CE 1023

IVD

For Professional Use Only

AmpliSens® *CMV*-FEP

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***CMV*****-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of human cytomegalovirus (*CMV*) DNA in the clinical materials (urogenital swabs, urine samples, saliva, and human whole blood) by using end-point hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

CMV DNA detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens®** ***CMV*****-FEP** PCR kit is a qualitative test that contains the Internal Control (IC). It 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®** ***CMV*****-FEP** 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 wax layer. Wax melts and reaction components mix only at 95 ºC.

3. CONTENT

AmpliSens® ***CMV*****-FEP** PCR kit is produced in 2 forms:

AmpliSens® *CMV*-FEP PCR kit variant FEP (0.5-ml tubes) **REF** V7-100-R0,5-FEP-CE.

AmpliSens® *CMV*-FEP PCR kit variant FEP (0.2-ml tubes) **REF** V7-100-R0,2-FEP-CE.

AmpliSens® *CMV*-FEP PCR kit variant FEP includes:

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FL *CMV* ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.01	110 tubes of 0.2 ml or 0.5 volume
PCR-mix-2-FL-red	red clear liquid	1.1	1 tube
PCR-mix-Background-red	red clear liquid	0.6	1 tube
Mineral oil for PCR*	colorless viscous liquid	4.0	1 dropper bottle
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)**	colorless clear liquid	1.2	1 tube
Internal Control-FL***	colorless clear liquid	1.0	1 tube

* must be used for thermocyclers without a constant-temperature lid.

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

*** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-AM **REF** K1-12-100-CE protocol).

AmpliSens® *CMV*-FEP PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes.
- Disposable tips with aerosol barriers (up to 100 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), MaxyGene (Axygen, USA), or equivalent).
- Fluorometer (for example, ALA-1/4 (Biosan, Latvia), or equivalent).
- Disposable polypropylene microtubes for PCR (0.2- or 0.5-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 mucosa. If skin, eyes and mucosa 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 move 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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *CMV*-FEP PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from scrapes from mucous membranes of urogenital tract, urine samples, saliva and whole human blood.

7. WORKING CONDITIONS

AmpliSens® *CMV*-FEP 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:

- DNA-sorb-AM, **REF** K1-12-100-CE.
- Other nucleic acid extraction kits, recommended by Federal Budget Institution of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being (see **Guidelines**).

Extract DNA according to the manufacturer's instructions.

8.2. Preparing PCR

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

8.2.1 Preparing tubes for PCR

1. Prepare the required number of the tubes with **PCR-mix-1-FL *CMV*** and wax for amplification of DNA from test and control samples.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer of each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *CMV*.**
3. Add above **1** drop of **mineral oil for PCR** (~ **25 µl**).
4. Prepare 1 tube with **PCR-mix-1-FL *CMV*** and mark it as **Background**. Add **20 µl** of **PCR-mix-Background-red** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FL *CMV***. Add above **1** drop of **mineral oil for PCR.**

Use **mineral oil for PCR** if working with thermocyclers without a constant-temperature lid.

Use **PCR-mix-Background-red** solution only if DNA samples were isolated with DNA-sorb-AM or DNA-sorb-B kits. If any other nucleic acid extraction kits (recommended by FBIS CRIE) were used, follow the instructions provided by the manufacturer.

5. Using tips with aerosol barrier, add **10 µl** of **DNA samples** obtained from test or control samples at the DNA extraction stage.
6. Carry out the control amplification reactions:

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

C+ - Add **10 µl** of **Positive Control complex** to the tube labeled C+ (Positive Control

of Amplification).

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

8.2.2 Amplification

1. Run the program **"AmpliSens-1-FEP amplification program"** on the thermocycler (see Table 1).
2. When the temperature reaches 95ºC (pause regimen), insert tubes to cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 1

"AmpliSens-1-FEP amplification program"

	GeneAmp PCR System 2700			**Gradient Palm Cycler, MaxyGene**		
Cycle	Temperature, °C	Time	Cycle repeats	Temperature, °C	Time	Cycle repeats
0	**95**	Pause		**95**	Pause	
1	**95**	5 min	1	**95**	5 min	1
2	**95**	20 s	20	**95**	2 s	24
	65	25 s		**65**	10 s	
	72	30 s		**72**	10 s	
3	**95**	20 s	24	**95**	2 s	20
	60	30 s		**60**	15 s	
	72	30 s		**72**	10 s	
4	**95**	20 s	1	**95**	2 s	1
	60	30 s		**60**	15 s	
5	**10**	storage		**10**	storage	

3. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Detection is performed with florescence detector according to the protocol provided by the manufacturer (please read the Instrument Operating Manual before using this kit).

The fluorescent signal intensity is detected in two channels:

– The signal from the *CMV* DNA amplification product is detected in the FAM channel (or analogous, depending on the detector model);

– The signal from the Internal Control amplification product is detected in the HEX channel (or analogous, depending on the detector model).

Program the detector according to the **Manufacturer's manual** and **Important Product Information Bulletin**.

Results interpretation

1. Principle of interpretation:

— *CMV* DNA is **detected** in a sample if its signal in the FAM channel is greater than the

defined threshold value of the positive result.

— *CMV* DNA is **not detected** in a sample if its signal in the FAM channel is less than the defined threshold value of the negative result while the signal in the HEX channel is greater than the defined threshold value.

— The result is **invalid** if the signal of a sample in the FAM channel is less than defined threshold of the negative result and the signal in the HEX channel is less than the defined threshold value.

— The result is **equivocal** if the signal of a sample in the FAM channel is greater than the defined threshold value of the negative result but less than the threshold value of the positive result (the signal is between thresholds).

Run the PCR test for the sample once again if the result is **invalid** or **equivocal**.

2. 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 (Table 2).

Table 2

Results for controls

Control	Stage for control	Result on channel		Interpretation
		FAM	HEX	
C–	DNA extraction	**<** threshold of negative result	> threshold value	«–» or OK
NCA	Amplification	**<** threshold of negative result	< threshold value	«nd»
C+	Amplification	**>** threshold of positive result	> threshold value	«+» or OK

10. TROUBLESHOOTING

- If the signal of the positive control of amplification (C+) in the FAM channel is less than the threshold of the positive result, repeat PCR and detection for all samples in which *CMV* DNA was not found.
- If the signal of the Negative Control of extraction (C–) and/or Negative Control of amplification (NCA) in the FAM channel is more than the threshold of the positive result, repeat PCR analysis of all samples in which *CMV* DNA was found starting from the extraction stage.

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

11. TRANSPORTATION

AmpliSens® *CMV*-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *CMV*-FEP** PCR kit are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *CMV*-FEP** 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* is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *CMV*-FEP** PCR kit is the following:

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml*
Urogenital swabs	Transport Medium for Swabs or Transport Medium with Mucolytic	DNA-sorb-AM	10^3
Urine (pretreatment is required)	–	DNA-sorb-AM	$2x10^3$

***** Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.

13.2. Specificity

The analytical specificity of **AmpliSens® *CMV*-FEP** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The clinical specificity of **AmpliSens® *CMV*-FEP** 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 Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines “End-Point PCR Detection of STIs and Other Reproductive Tract Infections”, developed by Federal Budget Institution 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 Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *CMV*-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification

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 Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”