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For Professional Use Only

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of RNA of *tick-borne encephalitis virus* (*TBEV), Borrelia burgdorferi sl* (Ixodes tick-borne borreliosis (ITB) pathogen), *Ehrlichia chaffeensis* and *Ehrlichia muris (*human monocytic ehrlichiosis (HME) pathogens) and DNA of *Anaplasma phagocytophilum (*human granulocytic anaplasmosis (HGA) pathogen) in biological materials (ticks, blood, cerebrospinal fluid, and autopsy material) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris nucleic acid detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** 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**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris nucleic acid detection includes:

a) RNA/DNA extraction from biological material simultaneously with the Internal Control;

b) reverse transcription of cDNA on RNA template;

c) PCR with real-time detection of cDNA/DNA amplification products.

3. CONTENT

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT PCR kit is produced in 1 form:

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis* / *E.muris*-FRT PCR kit variant FRT-100 F (for use with RG, iQ, Mx, Dt), **REF** R-V59(RG,iQ,Mx,Dt)-CE.

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT PCR kit variant FRT-100 F includes:

Reagent	***Description***	***Volume (ml)***	***Amount***
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E. m.*	colorless clear liquid	0.6	2 tubes
PCR-mix-1-FRT *B.b. sl* / IC	colorless clear liquid	0.6	2 tubes
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	4 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	4 tubes
Positive Control cDNA *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI (C+*TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI)	colorless clear liquid	0.2	2 tubes
DNA-buffer	colorless clear liquid	0.5	2 tubes
Internal Control STI-87-rec (IC)*	colorless clear liquid	0.12	10 tubes

* add 10 µl of Internal Control-STI-87-rec during the RNA/DNA extraction directly to the sample/lysis mixture (see RIBO-prep **REF** K2-9-Et-100-CE protocol).

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis* / *E.muris*-FRT PCR kit is intended for 120 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- RNA/DNA extraction kit.
- Reverse transcription kit
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett

Research, Australia), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene), or equivalent).

- Disposable polypropylene microtubes for PCR (0.5- or 0.2-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 a 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 another 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 and then move to the Amplification and Detection Areas. Do not return samples, equipment, and reagents to the area in which the previous step was performed.

Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

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

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT PCR kit is intended to analyze RNA/DNA extracted with RNA/DNA extraction kits from:

- *Tick suspension*

Tick pools of no more than 10 specimens or a single tick (preferably for the *Dermacentor* genus) can be used for analysis.

Place ticks into Eppendorf tubes, add 500 µl of 96 % ethanol, and vortex. Spin the tube with ticks for 3-5 s then remove liquid with a vacuum aspirator. Add 500 µl of 0.15 M NaCl or phosphate buffer, vortex, and spin for 3-5 s. Remove liquid with a vacuum aspirator.

Use a sterile porcelain mortar and a pestle to prepare tick suspension. Homogenize ticks in 300 µl (a single *Ixodes* tick), 500 µl (a single *Dermacentor* tick), or 1 ml (tick pool) of 0.15 M NaCl or phosphate buffer then centrifuge at 5,000 rpm for 2 min. Take 100 µl of the supernatant for RNA/DNA extraction from *Ixodes* ticks or 50 µl of the supernatant for RNA/DNA extraction from *Dermacentor* ticks.

Add glycerol (10% v/v) to the tube with the remained suspension, stir, and freeze at or below minus 16 °C for further use.

- *Cerebrospinal fluid (CSF) and leukocyte fraction of blood*

Take a blood specimen in the morning after overnight fasting to a tube with 6 % EDTA in the ratio 1:20. Invert the closed tube several times. To obtain the leukocyte fraction of blood, transfer 1.5 ml of the blood with EDTA to an Eppendorf tube and centrifuge at 800 rpm for 10 min. Then transfer 500-600 µl of the upper plasma layer with leukocytes to an Eppendorf tube and centrifuge at 13,000 rpm for 10 min. Remove and discard the supernatant. Use cell pellet and 200 µl of supernatant above it for RNA/DNA extraction.

Centrifuge 1-1.5 ml of CSF at 13,000 rpm for 10 min. Remove and discard the supernatant. Use the cell pellet and 200 µl of supernatant above it for RNA/DNA extraction.

- *Internal organs of animals and autopsy material*

Homogenize internal organs of animals and autopsy material with a porcelain mortar and a pestle and prepare 10 % suspension using sterile saline (0.15 M NaCl) or phosphate buffer. Take 50 µl of the suspension for RNA/DNA extraction.

7. WORKING CONDITIONS

PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. Reverse transcription

It's recommended that the following reagent kit for complementary DNA (cDNA) synthesis from RNA is used:

- REVERTA-L, **REF** K3-4-100-CE.

8.2. RNA/DNA extraction

It is recommended that the following nucleic acid extraction kits are used:

- RIBO-prep, **REF** K2-9-Et-100-CE.
- Other nucleic acid extraction kits recommended by CRIE.

Extract RNA/DNA according to the instructions provided by the manufacturer.

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**.

All obtained cDNA samples should be examined in two tubes – one with **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and the other one with **PCR-mix-1-FRT *B.b. sl* / IC.**

1. Prepare the reaction mixture for the required number of reactions. To do this, mix **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.,* polymerase (TaqF),** and **RT-PCR-mix-2 FEP/FRT** in one tube and **PCR-mix-1-FRT *B.b. sl* / IC, polymerase (TaqF), and RT-PCR-mix-2 FEP/FRT** in the other tube.

   Reagent volumes per one reaction as follows:

   – **10 µl of PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.* or PCR-mix-1-FRT *B.b. sl* / IC,**
   – **5 µl of RT-PCR-mix-2 FEP/FRT,**
   – **0.5 µl of polymerase (TaqF).**

Do not store the prepared reaction mixture.

PCR run should include amplification reactions for six control points: negative control of extraction (C-), positive control of RT-PCR (C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$), and negative control of RT-PCR (NCA) for two reaction mixtures (**PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and **PCR-mix-1-FRT *B.b. sl* / IC**).

2. Transfer **15 µl** of the prepared mixture to each tube.
3. Add **10 µl** of **cDNA** obtained from clinical or control samples at the reverse transcription

stage to the prepared tubes using tips with aerosol barrier.

4. Carry out the control amplification reactions:

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

C+*TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI -Add **10 µl** of **Positive Control cDNA *TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI** to the tube labeled C+*TBEV, B.b. sl, A.ph., E.ch. / E.m.* / STI (Positive Control of Amplification).

Perform the amplification reaction immediately after cDNA samples and controls are added to the reaction mixture.

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines.

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

Table 1

Amplification program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Cycles**	***Temperature, °C***	***Time***	**Cycles**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	10 s	5	**95**	10 s	5
	60	30 s		**60**	35 s	
	72	15 s		**72**	15 s	
3	**95**	10 s	40	**95**	10 s	40
	56	30 s *fluorescent signal detection*		**56**	35 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescent signal detection is enabled in the channels designed for the FAM/Green, JOE/Yellow/HEX, and ROX/Orange fluorophores for the tubes with the **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and for the FAM/Green and JOE/Yellow/HEX fluorophores for the tubes with the **PCR-mix-1-FRT *B.b. sl* / IC**.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin.*
3. Insert the tubes into the reaction module of the instrument. If amplification is carried out simultaneously for both PCR-mixes-1, the tubes with PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.* should be inserted first.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

[1] For example,Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q, or equivalent.
[2] For example, iCycler iQ5, Mx3000P, Mx3000, DT-96, or equivalent.

9. DATA ANALYSIS

The fluorescent signal intensity is detected in two or three channels depending on the PCR-mix-1 used.

PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*

- The signal from the *TBEV* cDNA amplification product is detected in the FAM/Green channel;
- The signal from the *A.phagocytophilum* DNA amplification product is detected in the JOE/Yellow/HEX channel;
- The signal from the *E.chaffeensis* / *E.muris* cDNA amplification product is detected in the ROX/Orange channel.

PCR-mix-1-FRT *B.b. sl* / IC

- The signal from the Internal Control cDNA amplification product is detected in the FAM/Green channel;
- The signal from the *Borrelia burgdorfer sl.* cDNA amplification product is detected in the JOE/Yellow/HEX channel.

Result interpretation

The results are interpreted by the Instrument software by the crossing (or not-crossing) of the fluorescence curve with a threshold line and are shown as the presence (or absence) of Ct (threshold cycle) in the result grid.

Principle of interpretation:

- *TBEV* cDNA is **detected** in a sample if its Ct is defined in the result grid in the FAM/Green channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *A.phagocytophilum* DNA is **detected** in a sample if its Ct is defined in the result grid in the JOE/Yellow/HEX channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *E.chaffeensis* / *E.muris* cDNA is **detected** in a sample if its Ct is defined in the result grid in the ROX/Orange channel (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *Borrelia burgdorferi sl.* cDNA is **detected** in a sample if its Ct is defined in the result grid in the JOE/Yellow/HEX channel (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**).

Moreover, the fluorescence curve of every studied sample should cross the threshold line at the exponential growth stage.

- *Borrelia burgdorferi sl.* cDNA is **not detected** in a sample if its Ct is not defined in the result grid in the JOE/Yellow/HEX channel while Ct in the FAM/Green channel is less

than the specified boundary value (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**).

- *TBEV A.phagocytophilum, and E.chaffeensis* / *E.muris* cDNA/DNA are **not detected** in a sample if Ct values are not defined in the result grid in the appropriate channels (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- The result is **invalid** if Ct of a sample is absent in the channels for specific pathogen detection whereas in the FAM/Green channel (with the use of **PCR-mix-1-FRT *B.b. sl* / IC)** Ct is also absent or greater than the specified boundary value. It is necessary to repeat the PCR test for such samples.

Ct boundary values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

The result of the analysis is considered reliable only if the results of both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (Table 2).

Table 2

Results for controls

PCR-mix-1	Control	Stage for control	Ct value (all channels)	Interpretation
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*	**C–**	RNA/DNA extraction	Neg (absent in all channels)	OK
	NCA	Amplification	Neg (absent in all channels)	OK
	C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$	Amplification	Pos (**<boundary value*** in all channels)	OK
PCR-mix-1-FRT *B.b. sl* / IC	**C–**	RNA/DNA extraction	**absent** (JOE/Yellow/HEX) **<boundary value*** (FAM/Green)	OK
	NCA	Amplification	Neg (absent in all channels)	OK
	C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$	Amplification	Pos (**<boundary value*** in all channels)	OK

*For boundary values, see the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

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

- If the Ct value of the Positive Control of amplification (C+$_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$) is absent or greater than the specified boundary value in the FAM, JOE, or ROX channels (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***) or in the FAM and JOE channels (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**), PCR should be repeated for all

samples in which specific cDNA/DNA detected in the appropriate channel was not found.

- If the Ct value of the Negative Control of extraction (C-) (in the FAM, JOE, ROX channels with **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and in the JOE channel with **PCR-mix-1-FRT *B.b. sl* / IC**) and/or Negative Control of amplification (NCA) (in all channels) is defined in the result grid, PCR should be repeated for all samples in which specific cDNA DNA detected in the appropriate channel was found.
- If no signal is detected for the positive controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components did not comply with the manufacturer's instruction, or that the reagent kit expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be repeated (if iCycler iQ or iQ5 instruments are used).

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

11. TRANSPORTATION

AmpliSens® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit (except for PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*, PCR-mix-1-FRT *B.b. sl* / IC, polymerase (TaqF), and RT-PCR-mix-2-FEP/FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*, PCR-mix-1-FRT *B.b. sl* / IC, polymerase (TaqF), and RT-PCR-mix-2-FEP/FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*, and PCR-mix-1-FRT *B.b. sl* / IC are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens**® ***TBEV, B. burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Reverse transcription kit	PCR kit	Analytical sensitivity, GE/ml*	Pretreatment of biological material
Ticks of *Ixodes* and *Dermacentor* genera	RIBO-prep	REVERTA-L	PCR kit variant FRT-100 F	5×10^3	Indicated sensitivity can be reached only if the specified pretreatment instructions are followed and the specified specimen volume is used

* Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample.

13.2. Specificity

The analytical specificity of **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E. chaffeensis / E.muris*-FRT** PCR kit is ensured by selection of specific primers and probes as well as by selection of strict reaction conditions. The primers and probes were checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis. Analitival specificity was studied on the following microorganisms:

– *flaviviruses* (*West Nile*, *Langat, Powassan, Japanese encephalitis, and Omsk hemorrhagic fever viruses*);
– *spirochaetes* (*Borrelia miyamotoi; Treponema pallidum; Leptospira interrogans, L.kirshneri; and L. borgpetersenii*);
– *rickettsiae* of spotted fever group (*Rickettsia conorii* spp*. caspia* and *R.heilongiangensis*; *Coxiella burnetii*; and *Bartonella henselae* and *B.quintana).*

No false-positive results were observed during examination of DNA of the above-mentioned organisms, ticks (*Ixodes persulcatus*, *I. ricinus*, *Dermacentor reticulatus*, and *D. marginatus*), rodents (*Clethrionomys glareolus* and *Apodemus agrarius)*, as well as human DNA.

The clinical specificity of **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics” developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights

Protection and Human Well-Being, Moscow, 2008.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens**® ***TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-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
EC REP	Authorised representative in the European Community	**C+** ***TBEV, B.b. sl, A.ph., E.ch / E.m.*** **/ STI**	Positive control of Amplification
IC	Internal control		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
29.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”