

# AmpliSens<sup>®</sup> CMV-screen/monitor-FRT PCR kit



For Professional Use Only

## Instruction Manual

### KEY TO SYMBOLS USED

	Catalogue number		Sufficient for
	Batch code		Use-by Date
	<i>In vitro</i> diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limit	<b>NCA</b>	Negative control of amplification
	Manufacturer	<b>C-</b>	Negative control of extraction
	Date of manufacture	<b>C+</b>	Positive control of amplification
	Authorized representative in the European Community	<b>PCE</b>	Positive Control of Extraction
	Caution	<b>KSG1, KSG2</b>	DNA Calibrators
		<b>IC</b>	Internal control

### 1. INTENDED USE

AmpliSens<sup>®</sup> CMV-screen/monitor-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *human cytomegalovirus* (CMV) DNA in the clinical material (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material) using real-time hybridization-fluorescence detection of amplified products.

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

### 2. PRINCIPLE OF PCR DETECTION

CMV detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special CMV 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<sup>®</sup> CMV-screen/monitor-FRT PCR kit is a qualitative and quantitative test which is used with two internal controls: exogenous and endogenous.

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), 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  $\beta$ -globin gene is amplified. DNA fragment of  $\beta$ -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<sup>®</sup> CMV-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.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because deoxyuridine triphosphate is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR. The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE	ROX
DNA-target	IC Glob	CMV	Internal Control STI-87 (IC)
Target gene	$\beta$ -globin gene	Pol gene	genetically engineered construction

### 3. CONTENT

AmpliSens<sup>®</sup> CMV-screen/monitor-FRT PCR kit is produced in 1 form:

variant FRT-100 F R-V7-100-S(RG,iQ,Mx)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL CMV screen/monitor	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
RNA-buffer	colorless clear liquid	0.6	1 tube
DNA calibrator KSG1	colorless clear liquid	0.2	1 tube
DNA calibrator KSG2	colorless clear liquid	0.2	1 tube
RNA-buffer	colorless clear liquid	1.2	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	2 tubes
Positive Control DNA CMV 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  $\mu$ l of Internal Control STI-87 (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep K2-9-Et-100-CE and DNA-sorb-B K1-2-100-CE protocols).

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

### 4. ADDITIONAL REQUIREMENTS

- Hemolytic.
- Disposable polypropylene screwed or tightly closed 1.5-ml tubes.
- DNA extraction kit or the DNA extraction automatic station.
- Additional requirements for DNA extraction kit or DNA extraction automatic station.
- Disposable polypropylene screwed or tightly closed 2.0-ml tubes.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 100 and 200  $\mu$ l).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany) iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  - a) 0.2-ml PCR tubes with domed 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 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

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

**AmpliSens<sup>®</sup> CMV-screen/monitor-FRT** PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), 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. Add 1.0 ml of **Hemolytic** (REF 137-CE, manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") and 0.25 ml of whole blood to 1.5-ml Eppendorf tube using an individual tip. Carefully vortex the contents of the tube and incubate it for 10 min with periodic stirring. Centrifuge tubes at 8,000 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. Thus obtained leukocyte pellet should be lysed immediately (in case of RIBO-prep extraction, add 300 µl of Solution for Lysis and then extract DNA according to the 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

It is obtained from 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 800-1,600 g (3,000 rpm) for 20 min. Then, collect the white film formed on the surface of the supernatant and carry out the pretreatment as described for whole peripheral and umbilical blood. White cells of peripheral and umbilical cord blood can be stored at ≤ -68 °C for a long time.

## 7. WORKING CONDITIONS

**AmpliSens<sup>®</sup> 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 (for details see Guidelines [2]).
- The DNA extraction of each clinical sample is carried out in the presence of **Internal Control STI-87 (IC)** (add 10 µl of **Internal Control STI-87 (IC)** into each sample).

**NOTE:** Addition of **Internal Control STI-87 (IC)** 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 (C-)** and 10 µl of **Positive Control DNA CMV and human DNA** to the tube labeled PCE (Positive Control of Extraction).

**NOTE:** Extract DNA according to the manufacturer's protocols.

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for PCR

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl. The type of tubes depends on the type of PCR real-time instrument. Use disposable filter tips for adding reagents, cDNA and control samples into tubes.

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. Mark the tube with the mixture preparation date.

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

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

2. Prepare the reaction mixture. Note that, for analysis of even 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)**. For analysis of even one DNA sample in the **quantitative format**, it is necessary to run **five controls** of amplification: two DNA calibrators (**KSG1** and **KSG2**) in two replicates and the **Negative Control of Amplification (RNA-buffer)**. In addition, 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 earlier 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 including the test and control samples (see Table 2).

Scheme of reaction mixture preparation

Total reaction volume is 25 µl including the volume of DNA sample – 10 µl.			
Reagent volume per 1 reaction, µl		10,0	5,0
Number of clinical samples		PCR-mix-1-FL CMV screen/monitor <sup>1</sup>	Mixture of PCR-mix-2-FRT and polymerase (TaqF) <sup>1</sup>
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

**NOTE:** If 60 samples are analyzed simultaneously, a simplified scheme of mixture preparation can be used. 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 CMV screen/monitor**.

- 4 Take the required quantity of tubes for amplification of test and control DNA samples. Transfer 15 µl of the prepared mixture to each tube.

- 5 Add 10 µl of **DNA** obtained at the DNA extraction stage into each tube 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 **DNA calibrator 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 CMV 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).

- DNA calibrators KSG1 and KSG2** – Add 10 µl of **KSG1** to two tubes and 10 µl of **KSG2** to two other 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 CMV and human DNA** reagent to the tube labeled PCE (Positive control of Extraction).

### 8.2.2. Amplification

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

AmpliSens-1 amplification program

Step	Rotor-type instruments <sup>2</sup>			Plate-type instruments <sup>3</sup>		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
72	15 s	72		15 s		
Cycling 1	95	5 s	40	95	5 s	40
	60	20 s		60	30 s	
	72	15 s		72	15 s	
	72	15 s		72	15 s	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines [2].

3. Insert the tubes into the reaction module of the instrument.

4. Run the amplification program with fluorescence detection.

5. Analyze results after the amplification program is completed.

<sup>1</sup> Values are given with account of one extra reaction and five controls (2 DNA calibrators KSG1 and KSG2 (in two replicates), negative control (RNA-buffer) for quantitative analysis of CMV DNA, and two controls (positive and negative) for qualitative analysis of CMV DNA.

<sup>2</sup> For example, Rotor-Gene 3000, Rotor-Gene 6000 or equivalent.

<sup>3</sup> For example, iCycler iQ5, Mx3000P or equivalent.

## 9. DATA ANALYSIS

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

- The signal of **β-globin gene DNA (IC Glob)** amplification product is detected in the channel for the FAM fluorophore.
- The signal of the **CMV DNA (Positive Control DNA CMV 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.

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

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

The results are analyzed in two channels:

- the signal of β-globin gene DNA (IC Glob) amplification product is detected in the channel for the FAM fluorophore,
- the signal of CMV DNA (Positive Control DNA CMV and human DNA) amplification product is detected in the channel for the JOE fluorophore.

Principle of interpretation is the following:

- CMV 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.
- CMV 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 greater than 2000 copies/reaction (for quantitative analysis).
- 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.
- 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 carry out 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**.
- For qualitative analysis, the negative result is considered **unreliable** if the Ct value in the channel for the FAM fluorophores is greater than the boundary value specified in the *Important Product Information Bulletin*. For quantitative analysis, the quantitative positive or negative results are considered **unreliable** if the quantity of IC Glob DNA is less than 2000 copies/reaction.

**NOTE:** Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit. See also Guidelines [2]

The result of the analysis is considered reliable only if the results obtained for controls C–, PCE, NCA, C+, KSG1, and KSG2 are correct (see Table 4). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin*.

Table 4

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

Control	Stage for control	Amplification results in the channel for the fluorophore			
		FAM		JOE	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction, PCR	Absent	Absent	Absent	Absent
PCE	DNA extraction, PCR	Ct < boundary value	Ct < boundary value	Ct < boundary value	concentration value falls in the range specified in the <i>Important Product Information Bulletin</i>
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	Ct < boundary value	–	Ct < 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 CMV 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:

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

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

$$10^5 \text{ of cells} = 2 \cdot 10^5 \text{ human genomes}$$

### 9.2. Interpretation of results for DNA extracted from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), bronchoalveolar lavage with internal control sample

The results are analyzed in two channels:

- the signal of the CMV DNA (Positive Control DNA CMV 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.

Principle of interpretation is the following:

- CMV 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.
- CMV 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*.
- 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.
- 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 controls C–, PCE, NCA, C+, are correct (see Table 4). For quantitative analysis the results for Positive Control should fall in the concentration range specified in the *Important Product Information Bulletin*.

Table 4

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

Control	Stage for control	Amplification results in the channel for the fluorophore			
		JOE		ROX	
		Qualitative format	Quantitative format	Qualitative format	Quantitative format
C–	DNA extraction, PCR	Absent	Absent	Ct < boundary value	Ct < boundary value
PCE	DNA extraction, PCR	Ct < boundary value	concentration value falls in the range specified in the <i>Important Product Information Bulletin</i>	Ct < boundary value	Ct < boundary value
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	Ct < boundary value	–	Ct < boundary value	–
KSG1, KSG2	PCR	–	Ct value and calculated concentration are determined	–	Ct value and calculated concentration are determined

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

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

$K_{\text{CMV DNA}}$  – quantity of CMV 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 CMV 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. If any Ct value appears in the channel for ROX fluorophore for the Negative Control of Amplification (NCA), in the channels for FAM and JOE fluorophores for Negative Control of Amplification (NCA) and Negative Control of Extraction (C–), these results testify the presence of contamination of reagents or samples. In that case the experiment needs to be repeated for all samples, in which DNA was found, starting with the extraction stage.
  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 channels for JOE (CMV), FAM or ROX fluorophores, the amplification must be repeated for all samples where **CMV 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 CMV and human DNA** – in the channels for JOE (CMV), 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 the channel for JOE fluorophore channel and the Ct value in the channels for FAM or ROX fluorophores 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 the channel for JOE fluorophore and the Ct value in the channels for FAM or ROX fluorophores 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 quantitative analysis the copies/reaction values in DNA calibrators differ by more than 30 % from the set values, it is necessary to check the tube order in the rotor (calibrators should be placed in the wells indicated as **Standard** in sample table, concentration should correspond to concentration specified in the *Important Product Information Bulletin*, well no.1 must be filled with some test tube (not empty)).
  7. If the correlation coefficient R in **Standard Curve** window is less than 0.9 (in case of quantitative analysis), it means that calibration failed. Check the settings of calibrators and correct inaccuracies, if no effect, repeat PCR for all samples and calibrators.
- If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

## 11. TRANSPORTATION

AmpliSens® 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.

**NOTE:** 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.

**NOTE:** 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), bronchoalveolar lavage	RIBO-prep	400 copies per ml
Whole human blood, white blood cells, viscera biopsy material	RIBO-prep	5 copies per 10 <sup>5</sup> cells

### 13.2. Specificity

The analytical specificity of **AmpliSens® CMV-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® 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

- 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.
- Guidelines to **AmpliSens® CMV-screen/monitor-FRT** PCR kit for qualitative detection and quantification of human cytomegalovirus (*CMV*) DNA in the clinical materials by using real-time hybridization-fluorescence detection, 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 Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® CMV-screen/monitor-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
23.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science "Central Research Institute for Epidemiology"
19.06.15 ME	Through the text	Corrections according to the template. Grammar corrections
	2. Principle of PCR detection	The section was rewritten
	8.1. DNA Extraction	Information about controls of extraction was added. 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
28.12.15 PM	9. Data analysis	The sections were rewritten
	10. Troubleshooting	
27.12.17 PM	Through the text	The clinical material saliva, oropharyngeal swabs, urine samples was deleted
05.12.18 PM	3. Content	The color of the reagent was specified
27.02.20 PM	2. Principle of PCR detection	The table with targets and the information about the enzyme UDG were added
	Through the text	The text formatting was changed
01.03.21 MM	Footer	The phrase "Not for use in the Russian Federation" was added
—	—	The name, address and contact information for Authorized representative in the European Community was changed

**AmpliSens®**



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