



For Professional Use Only

AmpliSens[®] *HHV6*-screen-titre-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens[®] HHV6-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of *human herpes virus* type 6 (*HHV6*) DNA in the clinical material (whole human blood, white blood cells, viscera biopsy material, saliva, oropharyngeal swabs and cerebrospinal fluid (liquor)) using real-time 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

HHV6 DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HHV6* primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens[®] HHV6-screen-titre-FRT PCR kit is a qualitative test 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. 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[®] HHV6-screen-titre-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 using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens[®] HHV6-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens[®] HHV6-screen-titre-FRT PCR kit variant FRT-100 F **REF** R-V10-T(RG,iQ,Mx)-CE.

AmpliSens® HHV6-screen-titre-FRT PCR kit variant FRT-100 F includes:

<i>Reagent</i>		<i>Description</i>	<i>Volume, ml</i>	<i>Quantity</i>
PCR-mix-1-FRT HHV-6 / Glob		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
Negative Control (C-)*		colorless clear liquid	1.2	2 tubes
Positive Control DNA HHV-6 and human DNA**		colorless clear liquid	0.2	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).

AmpliSens® HHV6-screen-titre-FRT PCR kit is intended for 110 reactions (including controls and DNA calibrators).

4. ADDITIONAL REQUIREMENTS

- Hemolytic.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 200 µl).
- 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 iQ or 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 at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir 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 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 an assay.
- 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 afterwards.
- 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 accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another 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 in the area where 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 the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® HHV6-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the clinical material (whole human blood, white blood cells, viscera biopsy material, saliva, oropharyngeal swabs, and cerebrospinal fluid (liquor)).

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® HHV6-screen-titre-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.
- DNA-sorb-C, **REF** K1-6-100-CE (for 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 HHV-6 and human DNA** to the tube labeled **PCE** (Positive Control of Extraction).



Extract DNA according to the manufacturer's instructions.

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 one tube with **Polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT (300 µl)**. Vortex carefully avoiding foaming. Mark the tube with the mixture preparation date.



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



If the mixture cannot be used up for 3 months, prepare the 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-FRT HHV-6 /Glob** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)** prepared earlier in an individual tube in the following proportion:
 - **10 µl of PCR-mix-1-FRT HHV-6 /Glob**,

– 5 µl of PCR-mix-2-FRT and polymerase (TaqF) mixture.

Calculate the required number of reactions including the test and control samples (see Table 1).

Table 1

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-FRT <i>HHV-6</i> / Glob ¹	Mixture of PCR-mix-2-FRT and polymerase (TaqF) ¹
For quantitative analysis	For qualitative analysis		
1	4	70	35
2	5	80	40
3	6	90	45
4	7	100	50
5	8	110	55
6	9	120	60
7	10	130	65
8	11	140	70
9	12	150	75
10	13	160	80
11	14	170	85
12	15	180	90
13	16	190	95
14	17	200	100
15	18	210	105
16	19	220	110
17	20	230	115
18	21	240	120
19	22	250	125
20	23	260	130
21	24	270	135
22	25	280	140
23	26	290	145
24	27	300	150
25	28	310	155
30	33	360	180

¹ 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 *HHV6* DNA, and two controls (positive and negative) for qualitative analysis of *HHV6* DNA.



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-FRT HHV-6 /Glob.**

4. Take the required number 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 samples** obtained at the DNA extraction stage into each tube 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 **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 HHV-6 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 add **10 µl** of **KSG2** to other two tubes.

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

PCE – Add **10 µl** of **the sample extracted from the Positive control DNA HHV-6 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 ²			Plate-type instruments ³		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
Hold	95	15 min	1	95	15 min	1
Cycling 1	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
Cycling 2	95	5 s	40	95	5 s	40
	60	20 s <i>Fluorescence acquiring</i>		60	30 s <i>Fluorescence acquiring</i>	
	72	15 s		72	15 s	

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

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in 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 **HHV6 DNA (Positive Control DNA HHV-6 and human DNA)** amplification product is detected in the channel for the JOE 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.

Principle of interpretation is the following:

- **HHV6 DNA is detected** if the *Ct* value determined in the results grid in the channel for the JOE fluorophore does not exceed the boundary value. Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

² For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.

³ For example, iCycler iQ5, Mx3000P, Mx3000 or equivalent.

- *HHV6* 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 for qualitative analysis the *Ct* value in the channel for the FAM fluorophore does not exceed the boundary *Ct* value specified in the *Important Product Information Bulletin* and for quantitative analysis the quantity of IC Glob DNA is greater than 2000 copies per reaction in the case of whole blood, white blood cells, and viscera biopsy material and greater than 500 copies per reaction in the case of saliva and oropharyngeal swabs. For cerebrospinal fluid (liquor), the *Ct* value in the results grid in the channel for the FAM fluorophore can be greater than the boundary *Ct* value specified in *the Important Product Information Bulletin* or the quantity of IC Glob DNA can be less than 500 copies per reaction in the case of quantitative analysis because cerebrospinal fluid samples may contain a very small number of cells.
- The result of analysis is **invalid** if the *Ct* value is not determined (absent) in the results grid or if it is greater than the boundary *Ct* value in the channel for the JOE fluorophore whereas for qualitative analysis the *Ct* value in the channel for the FAM fluorophore exceeds the *Ct* value specified in the *Important Product Information Bulletin* and for quantitative analysis the quantity of IC Glob DNA is less than 2000 copies per reaction for whole blood, white blood cells, viscera biopsy material and less than 500 copies per reaction for saliva, oropharyngeal swabs. In such case the PCR analysis should be repeated for required sample.
- The result of analysis is **equivocal** if the *Ct* value in the channel for the JOE fluorophore exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin*. It is necessary to carry out the additional analysis for such sample with two repeats. If a reproducible positive result is obtained, the result is considered positive. If the obtained *Ct* values are not reproducible 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 fluorophore exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin*. For quantitative analysis, the quantitative positive or negative result is considered **unreliable** if the quantity of IC Glob DNA is less than 2000 copies per reaction in the case of whole blood, white blood cells, and viscera biopsy material or less than 500 copies per reaction in the case of saliva and oropharyngeal swabs.



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

Table 3

Results for controls

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	$Ct < \text{boundary value}$	$Ct < \text{boundary value}$	$Ct < \text{boundary value}$	concentration value falls in the range specified in the <i>Important Product Information Bulletin</i>
NCA	PCR	Absent	Absent	Absent	Absent
C+	PCR	$Ct < \text{boundary value}$	–	$Ct < \text{boundary value}$	–
KSG1, KSG2	PCR	–	Ct value and calculated concentration are defined	–	Ct value and calculated concentration are defined

For whole human blood, white blood cells, or viscera biopsy material calculate the concentration in logarithm of *HHV6* DNA copies per the standard cell quantity (10^5) in control and test samples using the following formula:

$$\lg \left\{ \frac{\text{number of } HHV6 \text{ DNA copies in PCR sample}}{\text{number of Glob DNA copies in PCR sample}} \times 2 \cdot 10^5 \right\} = \lg \{ HHV6 \text{ DNA copies} / 10^5 \text{ cells} \}$$

For saliva, oropharyngeal swabs, and cerebrospinal fluid (liquor) calculate the concentration of *HHV6* DNA per ml of sample (CS_{HHV6} DNA) using the following formula:

$$CS_{HHV6} \text{ DNA} = K_{HHV6} \text{ DNA} \times 100 \text{ (copies/ml)}$$

K_{HHV6} DNA is the quantity of *HHV6* DNA copies in the DNA sample.

10. TROUBLESHOOTING

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

1. If any *Ct* value appears in the results grid in the channel for the JOE fluorophore and the *Ct* value determined in the channel for the FAM fluorophore for Negative Control of Amplification (NCA) and for Negative Control of Extraction (C–) is less than the value specified in the *Important Product Information Bulletin*, it indicates contamination of reagents or samples. Repeat PCR analysis for all samples in which *HHV6* DNA was detected starting from the DNA extraction stage.
2. In qualitative analysis, if the *Ct* value for the Positive Control of Amplification (C+) – **KGS2** – in the channel for the JOE (*HHV6*) or FAM fluorophore is absent or greater than the boundary value in the results grid, repeat amplification of all samples in which *HHV6* DNA was not detected.
3. If the *Ct* value for the Positive Control of Extraction (PCE) – **Positive Control DNA *HHV-6* and human DNA** – in the channel for the JOE (*HHV6*), FAM, or ROX fluorophore is absent or greater than the boundary value, the results of analysis of all samples are considered invalid. Repeat analysis of all samples starting from the DNA amplification stage.
4. If the *Ct* value for a sample in the channel for the JOE fluorophore is absent or exceeds the boundary *Ct* value and the *Ct* value for the same sample in the channel for the FAM fluorophore exceeds the boundary *Ct* value specified for Internal Control, repeat analysis of the sample starting from the DNA extraction stage. This may be caused by errors in preparation of clinical material, which entailed the loss of DNA, or by the presence of inhibitors.
5. If the *Ct* value for a clinical sample in the channel for the JOE fluorophore (*HHV6* DNA) exceeds the boundary *Ct* value, the result of analysis is considered **equivocal**. Repeat analysis of such samples in duplicate. If a positive result is obtained in both replicates, the result of analysis is considered as **positive**. If the result is not reproduced in two replicates, the result of analysis of such samples is considered **equivocal**.
6. If the number of copies per reaction in DNA calibrators in quantitative tests exceeds the specified value by more than 30%, check the order of the tubes in the rotor (DNA calibrators should be inserted into the cells named **Standard** in the table of samples, the concentration of samples should correspond to the concentration specified in the **Important Product Information Bulletin**, and cell no. 1 in rotor-type instruments should not be empty (fill it with any test tube).
7. If the correlation coefficient **R** in the **Standard Curve** window in quantitative tests is less than 0.9, this indicates error in calibration. Check whether the settings for DNA calibrators are correct and change them, if necessary. If this does not help, run PCR for

all samples and calibrators.

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

11. TRANSPORTATION

AmpliSens® HHV6-screen-titre-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® HHV6-screen-titre-FRT** PCR kit (except for PCR-mix-1-FRT *HHV-6* / Glob, polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® HHV6-screen-titre-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-FRT *HHV-6* / Glob, polymerase (TaqF), and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.



PCR-mix-1-FRT *HHV-6* / Glob should be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® HHV6-screen-titre-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Sensitivity
Cerebrospinal fluid (liquor), saliva, oropharyngeal swabs	RIBO-prep	400 copies per 1 ml
Whole human blood, white blood cells and viscera biopsy material	RIBO-prep	5 copies per 10 ⁵ cells

Linear range of **AmpliSens® HHV6-screen-titre-FRT** PCR kit is **500–10.000.000 copies/ml**.

13.2. Specificity

The analytical specificity of **AmpliSens® HHV6-screen-titre-FRT** PCR kit is ensured by the 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.

Nonspecific reactions were absent in tests with DNA of other viruses (herpes simplex virus types 1 and 2, Epstein-Barr human virus, human herpes virus type 8, varicella-zoster virus, parvovirus B19, and others), bacterial pathogens (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and others) and human DNA.

The clinical specificity of **AmpliSens® HHV6-screen-titre-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 State Institute of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2010.
2. Guidelines to the **AmpliSens® HHV6-screen-titre-FRT** PCR kit for qualitative and quantitative detection of *human herpes virus* type 6 (*HHV6*) DNA in clinical materials by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® HHV6-screen-titre-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	IC	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
07.12.11 VV	Specifications, Sensitivity	The linear measurement range was added
27.04.15 ME	Through the text	Corrections according to the template. Grammar corrections
	8.2.1 Preparing tubes for PCR	Appendix 1 was integrated into the text of the instruction manual as Table 1
	9. Data analysis 10. Troubleshooting	The sections were rewritten