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

CE IVD

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorized representative in the European Community	**C+***TBEV, B.b. sl, A.ph., E.ch. / E.m.* **/ STI**	Positive control of amplification
		IC	Internal control

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 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 the biological material (ticks, blood, cerebrospinal fluid, and autopsy material) by 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

TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris detection by the polymerase chain reaction (PCR) is based on the amplification of the 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 (Internal Control STI-87-rec (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 using a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E. m.*			
cDNA-target	*TBEV* cDNA	*A.phagocytophilum* cDNA	*E.chaffeensis/E.muris* cDNA
Target gene	C gene	msp2 gene	16S RNA
PCR-mix-1-FRT *B.b. sl* / IC			
cDNA-target	Internal Control STI-87-rec (IC) cDNA	*B.burgdorferi sl* cDNA	—
Target gene	Artificially synthesized sequence	16S RNA	—

3. CONTENT

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

variant FRT-100 F, REF R-V59(RG,iQ,Mx,Dt)-CE.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E. m.*	clear liquid from colorless to light lilac colour	0.6	2 tubes
PCR-mix-1-FRT *B.b. sl* / IC	clear liquid from colorless to light lilac colour	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 (IC)** during the RNA/DNA extraction directly to the sample/lysis mixture (see **RIBO-prep** REF K2-9-Et-50-CE or REF K2-9-Et-100-CE protocol).

Variant FRT-100 F is intended for 120 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- 0,15 M NaCl or Phosphate buffered saline (PBS), 96 % ethanol for pretreatment of ticks and autopsy material, glycerol.
- 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 3000/6000 (Corbett Research, Australia), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml):
  a) 0.2-ml PCR tubes with optical transparent domed or flat 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 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

NOTE: 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. Vortex the tube with ticks for 3-5 s, then remove liquid using a vacuum aspirator. Add 500 µl of 0.15 M NaCl or phosphate buffer, vortex, and centrifuge for 3-5 s to remove drops from the inner surface of the tubes caps. 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% by volume) to the tube with the remained suspension, stir, and freeze at the temperature not higher than 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

AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA/RNA extraction

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

- **RIBO-prep**, REF K2-9-Et-50-CE or REF K2-9-Et-100-CE.

Extract RNA/DNA according to the manufacturer's protocol taking into account next additions and improvements:

- Add **300 µl** of **Solution for Lysis** to the tubes with test material (**concentrated cell pellet of CSF (cerebrospinal fluid), white cell pellet, homogenate of internal organs, clarified tick suspension**) and to the tube labeled C- (Negative Control of extraction). Label the tubes. Mix the content of the tubes thoroughly. Centrifuge the tubes at **5,000 rpm** for **5 s** to sediment the drops from the caps.

NOTE:

- Add 10 µl of **Internal Control STI-87-rec (IC)** into each tube using individual tips. Thoroughly vortex the tubes and incubate them at 65 °C for 5 min.
- After each washing use a new one **200-µl** tip for each sample.
- In case of DNA/RNA extraction from the concentrated cell pellet of CSF or white cell pellet add **100 µl** of **RNA-buffer** to the tubes.
  In case of DNA/RNA extraction from the homogenate of tissues or tick suspension - add **50 µl** of **RNA-buffer** to the tubes**.**

8.2. Reverse transcription

It is recommended to use the following kit for the complementary DNA (cDNA) synthesis from the RNA:

- **REVERTA-L**, REF K3-4-50-CE or REF K3-4-100-CE.

NOTE: Carry out the reverse transcription according to the manufacturer's protocol.

8.3. Preparing PCR

8.3.1 Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of the **cDNA/DNA** is **10 µl**.

NOTE: All obtained cDNA/DNA 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:
   - **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).**

NOTE: Do not store the prepared reaction mixture.

NOTE: 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/DNA samples** to the prepared tubes using tips with filters.
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).

C– – Add **10 µl of cDNA obtained by extraction and reverse transcription of the Negative control of Extraction (containing the Internal Control STI-87-rec (IC) reagent only)** to the tube labeled C–.

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

8.3.2. Amplification

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

Table 2

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 fluorescence detection**		**56**	**35 s fluorescence detection**	
	72	**15 s**		**72**	**15 s**	

Fluorescent signal detection is assigned in the channels for the FAM, JOE, and ROX fluorophores for the tubes with the **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.*** and in the channels for the FAM and JOE 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* and Guidelines [2].
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.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three or two channels respectively for each PCR-mix:

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

- The signal of the *TBEV* cDNA amplification product is detected in the channel for the FAM fluorophore;
- The signal of the *A.phagocytophilum* DNA amplification product is detected in the channel for the JOE fluorophore;
- The signal of the *E.chaffeensis* / *E.muris* cDNA amplification product is detected in the channel for the ROX.

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

- The signal of the Internal Control cDNA amplification product is detected in the channel for the FAM fluorophore;
- The signal of the *Borrelia burgdorfer sl.* 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 the specific level that corresponds to the presence (or absence) of a *Ct* value of the cDNA/DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- *TBEV* cDNA is **detected** if *Ct* value is determined in the channel for the FAM fluorophore (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *A.phagocytophilum* DNA is **detected** if *Ct* value is determined in the channel for the JOE fluorophore (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *E.chaffeensis* / *E.muris* cDNA is **detected** if *Ct* value is determined in the channel for the ROX fluorophore (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- *Borrelia burgdorferi sl.* cDNA is **detected** if *Ct* value is determined in the channel for the JOE (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**).

Moreover, the fluorescence curve of the sample should cross the threshold line in the area of typical exponential growth of fluorescence.

- *Borrelia burgdorferi sl.* cDNA is **not detected** if the *Ct* value is not determined (absent) in the channel for the JOE fluorophore, whereas the *Ct* value determined in the channel for the FAM fluorophore is less than the specified boundary *Ct* 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** if the *Ct* value is not determined (absent) in the appropriate channels enabled for detection of specific signal (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***).
- The result is **invalid** if the *Ct* value is not determined (absent) in the channels for detection of specific signal, whereas the *Ct* value in the channel for the FAM fluorophore (with the use of **PCR-mix-1-FRT *B.b. sl* / IC)** is also not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated for such samples.

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 of both Positive and Negative Controls of amplification as well as Negative Control of extraction are correct (see Table 3).

Table 3

Results for controls

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

[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.

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 ($C+_{TBEV,\ B.b.\ sl,\ A.ph.,\ E.ch.\ /\ E.m.\ /\ STI}$) is absent or greater than the specified boundary *Ct* value in the channels for FAM, JOE, or ROX fluorophores (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.***) or in the channels for FAM and JOE fluorophores (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**), the amplification should be repeated for all samples in which specific cDNA/DNA was not detected in the appropriate channel.
2. If the *Ct* value for the Negative Control of extraction (C-) in the channels for FAM, JOE, ROX fluorophores (with the use of **PCR-mix-1-FRT *TBEV, A.ph., E.ch. / E.m.)*** and in the channel for the JOE fluorophores (with the use of **PCR-mix-1-FRT *B.b. sl* / IC**) and/or Negative Control of amplification (NCA) (in all channels) is determined in the result grid, PCR analysis should be repeated for all samples in which specific cDNA DNA was detected in the appropriate channel.

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 are to be stored at 2–8 °C when not in use (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). All components of the **AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit are stable until the expiry 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-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.

NOTE: 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

Biological material	Nucleic acid extraction kit	Reverse transcription kit	PCR kit	Analytical sensitivity, GE/ml[3]	Pretreatment of biological material
Ticks of *Ixodes* and *Dermacent or* genera	RIBO-prep	REVERTA-L	variant FRT-100 F	5×10^3	The claimed sensitivity is achieved while respecting the rules specified in the section *Sampling and Handling* and the specified sample volume

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. Analytical 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*, *Ixodes ricinus*, *Dermacentor reticulatus*, *Dermacentor 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.
2. Guidelines to the **AmpliSens® *TBEV, B.burgdorferi sl, A.phagocytophilum, E.chaffeensis / E.muris*-FRT** PCR kit for 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 the biological material by 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 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.

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”
18.06.15 PM	Footer	REF R-V59-50-F(RG,iQ,Mx,Dt)-CE was added.
	Through the text	Description of working with the PCR kit variant FRT-50 F was added. Corrections according to the template.
	8.1. DNA/RNA extraction	The extraction procedure was clarified
18.12.17 PM	3. Content	The colour of the reagent was specified
22.02.19 PM	3. Content	The colour of the reagent was specified
30.04.20 MA	Through the text	The text formatting was changed
	Footer	The phrase “Not for use in the Russian Federation” was added
	2. Principle of PCR detection	The table with targets was added
23.10.20 MA	Through the text, Footer	The information about **variant FRT-50 F** REF R-V59-50-F(RG,iQ,Mx,Dt)-CE was deleted
11.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed

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