

AmpliSens® *Borrelia miyamotoi*-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

	Catalogue number		Contains sufficient for <n> tests
	Batch code		Use-by Date
	In vitro diagnostic medical device		Consult instructions for use
	Version		Keep away from sunlight
	Temperature limit		Keep dry
	Manufacturer		Negative control of amplification
	Date of manufacture		Negative control of extraction
	Authorized representative in the European Community		Positive control of amplification
	Caution		Internal control

1. INTENDED USE

AmpliSens® *Borrelia miyamotoi*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Borrelia miyamotoi* DNA in the biological material (blood, tissue (autopsy, biopsy) material, cerebrospinal fluid, ticks) using real-time hybridization-fluorescence detection of amplified products.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material, taken from the persons suspected of ixodic tick-borne borreliosis without distinction of form and presence of manifestation, and ticks.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

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

2. PRINCIPLE OF PCR DETECTION

Borrelia miyamotoi detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *Borrelia miyamotoi* 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® *Borrelia miyamotoi*-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control-FL (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® *Borrelia miyamotoi*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit variant FRT-50 FN contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

At the amplification stage 2 reactions are carried out in one tube simultaneously: amplification of *Borrelia miyamotoi* DNA as well as amplification of Internal Control-FL (IC) DNA. The results of amplification of *Borrelia miyamotoi* DNA and Internal Control-FL (IC) DNA are registered in 2 different fluorescence channels.

Table 1

Channel for fluorophore	FAM	ROX
DNA-target	IC DNA	<i>Borrelia miyamotoi</i> DNA
Target gene	Artificial nucleotide sequence	gIpQ gene

3. CONTENT

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

variant FRT-50 FN, H-2791-1-CE,

variant FRT-L, H-2792-1-4-CE.

Variant FRT-50 FN includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL <i>Borrelia miyamotoi</i>	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-H	colorless clear liquid	0.3	1 tube
C+ <i>Borrelia miyamotoi</i>	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-FL (IC)**	colorless clear liquid	0.5	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see MAGNO-sorb, K2-16-200-CE; K2-16-1000-CE protocol).

** add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, K2-9-Et-50-CE, MAGNO-sorb, K2-16-200-CE; K2-16-1000-CE protocol).

Variant FRT-50 FN is intended for 55 reactions (including controls).

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix <i>Borrelia miyamotoi</i> -Lyo	white powder	–	48 tubes of 0.2 ml
C+ <i>Borrelia miyamotoi</i>	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-FL (IC)**	colorless clear liquid	0.5	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (see MAGNO-sorb, K2-16-200-CE; K2-16-1000-CE protocol).

** add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see RIBO-prep, K2-9-Et-50-CE, MAGNO-sorb, K2-16-200-CE; K2-16-1000-CE protocol).

Variant FRT-L is intended for 48 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2.7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7.5±0.2).
- 96 % ethanol for ticks pretreatment.
- Glycerin for the storage of pretreated ticks.
- Vacuum blood collection system.
- Puncture needles.
- Sterile plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- Flocked swab for collection, transportation and storage of biological samples.
- Sterile tools (individual for each sample) for homogenization (porcelain mortar and mallet) or homogenizer for pretreatment of tissue material and ticks.
- Vacuum aspirator with flask for removing supernatant.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100, 200, 1,000 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes:
 - a) tightly closed 1.5 and 2-ml tubes for sampling.
 - b) screwed or tightly closed 1.5 and 2-ml tubes for pretreatment.
 - c) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
 - d) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
 - e) thin-walled 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 filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls) away from all other reagents and add it to the reaction mix in a distantly separated facility. Store and handle amplicons away from all other reagents.
- 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 the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- 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 inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- 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

AmpliSens® Borrelia miyamotoi-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (blood, tissue (autopsy, biopsy) material, cerebrospinal fluid, ticks.

Sampling

Blood. For the method sensitivity increasing, the bacterial pellet of blood is analyzed. To obtain the pellet, blood should be taken after overnight fasting or in 3 hour after eating by a disposable 0.8-1.1 mm diameter needle into the tube (special vacuum system) with EDTA or sodium citrate solution as anticoagulant. After blood sampling the tube should be smoothly rotated several times for the thoroughly mixing with the anticoagulant. (Otherwise, blood will coagulate and DNA extraction will be impossible!). Place the tube in the rack after rotating.

Blood samples can be stored before the obtaining and preparing the bacterial pellet:

- at the temperature from 20 to 25 °C – for 2 hour;
- at the temperature from 2 to 8 °C – for 12 hour;

Freezing of whole blood samples is unallowable!

The samples are to be prepared no later than the specified time.

Tissue (autopsy, biopsy) material. The material is taken from the area of probable location of infection agent, from the lesional tissue or the area surrounding lesional tissue or from the unaltered fragments of organ tissues: brain, heart, lung, liver, spleen, nephros, by a sterile tool (for example, tweezers) into a sterile plastic 50-ml container with tightly closed cap or 2 ml tube. The tube is to be closed tightly.

The tissue material samples can be stored:

- at room temperature – for 6 hour,
- at the temperature from 2 to 8 °C – for 3 days,
- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature ≤ -68 °C – for a long time.

Cerebrospinal fluid is collected in an amount no less than 1 ml by sterile needle into disposable 2.0-ml tubes.

The cerebrospinal fluid samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 1 day,
- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature ≤ -68 °C – for a long time.

Only one freeze-thawing cycle is required.

Ticks. The collected material is sorted into species, sex, places and dates of collection and placed into the dry sterile 2.0-ml tube. Number of ticks in pool for analysis should not exceed 10.

The material samples can be stored after sorting and samples formation:

- at the temperature from minus 24 to minus 16 °C – for 1 month;
- at the temperature not more than minus 68 °C or in the Dewar flask with liquid nitrogen – for a long time.

Only one freeze-thawing cycle is required.

The above mentioned material can be transported at the temperature from 2 to 8 °C for 1 day.

Pretreatment

To obtain the *bacterial pellet* of blood the pretreatment of blood samples is required.

Using a filter tip transfer 1.5 ml of blood with EDTA into the sterile disposable 2.0-ml tube. Centrifuge at 40 g (for example, 800 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min. Using a new one filter tip transfer 500-600 µl of supernatant (plasma with leucocytes) into sterile disposable 1.5-ml tube (do not take the pellet with erythrocytes). Centrifuge at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min.

The bacterial pellet samples can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

Tissue (autopsy, biopsy) material is to be pretreated. For DNA extraction take 30-50 mg (µl) of the material and homogenize it by trituration using precooled sterile porcelain mortar and mallet or homogenizer. Prepare 10 % suspension using grinded tissue and precooled 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). For this, add 9 volumes of saline solution or phosphate buffer to 1 volume of grinded tissue. Use 100 µl of obtained suspension for DNA extraction.

The pretreated biopsy material samples can be stored:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

Cerebrospinal fluid is to be pretreated.

Centrifuge 1-1.5 ml of cerebrospinal fluid at 10,000 g (for example, 12,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 10 min. Discard the supernatant into the reservoir for the waste dispose. Use the cells pellet in 100 µl of supernatant for DNA extraction.

The cerebrospinal fluid samples can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C – for a long time.

The material freeze-thawing of is not allowed without the DNA extraction procedure.

Ticks are to be pretreated. If pools of ticks are used for the analysis, number of ticks in one pool should not exceed 10. For ixodic tick of all genus (except for *Ixodes*) it is preferable to analyze individual ticks. Place the ticks in 1.5-ml tubes, add 500 µl of 96 % ethanol. Mix and sediment on vortex. Discard ethanol from each tube by a separate tip without filter using vacuum aspirator. Add 500 µl of 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). Mix and sediment on vortex. Discard supernatant by a separate tip without filter using vacuum aspirator. Transfer the ticks into the sterile porcelain mortar, add 300 µl (if the sample consists of 1 *Ixodes* tick), 600 µl (if the sample consists of ixodic tick of any genus, except for *Ixodes*) and 1 ml (if a pool of ticks is analyzed) of 0.9 % sodium chloride solution (sterile saline solution) or phosphate buffer (PBS). Homogenize the sample.

Using a separate filter tip transfer the sample into 1.5-ml tube and centrifuge at 2,000 g (for example, 5,000 rpm for the MiniSpin Eppendorf microcentrifuge) for 2 min for the clarification of the sample. Remove 100 µl of supernatant. Add glycerin into the rest of suspension (10 % from the volume of the rest of suspension). Mix the sample and freeze it at the temperature from minus 24 to minus 16 °C for the subsequent PCR analysis.

The pretreated ticks can be stored before the PCR analysis:

- at the temperature from minus 24 to minus 16 °C – for 1 week,
- at the temperature not more than minus 68 °C or in the Dewar flask with liquid nitrogen – for a long time.

Only one freeze-thawing cycle is required.

Interfering substances and limitations of using test material samples

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

The blood samples, collected in the tubes with heparin as anticoagulant are inapplicable for analysis.

7. WORKING CONDITIONS

AmpliSens® Borrelia miyamotoi-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA extraction

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

- **RIBO-prep**, **REF** K2-9-Et-50-CE – for DNA extraction from blood, cerebrospinal fluid, ticks, tissue (autopsy and biopsy) material;
- **MAGNO-sorb**, **REF** K2-16-200-CE; **REF** K2-16-1000-CE – for DNA extraction from ticks.

NOTE: **If using the RIBO-prep kit** extract the DNA according to the manufacturer's protocol.

The volumes of reagents and samples when the DNA is extracted by the RIBO-prep reagent kit:

The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC)**.

Add **10 µl of Internal Control-FL (IC)** to each tube.

The volume of the test sample is **100 µl**. The pellet and **100 µl** of supernatant are used for the analysis of blood and cerebrospinal fluid. **100 µl** of suspension is used for the analysis of tissue (autopsy, biopsy) material, suspension of ixodes ticks.

Do not add the **Negative Control (C-)** reagent into the tube labeled C- (Negative Control of Extraction).

The volume of elution is **50 µl**.

In case of using PCR kit variant FRT-L for the amplification carry out the DNA elution in **100 µl** of Buffer for elution

NOTE: **If using the MAGNO-sorb kit** extract the DNA according to the Section 8.1.1. The volumes of reagents and samples when the DNA is extracted by the MAGNO-sorb reagent kit:

The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC)**.

Add **10 µl of Internal Control-FL (IC)** to each tube.

The volume of the test sample is **100 µl** of ixodes tick suspension.

Add **100 µl of Negative Control (C-)** to the tube labeled C- (Negative Control of Extraction).

The volume of elution is **100 µl**.

8.1.1 DNA extraction from tick suspension using MAGNO-sorb nucleic acid extraction kit.

1. Warm up **Lysis Solution MAGNO-sorb** and **Washing Solution 5** at 60 °C until crystals disappear.
2. Prepare the required number of 1.5-ml tubes including the tube for Negative Control of Extraction. Mark the tubes.
3. Mix in a disposable 1.5-ml tube **Internal Control (IC)** (if it is provided for analysis of given infectious agent), **Component A**, and **Magnetized silica** in the following proportion calculated per one sample: **10 µl of IC**, **10 µl of Component A**, and **20 µl of Magnetized Silica**. Do not forget to add extra volumes for one more reaction. For example:

Number of samples for DNA extraction	Internal Control-FL (IC), µl	Component A, µl	Magnetized silica, µl
6	70	70	140
12	130	130	260
18	190	190	380
24	250	250	500

4. Add **40 µl** of the prepared mixture of **Internal Control-FL (IC)**, **Component A** and **Magnetized silica** into each tube.
5. Add **900 µl** of **Lysis Solution MAGNO-sorb** into the tubes.
6. Add **100 µl** of **test sample** into each prepared tube.
7. Add **100 µl** of **Negative Control (C-)** into the tube for the Negative Control of extraction (C-) (for each panel).
8. Tightly close the tubes. Vortex. Incubate the tubes at 60 °C for **10 min** in a thermostat.
9. Sediment the drops on a vortex. Transfer the tubes to a magnetic rack, and incubate for **2 min**.
10. Carefully remove the supernatant inserting the tip near the internal tube wall and using

- vacuum aspirator. Take a new tip for each sample. Transfer the tubes to a regular rack.
- Add **700 µl of Washing Solution 5** to the tubes.
 - Wash the magnetized silica mixing on vortex. Then sediment the drops on vortex.
 - Transfer the tubes to a regular rack, open the caps and transfer to a magnetic rack for **2 min**.
 - Remove the supernatant and transfer the tubes to a regular rack.
 - Repeat washing procedure with **Washing Solution 5** (steps 11-14).
 - Carry out washing procedure with **700 µl of Washing Solution 6** as described above.
 - Add **200 µl of Washing Solution 7**, mix, and vortex shortly to sediment drops. Place the tubes to a regular rack and open the tubes.
 - Transfer the tubes to the magnetic rack for **1 min** and then remove the supernatant.
 - Dry the sorbent. To do this, open the tubes and incubate them in the magnetic rack for **10 min**.
 - Add **100 µl of Buffer for elution** to each tube and vortex.
 - Incubate the tubes at **60 °C** for 5 min. Vortex the tubes 2 min later.
 - Vortex the tubes shortly and transfer them to the magnetic rack. Incubate for 2 min. Supernatant contains purified DNA.

be increased up to 30 s in the third step.

Table 3
AmpliSens unified amplification program for rotor-type² and plate-type³ instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	95	15 min	–	1
2	95	10 s	–	45
	60	30 s	FAM, ROX	

- Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
- 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.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.
- Run the amplification program with fluorescence detection.
- 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 2 channels:

Table 3

Channel for the fluorophore	FAM	ROX
Signal registration, indicating the amplification product accumulation	Internal Control-FL (IC) DNA	<i>Borrelia miyamotoi</i> DNA

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) 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:

Table 5

Results interpretation		
<i>Ct</i> value in the channel for the fluorophore		Result
FAM	ROX	
< boundary value	absent	<i>Borrelia miyamotoi</i> DNA is not detected
determined or absent	< boundary value	<i>Borrelia miyamotoi</i> DNA is detected
absent or > boundary value	absent or > boundary value	Invalid result*
< boundary value	> boundary value	Equivocal result**

* In case of invalid result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage.

** In case of equivocal result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage. If the same result is obtained, the sample is considered positive. If the negative result is obtained in the second run, the sample is considered equivocal and re-sampling of the material for analysis is recommended.

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

The result of the PCR analysis is considered reliable only if the results obtained for the controls of amplification and extraction are correct (see Table 6).

Table 6

Results for controls			
Control	Stage for control	<i>Ct</i> value in the channel for fluorophore	
		FAM	ROX
C–	DNA extraction	< boundary value	Absent
NCA	PCR	Absent	Absent
C+	PCR	< boundary value	< boundary value

10. TROUBLESHOOTING

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

- The *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the FAM and/or ROX fluorophores is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which the specific DNA was not detected.
- The *Ct* is determined for the Negative Control of Extraction (C–) in the channel for the ROX fluorophore, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
- The *Ct* value is determined for the Negative Control of Amplification (NCA) in the channels for the FAM and/or ROX fluorophores, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.
- The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check that threshold line or parameters of threshold line measurement are correct. If the result has been obtained with the correct threshold line level, the amplification and detection should be repeated for this sample.

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

11. TRANSPORTATION

AmpliSens® *Borrelia miyamotoi*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

Variant FRT-50 FN

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

- Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
 - 10 µl of PCR-mix-FL *Borrelia miyamotoi*,
 - 5 µl of PCR-buffer-H.

Prepare the reaction mixture for the total number of test and control samples plus one extra reaction. See numbers of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

- Thaw the tube with PCR-mix-FL *Borrelia miyamotoi*. Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
- In a new tube prepare the reaction mixture. Mix the required quantities of PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H. Sediment the drops by vortex.
- Take the required number of the tubes or strips taking into account the number of test samples and control samples.
- Transfer 15 µl of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
- Add 10 µl of DNA samples extracted from test samples at the DNA extraction stage using tips with filter.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted by magnetic separation method.

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control reactions:

- NCA** – Add **10 µl of TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).
- C+** – Add **10 µl of C+ *Borrelia miyamotoi*** to the tube labeled **C+** (Positive Control of Amplification).
- C–** – Add **10 µl of the sample extracted from the C– sample** to the tube labeled **C–** (Negative Control of Extraction).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Carry out the PCR just after the mix of reaction mixture and DNA-samples and controls.

Variant FRT-L

The total reaction volume is 25 µl, the volume of the DNA sample is 25 µl. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

- Take the required number of the tubes with ready-to-use lyophilized reaction mixture PCR-mix *Borrelia miyamotoi*-Lyo for amplification of DNA from test and control samples (see numbers of control samples in point 3).
- Add **25 µl of DNA samples** extracted from test samples into the prepared tubes.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted by magnetic separation method.

3. Carry out the control reactions:

- NCA** – Add **25 µl of TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).
- C+** – Add **25 µl of C+ *Borrelia miyamotoi*** to the tube labeled **C+** (Positive Control of Amplification).
- C–** – Add **25 µl of the sample extracted from the C– sample** to the tube labeled **C–** (Negative Control of Extraction).

NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

NOTE: Carry out the PCR just after the mix of reaction mixture and DNA-samples and controls. Time of the addition of samples to the reaction mixture and the reaction run on the instrument cannot be more than 10-15 min.

8.2.2. Amplification

- Create a temperature profile on your instrument as follows¹:
 - REVERTA-L, REF K3-4-50-CE.

Table 2

AmpliSens unified amplification program for rotor-type² and plate-type³ instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
	95	10 s	–	
3	60	20 s	FAM, ROX	45

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

Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiplex" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If in one instrument only the tests for the DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted for time saving, but the iteration at 60 °C should

¹ It is preferable to use the amplification program in Table 3, if there is no need to use the unified amplification program.

² For example, Rotor-Gene Q (QIAGEN, Germany).

³ For example, CFX 96 (Bio-Rad, USA).

12. STABILITY AND STORAGE

All components of the **AmpliSens® Borrelia miyamotoi-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H). All components of the **AmpliSens® Borrelia miyamotoi-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.

NOTE: PCR-mix-FL *Borrelia miyamotoi* and PCR-buffer-H are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FL *Borrelia miyamotoi* is to be kept away from light

NOTE: PCR-mix *Borrelia miyamotoi*-Lyo is to be kept in packages with a desiccant away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 7

Biological material	The volume of sample for extraction, µl	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), copies/ml
Blood	Pellet + 100	RIBO-prep	variant FRT-50 FN, variant FRT-L	10 ³
Tissue (autopsy, biopsy) material	100	RIBO-prep	variant FRT-50 FN, variant FRT-L	10 ³
Cerebrospinal fluid	Pellet + 100	RIBO-prep	variant FRT-50 FN, variant FRT-L	10 ³
Ticks	100	RIBO-prep	variant FRT-50 FN, variant FRT-L	10 ³
		MAGNO-sorb	variant FRT-50 FN, variant FRT-L	10 ³

The claimed features are achieved while respecting the rules specified in the section "Sampling and Handling".

13.2. Analytical specificity

The analytical specificity of **AmpliSens® Borrelia miyamotoi-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The reagent kit detects of DNA fragments claimed microorganisms. The analytical specificity of the reagent kit was proved in the study of the following strains of microorganisms: *Borrelia afzelii*, *B. garinii*, *B. burgdorferi sensu stricto*, *Leptospira interrogans*, *Coxiella burnetii*, *Bartonella quintana*, *B. henselae*, *Rickettsia conorii*, *R. sibirica*, *Babesia microti*, *Treponema pallidum*, and human genomic DNA.

When testing the DNA samples of the above microorganisms, and human DNA, DNA of ticks and DNA of rodents of nonspecific reactions was not revealed.

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

13.3. Diagnostic characteristics

To evaluate of diagnostic characteristics were used:

- 131 blood samples, obtained in the Izhevsk Regional Infectious Diseases Hospital and the "New Hospital" of Ekaterinburg from the patients with suspected disease of ixodes tick-borne borreliosis, and 50 blood samples, taken during intraepidemic period of ixodes tick-borne infections from patients of Infectious Diseases Hospitals No. 1 and No. 2 in Moscow with diseases of a different etiology;
- 200 samples of *I. ricinus* mites collected in Moscow and Moscow region;
- 50 samples each of cerebrospinal fluid and autopsy material from the patients of Infectious Diseases Hospitals No. 1 and No. 2 in Moscow with diseases of a different etiology;
- 20 model samples each of cerebrospinal fluid, autopsy material and ticks, contaminated with the strain *Borrelia miyamotoi* Izh-4 from the State Collection of Pathogenic Microorganisms and Cell Cultures "GKPM-Obolensk".

A reagent kit for *Borrelia miyamotoi* DNA detection with real-time polymerase chain reaction (RealBest DNA *Borrelia miyamotoi*) produced by Vector-Best JSC, Russia was used as the kit for comparison.

Table 8

Diagnostic characteristics of **AmpliSens® Borrelia miyamotoi-FRT** PCR kit

Samples type	Diagnostic sensitivity ⁴ , (with a confidence level of 90 % in the interval (%))	Diagnostic specificity ⁵ , (with a confidence level of 90 % in the interval (%))
Blood	91.2 – 100.0	94.0 – 99.8
Ticks	87.1 – 100.0	98.1 – 100.0
Tissue (autopsy, biopsy) material	83.9 – 100.0	92.9 – 100.0
Cerebrospinal fluid	83.9 – 100.0	92.9 – 100.0

14. REFERENCES

1. Krause P., Fish D., Narasimhan S., Barbour A. *Borrelia miyamotoi* infection in nature and in humans/Clin Microbiol Infect. - 2015. – Vol.21. - P.631–639.

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® Borrelia miyamotoi-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®



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List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
30.08.19 DV	Through the text	The text formatting was changed
	1. Intended use	The subsection Indications and contra-indications for use of the reagent kit was added
	6. Sampling and handling	The information about transportation of biological material was added
	13. Specifications	The section was rewritten
11.06.20 MM	14. References	The section was actualized
	Page footer	Phrase "Not for use in the Russian Federation" was added
	3. Content	The reagents volumes are corrected
	13.2 Analytical specificity	The section was actualized
17.03.21 VA	13.3. Diagnostic characteristics	In the table 8 values of diagnostic characteristics for tissue (autopsy, biopsy) material and cerebrospinal fluid were added
	—	The name, address and contact information for Authorized representative in the European Community was changed

⁴ Diagnostic sensitivity in comparison with a PCR reagent kit.

⁵ Diagnostic specificity in comparison with a PCR reagent kit.