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For Professional Use Only

AmpliSens® *Enterovirus*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *Enterovirus*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of the *Human enterovirus* RNA in the biological material (cerebrospinal fluid, fecal samples), taken from the persons suspected of enteroviral infection without distinction of form and presence of manifestation, and natural environments (concentrated water samples) 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

Human enterovirus detection by the polymerase chain reaction (PCR) includes three stages: the RNA extraction from the test samples, combined stage of the RNA reverse transcription and the given microorganism cDNA fragment amplification and real-time hybridization-fluorescence detection.

Human enterovirus detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Human enterovirus* 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® *Enterovirus*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87-rec). 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® *Enterovirus*-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 chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *Enterovirus*-FRT PCR kit is produced in 2 forms:

AmpliSens® *Enterovirus*-FRT PCR kit variant FRT-50 F, **REF** R-V16-F-CE.

AmpliSens® *Enterovirus*-FRT PCR kit variant FRT-50 F in bulk[1], **REF** R-V16-F-CE-B.

AmpliSens® *Enterovirus*-FRT PCR kit variant FRT-50 F includes:

Reagent	*Description*	*Volume, ml*	*Quantity*
PCR-mix-FL *Enterovirus* / STI	colorless clear liquid	0.6	1 tube
PCR-buffer-C	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
RT-G-mix-2	colorless clear liquid	0.015	1 tube
Positive Control *Enterovirus* / STI (C+$_{Enterovirus\ /\ STI}$)	colorless clear liquid	0,2	1 tube
TE-buffer	colorless clear liquid	0,2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control STI-87-rec (IC)**	colorless clear liquid	0,5	1 tube

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

** add 10 µl of Internal Control during the RNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, **REF** K2-9-Et-50-CE protocol).

AmpliSens® *Enterovirus*-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- 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), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat caps , or tubes (0.2 ml) with transparent caps from the eight-pieces-strips 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 with the temperature range from 2 to 8 °C.
- Deep-freezer with the temperature range 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 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® *Enterovirus*-FRT PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from the biological material (cerebrospinal fluid, fecal samples) and natural environments (concentrated water samples).

The material is to be stored at 2 to 8 °C within 1 day, at minus 24 to minus 16 °C within 1 week.

Only one freeze-thaw cycle is allowed.

Pretreatment

The pretreatment of cerebrospinal fluid and concentrated water samples is not required.

Fecal samples are to be pretreated.

Samples pretreatment is carried out in accordance with the manufacturer's handbook [1].

7. WORKING CONDITIONS

AmpliSens® *Enterovirus*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It is recommended to use the following nucleic acid extraction kits:

– RIBO-prep, REF K2-9-Et-50-CE;

Extract the RNA according to the manufacturer's protocol.

8.2. Preparing reverse transcription and PCR

8.2.1 Preparing tubes for RT-PCR

The total reaction volume is 25 µl, the volume of RNA sample is 10 µl.

1. Prepare the reaction mixture just before use. Prepare the reaction mixture for required number of reactions (including clinical and control samples) as specified in Table 1.

Carry out all control reactions of reverse transcriptions and amplification (positive (C+) and negative (NCA)) for testing even one test sample. Prepare the reagent mixture for an even number of reactions to attain more precise dispensing.

2. Take the required number of the tubes taking into account the number of test samples and control samples. Select the type of the tubes, stripes and plates according to used device.
3. To prepare the reaction mixture add to a new sterile tube **PCR-mix-FL *Enterovirus* / STI**, **PCR-buffer-C**, **RT-G-mix-2, polymerase (TaqF)** and **TM-Revertase (MMlv)** in accordance to Table 1. Thoroughly vortex the tubes and sediment the drops from the caps of the tubes.
4. Transfer **15 µl** of the prepared mixture to each tube.

Table 1

Scheme of reaction mixture preparation

		Reagent volume for the specified number of reactions, µl				
Reagent volume per one reaction, µl		**10.00**	**5.00**	**0.25**	**0.50**	**0.25**
Number of test samples	**Number of reactions[2]**	**PCR-mix-FL *Enterovirus* / STI**	**PCR-buffer-C**	**RT-G-mix-2**	**Polymerase (TaqF)**	**TM-Revertase (MMlv)**
2	**6**	**60**	**30**	**1.5**	**3.0**	**1.5**
4	**8**	**80**	**40**	**2.0**	**4.0**	**2.0**
6	**10**	**100**	**50**	**2.5**	**5.0**	**2.5**
8	**12**	**120**	**60**	**3.0**	**6.0**	**3.0**
10	**14**	**140**	**70**	**3.5**	**7.0**	**3.5**
12	**16**	**160**	**80**	**4.0**	**8.0**	**4.0**
14	**18**	**180**	**90**	**4.5**	**9.0**	**4.5**
16	**20**	**200**	**100**	**5.0**	**10.0**	**5.0**
18	**22**	**220**	**110**	**5.5**	**11.0**	**5.5**
20	**24**	**240**	**120**	**6.0**	**12.0**	**6.0**
22	**26**	**260**	**130**	**6.5**	**13.0**	**6.5**
24	**28**	**280**	**140**	**7.0**	**14.0**	**7.0**
26	**30**	**300**	**150**	**7.5**	**15.0**	**7.5**
28	**32**	**320**	**160**	**8.0**	**16.0**	**8.0**

5. Add **10 µl** of **RNA samples** extracted from test or control samples of RNA extraction stage using tips with filter. Discard the unused reaction mixture.

[2] Number of test samples (N) + 1 control of RNA extraction + 2 controls of RT-PCR + 1 extra reaction (N+1+2+1).

Avoid transferring of sorbent together with the RNA samples extracted by RIBO-sorb kit.

6. Carry out the control reactions:

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

C+$_{EV\ 71\ /\ STI}$ - Add **10 µl** of **Positive Control *Enterovirus* / STI (C+$_{Enterovirus\ /\ STI}$)** to the tube labeled (C+$_{Enterovirus\ /\ STI}$) (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).

8.2.2. Reverse transcription and amplification

Make sure that the amplification run starts within 10-15 min after the addition of RNA to the reaction mixture

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

Table 2

AmpliSens unified amplification program for rotor-type[3] and plate-type instruments[4]

Step	*Temperature, °C*	*Time*	*Fluorescent signal detection*	*Cycles*
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 c	–	45
	60	20 c	Fluorescence acquiring	

Any combination of the tests can be performed in one instrument simultaneously with the use of the unified amplification program.

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

Note – When several tests are performed simultaneously the detection in other used channels is enabled.

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin* and Guidelines [2].
3. Insert tubes into the reaction module of the device.

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

[3] For example, Rotor-Gene 3000, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).
[4] For example, iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene USA).

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

– The signal of the IC cDNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Enterovirus* cDNA 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 a specific level that corresponds to the presence (or absence) of a *Ct* value of a cDNA sample in the corresponding column of the result grid.

The results are interpreted in accordance with the table 3 and *Important Product Information Bulletin*.

Table 3

Results interpretation

***Ct* value**		**Result**
Channel for the FAM fluorophore	**Channel for the JOE fluorophore**	
< boundary value	The value is absent or > boundary value	*Human enterovirus* RNA **is not detected**
> or < boundary value	< boundary value	*Human enterovirus* RNA **is detected**
The value is absent or > boundary value	The value is absent or > boundary value	**Invalid** result Repeat the extraction and amplification

Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed in the PCR kit. See also Guidelines [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 4).

Table 4

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	**JOE**
C–	RNA extraction	< boundary value	The value is absent or > boundary value
NCA	Reverse transcription and amplification	The value is absent or > boundary value	The value is absent or > boundary value
C+	Reverse transcription and amplification	< boundary value	< boundary value

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channel for **JOE** fluorophore is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which the *Human enterovirus* RNA was not detected.
2. If the *Ct* value determined for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the channel for **JOE** fluorophore is less than the boundary *Ct* value, PCR analysis (beginning with RNA extraction stage) should be repeated for all samples in which the *Human enterovirus* RNA was detected.

11. TRANSPORTATION

AmpliSens® *Enterovirus*-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® *Enterovirus*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Enterovirus* / STI, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMlv), RT-G-mix-2). All components of the **AmpliSens® *Enterovirus*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-FL *Enterovirus* / STI, PCR-buffer-C, polymerase (TaqF), TM-Revertase (MMlv) and RT-G-mix-2 are to be stored at the temperature from minus 24 to minus 16 °C

PCR-mix-FL *Enterovirus* / STI is to be kept away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity

Biological material	Nucleic acid extraction kit	PCR kit	Sensitivity, GE/ml[5]
Cerebrospinal fluid, concentrated water samples	RIBO-prep	PCR kit variant FRT-50 F	5×10^3
Fecal samples	RIBO-prep	PCR kit variant FRT-50 F	1×10^4

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *Enterovirus*-FRT** 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 specificity was proved on the follows strains of microorganisms: *Human enterovirus* (representatives of different genetic clusters – *Human echovirus* 2, 6, 9, 11, 14, 15, 16, 17, 18, 30; *Human coxsackievirus* A4, A5, A6, A9, A16, B4, B5*, Human poliovirus* 1, 2, 3 (Sabin1, Sabin2, Sabin3)); *Influenza viruses* A (H13N2, H9N2, H8N4, H2N3, H4N6, H11N6, H12N5, H3N8, H1N1, H6N2, H10N7, H5N1), B, *Rhinovirus*, *RS* viruses, *Human adenovirus* types 3, 5, 7, 37, 40, 41 (clinical isolates, the specificity was proved by direct sequencing of nucleic sequences); strains of *Acinetobacter baumanii* ATCC® 19606™, *Bacteroides fragilis* ATCC® 25285™, *Bordetella bronchiseptica* ATCC® 10580™, *Bordetella bronchiseptica* ATCC® 4617™, *Bordetella pertussis* ATCC® 9340™, *Candida albicans* ATCC® 14053™, *Candida guilliermondii* ATCC® 6260™, *Candida krusei* ATCC® 14243™, *Clostridium difficile* ATCC® 9689™, *Clostridium septicum* ATCC® 12464™, *Corynebacterium jeikeium* ATCC® 43734™, *Corynebacterium xerosis* ATCC® 373™, *Eggerthella lenta (Eubacterium lentum)* ATCC® 43055™, *Enterobacter aerogenes* ATCC® 13048™, *Enterobacter cloacae* ATCC® 13047™, *Enterococcus faecalis* ATCC® 29212™, *Enterococcus faecalis (vancomycin resistant)* ATCC® 51299™, *Enterococcus faecium* ATCC® 35667™, *Erysipelothrix rhusiopathiae* ATCC® 19414™, *Escherichia coli* ATCC® 25922™, *Escherichia coli* ATCC® 35218™, *Fluoribacter (Legionella) dumoffii* ATCC® 33279™, *Haemophilus infuenzae* ATCC® 33930™, *Haemophilus influenzae* ATCC® 9006™, *Haemophilus influenzae* ATCC® 10211™, *Haemophilus parainfluenzae* ATCC® 7901™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* ATCC®

[5]Genome equivalents (GE) of the pathogen agent per 1 ml of a sample.

27736™, *Legionella pneumophila* ATCC® 33152™, *Listeria grayi (murrayi)* ATCC® 25401™, *Listeria innocua* ATCC® 33090™, *Listeria monocytogenes* ATCC® 7644™, *Moraxella (Branhamella) catarrhalis* ATCC® 25238™, *Moraxella (Branhamella) catarrhalis* ATCC® 8176™, *Neisseria meningitidis* ATCC® 13102™, *Neisseria meningitidis* ATCC® 13090™, *Neisseria lactamica* ATCC® 23970™, *Neisseria gonorrhoeae* ATCC® 19424™, *Neisseria gonorrhoeae* ATCC® 49926™, *Peptoniphilus (Peptostreptococcus) anaerobius* ATCC® 27337™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Propionibacterium acnes* ATCC® 11827™, *Pseudomonas aeruginosa* ATCC® 15442™, *Rhodococcus equi* ATCC® 6939™, *Salmonella enterica subsp. enterica serovar Typhimurium* ATCC® 14028™, *Serratia marcescens* ATCC® 14756™, *Staphylococcus aureus* ATCC® 6538P™, *Staphylococcus aureus* (MRSA) ATCC® 43300™, *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus aureus* ATCC® 25923™, *Staphylococcus aureus* ATCC® 33862™, *Staphylococcus aureus* (MRSA) ATCC® 33591™, *Staphylococcus aureus subsp. aureus* ATCC® 12600™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus haemolyticus* ATCC® 29970™, *Staphylococcus saprophyticus* ATCC® 49907™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Streptococcus agalactiae* ATCC® 12386™, *Streptococcus agalactiae* ATCC® 13813™, *Streptococcus equisimilis* ATCC® 12388™, *Streptococcus equi subsp. equi* ATCC® 9528™, *Streptococcus bovis* (Group D) ATCC® 9809™, *Streptococcus mutans* ATCC® 35668™, *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus pneumoniae* ATCC® 6303™, *Streptococcus pneumoniae* ATCC® 27336™, *Streptococcus pneumoniae* ATCC® 6305™, *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus salivarius* ATCC® 13419™, *Streptococcus uberis* ATCC® 700407™, *Trichophyton mentagrophytes* ATCC® 9533™, *Trichophyton mentagrophytes* ATCC® 9533™, *Vibrio parahaemolyticus* ATCC® 17802™, *Vibrio vulnificus* ATCC® 27562™, *Moraxella catarrhalis* ATCC® 25240™. Nonspecific responses were absent in tests of DNA samples of this microorganisms and human DNA samples.

The clinical specificity of **AmpliSens®** ***Enterovirus*-FRT** PCR kit was confirmed in laboratory clinical trials.

13.3. Reproducibility and repeatability

Biological material	Number of repeats	Coefficient of variation CV, %
Dispersion of values in a single test		
Fecal samples	8	1.71
Concentrated water samples	8	2.63
Cerebrospinal fluid	8	1.20
Dispersion of values between tests, carried out in different days		
Fecal samples	16	1.47
Concentrated water samples	16	2.57
Cerebrospinal fluid	16	2.04

13.4. Diagnostic characteristics

Comparative characteristics of reagent kits:

Samples type	Number of samples	Results of using reference essay[6]	Results of using AmpliSens® *Enterovirus* 71-FRT PCR kit
Cerebrospinal fluid [7]	140	Positive 50 Negative 90	Positive 52[8] Negative 88
Fecal samples [9]	402	Positive 52 Negative 350	Positive 56[8] Negative 146
Concentrated water samples [10]	100	Positive 28 Negative 72	Positive 30[8] Negative 70

In accordance with the submitted data the diagnostic sensitivity of the **AmpliSens® *Enterovirus*-FRT** PCR kit (relative sensitivity in comparison with the used reference essay) is no less than 95 % with a confidence coefficient of 90 % for cerebrospinal fluid, no less than 93 % with a confidence coefficient of 90 % for concentrated water samples and no less than 96 % with a confidence coefficient of 90 % for fecal samples.

[6] AmpliSens® *Enterovirus*-EPh PCR kit, manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology", was used as a reference essay.

[7] 12 samples of cerebrospinal fluid from the patients from hotbed of *EV* 71 group disease and 38 model samples of cerebrospinal fluid with average clinical *EV* concentrations were used. Negative cerebrospinal fluid samples (*EV* was absent) were taken from the patients with serous and purulent meningitis within 2011-2013 years.

[8] Containing of *EV* RNA in discordant samples (2 cerebrospinal fluid samples, 4 fecal samples and 2 concentrated water samples) was proved by direct sequencing of amplification product that allows connecting its appearance with the *Enteroviruses* presence in test samples in concentrations less than analytical sensitivity of the used reference essay.

[9] 52 fecal samples from the patients with serous meningitis of *enterovirus* etiology and from clinically healthy persons, excreted *enteroviruses,* were used. Negative fecal samples (*EV* was absent) were taken from the patients with acute intestinal infections (n=200) and clinically healthy persons (n=150) within 2011-2013 years.

[10] 30 model concentrated water samples (filtration module with membranes with positive charge to 40 mV/cm^2, elution beef extract (Sigma-Aldrich, USA)), contaminated by *EV* (Coxsackievirus B5) in statistically average concentration specific to *EV* content in waste water, and 70 negative concentrated water samples (*EV* was absent) were used.

The **diagnostic specificity** of the **AmpliSens®** ***Enterovirus*** **71-FRT** PCR kit (relative specificity in comparison with the used reference essay) is no less than 95 % with a confidence coefficient of 90 % for cerebrospinal fluid, no less than 93 % with a confidence coefficient of 90 % for concentrated water samples and no less than 96 % with a confidence coefficient of 90 % for fecal samples.

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, 2010.
2. Guidelines to the **AmpliSens®** ***Enterovirus*****-FRT** PCR kit for qualitative detection of the *Human enterovirus* RNA in the biological material (cerebrospinal fluid, fecal samples) and natural environments (concentrated water samples) by 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 the **AmpliSens® *Enterovirus*-FRT** 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
		C+$_{\textit{Enterovirus / STI}}$	Positive control of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
08.08.14 ChA	Footer	**REF** R-V16-F-CE-B was added
	Content	The form in bulk was added