirus type 5 specific DNA fragments and the *ALB* gene section (hereinafter SVC).

Channel	Concentration (copies/ml)	
	PC-1	PC-2
FAM/Green (CMV)	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. For the qualitative analysis only PC-1 is used in one repetition. For the quantitative analysis PC-1 and PC-2 are used, each in two repetitions.

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

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

4. Reagent kit characteristics

4.1. Technical and functional characteristics

Table 3 – 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 lilac shade
PC-1	Transparent colorless liquid
PC-2	Transparent colorless liquid
NC	Transparent colorless liquid
1.2. Completeness	According to Clause 1.4 TS 21.20.23-058-97638376-2022
1.3. Labeling	According to Clause 4 TS 21.20.23-058-97638376-2022
1.4. Packaging	According to Clause 5 TS 21.20.23-058-97638376-2022
2. Functional characteristics	
Positive result with PC-1	Fluorescence signal growth registration in tubes with PC-1 in the FAM, HEX channels Ct ≤ 28
Positive result with	Fluorescence signal growth registration in tubes with

PC-2	PC-2 in the FAM, HEX channel Ct ≤ 32
Negative result with NC	In tubes with NC in the FAM and HEX channels Ct is not indicated (i.e. there is no fluorescence accumulation curve) or > 35
Reaction in tubes with ESS-SC	In tubes with ESS-SC, Ct is not indicated in the FAM channel (i.e. there is no fluorescence accumulation curve), and Ct ≤ 32 via the HEX channel.
Reaction in tubes with ESS-SenC-5	In tubes with ESS-SenC-5, the Ct ≤ 35 in the FAM channel, and Ct is not indicated via the HEX channel (i.e. there is no fluorescence accumulation curve)
"Linearity" test	Correlation coefficient of PC-1, PC-2 and a standard sample (SS) is at least 0.98
Precision test: coefficient of variation (CV) under repeatability conditions	The coefficient of variation Ct for repetitions of each calibration sample PC-1 and PC-2 (at least 4) under repeatability conditions is not more than 5%.
Accuracy of concentration detection test	The obtained value of human herpesvirus type 5 DNA concentration must correspond to the concentration given in the standard sample certificate ESS-1 (6 log10 copies/ml) and ESS-2 (4 log10 copies/ml) with a tolerance of ± 0.5 log10 copies/ml concentration

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

4.2. Analytical efficiency characteristics

4.2.1. Analytical specificity

The kit is specific to human herpesviruses type 5 DNA.

There were no nonspecific positive amplification results in the presence of the following organisms and viruses in the genomic DNA sample: herpes simplex virus type 1 and 2, herpes simplex virus type 4, 6 and 8, Varicella zoster virus, Parvovirus B19, *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus agalactiae*.

4.2.2 Limit of detection

According to GOST R 51352-2013 and taking into account the international recommendations **CLSI EP-17A2** the detection limit (LOD) was determined by the standard sample AMPLIRUN

CYTOMEGALOVIRUS DNA CONTROL MBC016, manufactured by Vircell, Spain, dilution analysis method.

According to the analysis results, the detection limit of human cytomegalovirus DNA in 100 µl samples with 95% detection rate when using isolation kits NA-Extra (RU No. RZN 2021/15428 dated June 05, 2023) and Ribo-Sorb (RU No. FSR 2008/03993 dated February 22, 2019) for each cycler is:

Cycler used	A reagent kit for DNA/RNA isolation from the clinical material "NA-Extra" according to TS 21.20.23-013-97638376-2019		A reagent kit for RNA/DNA isolation from clinical material Ribo-Sorb according to TS 9398-004-01897593-2008	
	Concentration, copies/ml (LOD) with 95% confidence probability	Confidence interval with 95% confidence probability	Concentration, copies/ml (LOD) with 95% confidence probability	Confidence interval with 95% confidence probability
DTprime	382	328.32-435.68	364	310.32-417.68
CFX 96	374	320.32-427.68	377	323.32-430.68
Rotor-Gene Q	379	325.32-432.68	376	322.32-429.68
Quant Studio 5	384	330.32-437.68	381	327.32-434.68
FLUORITE	371	317.32-424.68	373	319.32-426.68

4.2.3 Limit of quantitation (LOQ)

In accordance with GOST R 51352-2013 and taking into account the international recommendations **CLSI EP-17A2**, the limit of quantitation (LOQ) was determined by the standard sample AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL MBC016, manufactured by Vircell, Spain, dilution analysis method.

According to the analysis results, limit of quantification (LOQ) of human cytomegalovirus DNA in 100 µl samples with 95% detection rate when using isolation kits NA-Extra (RU No. RZN 2021/15428 dated June 05, 2023) and Ribo-Sorb (RU No. FSR 2008/03993 dated 02/22/2019) for each cycler is:

Cycler used	A reagent kit for DNA/RNA isolation from the clinical material "NA-Extra" according to TS 21.20.23-013-97638376-2019		A reagent kit for RNA/DNA isolation from clinical material Ribo-Sorb according to TS 9398-004-01897593-2008	
	Concentration, copies/ml (LOQ) with 95% confidence probability	Confidence interval with 95% confidence probability	Concentration, copies/ml (LOQ) with 95% confidence probability	Confidence interval with 95% confidence probability
DTprime	800.4	746.72-854.08	800.7	747.02-854.38
CFX 96	803.8	750.12-857.48	801.2	747.52-854.88
Rotor-Gene Q	803.7	750.02-857.38	799.8	746.12-853.48

Quant Studio 5	802.2	748.52-855.88	796.1	742.42-849.78
FLUORITE	800.4	746.72-854.08	800.7	747.02-854.38

4.2.4 Linear measuring range

The linear measurement range was verified using the standard sample AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL MBC016, manufactured by Vircell, Spain.

According to GOST R 51352-2013 and taking into account the international recommendations **CLSI EP09-A3** using the regression and correlation method, the correlation coefficients for each cycler when using NA-Extra isolation kits (RU No. RZN 2021/15428 dated June 05, 2023) and Ribo-Sorb (RU No. FSR 2008/03993 dated February 22, 2019) correspond to strong correlation of human cytomegalovirus DNA concentration in control samples.

Based on the results of the linear range assay, it can be concluded that for 100 µl samples, the testing results with CMV-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 of not 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 not more than 3%;

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

4.2.6 Interfering substances influence and limitations on the test material use

The effect of potentially interfering substances on the reagent kit performance has been tested for potentially interfering substances that may occur during the reagent kit normal use, and presumably affect the reagent kit ability to give reliable results.

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

1) substances used in a 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 tested 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, leukocytes, oropharyngeal swabs, saliva, biopsies samples 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		
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
Medications prescribed for herpesvirus infection		
whole blood, leukocytes, oropharyngeal swabs, saliva, biopsies samples of internal organs, cerebrospinal fluid	Acyclovir	2.37 µg/100 µl
	Lactoferrin	0.1 µg/100 µl

Based on the assay results, anticoagulants – heparin at 0.015 IU/100 µl concentration and sodium citrate at 0.01 mM/100 µl concentration - were classified as PCR inhibitors during the assay. It is not allowed to use heparin and sodium citrate as an anticoagulant when taking human venous blood.

Limitations on test material use:

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

- do not use the test material in case of storage and transportation conditions violation (temperature, duration, repeated freezing and thawing);

- do not use samples contaminated with extraneous biological material;

- do not use hemolyzed and chylous blood. The testing of such samples may result in unreliable results!

4.2.7 Metrological traceability of the end–user kit IVD calibrators – PC-1, PC-2, included in CMV-test reagent kit, and the used calibrators ESS-1, ESS-2 and ESS-Senc-5 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 general calibration hierarchy, indicating the measurement uncertainty at each stage, is shown in Table 5.

Table 5 – Calibration hierarchy results

Sample type	Sample	Measurement methods	Measurement uncertainty
Primary standard sample	AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain	Flow cytometry, FCM	$u_{ref} = 0.5$
Primary calibrator	Rehydrated standard sample AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016)	The primary calibrator is prepared by rehydrating the lyophilized SS AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain	$u_{p.2} = 0.1$
Secondary calibrator	Dilution panel of the rehydrated sample AMPLIRUN CYTOMEGALOVIRUS DNA CONTROL (MBC016), manufactured by Vircell, Spain	It was prepared by the primary calibrator dilution, in accordance with the SS certificate	$u_{p.3} = 0.1$
Used calibrator	ESS-SenC-5, ESS-1, ESS-2	Manufacturer's measurement method	$u_{p.4} = 0.20$

		- quantitative PCR with real-time hybridization-fluorescence detection	
Calibrator kit IVD of the end-user	PC-1, PC-2	Manufacturer's measurement method - quantitative PCR with real-time hybridization-fluorescence detection	$u_{p.5} = 0.17$ $u_{cal} = 0.5$
Combined standard uncertainty			$u(y) = 0.5$
Expanded combined uncertainty			$U(y) = 1$
Maximum acceptable measurement uncertainty			*Umax(y)* = 1

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

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

4.2.8 Biological reference intervals

The biological reference interval for CMV DNA level among 211 patients aged 7 to 45 years, as determined by the clinical trials results, is from 2.71 to 5.44 log10 copies/ml. CMV DNA concentration median in the sample is 4.11 log10 copies/ml.

4.3. Clinical efficiency characteristics

For clinical assays, 212 samples of human clinical material were used (47 - whole blood, 45 - leukocytes, 45 - oropharyngeal swabs, 45 - saliva, 15 - biopsies samples of internal organs, 15 - cerebrospinal fluid), from patients diagnosed with herpesvirus infection caused by the Human Herpes Virus type 5 (CMV), regardless of the disease form and stage in all population groups.

To evaluate diagnostic specificity and cross-reactivity in clinical assays 116 samples of human clinical material were also tested using CMV-test reagent kit (25 - whole blood, 23 - leukocytes, 24 - oropharyngeal swabs, 24 - saliva, 10 - biopsies of internal organs, 10 - cerebrospinal fluid) not containing HHV-5 DNA, but with confirmed

genomic NA positive presence of the following organisms and viruses: herpes simplex virus type 1 and 2, herpes simplex virus type 8, Varicella zoster virus, *Parvovirus* B19, *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus agalactiae*.

CLSI EP09-A3 International Guideline recommends to conduct clinical assays on at least 40 clinical samples. **According to CLSI EP09-A3 recommendations to conduct clinical assays using internal organ and cerebrospinal fluid biopsy samples on a larger sample size**, **each sample was tested in 3 repetitions** starting from the DNA isolation procedure.

Each sample was tested in two series with a "Reagent kit for the qualitative and quantitative determination of DNA of the human herpesvirus type 5 (CMV) by polymerase chain reaction with real-time detection "CMV-test" according to TS 21.20.23-058-97638376-2022", produced by TestGene LLC and a comparison kit:

- "Reagent kit for the qualitative and quantitative detection of human cytomegalovirus (*CMV*) DNA in the clinical material by polymerase chain reaction (PCR) with hybridization-fluorescence detection «AmpliSens® *CMV*-screen/monitor-FL", manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (RU No. FSR 2010/09504 dated 04.03.2019).

The proof of the studied medical device correct operation was the results compliance.

The following cyclers, recommended by the studied reagent kit manufacturer, were used for PCR assay:

- 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 are calculated using the Clopper-Pearson Confidence Interval (Clopper, C., & Pearson, E. (1934)). The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. Biometrika,26(4), 404-413. doi:10.2307/2331986). The diagnostic characteristics of the studied reagent kit were calculated with 95% confidence probability.

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

Test material	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	94	50	100% (95% CI:96.15%-100%)	100% (95% CI:92.89%-100%)
Leukocytes	90	46	100% (95% CI:95.98%-100%)	100% (95% CI:92.29%-100%)
Oropharyngeal swabs	90	48	100% (95% CI:95.98%-100%)	100% (95% CI:92.60%-100%)
Saliva	90	48	100% (95% CI:95.98%-100%)	100% (95% CI:92.60%-100%)
Biopsies samples of internal organs	90	20	100% (95% CI:95.98%-100%)	100% (95% CI:83.16%-100%)
Cerebrospinal fluid	90	20	100% (95% CI:95.98%-100%)	100% (95% CI:83.16%-100%)

4.3.2 Methods comparison: accuracy

Data obtained from testing **212 samples of human clinical material** (47 - whole blood, 45 - leukocytes, 45 - oropharyngeal swabs, 45 - saliva, 15 - biopsies samples of internal organs, 15 - cerebrospinal fluid) from patients diagnosed with herpesvirus infection caused by human herpesvirus type 5 (CMV), allow to conclude on the reliable conformity of the results of human herpesvirus type 5 (CMV) DNA concentration quantitative detection in clinical samples obtained using the **tested medical device** "Reagent kit for the qualitative and quantitative determination of DNA of the human herpesvirus type 5 (CMV) by the polymerase chain reaction with real-time detection CMV-test according to TS 21.20.23-058-97638376-2022", produced by TestGene LLC and a **comparison kit:**

- Reagent kit for the qualitative and quantitative detection of human cytomegalovirus (*CMV*) DNA in the clinical material by polymerase

chain reaction (PCR) with hybridization-fluorescence detection «AmpliSens® *CMV*-screen/monitor-FL», manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor, Russia (RU No. FSR 2010/09504 dated 04.03.2019).

Cyclers used for carrying out PCR testing**:**

- Detecting cycler DTprime (NPO DNA Technology LLC, Russia), registration certificate No. FSR 2011/10228 dated March 03, 2011;
- CFX 96 cycler (Bio-Rad, USA), registration certificate No. FSZ 2008/03399 dated June 21, 2016;
- Rotor-Gene Q cycler (Qiagen, Germany), registration certificate No. FSZ 2010/07595 dated August 10, 2010;
- QuantStudio 5 cycler (Thermo Fisher Scientific, USA), registration certificate No. RZN 2019/8446 dated June 06, 2019;
- FLUORITE cycler (Xian TianLong Science and Technology Co, China, registration certificate No. RZN 2022/16415 dated January 24, 2022).

The systematic error in measuring the logarithm of CMV DNA concentration does not exceed 3%.

The statistical processing results of the obtained data on methods comparison (accuracy) according to CLSI EP09-A3 document recommendations using the regression and correlation method.

	Sample type	**Unit**	**Cycler used**	**Number of samples**	**Correlation ratio**	**Intersection**	**Slope**
CMV-test reagent kit, manufactured by TestGene LLC, **in comparison** with "AmpliSens® *CMV*-screen/monitor-FL" reagent kit, manufactured by the Central Research Institute of Epidemiology of Rospotrebnadzor,	Whole blood	log10 copies /ml	DTprime	47	0.9977	0.036	1.0093
			CFX 96	47	0.997	0.0245	1.0064
			Rotor-Gene Q	47	0.9977	0.0572	1.0136
			Quant Studio 5	47	0.9975	0.0401	1.0103
			FLUORITE	47	0.998	0.007	1.0024
	Leukocytes	log10 copies /ml	DTprime	45	0.9952	0.0714	0.9856
			CFX 96	45	0.9968	0.0579	0.9874
			Rotor-Gene Q	45	0.9959	0.0534	0.9891
			Quant Studio 5	45	0.9955	0.0487	0.9913
			FLUORITE	45	0.9949	0.063	0.9874
	Oropharyngeal swabs	log10 copies	DTprime	45	0.9966	0.0154	0.9985
			CFX 96	45	0.9977	0.0192	1.0052

Russia, (RU No. FSR 2010/09504 dated 04.03.2019)		/ml	Rotor-Gene Q	45	0.9963	0.0225	0.9974
			Quant Studio 5	45	0.9974	0.0207	1.0064
			FLUORITE	45	0.9976	0.0125	1.004
	Saliva	log10 copies /ml	DTprime	45	0.9965	0.1184	0.9757
			CFX 96	45	0.9969	0.0929	0.9801
			Rotor-Gene Q	45	0.9969	0.0827	0.983
			Quant Studio 5	45	0.9969	0.0629	0.9862
			FLUORITE	45	0.9965	0.1048	0.9773
	Biopsies samples of internal organs	log10 copies /ml	DTprime	45	0.9941	0.0365	0.9915
			CFX 96	45	0.9939	0.0189	0.9955
			Rotor-Gene Q	45	0.9955	0.0361	0.9936
			Quant Studio 5	45	0.9959	0.019	0.9973
			FLUORITE	45	0.9952	0.0172	0.9971
	Cerebro spinal fluid	log10 copies /ml	DTprime	45	0.9961	0.0164	0.9952
			CFX 96	45	0.9968	0.0558	0.9856
			Rotor-Gene Q	45	0.9972	0.0309	0.9929
			Quant Studio 5	45	0.9975	0.0412	0.9902
			FLUORITE	45	0.997	0.0261	0.9936

4.3.3 The interlot correlation detection results

To **determine the interlot correlation** of measurement results in clinical samples in accordance with the international guideline CLSI EP09-A3, a scattering diagram of the dependent variable X – CMV DNA concentration was constructed using the test kit CMV-test manufactured by TestGene LLC, **LOT: 202309-303**, and Y – CMV DNA concentration using the test kit CMV-test, manufactured by TestGene LLC, **LOT: 202309-304**.

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

Sample type	Unit	Cycler used	Number of samples	Correlation ratio	Intersection	Slope
Whole blood	log10 copies/ml	DTprime	47	0.9968	0.0207	0.9936
		CFX 96	47	0.9964	0.0313	0.9937
		Rotor-Gene Q	47	0.9973	0.0095	0.9982
		Quant Studio 5	47	0.997	0.0141	0.9984
		FLUORITE	47	0.9975	0.0312	0.9938
Leukocytes	log10 copies/ml	DTprime	45	0.9945	0.06	1.0153
		CFX 96	45	0.9935	0.0397	0.9934
		Rotor-Gene Q	45	0.9936	0.0003	1.0007
		Quant Studio 5	45	0.9938	0.0317	0.992
		FLUORITE	45	0.994	0.0012	0.9999
Oropharyngeal swabs	log10 copies/ml	DTprime	45	0.9957	0.0027	0.9999
		CFX 96	45	0.9956	0.0114	1.0028
		Rotor-Gene Q	45	0.9955	0.005	0.9994
		Quant Studio 5	45	0.996	0.0343	0.9969
		FLUORITE	45	0.9969	0.0282	1.0083
Saliva	log10 copies/ml	DTprime	45	0.9967	0.0164	1.005
		CFX 96	45	0.9968	0.0639	0.9854
		Rotor-Gene Q	45	0.9952	0.0062	0.9993
		Quant Studio 5	45	0.9949	0.0213	0.996
		FLUORITE	45	0.9961	0.0215	1.0074
Biopsies samples of internal organs	log10 copies/ml	DTprime	15	0.9944	0.0348	0.99
		CFX 96	15	0.9933	0.0222	0.9973
		Rotor-Gene Q	15	0.9945	0.0179	0.9978
		Quant Studio 5	15	0.9934	0.0456	1.0092
		FLUORITE	15	0.9945	0.0134	1.0021

Cerebrospinal fluid	log10 copies/ml	DTprime	15	0.997	0.0035	0.999
		CFX 96	15	0.9959	0.0266	0.9947
		Rotor-Gene Q	15	0.9967	0.0384	1.0096
		Quant Studio 5	15	0.997	0.0619	1.0153
		FLUORITE	15	0.9953	0.0093	0.9988

The obtained data allow to conclude on the reliable conformity of the results of CMV DNA concentration quantitative determination in clinical samples obtained with **different lots** of the studied "Reagent kit for the qualitative and quantitative determination of DNA of the human herpesvirus type 5 (CMV) by polymerase chain reaction with real-time detection "CMV-test" according to TS 21.20.23-058-97638376-2022", 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 operation under inappropriate conditions;
2. Clinical material contamination with inhibitory substances in concentrations exceeding permissible levels;
3. Contamination of reaction mixtures and test DNA samples with contents from PC-1 and PC-2 tubes or amplification products;
4. Testing with 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 the 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 is acceptable, and the benefits of its use exceed the risk.

6. Safety precautions

Potential risk Class — 2b — in accordance with Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation No.4n dated June 6, 2012.

The kit reagents are non-flammable. The outer packaging is not self-igniting or explosive. The reagents included in CMV-test kit have low vapor pressure and exclude the possibility of inhalation poisoning.

The reagents included in CMV-test kit are non-toxic, as they are prepared by mixing individual non-toxic components.

Work with infected or suspected of being infected material is carried out in accordance with the requirements of SanPiN 3.3686-21 "Sanitary and epidemiological requirements for the prevention of infectious diseases", MU "Organization of work of laboratories using methods of nucleic acid amplification when working with material containing microorganisms of pathogenicity groups I–IV" (MU 1.3.2569-09).

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

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

The following requirements should always be met when working:

- remove unused reagents in accordance with applicable rules and regulations;

ATTENTION! When removing waste after amplification (tubes containing PCR products), it is forbidden 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. Carried out the testing in separate rooms (areas). Start the work 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. Throw disposable plastic items in a special container with a disinfectant that can be used to disinfect medical waste;

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

- use the kit strictly according to its intended purpose and these Instructions for Use;

- 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;

- allow only specially trained personnel to work with the kit (a specialist with higher medical education who has been trained 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 concentrations used. In case of the kit components contact with the skin or mucous membranes, rinse immediately the affected area with water and seek medical assistance.

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

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

7. Required equipment and materials

Work with a reagent kit is carried out in 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 Laminar Systems, RU No. FSR 2012/13259 dated July 29, 2021 or Cabinet for sterile work DNA/RNA UV-Cleaner UVC/T-M-AR, Biosan, Latvia, RU No. RZN 2023/19369 dated January 18, 2023);

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

3. Variable volume dispensers allowing 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, RU No. FSZ 2011/11028 dated November 15, 2011 or Biohit, Finland, RU No. FSZ 2012/12201 dated May 18, 2012);

4. Refrigerator from +2°C to +8°C with freezer for 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, RU No. RZN 2016/4043 dated May 08, 2019);

5. Cycler[2] with real-time fluorescence detection in the channels corresponding to the FAM/Green, HEX/Yellow fluorophores:

- CFX96 (BioRad, USA, RU No. FSZ 2008/03399 dated June 21, 2016),
- DTprime (NPO DNA Technology LLC, Russia, RU No. FSR 2011/10229 dated March 03, 2011),
- Rotor-Gene Q (Qiagen, Germany, RU No. FSZ 2010/07595 dated August 10, 2010),
- QuantStudio 5 (Thermo Fisher Scientific, USA, RU No. RZN 2019/8446 dated June 06, 2019),

[2] 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.

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

Materials and reagents not included in the device:

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

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

2. Disposable Eppendorf type 1.5–2.0 ml tubes (Axygen, USA, RU No. FSZ 2012/11892 dated 26.08.2014);

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

- 0.2 ml PCR tubes,
- 0.1–0.2 ml PCR strip tubes,
- 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 "Disposable sterile medical probe according to TS 32.50.13-002-28731857-2020", manufactured by Pharmedpolis RT LLC, Russia (registration certificate No. RZN 2021/13989 dated December 09, 2022);

8. To take a swab from the oropharynx – use sterile saline solution or phosphate buffer (PBS) solution;

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

8. Test samples

Test sample type

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

Material sampling for testing

ATTENTION! Before starting work, study the guidelines "Sampling, transportation and storage of clinical material for PCR diagnostics" developed by the Central Research Institute of Epidemiology of Rospotrebnadzor, Moscow, 2012.

Clinical material collection and its packaging is carried out by a medical organization employee trained in the requirements and rules of biological safety when working and collecting material suspected of infection with pathogenicity group III microorganisms.

8.1. Human whole peripheral venous blood sampling

To obtain plasma, take peripheral venous blood (at least 4-5 ml) into a tube with EDTA-K2 added as an anticoagulant. Turn the tube upside-down 2-3 times to mix the blood with the anticoagulant after the material collection.

ATTENTION! Do not use heparin and sodium citrate as an anticoagulant.

Initial clinical material transportation and storage conditions:

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

ATTENTION! Avoid freezing and heating a tube containing blood above 25°C.

Do not use hemolyzed and chylous blood. The testing of such samples may lead to unreliable results!

8.2. Leukocytes obtaining procedure

They are obtained from whole peripheral and/or cord blood. Blood can be stored at room temperature up to 6 hours from the collection moment. To obtain leukocytes, centrifuge the tube with blood for 20 minutes at 3 000 rpm. Carefully collect 0.2 ml of the leukocyte mass from the cell sediment surface and transfer into a sterile 1.5-2.0 ml tube using a filter tip.

Storage conditions:

- at below -70°C for a long time.

8.3 Cerebrospinal fluid sampling

Collect at least 1.0 ml of cerebrospinal fluid with disposable needles into disposable plastic 1.5 or 2.0 ml tubes.

ATTENTION! Sample pre-processing is not required.

Material storage and transportation conditions:

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

Only single material freezing/thawing is allowed.

8.4 Oropharyngeal swabbing

Swabs are taken using dry cotton swabs with a plastic base by rotational movements from the surface of the tonsils, palatine arches and the posterior wall of the oropharynx.

After taking the material, 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 buffer solution (PBS) and carefully break off 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 2°C... 8°C – up to 3 days;
- at -20°C – up to 1 week;
- at -70°C – for a long time.

Only single material freezing/thawing is allowed.

8.5 Saliva sampling procedure

Before collecting saliva, rinse the oral cavity three times with saline solution. Collect at least 1.0 ml of saliva into disposable sterile plastic 2-5 ml 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 2°C... 8°C – up to 1 day;
at -20°C – up to 1 week;
at -70°C – for a long time.

Only single material freezing/thawing is allowed.

8.6 Biopsies samples collection and preparation

Place puncture samples (microbioptates) into 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 into 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 into sterile microtubes using a sterile filter tip.

8.7 DNA isolation procedure from biological material

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

- when using blood and oropharyngeal swabs as clinical material: Reagent kit for DNA/RNA isolation from the clinical material "NA-Extra" according to TS 21.20.23-013-97638376-2019, manufactured by TestGene LLC, Russia (registration certificate No. RZN 2021/15428 dated June 05, 2023);

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

ATTENTION! 100 µl of a negative control sample (NC), included in the reagent kit, must undergo all sample preparation stages, simultaneously with DNA isolation from the clinical test samples.

Test DNA samples storage conditions:

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

9. Kit components preparation for testing

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

ATTENTION! It is required to use only disposable sterile DNase-free plastic consumables 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 according to Table 6 in PCR tubes before testing.

Kit components preparation for testing

1. Thoroughly mix the tubes contents with DNA isolated for testing, 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 over each tube 10 times or mixing using vortex at low speed for 3-5 seconds, and then remove the drops from the tube lids by short centrifugation;

2. Take the required number of strips or tubes for the test and control DNA 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, it is required to wet clean the PCR cabinet, 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 testing consists of the following stages:

1. PCR preparation;
2. DNA amplification with hybridization-fluorescence detection of amplification products in real time;
3. Result 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, the following components are 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.

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

- Label 0.1-0.2 µl tubes for PCR;
- 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 vortex at low speed for 3-5 seconds, and then remove the 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 into the 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 vortex microcentrifuge.

Table – 6 Tubes layout for qualitative analysis

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

FOR QUANTITATIVE ANALYSIS

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

- Label 0.1-0.2 µl tubes for PCR;
- In a separate disposable sterile 1.5-2.0 ml Eppendorf tube prepare the reaction mixture: (N+6) x5 µl of PCR buffer + (N+6)x5 µl of Primer Mix, where N is a number of test samples. Mix using vortex at low speed for 3-5 seconds, and then remove the 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 into the 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 on 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 hybridization-fluorescence detection of amplification products in real time

(carried out in PCR AREA, a room for PCR amplification)

1. Place the tubes in a reaction module of a PCR device in "real time" mode. It is recommended to place the tubes in the center of a thermal block to evenly press the tubes with the heating lid;

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

Table 8 – PCR protocol

Stage	**Temperature, °C**	**Time, min.:sec.**	**Detection channels**	**Total number of 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 the number and identifiers of the samples, PC-1 and PC-2 standards, indicating 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 (CMV)	1 000 000 = 10^6	10 000 = 10^4
HEX/Yellow (*ALB*)		

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

FAM/Green and HEX/Yellow;

5. Run PCR with a fluorescent signal detection;

6. Upon the program completion, start analyzing the results.

11. Results registration and interpretation

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

Recommendations on setting a 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.

The results interpretation is carried out using the Ct values of channels listed in Table 1. Only Ct values obtained at the PCR stage with fluorescence detection can be taken into account (i.e., corresponding to stage 3 – see Table 8).

The reaction and Ct values in the control samples are evaluated first. The test samples results interpretation is carried out only after the correct PC-1, PC-2 and NC reactions.

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

Results interpretation in control samples

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

Table 10 – Test results for PC and NC

Control sample	Channel corresponding to a fluorophore	
	FAM/Green	HEX/Yellow

NC	Ct is not indicated or > 35
PC-1 and PC-2	Ct ≤ 32

When obtaining NC values that differ from those indicated in Table 10, the entire testing series results are considered unreliable. In this case, take special measures to eliminate possible contamination.

When obtaining PC values that differ from those indicated in Table 10, it is required to repeat amplification of the entire sample batch.

When reobtaining PC values that differ from those indicated in Table 10, the reagents must be replaced.

Result interpretation in DNA test samples

Result evaluation during the qualitative analysis is shown in Table 11.

Table 11 – The result interpretation principle during qualitative analysis

Ct values		Result
FAM/Green (CMV)	HEX/Yellow (SVC)	
Ct ≤ 35	Not considered	Human herpesvirus type 5 (HHV5) DNA is **detected**
Ct is absent or Ct > 35	Ct is absent or Ct ≤ 35	Human herpesvirus type 5 DNA is **not detected or is below the detection limit**
Ct is absent or Ct > 35		**Invalid result**

Results evaluation during quantitative analysis.

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

Based on the obtained Ct values for calibration samples and their concentrations, it is required to draw a calibration line. The test samples absolute concentrations are calculated when using the calibration line. Ct values ≤ 35 in the FAM channel are considered for the samples.

PCR efficiency should be within 90-110% range, and the difference between the Ct values of the repeats of each positive control sample, PC-1 and PC-2, should be no more than 1.5. Otherwise, it is required to carry out the assay, starting from the DNA isolation stage.

If the target analyte concentration is within $8x10^2$ – $1x10^7$ copies/ml range, indicate the exact concentration in copies/ml. If the concentration is less or greater than the specified range, the given 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 for the viral load assessment for 10^5 human cells is calculated using the following formula:

$$\frac{CMV \text{ DNA copy number in ml}}{\text{human DNA copy number in ml}} * 2 * 10^5$$

Invalid result may obtained be due to the presence of inhibitors in the DNA obtained from clinical material, incorrect assay protocol 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, retest with a reagent kit from another manufacturer or by another method.

Diagnostic value of the obtained test result:

The test result can be used by a qualified specialist (doctor), taking into account the data of the clinical picture and other types of studies combined, for the 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 the detected human herpesvirus type 5.

Combine the results obtained using the test kit with other data: symptoms, the general clinical picture, the testing results by other test systems and the used therapy.

12. Reagent kit storage, transportation and operation conditions

Storage

Store CMV-test reagent kit in the manufacturer's packaging at

-16°C... -24°C during the kit entire shelf life, it can be stored at 2°C... 8°C up to 14 days.

It is allowed to freeze / thaw the kit up to 10 times.

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

Transportation

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

Transport at -16°C...-24°C during the kit entire 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 within the entire transportation period, place a reagent kit in a reusable polyurethane foam thermal container for temporary storage and transportation with prepared iced packs. The type, volume and quantity of iced 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 reagent kit shelf life is 12 months from the acceptance date of the manufacturer's QCD, if all transportation, storage and operation conditions are met. The 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°C... -24°C.

Ready for usage kit components shelf life is 1 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, living quarters and the operation of production facilities, public facilities, organization and conduct of sanitary and anti-epidemic (preventive) measures".

According to the classification of medical waste, the kits belong to Class A (epidemiologically safe waste, similar in composition to solid household waste). Unused reagents in accordance with SanPiN 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, atmospheric air, soils, residential premises, operation of industrial and public premises, organization and 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.

The tubes and packaging of the 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 the reagent kit quality and safety during the shelf life in compliant with the kit transportation and storage requirements, as well as rules of operation.

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

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

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

Annex A

Designation	Document name
GOST ISO 14971-2021	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

Symbol	Explanation
	Refer to the Instructions for Use
	In vitro diagnostics medical device
	Temperature range
	Batch code
	Use before
	Manufacture date
	Fragile, handle with care
	This side up