CE

IVD

For Professional Use Only

AmpliSens® *EBV*-screen/monitor-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040

Federal Budget Institute of
Science “Central Research
Institute for Epidemiology”
3A Novogireevskaya Street
Moscow 111123 Russia

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1. INTENDED USE

AmpliSens® *EBV*-screen/monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantitation of *Epstein-Barr virus* (*EBV*) DNA in the clinical materials (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 determination by the polymerase chain reaction (PCR) with hybridization fluorescent detection consists of 2 stages: DNA extraction from clinical samples and PCR amplification of pathogen genome specific region with real-time hybridization fluorescent 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 used is a qualitative test which is used with two controls. The Internal Control STI-87 (IC) must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit is also based on the use of an endogenous control, the β-globin gene. The DNA target selected as an endogenous internal control is a human genome fragment that is present in sample in a sufficient quantity equivalent to that of cells in the sample. Endogenous internal control (IC Glob) allows controlling PCR-analysis stages (DNA extraction and PCR-amplification), material sampling and storage adequacy. **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). 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 (for use with RG, iQ, Mx) **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 laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene™ 3000 or Rotor-Gene™ 6000

(Corbett Research, Australia), Rotor-Gene Q (Qiagen, Germany), iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), DT-96 (DNA-Technology, Russia), or equivalent).

- Disposable polypropylene microtubes for PCR (0.2- or 0.1-ml; for example, Axygen, USA; Corbett Research, Australia; Qiagen, Germany).
- Refrigerator for 2-8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin 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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while handling samples and reagents. 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water and seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. 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 by using DNA extraction kits from 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 with a vacuum aspirator. Do not capture the pellet. The pellet should be white after washing. A small quantity of pinkish thin coat (destroyed erythrocytes) above the pellet is allowed. Washing with hemolytic can be repeated if required. 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.

White blood cells (leukocyte mass) 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.
- Automatic instrument NucliSENS easyMAG can also be used.

Extract DNA according to the manufacturer's instructions.

Add **10 µl** of **Internal Control STI-87** into each sample.

Carry out the control extraction reactions.
Add **100 µl** of **Negative Control** to the tube labeled **C–**.
Add **90 µl** of **Negative Control** and **10 µl** of **Positive Control DNA *EBV* and human DNA** to the tube labeled **PCE**.

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. Use when needed.

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

Even for analysis of **one test** 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 **one test** DNA sample in the **quantitative format**, it is necessary to run **five controls** of amplification: two calibrators (KSG1 and KSG2) in duplicates 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 PCR-mix-2-FRT and polymerase (TaqF).**

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

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.

4. Collect the required quantity of tubes for amplification of clinical and control DNA samples. Transfer **15 µl** of the prepared mix into each tube.
5. Add **10 µl** of **DNA** obtained from clinical or control samples into tubes with the reaction mixture.
6. <u>For qualitative analysis:</u>

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).

<u>For quantitative analysis:</u>

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

8.2.2. Amplification

1. Program the thermocycler according to **Manufacturer's manual**, **Guidelines** [2], and **Tables 1a** and **1b**.
2. Create a temperature profile on your instrument as follows:

Table 1a

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

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
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	–	

[1] For example, Rotor-Gene 3000 and Rotor-Gene 6000 (Corbett Research, Australia) or equivalent

Table 1b

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

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycle repeats*
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	–	

Fluorescence is detected on the 2-nd step of Cycling 2 **(60 °C)** in FAM/Green, HEX/JOE/Yellow and ROX/Orange fluorometer channels.

9. DATA ANALYSIS

β-globin gene DNA (IC Glob) is detected in the FAM/Green channel, *EBV* DNA (Positive Control DNA *EBV* and human DNA) is detected in the JOE/HEX/Yellow channel, Internal Control STI-87 (IC) DNA is detected in the ROX/Orange channel.

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.

9.1. Interpretation of results for DNA extracted from cell suspension

The results are detected in two channels – β-globin gene DNA (IC Glob) in the FAM/Green channel, *EBV* DNA (Positive Control DNA *EBV* and human DNA) in the JOE/HEX/Yellow channel.

If the total DNA from cell suspension (whole human blood, white blood cells, viscera biopsy material) is extracted, the results are interpreted as follows:

1. The sample is considered to be **positive** for *EBV* DNA if its Ct value in the results grid in the JOE/HEX/Yellow channel is defined and does not exceed the threshold value of the positive result.
2. The sample is considered to be **negative** for *EBV* DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel, and the Ct value in the results grid in the FAM/Green channel does not exceed the Ct value indicated in the **Important product information bulletin** (for quantitative analysis) or the quantity of IC Glob DNA is more than 2000 copies per reaction (for qualitative analysis).
3. The result is considered to be **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow

[2] For example, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA) or equivalent

channel and the Ct value in the results grid in the FAM/Green channel exceeds the Ct value indicated in the **Important product information bulletin** (for quantitative analysis) or the quantity of IC Glob DNA is 2000 copies or less per reaction (for qualitative analysis).

4. The result is considered to be **equivocal** if the Ct value in the JOE/HEX/Yellow channel exceeds the Ct value indicated in the **Important product information bulletin**. It is necessary to repeat the analysis in duplicates. If the result is not reproduced for both replicates, the result is considered to be **equivocal**.

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 channel				Interpre-tation
		FAM/Green		JOE/HEX/Yellow		
		Qualitative format	Quantitative format	Qualitative format	Quantitative format	
C–	DNA extraction, amplification	Neg	Neg	Neg	Neg	OK
PCE	DNA extraction, amplification	Pos (< boundary value)	Pos (< boundary value)	Pos (< boundary value)	Ct value is in the range indicated in Important product information bulletin	OK
NCA	Amplification	Neg	Neg	Neg	Neg	OK
C+	Amplification	Pos (< boundary value)	-	Pos (< boundary value)	-	OK
KSG1, KSG2	Amplification	-	Ct value and rated concentration are defined	-	Ct value and rated concentration are defined	OK

9.1.1. Qualitative analysis

For qualitative analysis, if the Ct value in the FAM/Green channel exceeds the Ct value indicated in the **Important product information bulletin**, the negative result is considered to be invalid.

9.1.2. Quantitative analysis

For quantitative analysis, if the quantity of IC Glob DNA is less than 2000 copies/reaction, the quantitative positive or negative result is considered to be invalid.

Results are accepted as relevant if the results for all controls of amplification and extraction are correct. For quantitative analysis, the results for PCE are to be in the concentration range indicated in the **Important product information bulletin**.

The concentration in log units 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 by the following formula:

$$\log \{ \frac{\textit{EBV DNA copies in PCR sample}}{\text{Glob DNA copies in PCR sample}} \times 2 * 10^5 \} = \log \{ \textit{EBV} \text{ DNA copies}/10^5 \text{ of cells} \}.$$

To express relative *EBV* DNA concentration in copies per standard cell 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 another material

The results are detected in two channels – *EBV* DNA (Positive Control DNA *EBV* and human DNA) in the JOE/HEX/Yellow channel, Internal Control STI-87 (IC) DNA in the ROX/Orange channel.

If the total DNA from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, bronchoalveolar lavage with internal control sample is extracted, the results are interpreted as follows:

1. The sample is considered to be **positive** for *EBV* DNA if its Ct value in the results grid in the JOE/HEX/Yellow channel is defined and does not exceed the threshold value of the positive result.
2. The sample is considered to be **negative** for *EBV* DNA if its Ct value in the JOE/HEX/Yellow channel is not defined in the results grid (the fluorescence curve does not cross the threshold line) and the Ct value in the results grid in the ROX/Orange channel does not exceed the Ct value indicated in the **Important product information bulletin**.
3. The analysis result is considered to be **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel and the Ct value in the results grid in the ROX/Orange channel is absent or exceeds the Ct value indicated in the **Important product information bulletin**.
4. The result is considered to be **equivocal** if the Ct value in the JOE/HEX/Yellow channel exceeds the Ct value indicated in the **Important product information bulletin**. It is necessary to repeat the analysis in duplicates. If the result is not reproduced for both replicates, the result is considered to be **equivocal**.

Results are accepted as relevant if the results for all controls of amplification and controls of extraction are correct. For quantitative analysis, the results for PCE are to be in the concentration range of concentrations indicated 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 channel				Interpretation
		JOE/HEX/Yellow		ROX/Orange		
		Qualitative format	Qualitative format	Qualitative format	Qualitative format	
C–	DNA extraction, Amplification	Neg	Neg	Pos (< boundary value)	Pos (< boundary value)	OK
PCE	DNA extraction, Amplification	Pos (< boundary value)	Ct value is in the range indicated in Important product information bulletin	Pos (< boundary value)	Pos (< boundary value)	OK
NCA	Amplification	Neg	Neg	Neg	Neg	OK
C+	Amplification	Pos (< boundary value)	-	Pos (< boundary value)	-	OK
КSG1, КSG2	Amplification	-	Ct value and rated concentration are defined	-	Ct value and rated concentration are defined	OK

9.2.1. Qualitative analysis

For qualitative analysis, if the Ct value in the FAM/Green channel exceeds the Ct value indicated in the **Important product information bulletin**, the negative result is considered to be invalid.

9.2.2. Quantitative analysis

For quantitative analysis, if the quantity of IC Glob DNA is less than 2000 copies/reaction, the quantitative positive or negative result is considered to be invalid.

Results are accepted as relevant if the results of all controls of amplification and extraction are correct. For quantitative analysis, the results for PCE are to be in the concentration range indicated in the **Important product information bulletin**.

The concentration of *EBV* DNA (**КР *EBV* DNA**) per ml of sample for peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage is calculated by the following formula:

$$КР_{EBV\ DNA} = К_{EBV\ DNA} / К_{STI\text{-}87} \times \text{IC coefficient (copies/ml)}$$

$К_{EBV\ DNA}$ − quantity of copies of *EBV* DNA in DNA-sample;

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

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

IC coefficient, Positive Control DNA *EBV* and human DNA, Internal Control STI-87 and DNA calibrators concentrations are indicated in the **Important product information bulletin**.

10. TROUBLESHOOTING

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

1. If the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel and the Ct value in the results grid in the ROX/Orange channel is absent or exceeds the Ct value indicated in the **Important product information bulletin**, it is necessary to repeat the analysis starting from the DNA extraction stage.
2. If the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel and the Ct value in the results grid in the FAM/Green channel exceeds the Ct value indicated in the **Important product information bulletin** (for quantitative analysis) or if the quantity of IC Glob DNA is not more than 2000 copies per reaction (for qualitative analysis), it is necessary to repeat the analysis starting from the DNA extraction stage.

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 (except for PCR-mix-1-FL *EBV* screen/monitor, PCR-mix-2-FRT, and polymerase (TaqF)) are to be stored at 2-8 °C when not in use. 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 when not in use.

PCR-mix-1-FL *EBV* screen/monitor is to be stored 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***.

Analytical Sensitivity of **AmpliSens® *EBV*-screen/monitor-FRT** PCR kit is given in the table below.

Type of clinical material	Nucleic acid extraction kit	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

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, 2008.

2. Guidelines "Real-time Fluorescence PCR Detection and Quantitation of *EBV* DNA in Various Clinical Samples", 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.

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 **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
KSG1, KSG2	DNA calibrators	**PCE**	Positive Control of Extraction
	Caution		

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”