CE

IVD

For Professional Use Only

AmpliSens® *EBV*-screen/monitor-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***EBV*****-screen/monitor-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of the *Epstein-Barr virus* (*EBV*) DNA in the clinical material (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

EBV detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *EBV* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time PCR monitoring of the 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*****-screen/monitor-FRT** PCR kit is a qualitative and quantitative test which is used with two internal controls: exogenous and endogenous.

The Internal Control STI-87 (IC) – the internal exogenous control – must be used in the extraction procedure of peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage in order to monitor test stages of each individual sample and to identify possible reaction inhibition.

When the clinical material containing cells (whole human blood, white blood cells, and viscera biopsy material) is extracted then the DNA fragment of β-globin gene is amplified. DNA fragment of β-globin gene is used as an internal endogenous control (a human genome fragment). Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of clinical material.

AmpliSens® ***EBV*****-screen/monitor-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

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

AmpliSens® *EBV*-screen/monitor-FRT PCR kit variant FRT-100 F **REF** R-V9-100-S(RG,iQ,Mx)-CE.

AmpliSens® *EBV*-screen/monitor-FRT PCR kit, variant FRT-100 F includes:

Reagent		*Description*	*Volume, ml*	*Quantity*
PCR-mix-1-FL *EBV* screen/monitor		colorless clear liquid	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 calibrators	**KSG1**	colorless clear liquid	0.2	1 tube
	KSG2	colorless clear liquid	0.2	1 tube
RNA-buffer		colorless clear liquid	1.2	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	2 tubes
Positive Control DNA *EBV* and human DNA **		colorless clear liquid	0.1	2 tubes
Internal Control STI-87 (IC)***		colorless clear liquid	0.6	2 tubes

* must be used in the extraction procedure as Negative Control of Extraction (C–).

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

*** add 10 µl of Internal Control STI-87 (IC) during the DNA extraction procedure directly to the sample/lysis mixture.

AmpliSens® *EBV*-screen/monitor-FRT PCR kit variant FRT-100 F is intended for 110 reactions, including controls and DNA calibrators.

4. ADDITIONAL REQUIREMENTS

- Hemolytic.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with 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 iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) when working with PCR kit variant FRT-100 F:
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps if a plate-type instrument is used;
  b) 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.
- Refrigerator for 2-8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers 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 detection.
- 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 afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in compliance with local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) are available on request.
- 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 to the area in which 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

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

AmpliSens® *EBV*-screen/monitor-FRT PCR kit is intended for the analysis of DNA extracted using DNA extraction kits from the clinical material (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material).

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 into 1.5-ml Eppendorf-type tube using an individual tip. Carefully vortex the content of the tube and incubate it for 10 min with periodic stirring. Centrifuge tubes at 8000 rpm for 2 min. Remove the supernatant using vacuum aspirator. Do not disturb the pellet. After washing, the pellet should be white. A small quantity of a pinkish film above the pellet (erythrocyte debris) is allowed. Washing with Hemolytic can be repeated, if necessary. The obtained pellet of leukocytes should be lysed immediately (in case of "RIBO-prep" extraction, add 300 µl of Solution for Lysis and then extract DNA according to "RIBO-prep" instruction manual; do not add Solution for Lysis again) or it can be stored at ≤ –68 °C for a long time.

Packed white cells of peripheral and/or umbilical blood

Blood can be stored for 6 hours after sampling at room temperature. To obtain white cells, centrifuge tube with blood at 800-1600 g (3000 rpm) for 20 min. Then remove the white film formed on the surface of the blood and carry out the pretreatment as described for whole peripheral and umbilical blood. White blood cells of peripheral and umbilical blood can be stored at ≤ –68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *EBV*-screen/monitor-FRT PCR kit should be used at 18–25 °C.

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.
- NucliSENS easyMAG automated system (for details see Guidelines [2]).

The DNA extraction of each clinical sample is carried out in the presence of **Internal Control STI-87** (add **10 µl** of **Internal Control STI-87** into each sample).

Addition of **Internal Control STI-87** is not necessary for the samples of whole human blood, white blood cells, and viscera biopsy material.

In the extraction procedure it is necessary to carry out the control reaction:

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

PCE – Add **90 µl** of **Negative Control** and **10 µl** of **Positive Control DNA *EBV* and human DNA** to the tube labeled **PCE** (Positive Control of Extraction)**.**

Extract the DNA according to the manufacturer's protocol.

8.2. Preparing the PCR

The total reaction volume is **25 µl**, the volume of 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 **30 µl** of **polymerase (TaqF)** into the tube with **PCR-mix-2-FRT** and vortex without foam forming.

The prepared mixture is intended for analysis of 60 samples. The mixture is to be stored at the temperature 2-8 °C for 3 months and used as needed.

If the mixture cannot be used up for 3 months, it is necessary to prepare a mixture for fewer 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.

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-FL *EBV* screen/monitor** and the mixture of **PCR-mix-2-FRT** and

polymerase (TaqF) prepared before in the individual tube in the following proportion:

- **10 µl of PCR-mix-1-FL *EBV* screen/monitor,**
- **5 µl of** mixture of **PCR-mix-2-FRT and polymerase (TaqF).**

Calculate the required reaction number including clinical and control samples (see Table 1).

Table 1

Scheme of reaction mixture preparation for variant FRT-100 F

Total reaction volume is 25 µl, including the volume of DNA sample - 10 µl			
Reagent volume for 1 reaction (µl)		**10.0**	**5.0**
Quantity of clinical samples		**PCR-mix-1-FL *EBV* screen/monitor[1]**	**Mixture 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**

If 60 samples are analyzed simultaneously, you can use a simplified variant of mixture preparation: transfer the content of one tube with PCR-mix-2-FRT and the content of one tube with polymerase (TaqF) into the tube with PCR-mix-1-FL *EBV* screen/monitor.

[1] Values are given with account of one extra reaction and five controls (2 DNA-calibrators КSG1 and КSG2 (in two replicates), negative control (RNA-buffer) for quantitative analysis of *EBV* DNA, and two controls (positive and negative) for qualitative analysis of *EBV* DNA.

4. Take the required number of tubes for amplification for the clinical and control samples. Transfer **15 µl** of the prepared mix into each tube.
5. Add **10 µl** of **DNA** obtained at the DNA extraction stage into tubes with the reaction mixture.
6. Carry out the control amplification 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 **KSG2** to the tube labeled C+ (Positive Control of Amplification).

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

PCE - Add **10 µl** of **the sample extracted from the Positive control DNA *EBV* 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)

KSG1
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 (C–) reagent** to the tube labeled C– (Negative control of Extraction).

PCE - Add **10 µl** of **the sample extracted from the Positive control DNA *EBV* and human DNA reagent** to the tube labeled PCE (Positive control of Extraction).

8.2.2. Amplification

1. Insert tubes into the reaction module of the device
2. Create a temperature profile on your instrument as follows:

Table 1a

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	
	72	15 s	–	

Table 1b

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

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	30 s	FAM, HEX/JOE, ROX	
	72	15 s	–	

Fluorescent signal is detected in the channels for the FAM, JOE and ROX fluorophores

3. Run the amplification program with fluorescence detection.
4. 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 three channels:

- The signal of β-globin gene DNA (IC Glob) amplification product is detected in the channel for the FAM fluorophore,
- The signal of *EBV* DNA (Positive Control DNA *EBV* and human DNA) amplification product is detected in the channel for the JOE fluorophore,
- The signal of Internal Control STI-87 (IC) DNA amplification product is detected in the channel for the ROX fluorophore.

The results are interpreted by the software of the used instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

[2] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia) or equivalent
[3] For example, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent

9.1. Interpretation of results for DNA extracted from cell suspension (whole human blood, white blood cells, viscera biopsy material)

The results are analysed in two channels:

– the signal of β-globin gene DNA (IC Glob) amplification product is detected in in the channel for the FAM fluorophore,

– the signal of *EBV* DNA (Positive Control DNA *EBV* and human DNA) amplification product is detected in the in the channel for the JOE fluorophore.

If the total DNA is extracted from cell suspension (whole human blood, white blood cells, viscera biopsy material) the principle of interpretation is the following:

1. *EBV* DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the threshold value of the positive result (for details see Guidelines [2]). Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
2. *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), whereas the *Ct* value in the channel for the FAM fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin* (for qualitative analysis) or the quantity of IC Glob DNA is more than 2000 copies/reaction (for quantitative analysis).
3. The result is **invalid** if the *Ct* value is not determined (absent) in the channel for JOE fluorophore, whereas 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) or the quantity of IC Glob DNA is less than 2000 copies/reaction (for quantitative analysis). In such case the PCR analysis should be repeated for required sample.
4. The result is **equivocal** if the *Ct* value in the channel for JOE fluorophore exceeds 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.**
5. The negative result is considered **unreliable** if the *Ct* value in the channel for **FAM** fluorophores is greater than the boundary value specified in the *Important Product Information Bulletin* (for qualitative analysis). The quantitative positive or negative results are considered **unreliable** if the quantity of IC Glob DNA is less than 2000

copies/reaction.

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 2a). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin.*

Table 2a

Results for controls for DNA extracted from cell suspension (whole human blood, white blood cells, and viscera biopsy material)

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

For quantitative analysis the concentration in logarithm of *EBV* DNA copies per standard cell quantity (10^5) in control and clinical samples (whole human blood, white blood cells, and viscera biopsy material) is calculated according to the following formula:

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

To express relative concentration of *EBV* DNA in copies per standard cells quantity (for example, 10^5), use the scaling ratio:

10^5 of cells = $2{*}10^5$ human genomes

9.2. Interpretation of results for DNA extracted from peripheral blood plasma,

amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage with internal control sample

The results are analysed in two channels:

– the signal of the *EBV* DNA (Positive Control DNA *EBV* and human DNA) amplification product is detected in the channel for the JOE fluorophore,
– the signal of the Internal Control STI-87 (IC) DNA amplification product is detected in the channel for the ROX fluorophore.

If the total DNA is extracted from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage with internal control sample, principle of interpretation is the following:

1. *EBV* DNA is **detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the threshold value of the positive result (for details see Guidelines [2]). Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.
2. *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), whereas the *Ct* value in the channel for the ROX fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin*.
3. The result is **invalid** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value in the channel for the ROX fluorophore is not determined (absent) or greater than the boundary *Ct* value specified in the *Important Product Information Bulletin.* In such cases, the PCR analysis should be repeated for required sample.
4. The result is considered to be **equivocal** if the Ct value in the channel for the JOE fluorophore exceeds 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 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 2b). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin*.

Table 2b

Results for controls for DNA extracted from peripheral blood, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage with internal control

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

For quantitative analysis the concentration of *EBV* DNA (**CS *EBV* DNA**) per ml of sample (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage) is calculated according to the following formula:

$$\text{CS}_{EBV\ \text{DNA}} = [K_{EBV\ \text{DNA}} / K_{\text{STI-87}}] \times \text{IC coefficient (copies/ml)}$$

$K_{EBV\ \text{DNA}}$ – quantity of *EBV* DNA copies in DNA-sample;

$K_{\text{STI-87}}$ – quantity of STI-87 DNA copies in DNA-sample;

IC coefficient – quantity of Internal Control STI-87 DNA copies in DNA-sample.

IC coefficient, Positive Control DNA *EBV* and human DNA, Internal Control STI-87 and DNA calibrators concentrations as well as boundary *Ct* values are specified in the *Important Product Information Bulletin*.

10. TROUBLESHOOTING

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

1. The *Ct* value determined in the channel for ROX fluorophore for the Negative Control of Amplification (NCA); in the channel for JOE fluorophore for Negative Control of Amplification (NCA), and for Negative Control of Extraction (C–); also, if any *Ct* value that appears in the channel for FAM fluorophore for the Negative Control of

Amplification (NCA) and for Negative Control of Extraction (C–) is less than the boundary *Ct* values specified in the *Important Product Information Bulletin*. The results testify the presence of contamination of reagents or samples. In that case the PCR analysis (beginning with the extraction stage) should be repeated for all samples, in which DNA was found,

2. If *Ct* value is absent or greater than the threshold in the results grid for the Positive Control of Amplification (C+) – **KSG2** – in the channel for JOE (*EBV*), FAM or ROX fluorophores, the amplification should be repeated for all samples where ***EBV* DNA** was not detected.
3. If the *Ct* value is absent or greater than threshold for the Positive Control of Extraction (PCE) – **Positive Control DNA *EBV* and human DNA –** in the channel for JOE (*EBV*), FAM or ROX 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 is absent or is greater than the specified boundary value in channel for JOE and the *Ct* value in the channels for FAM or ROX fluorophore is greater than the maximal value for **IC**, the experiment should be repeated starting from DNA extraction stage.
5. If the *Ct* value is greater than the specified boundary value in channel for JOE and the *Ct* value in the channels for FAM or ROX is less than the specified boundary value, 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 quantitation analysis the copies/reaction values in DNA calibrators differ by more than for 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*-screen/monitor-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit are to be stored at 2-8 °C when not in use(except for PCR-mix-1-FL *EBV* screen/monitor, PCR-mix-2-FRT, and polymerase (TaqF)). All components of the **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FL *EBV* screen/monitor, PCR-mix-2-FRT and polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 ºC

PCR-mix-1-FL *EBV* screen/monitor is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The linear range of **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit is **500 – 10.000.000 copies/ml**. If the result is more than 10.000.000 copies/ml, it is indicated as ***the result is more than 10.000.000 EBV DNA copies/ml***. If the result is less than 500 copies/ml, it is indicated as ***the result is less than 500 EBV DNA copies/ml***.

Clinical material	DNA extraction kit	Analytical sensitivity
Peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage	RIBO-prep	**400 copies/ml**
Whole human blood, white blood cells, viscera biopsy material	RIBO-prep	**5 *EBV* DNA copies per 10^5 cells**

13.2. Specificity

The analytical specificity of **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

AmpliSens® *EBV*-screen/monitor-FRT PCR kit is intended for *Epstein-Barr virus* DNA fragment detection. The specific activity of **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit is proved by analyzing QCMD panels as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplification fragments. The activity of PCR kit components with respect to DNA of other viruses (*human cytomegalovirus, herpes simplex virus types 1 and 2, human herpes virus types 6 and 8, Varicella Zoster Virus, Parvovirus* B19 and others), bacterial pathogens (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae* and others), and human DNA is absent.

The clinical specificity of **AmpliSens® *EBV*-screen/monitor-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, Moscow, 2010.
2. Guidelines to the **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit for qualitative detection and quantitation of *Epstein-Barr virus* (*EBV*) DNA in the clinical materials by using real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

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*-screen/monitor-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative Control of Extraction
	Date of manufacture	**C+**	Positive Control of Amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution	**PCE**	Positive Control of Extraction
		KSG1, KSG2	DNA calibrators

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
17.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
25.05.15 ME	Through the text	Corrections according to the template. Grammar corrections
	2. Principle of PCR detection	The additions about using endogenous and exogenous internal controls
	8.1. DNA extraction	The phrase: “Addition of Internal Control STI-87 is not necessary for the samples of whole human blood, white blood cells, and viscera biopsy material” was added
	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 sections were rewritten
	10. Troubleshooting	