AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit

CE 1023

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by-date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
EC REP	Authorized representative in the European Community	C+	Positive control of amplification
		PCE	Positive Control of Extraction

1. INTENDED USE

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *Epstein-Barr virus* (*EBV*) DNA, *Human Herpes virus* type 6 (*HHV*6) DNA and *human cytomegalovirus* (*CMV*) DNA in clinical material (whole blood, white blood cells, viscera biopsy material and cerebrospinal fluid) using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material and the simultaneous amplification of DNA fragments of the detected microorganism and DNA of the human β-globin gene with hybridization-fluorescence detection. DNA of the β-globin gene is used as an endogenous internal control (IC Glob) and allows not only to control all stages of the PCR study for each sample, but also to evaluate the adequacy of the material and its storage.

Amplification of DNA fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 ºC for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
DNA-target	IC Glob DNA	*EBV* DNA	*CMV* DNA	*HHV*6 DNA
Target gene	β-globin gene	LMP-gene	exon 4 of MIE (major immediate early) gene	DNA polymerase catalytic subunit

3. CONTENT

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit is produced in 1 form:

variant FRT-100 F REF R-V48(RG,iQ,Mx)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
RNA-buffer	colorless clear liquid	0.6	1 tube
DNA calibrator KSG1	colorless clear liquid	0.2	1 tube
DNA calibrator KSG2	colorless clear liquid	0.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	2 tubes
Positive Control DNA *EBV / CMV / HHV*6 and human DNA**	colorless clear liquid	0.1	2 tubes

* must be used in the extraction procedure as Negative Control of Extraction.

** must be used in the extraction procedure as Positive Control of Extraction (PCE).

Variant FRT-100 F is intended for 110 reactions (including controls).

The software in Microsoft® Excel format for data processing and result generation.

4. ADDITIONAL REQUIREMENTS

For pretreatment

- Reagent for pretreatment of whole or cord blood
- Disposable screwed or tightly closed 1.5-ml tubes

For DNA extraction and amplification

- DNA extraction kit.
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ or iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent).
- Disposable polypropylene tubes:
  a) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and

moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (whole blood, white blood cells, viscera biopsy material and cerebrospinal fluid).

Whole peripheral and umbilical blood

Before extraction, it is necessary to pretreat blood. Transfer 1.0 ml of **Hemolytic** (REF 137-CE, manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") and 0.25 ml of whole blood to 1.5 ml Eppendorf-type tube using a new tip. Carefully mix the contents of the tube by vortexing and incubate it for 10 min under periodic stirring. Centrifuge tubes at 8,000 rpm for 2 min. Remove the supernatant with a vacuum aspirator. Do not disturb the pellet. After washing, the pellet should be white. A small quantity of pinkish film above the pellet (erythrocyte debris) is allowed. Washing with hemolytic can be repeated if required. Thus obtained leukocyte pellet should be lysed immediately (in case of extraction with RIBO-prep, add 300 µl of Solution for Lysis and then isolate DNA according to the RIBO-prep Instruction manual; do not add Solution for Lysis again). The pellet can be also stored at the temperature not more than minus 68 °C for a long time.

White blood cells (leukocyte mass) of peripheral and/or umbilical blood

White blood cells are obtained from peripheral and/or umbilical blood. Blood can be stored for 6 hours after sampling at room temperature. To obtain white cells, centrifuge blood at 800–1,600 g (3,000 rpm) for 20 min. Then, collect the white film formed on the surface of the supernatant and pretreat it as described for whole peripheral and umbilical blood. White cells of peripheral and umbilical blood can be stored at the temperature not more than minus 68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA Extraction

It is recommended to use the following nucleic acid extraction kits:

- **RIBO-prep**, REF K2-9-Et-100-CE.
- **DNA-sorb-B**, REF K1-2-100-CE.

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

PCE – Add **90 µl of Negative Control (C–)** and **10 µl of Positive Control DNA *EBV / CMV / HHV*6 and human DNA** to the tube labelled PCE (Positive Control of Extraction).

NOTE: Extract the DNA according to the manufacturer's protocol.

8.2. Preparing PCR

The total reaction volume is **25 µl**, the volume of the DNA sample is **10 µl**.

8.2.1 Preparing tubes for PCR

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. For this purpose transfer the content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and mix by vortexing avoiding forming. Mark the tube by the date of mixture preparation.

NOTE: The prepared mixture is intended for analysis of 60 samples. The mixture is to be stored at 2–8 ºC for 3 months. Use when needed.

NOTE: If the mixture cannot be used up for 3 months, prepare the mixture for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF).** The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture.

NOTE: Even for analysis of **one** DNA sample in the **qualitative format**, it is necessary to run **two controls** of amplification: the Positive Control of Amplification (**KSG2**) and the Negative Control of Amplification (**RNA-buffer**). And even for analysis of **one** DNA sample in the **quantitative format**, it is necessary to run **five controls** of amplification: two calibrators (**KSG1** and **KSG2**) in two replicates and the Negative Control of Amplification (**RNA-buffer**). In addition, you should take reagents for one extra reaction.

3. Mix **PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** prepared before in an individual tube in the following proportion:
   - **10 µl of PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob,**
   - **5 µl of** mixture of **PCR-mix-2-FRT and polymerase (TaqF).**

   Calculate the required number of reaction including test and control samples, see Table 2.

Table 2

Scheme of reaction mixture preparation

Total reaction volume is 25 µl, volume of DNA sample is 10 µl			
Reagent volume for 1 reaction (µl)		10.0	5.0
Quantity of clinical samples		PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob[1]	mix of PCR-mix-2-FRT and polymerase (TaqF)[1]
For quantitative analysis	For qualitative analysis		
1	4	70	35
2	5	80	40
3	6	90	45
4	7	100	50
5	8	110	55
6	9	120	60
7	10	130	65
8	11	140	70
9	12	150	75
10	13	160	80
11	14	170	85
12	15	180	90
13	16	190	95
14	17	200	100
15	18	210	105
16	19	220	110
17	20	230	115
18	21	240	120
19	22	250	125
20	23	260	130
21	24	270	135
22	25	280	140
23	26	290	145
24	27	300	150
25	28	310	155
30	33	360	180

4. Take the required number of tubes for amplification of test and control DNA samples. Transfer **15 µl** of the prepared mixture into each tube. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the tubes with the reaction mixture.
5. Carry out the control reactions:

   For qualitative analysis:

   NCA – Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

   C+ – Add **10 µl** of **DNA calibrator KSG2** to the tube labeled C+ (Positive Control of Amplification).

   C– – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

   PCE – Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV / CMV / HHV*6 and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

   For quantitative analysis:

   NCA – Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

   Calibrators KSG1 and KSG2 – Add **10 µl** of **KSG1** to two tubes and **10 µl** of **KSG2** to other two tubes.

   C– – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative control of Extraction).

   PCE – Add **10 µl** of **the sample extracted from the Positive Control DNA *EBV / CMV / HHV*6 and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 3a

AmpliSens-1 program for rotor-type instruments[2]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	95	15 min	–	1
Cycling 1	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

Table 3b

AmpliSens-1 program for plate-type instruments[3]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, JOE, ROX, Cy5	
	72	15 s	–	

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.

[1] Values are given with account of one extra reaction and five controls (2 DNA calibrators KSG1 and KSG2 (in two replicates), negative control (RNA-buffer) for quantitative analysis of DNA, and two controls (positive and negative) for qualitative analysis of DNA.
[2] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia) or equivalent
[3] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent.

5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in four channels:

– The signal of the β-Globin gene DNA (IC Glob) amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *EBV* DNA amplification product is detected in the channel for the JOE fluorophore.
– The signal of the *CMV* DNA is detected in the channel for the ROX fluorophore.
– The signal of the *HHV*6 DNA is detected in the channel for the Cy5 fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

– ***EBV* DNA** is **detected** if the *Ct* value determined in the results grid in the channel for the **JOE** fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin.* Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***CMV* DNA** is **detected** if the *Ct* value determined in the results grid in the channel for the **ROX** fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin.* Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***HHV*6 DNA** is **detected** if the *Ct* value determined in the results grid in the channel for the **Cy5** fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin.* Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
– ***EBV* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the JOE fluorophore (the fluorescence curve does not cross the threshold line); ***CMV* DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the ROX fluorophore (the fluorescence curve does not cross the threshold line); and ***HHV*6 DNA is not detected** if the *Ct* value is not determined (absent) in the results grid in the channel for the Cy5 fluorophore (the fluorescence curve does not cross the threshold line). Whereas for qualitative analysis the *Ct* value in the results grid in the channel for the FAM fluorophore should not exceed the *Ct* value specified in the *Important Product Information Bulletin,* and for quantitative analysis, the quantity of IC Glob DNA should be more than **2000** copies/reaction for whole blood, white blood cells, viscera biopsy material.

NOTE: For cerebrospinal fluid, the *Ct* value could be greater than the *Ct* value in the channel for FAM fluorophore specified in the *Important Product Information Bulletin* or the quantity of IC Glob DNA could be less than 500 copies/reaction in case of quantitative analysis because the cerebrospinal fluid samples may contain a very small number of cells.

– The result of analysis is **invalid** if the *Ct* value is not determined (absent) in the results grid or greater than the boundary *Ct* value in the channels for the JOE, ROX or Cy5 fluorophores. Whereas the *Ct* value in the results grid in the channel for the FAM fluorophore is greater than the *Ct* value specified in the *Important Product Information Bulletin* (for qualitative analysis) or the quantity of IC Glob DNA is less than **2000** copies/reaction for whole blood, white blood cells, viscera biopsy material (for quantitative analysis). In such case the PCR analysis should be repeated for required sample.
– The result is **equivocal** for the clinical samples with the *Ct* value determined in the channels for the ROX, JOE or Cy5 fluorophores greater than the boundary *Ct* value specified in the *Important Product Information Bulletin.* In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal.**
– The negative result is considered **unreliable** if the *Ct* value in the channel for the FAM fluorophore is greater than the boundary *Ct* value specified in the *Important Product Information Bulletin* (for qualitative analysis). The positive or negative results (the quantitative analysis) are considered **unreliable** if the quantity of IC Glob DNA is less than **2000** copies/reaction for whole blood, white blood cells, viscera biopsy material.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 4). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin* (see Table 5).

Table 4

Results for controls in qualitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	<boundary value	<boundary value	<boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+ (for qualitative analysis)	PCR	<boundary value	<boundary value	<boundary value	<boundary value

Table 5

Results for controls in quantitative analysis

Control	Stage for control	*Ct* in the channel for fluorophore			
		FAM	JOE	ROX	Cy5
C–	DNA extraction, PCR	Absent	Absent	Absent	Absent
PCE	DNA extraction, PCR	<boundary value	concentration value falls in the range specified in the *Important Product Information Bulletin*	concentration value falls in the range specified in the *Important Product Information Bulletin*	concentration value falls in the range specified in the *Important Product Information Bulletin*
NCA	PCR	Absent	Absent	Absent	Absent
KSG1, KSG2	PCR	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined	*Ct* value and calculated concentration are defined

For quantitative analysis, if total DNA is extracted from human whole blood, white blood cells, and viscera biopsy material, the concentration in log of DNA copies per standard cell quantity (10^5) in control and test samples is calculated according to the following formula:

For *CMV*:

$$\lg\left\{\frac{\text{number of } \textit{CMV} \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \times 2{\cdot}10^5\right\} = \lg\{\textit{CMV} \text{ DNA copies}/10^5 \text{ cells}\}$$

For *EBV*:

$$\lg\left\{\frac{\text{number of } \textit{EBV} \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \times 2{\cdot}10^5\right\} = \lg\{\textit{EBV} \text{ DNA copies}/10^5 \text{ cells}\}$$

For *HHV*6:

$$\lg\left\{\frac{\text{number of } \textit{HHV6} \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \times 2{\cdot}10^5\right\} = \lg\{\textit{HHV6} \text{ DNA copies}/10^5 \text{ cells}\}$$

If total DNA is extracted from cerebrospinal fluid (liquor), the concentration of DNA per ml of clinical sample (CS_{DNA}) is calculated according to the following formula:

$$CS_{DNA} = \text{number of DNA copies } \textit{CMV} (\textit{EBV}, \textit{HHV}6) \text{ in PCR sample} \times 100 \text{ (copies/ml)}$$

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If any *Ct* value appears in the channels for the FAM, JOE, ROX and Cy5 fluorophores for the Negative Control of Amplification (NCA) and Negative Control of Extraction (C–) these results testify the presence of contamination of reagents or samples. In that case the PCR-analysis should be repeated (beginning with the extraction stage) for all samples, in which DNA was detected.
2. If the *Ct* value is absent or greater than the boundary value in the results grid for the Positive Control of Amplification (C+) – **KSG2** – for the qualitative analysis in the channels for the JOE, FAM, ROX or Cy5 fluorophores, the amplification must be repeated for all samples where pathogen agent DNA was not detected.
3. If the *Ct* value is absent or greater than the boundary value for the Positive Control of Extraction (PCE) – **Positive Control DNA *EBV* / *CMV* / *HHV*6 and human DNA –** in the channels for the JOE, FAM, ROX or Cy5 fluorophores, the results of analysis must be considered as **invalid** for all samples. PCR should be repeated for all samples.
4. If the *Ct* value for given sample was not defined or the *Ct* value exceeds the boundary value in the channel for the JOE, ROX or Cy5 fluorophores, and *Ct* value defined in the channel for the FAM fluorophore exceeds the maximal value specified for IC, the experiment needs to be repeated, starting with the extraction stage. Possible reason is an error in the clinical material pretreatment procedure that leads to the DNA loss or the presence of PCR inhibitors.
5. If the *Ct* value for the clinical samples exceeds the maximal boundary value in the channel for the JOE, ROX or Cy5 fluorophore, the results of analysis must be considered as **equivocal**. In that case, it is necessary to conduct additional analysis for that DNA sample with two repeats. If the repeated positive *Ct* value is obtained, the result is considered positive. If the positive *Ct* value can't be reproduced in two repeats, the result is considered **equivocal**.
6. If in quantitative analysis the copies/reaction values in calibrators differ by more than 30 % from the set values, it is necessary to check the tube order in the rotor (calibrators should be placed in the wells indicated as ***Standard*** in sample table, concentration should correspond to concentration specified in the *Important Product Information Bulletin*, well no.1 must be filled with some test tube (not empty)).
7. If the correlation coefficient R in ***Standard Curve*** window is less than 0.9 (in case of quantitative analysis), it means that calibration failed. Check the settings of calibrators and correct inaccuracies, if no effect, repeat PCR for all samples and calibrators.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *EBV* / *CMV* / *HHV*6-screen-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit are to be stored at 2–8 ºC when not in use (except for PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob, PCR-mix-2-FRT and polymerase (TaqF)). All components of the **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob, PCR-mix-2-FRT$_T$ and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 ºC.

NOTE: PCR-mix-1-FRT *EBV / CMV / HHV*6 / Glob is to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity

Clinical material	Nucleic acid extraction kit	Analytical sensitivity
Cerebrospinal fluid (liquor)	**RIBO-prep**	400 copies/ml
Whole blood, white blood cells, viscera biopsy material	**RIBO-prep**	5 DNA copies per 10^5 cells

13.2. Analytical specificity

AmpliSens® *EBV / CMV / HHV*6-screen-FRT PCR kit is intended for *Epstein-Barr virus* (*EBV*) DNA, *Human Herpes Virus* type 6 (*HHV*6) DNA and *human cytomegalovirus* (*CMV*) DNA detection. Specific activity of **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit was confirmed by analysis of reference *CMV* strain AD 169, QCMD panel for *Epstein-Barr virus,* as well as by analysis of clinical material with subsequent confirmation of the results by sequencing the amplified fragments.

The activity of the PCR kit components with respect to DNA of other viruses (herpes simplex virus types 1 and 2, human herpes virus type 8, Varicella Zoster Virus, Parvovirus B19, and others), bacterial pathogens (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and others) and human DNA was absent.

The clinical specificity of **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit for qualitative and quantitative detection of *Epstein-Barr virus* (*EBV*) DNA, *Human Herpes virus* type 6 (*HHV*6) DNA and *human cytomegalovirus* (*CMV*) DNA in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *EBV / CMV / HHV*6-screen-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
30.03.15 PM	Through the text	Corrections according to the template. Grammar corrections
	8.2.1 Preparing tubes for PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	9. Data analysis	The section was rewritten
	10. Troubleshooting	The section has been supplemented
28.12.15 ME	Text	The clinical material saliva and oropharyngeal swabs was deleted
11.10.16 PM	9. Data analysis	The data analysis was clarified
05.12.18 PM	2. Principle of PCR detection	The table with targets and the information about the enzyme UDG were added
	Through the text	The text formatting was changed
27.02.20 PM	Footer	The phrase “Not for use in the Russian Federation” was added
22.09.20 EM	Through the text	Corrections according to the template Grammar corrections
	10. Troubleshooting	The information for Negative Control of Amplification (NCA) and Negative Control of Extraction (C–) was corrected
01.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

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