



For Professional Use Only

AmpliSens[®] CMV-screen/monitor-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens® CMV-screen/monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and quantification of human cytomegalovirus (*CMV*) DNA in the clinical materials (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, 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

CMV determination by the polymerase chain reaction (PCR) with hybridization fluorescent detection includes three stages: DNA extraction from clinical samples, PCR-amplification of pathogen genome specific region, and real-time hybridization fluorescent detection. DNA is extracted from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material in presence of Internal Control (IC STI-87), which allows monitoring of analysis of each sample. Endogenous internal control (IC Glob) allows monitoring of PCR analysis stages (DNA extraction and PCR amplification), material sampling and storage adequacy. Then, *CMV* DNA is amplified using specific primers and polymerase (TaqF). In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time 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® CMV-screen/monitor-FRT** 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® CMV-screen/monitor-FRT PCR kit is produced in 1 form:

AmpliSens® *CMV*-screen/monitor-FRT PCR kit variant FRT-100 F (for use with RG, iQ, Mx)

REF R-V7-100-S(RG,iQ,Mx)-CE.

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

<i>Reagent</i>	<i>Description</i>	<i>Volume (ml)</i>	<i>Quantity</i>
PCR-mix-1-FL <i>CMV</i> 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 calibrator KSG1	colorless clear liquid	0.2	1 tube
DNA calibrator 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 <i>CMV</i> 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.

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

***add 10 µl of Internal Control STI-87 during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep **REF** K2-9-Et-100-CE and DNA-sorb-B **REF** K1-2-100-CE protocols).

AmpliSens® *CMV*-screen/monitor-FRT PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol barriers (100 or 200 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany) iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.1-ml; for example, Axygen, USA).
- 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 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 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 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 mucous membranes. If skin, eyes, and mucous membranes contact, immediately flush with water, 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens[®] CMV-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, urine samples, 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, a reagent 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 mix the content of the tube by vortexing and incubate it for 10 min under periodic stirring. Centrifuge the 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 a pinkish film above the pellet (erythrocyte debris) is allowed. If necessary, washing with Hemolytic can be repeated. Thus obtained pellet of leukocytes 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) or it can be stored at temperature ≤ -68 °C for a long time.

Packed white cells of peripheral and/or umbilical blood

Packed white 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 temperature ≤ -68 °C for a long time.

7. WORKING CONDITIONS

AmpliSens® *CMV-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.
- DNA-sorb-B, **REF** K1-2-100-CE.
- NucliSENS easyMAG automated system can also be used.



Extract DNA according to the manufacturer’s instructions.



DNA is extracted from each clinical sample in the presence of internal control sample, **Internal Control STI-87** (10 µl of **Internal Control STI-87** is added to each sample). Transfer **100 µl** of **Negative Control** to the tube labeled C–. Transfer **90 µl** of **Negative Control** and **10 µl** of **Positive Control DNA CMV and human DNA** to the tube labeled PCE.

8.2. Preparing 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 the content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)** and mix by vortexing without foam forming.



The prepared mixture is intended for 60 samples analysis. Mixture is to be stored at the temperature between 2 °C and 8 °C for 3 months. Use when needed.



If the mixture can't be used up for 3 months it's necessary to prepare mixture for 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 that for analysis of even one clinical DNA sample in the qualitative format, it is necessary to run two controls of PCR amplification stage: positive control (DNA calibrator KSG2) and negative control of amplification (RNA-buffer). For analysis of even one clinical DNA sample in the quantitative format, it is necessary to run five controls of PCR amplification stage: 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 CMV screen/monitor** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** prepared before in a new tube in the following proportion:
 - **10 µl of PCR-mix-1-FL CMV screen/monitor,**
 - **5 µl of PCR-mix-2-FRT and polymerase (TaqF).**

Calculate the required number of reactions with allowance for the clinical and control samples. See Appendix 1.



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

4. Take the required quantity of tubes for amplification of clinical and control DNA samples. Transfer **15 µl** of the prepared mixture to each tube.
5. Add **10 µl** of **DNA** obtained from clinical or control samples to the tubes with the reaction mixture.
6. 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).

For quantitative analysis:

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

DNA calibrators KSG1 and KSG2 - Add **10 µl** of **KSG1** to two tubes and **10 µl** of **KSG2** to two other tubes.

8.2. 2. Amplification

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

Table 1

AmpliSens-1 program for rotor-type instruments¹

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	–	

Table 2

AmpliSens-1 program for plate-type instruments²

Step	Temperature, °C	Time	Fluorescence detection	Cycle repeats
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, HEX/JOE, ROX	
	72	15 s	–	

Fluorescence is detected on the 2nd step (**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, *CMV* DNA (Positive Control DNA *CMV* and human DNA) is detected in the JOE/HEX/Yellow channel, Internal Control STI-87 (IC) DNA is detected in the ROX/Orange channel.

If total DNA from cell suspension (whole human blood, white blood cells, viscera biopsy material) is isolated, the results are detected in two channels - β-globin gene DNA (IC Glob) in the FAM/Green channel, *CMV* DNA (Positive Control DNA *CMV* and human DNA) in the

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

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

JOE/HEX/Yellow channel.

If total DNA from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage are isolated with internal control sample, the results are detected in two channels: *CMV* DNA (Positive Control DNA *CMV* and human DNA) is detected in the JOE//Yellow/HEX channel, Internal Control STI-87 (IC) DNA is detected in the ROX/Orange channel.

Interpretation of results

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

If 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 *CMV* DNA if a Ct value in the JOE/Yellow/HEX channel, which does not exceed the Ct value of the positive result (see **Guidelines**), is defined in the results grid. The fluorescence curve should cross the threshold line in the exponential growth region.
2. For qualitative analysis, the sample is considered to be **negative** for *CMV* 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, the sample is considered to be negative for *CMV* 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 quantity of IC Glob DNA is greater than 2000 copies per reaction.
3. For qualitative analysis, 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 FAM/Green channel exceeds the Ct value specified in the **Important product information bulletin**. For quantitative analysis, the result of analysis 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 quantity of IC Glob DNA is less than 2000 copies per reaction. In such cases, PCR analysis of the sample should be repeated.
4. For clinical samples whose Ct values in the JOE/Yellow/HEX channel exceed the boundary Ct value specified in the Important product information bulletin, the result is considered to be **equivocal**. Such DNA samples should be additionally analyzed in duplicate. If a reproducible positive Ct value is obtained, the result should be considered as **positive**. If Ct values are not reproduced in two replicates, the result is considered as **equivocal**.

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**.

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

Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct. For quantitative analysis, the results for C+ should fall into the concentration range indicated in the **Important product information bulletin**.

Results for controls if total DNA was isolated from cell suspension (whole human blood, white blood cells, and viscera biopsy material)

Control	Stage for control	Ct in channel				Interpretation
		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 calculated concentration are determined	–	Ct value and calculated concentration are determined	OK

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

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

To express relative *CMV* DNA concentration in copies per the standard cell quantity (e.g., for 10^5), use the following recalculation ratio:

$$10^5 \text{ cells} = 2 \cdot 10^5 \text{ human genomic equivalents}$$

If total DNA from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage is

isolated together with the internal control sample, the results are interpreted as follows:

1. The sample is considered to be **positive** for *CMV* DNA if a Ct value in the JOE/Yellow/HEX channel, which does not exceed the Ct value of the positive result (see **Guidelines**), is defined in the results grid. The fluorescence curve should cross the threshold line in the exponential growth region.
2. the sample is considered to be **negative** for *CMV* 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 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**. PCR analysis of such clinical samples should be repeated.
4. For clinical samples whose Ct values in the JOE/Yellow/HEX channel exceed the boundary Ct value specified in the Important product information bulletin, the result is considered to be **equivocal**. Such DNA samples should be additionally analyzed in duplicate. If a reproducible positive Ct value is obtained, the result should be considered as **positive**. If Ct values are not reproduced in two replicates, the result is considered as **equivocal**.

Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct. For quantitative analysis, the results for C+ should fall into the concentration range indicated in the **Important product information bulletin**.

Results for controls if total DNA was isolated from peripheral blood, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage together with the internal control

Control	Stage for control	Ct in channel				Interpretation
		JOE/HEX/Yellow		ROX/Orange		
		Qualitative format	Quantitative format	Qualitative format	Quantitative 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
KSG1, KSG2	Amplification	–	Ct value and calculated concentration are determined	–	Ct value and calculated concentration are determined	OK

For quantitative analysis, the concentration of *CMV* DNA (**KP *CMV* 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:

$$\text{KP } CMV \text{ DNA} = K_{CMV \text{ DNA}} / K_{STI-87} \times IC \text{ coefficient (copies/ml)}$$

$K_{CMV \text{ DNA}}$ is the number of *CMV* DNA copies in DNA sample;

K_{STI-87} is the number of STI-87 DNA copies in DNA sample;

IC coefficient corresponds to the number of STI-87 DNA copies in DNA sample. It is specified in the Important product information bulletin provided with each lot of the reagent kit. This coefficient is specific for each lot.

10. TROUBLESHOOTING

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

1. The presence of any Ct value in JOE/Yellow/HEX, FAM/Green, and ROX/Orange channels in the results grid for the Negative Control of Amplification (NCA) and for the Negative Control of Extraction (C–) in JOE/Yellow/HEX channel indicates contamination of reagents or samples. In this case, PCR analysis should be repeated for all samples where *CMV* DNA was detected starting from the DNA extraction stage.
2. If, for qualitative analysis, the Ct value in the results grid for the Positive Control of Amplification (KSG2) in JOE/Yellow/HEX (*CMV*), FAM/Green, or ROX/Orange channels is absent, it is necessary to repeat amplification for all samples where *CMV* DNA was not detected.
3. If the Ct value in the results grid for the Positive Control of Extraction (Positive Control DNA *CMV* and human DNA) in JOE/Yellow/HEX (*CMV*), FAM/Green, or ROX/Orange channels is absent, the results of analysis of all samples are considered to be **invalid**. It is necessary to repeat PCR analysis for such samples.
4. If the Ct value for the sample in the JOE/Yellow/HEX channel is absent or exceeds the boundary Ct value specified in the **Important product information bulletin** and the Ct value for the sample is greater than the maximum Ct value for IC in FAM/Green and ROX/Orange channels, it is necessary to repeat the analysis starting from the DNA

extraction stage. This error may be due to incorrect treatment of clinical material, which resulted in the loss of DNA, or to the presence of PCR inhibitors.

5. If the Ct value for the sample in the JOE/Yellow/HEX channel is absent or exceeds the boundary Ct value specified in the **Important product information bulletin** and Ct value for the sample is less than the boundary Ct value in FAM/Green and ROX/Orange, the result is considered to be **equivocal**. It is necessary to repeat analysis of such samples in duplicate. If a reproducible positive Ct value is obtained, the result is considered to be **positive**; otherwise, the result is considered to be **equivocal**.

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

11. TRANSPORTATION

AmpliSens® CMV-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® CMV-screen/monitor-FRT** PCR kit (except for PCR-mix-1-FL *CMV* 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® CMV-screen/monitor-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.



PCR-mix-1-FL *CMV* screen/monitor, PCR-mix-2-FRT, and Polymerase (TaqF) are to be stored at temperature from minus 24 to minus 16 °C when not in use.



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

13. SPECIFICATIONS

13.1 Sensitivity

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

The analytical sensitivity of **AmpliSens® CMV-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, urine samples, bronchoalveolar lavage	RIBO-prep	400 copies/ml
Whole human blood, white blood cells, viscera biopsy material	RIBO-prep	5 CMV DNA copies per 10 ⁵ cells

13.2 Specificity

AmpliSens[®] CMV-screen/monitor-FRT PCR kit is intended for human cytomegalovirus (CMV) DNA detection. Specific activity of **AmpliSens[®] CMV-screen/monitor-FRT** PCR kit was confirmed in studies of the CMV reference strain AD 169 as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplification fragments. The activity of the PCR kit components with respect to DNA of other viruses (Epstein-Barr virus, 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[®] CMV-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 CMV 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[®] CMV-screen/monitor-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Expiration Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	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
	Authorised representative in the European Community	PCE	Positive Control of Extraction
	Caution	KSG1, KSG2	DNA Calibrators

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”