SCIENTIFIC REPORT

RealLine *Borrelia miyamotoi*

An Assay Kit for Detection of *Borrelia miyamotoi*, a Tick-Borne Relapsing Fever Pathogen, Using Real-Time PCR

In Eastern Europe, ixodes tick-borne borreliosis (ITB) are one of the leaders in prevalence among tick-borne zoonotic diseases. ITB pathogens are spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex: *B. afzelii*, *B. garinii, B. burgdorferi* sensu stricto (s.s.). Recently, spirochetes of *Borrelia miyamotoi* species, which do not belong to this complex, were found in ixodic ticks in several regions [1–3]. This Borrelia species was first isolated from *Ixodes persulcatus* ticks and identified in Japan in 1995 [4]. Later, *B. miyamotoi* was also found in ticks of *I. ricinus* species in Germany and Sweden, and in *I. pacificus* and *I. scapularis* ticks in the USA [5–8].

The rate of tick infection with *B. miyamotoi* is lower than that with *Borrelia* of the *B. burgdorferi* s.l. complex [6, 7]. However, this rate reaches 6.3 %, and even 16 % in some regions [3, 9].

To date, the transmission cycle of *B. miyamotoi* in nature has not been studied in details. The dominant natural reservoir for spirochetes of these species is apparently small mammals [5, 10]. It has been demonstrated that quite often small rodents, as well as ticks, may simultaneously contain *Borrelia* of different groups and other human-pathogenic microorganisms: tick-borne encephalitis virus (TBE), *Anaplasma phagocytophilum*, *Ehrlichia muris*, etc. [3, 11–13].

Although, based on its molecular and genetic characteristics, *B. miyamotoi* was assigned to the group of *Borrelia* causing tick-borne relapsing fever (TBRF), its pathogenicity for humans has long remained questionable. However, in 2009–2011, data were published on the detection of *B. miyamotoi* nucleic acids (NA) in blood of patients with non-typical for ITB relapsing fits of fever that appeared after taiga ticks bites [2, 9, 14, 15].

Typical TBRF pathogens, *B. duttonii*, *B. parkeri*, *B. turicatae*, *B. hermsii*, etc., are carried by argasid ticks commonly found in desert, semi-desert, and subtropic zones [16]. Usually, patients infected with these *Borrelia* species have almost no eschar at the bite site, no erythema, the disease starts with an acute shivering attack and temperature rises to 38 – 40 °C. TBRF is also characterised by multiple alternating periods of fever and apyrexia of varying duration [17].

The studies conducted in Russia demonstrated that, in most patients with a *B. miyamotoi* DNA detected in blood, the disease was accompanied by flu-like symptoms and a temperature above 39.5 °C, development of a pronounced intoxication syndrome, as well as relapsing fits of fever [9, 18]. Just recently, it was reported that meningoencephalitis in a patient with immunosuppression was caused by *Borrelia* of this species [19]. These data provide confirmation of *B. miyamotoi* pathogenicity for humans.

TBRF, apparently having *B. miyamotoi* as its pathogen, is currently almost unknown to medical specialists. And yet, this new and still insufficiently studied disease poses a serious threat to the populations given the extended habitat of ticks and their high rate of infection with *Borrelia* of this species. Absence of any pathognomonic symptoms (such as, e.g., erythema or inoculation eschar) for TBRF of this aetiology, and similar manifestations of *B. miyamotoi* infection and tick-borne encephalitis as well as non-erythemic forms of ITB complicate significantly its clinical differential diagnostics. The problem is even more complicated considering that a single tick may simultaneously carry two or more pathogens of the zoonotic diseases.

No criteria for laboratory diagnostics of pathological conditions related to *B. miyamotoi* infection have yet been specified. A study of pathogenesis of *Borrelia* of this species is complicated, in particular, by lack of established methods for their culture [10, 12].

There are still no commercially available kits for serological diagnostics of *B. miyamotoi* infection on the international diagnostic markets. It has been demonstrated that serological tests for detection of class M and G immunoglobulins to *Borrelia* that cause ITB also detect antibodies to *Borrelia* of the TBRF group, *Borrelia persica* and *B. miyamotoi*, in patient's blood, which is explained by application of protein antigens common to *Borrelia* of these groups in such tests [9, 20]. According to published data, glpQ protein, which is not present in *B. burgdorferi* s.l. complex, was used as an antigen to study seroconversion in patients infected with *B. miyamotoi* [21].

Polymerase chain reaction (PCR), with its exceptionally high specificity and sensitivity, is the most promising method for early laboratory diagnostics of *B. miyamotoi* infection. The ground for it is a relatively high concentration of *Borrelia* of the TBRF pathogenic group in blood of infected patients, as opposed to the *B. burgdorferi* s.l. complex. In particular, PCR allowed detecting *B. persica* DNA in 21 (100 %) patients with suspected TBRF, while a pathogen of this disease was detected in 90% of the cases (19 out of 21 patients) in a microscopic study [22]. According to the data of the Central Research Institute of Epidemiology of Russia, based on PCR studies, the concentration of *B. miyamotoi* in patient's blood at the peak of fever is 10^3 to 10^6 bacteria per ml [23].

The objective of this work is to develop and test an assay kit based on real-time PCR method for the detection of *B. miyamotoi* DNA in ticks, blood samples of small rodents (tick feeders), as well as patients bitten by ticks.

Materials and Methods. The study used 652 ticks (imago) of two species, *I. persulcatus* and *I. pavlovskyi*, of which 391 ticks (sampling No. 1) were collected by flagging in the Toguchin district of the Novosibirsk region (Russia) in spring 2011 and stored individually at -50 °C. In addition, 261 ticks (sampling No. 2) were picked off from people in Novosibirsk and its suburbs in June 2012, and were sent for analysis to the clinical diagnostic laboratory (SibLabServis Ltd, Novosibirsk, Russia).

Individual ticks from sampling No. 1 were manually homogenised using liquid nitrogen according to the Instruction Manual for the "RealLine *Borrelia burgdorferi s.l.*" assay kit (BIORON Diagnostics,). The obtained homogenate was suspended in 250 µl of the solution for sample preparation (SSP). Each tick from sampling No. 2 was placed into 250 µl of SSP and homogenised using MagNa Lyser (Roche Diagnostics, Switzerland). Nucleic acids for PCR were extracted using 100 µl of individual tick suspensions and the "RealLine Extraction 100" kit (BIORON Diagnostics), which includes an internal control sample [24]. The remaining tick suspension was stored at -50 °C.

Blood samples of 60 mouse-like rodents (red-backed mice genus Myodes) caught on the territory of the Omsk region in 2011, were generously provided by V.A. Rar and V.V. Yakimenko. Lysates of animal blood were obtained as described earlier [25]. To extract NA with the "RealLine Extraction 100" kit, 100 µl of each lysate was used.

In this work, we also studied blood samples of 125 patients that were admitted to Municipal Clinical Hospital for Infectious Diseases No. 1, Novosibirsk, in May–June 2012 with a feverish condition, a tick bite in their medical history, and a suspected tick-borne infection (TBI). NA was extracted from the leucocyte fraction of patients' blood by sampling 1.5 – 2.0 ml of whole blood into Monovette Haematology tubes (SARSTEDT, Germany) and centrifuging in Eppendorf microtubes for 10 minutes at 800 rpm in a MiniSpin centrifuge (Eppendorf, Germany). The upper layer of plasma of about 500 µl, containing leucocytes, was transferred to another tube and centrifuged for 10 minutes at 13,000 rpm. After removing most of the supernatant, the remaining 200 µl of liquid in the tube, together with the cell pellet, were re-suspended, and 100 µl of the suspension was used to extract NA with the "RealLine Extraction 100" kit.

The corresponding assay BIORON Diagnostics kits were used to detect DNA of *Borrelia burgdorferi s.l.*, *Anaplasma phagocytophilum*, *Ehrlichia muris*, *Ehrlichia chaffeensis*, as well as TBE RNA in the analysed samples.

To assess the specificity of *B. miyamotoi* DNA detection, we used *in vitro* generated normalised reference cultures of *Borrelia afzelii* (4 strains), *Borrelia garinii* (7 strains), and *Borrelia burgdorferi* s.s. (1 strain) with a concentration of 1.05 – 2.55 x 10^7 cell/ml (NPO Virion, Tomsk, Russia), as well as *Treponema pallidum* (Nichols strain) with a concentration of 1.5 x 10^7 cell/ml, as control materials. Furthermore, to this end, we analysed samples containing no less than 10^5 copies of NA of *A. phagocytophilum*, *E. muris*, the tick-borne encephalitis virus, which were extracted from ticks collected in Novosibirsk in 2011. As an additional control material, we used samples of total NA obtained from a leucocyte fraction of donor blood using the "RealLine Extraction 100" kit.

The Standard Reference Sample (SRS) used to determine the analytical sensitivity of the developed kit and to subsequently control its quality was prepared following an earlier described method [26], using a cloned DNA fragment of the glpQ gene of *B. miyamotoi* extracted from an infected tick. The nucleotide sequence of this fragment contained 680 base pairs and corresponded to entry Acc. No. FJ940729 of the GenBank database. SRS concentration was 4 x 10^5 copies of the DNA fragment in 1 ml.

Real-time PCR was performed using CFX 96 thermocycler with real-time fluorescence detection (Bio-Rad, USA). 50 µl of the total NA solution extracted from each test sample was placed in a tube with the ready master mix for PCR. The obtained results were evaluated.

Species identification in the test samples was based on detection of the nucleotide sequence in both DNA strands in the polymorphic regions of the glpQ and 23S rRNA genes. This study was performed on an automatic ABI Prism 3100 DNA Analyser (Applied Biosystems, USA). Phylogenetic analysis of the obtained nucleotide sequences was performed with MEGA 4.0 software [28]; sequences were aligned to GenBank data using BLAST search system.

Results and Discussion. The "RealLine *Borrelia miyamotoi*" assay kit, containing lyophilised ready master mix for PCR (RMM) with all the components required for real-time PCR, was developed in compliance with the general principles for designing the RealLine line of diagnostic kits [24]. To prevent false positive results obtained because of contamination with PCR product, *E. coli* uracil-DNA-glycosylase and deoxyuridine triphosphate were introduced into the RMM [26].

A unique fragment of the glpQ gene (Acc. No. AY368276), which is not present in species of *Borrelia burgdorferi s.l.* complex, was chosen as a target for amplification and subsequent detection of the *B. miyamotoi* DNA fragment [22]. Fluorescence of the amplification products of the analysed *B. miyamotoi* DNA region is registered in the ROX channel. Any losses of the studied nucleotide material in the course of extraction, as well as the degree of PCR inhibition, are accounted for by amplification of the internal control sample (IC) and independent evaluation of fluorescence in the FAM channel.

The analytical sensitivity of the "RealLine *Borrelia miyamotoi*" assay kit for detection of *B. miyamotoi* DNA was assessed using probit analysis based on the results of real-time PCR of four eight-fold dilutions of SRS containing from 800 to 4 x 10^5 copies of the DNA fragment of this pathogen in 1 ml. The sensitivity of analysis at the 95 % confidence level ($p < 0.05$) is 40 genome equivalents (g/e) per sample with amplification efficiency at 94.8 %. The value of the threshold cycle showed a linear dependence on the quantity of a DNA in the test samples in the range of 40 to 2 x 10^4 g/e per reaction (Fig. 1).

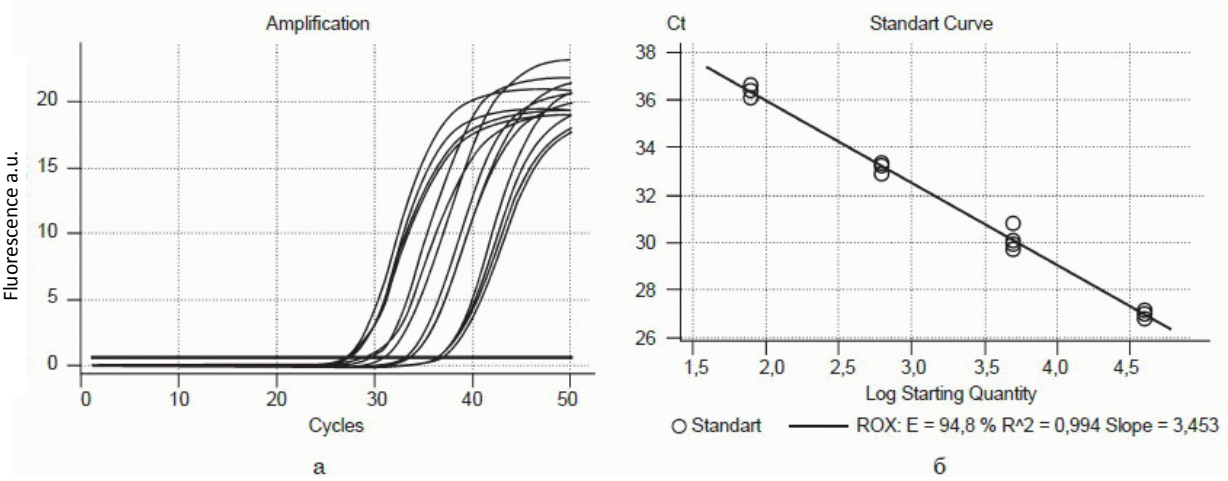

Figure 1. Assessment of the sensitivity of *B. miyamotoi* DNA detection and the linear range for its measurement using real-time PCR: a—fluorescence profile of four dilutions (40 to 2 x 10^4 g/e) of the reference sample of the *B. miyamotoi* genome fragment as a function of the number of PCR cycles (Cycles); b—standard correlation curve between the Ct (Threshold Cycle) values and log10 of the concentration of the specific matrix (Log Starting Quantity).

To assess the analytical specificity of the "RealLine *Borrelia miyamotoi*" assay kit, we tested samples containing no less than 10^6 g/e of a DNA of *B. afzelii*, *B. burgdorferi* s.s., and *B. garinii* extracted from 12 reference cultures, as well as from *T. pallidum*, using two individual samples of each strain for real-time PCR. Five test repeats for each sample yielded only negative results.

Analysis with a set of 40 samples of tick suspensions, 11 of which contained TBE RNA, 8 contained DNA of *A. phagocytophilum*, the human granulocytic anaplasmosis (HGA) pathogen, 21 contained DNA of *E. muris*, causing human monocytic Ehrlichiosis (HME), and 16 contained total DNA extracted from the leucocyte fraction of blood of healthy donors, also yielded negative results. Based on the data from these studies, we evaluated the specificity of the developed kit using the given set of samples at 100 %.

The "RealLine *Borrelia miyamotoi"* assay kit was tested by studying ticks, blood of small rodents, and a leucocyte fraction obtained from blood of patients with suspected TBI.

It should be noted that tick homogenisation step, which preceeds sample preparation, is one of the important steps in the detection of NA of TBI pathogens. In the majority of laboratories undertaking such studies, this rather time-consuming procedure is performed manually after freezing ticks with liquid nitrogen. We have demonstrated that manual homogenisation requires very accurate execution and is a major contributor to the errors in the laboratory tests on the detection of NA of TBI pathogens [28]. Furthermore, a poorly performed tick homogenisation cannot be tracked using the routinely tested IC. Another serious drawback of manual homogenisation is the possibility of contaminating the box room and the risk of infecting laboratory personnel with TBI pathogens contained in the ticks. It has been proven that TBE can be contracted via the olfactory tract [29], while human-pathogenic *Borrelia* can be contracted by accidental contact of eye conjunctiva with the contents of the tick's intestine and via skin microtraumas [30].

An alternative method for preparing a fine tick suspension is electromechanic homogenisation, which was performed in this work using MagNA Lyser (Roche Diagnostics, Switzerland) and Green Beads reagents for grinding (Roche Diagnostics, Switzerland). The procedure of tick grinding in this case is performed in closed tubes, with the duration of the procedure being on average 10 times less than that of the manual method; in addition, the elimination of the human factor at this step of sample preparation results in improved PCR sensitivity and specificity [28]. A clear advantage of electromechanic homogenisation of ticks is also the minimal risk of infection of laboratory personnel with TBI pathogens.

When testing the samples of total NA, individually extracted from 391 ticks collected by flagging (sampling No. 1) using the "RealLine *Borrelia miyamotoi*" assay kit, a positive result was obtained for 8 (2.1 %) samples, with no NA of other TBI pathogens found in the samples. In a similar test of 261 ticks picked off from people (sampling No. 2), *B. miyamotoi* DNA was detected in 9 (3.5 %) cases, with the DNA of the *B. burgdorferi* s.l. complex being additionally detected in two ticks, the DNA of *A. phagocytophilum* in one tick, and the DNA of the ITB, HGA, and HME pathogens in still other tick (Fig. 2). Thus, four of the nine ticks infected with *B. miyamotoi* simultaneously contained other human-pathogenic microorganisms, supporting the earlier published data on the high mixed-infection rate of these carriers with various TBI pathogens [11, 13].

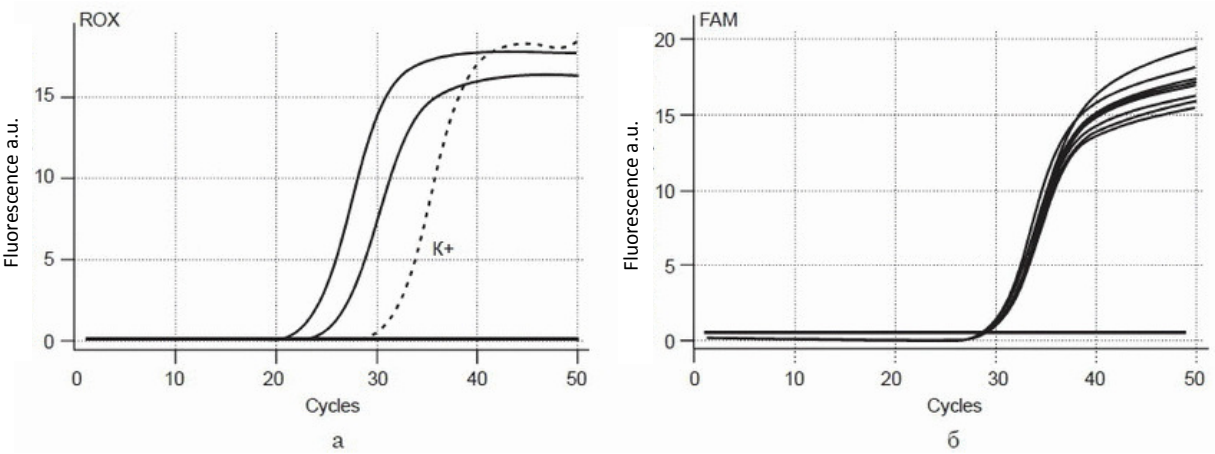

Figure 2. a) Detection of a *B. miyamotoi* DNA in two ticks picked off from people using the "RealLine DNA *Borrelia miyamotoi*" assay kit (detection in the ROX channel). b) Stability of detection of the IC added to the samples of tick suspensions upon extraction of the total NA (detection in the FAM channel).

During the next step, the "RealLine DNA *Borrelia miyamotoi*" assay kit was used to study 60 blood samples of red-backed mice genus Myodes, in which we had previously detected the DNA of the ITB, HGA, and HME pathogens, both individually and in various combinations [11]. According to the results of analysis, *B. miyamotoi* DNA was found in 5 (8.3 %) cases, with one of the 5 samples additionally containing the DNA of *E. muris*, and two other samples containing the DNA of *E. muris* and *A. phagocytophilum* (i.e., three pathogens simultaneously). The frequency of a *B. miyamotoi* DNA in the tested mice sampling corresponds to the earlier published data on the prevalence of Borrelia of this species in small rodents [31]. Furthermore, the results obtained with the developed PCR kit indicate that *B. miyamotoi* can circulate together with other human-pathogenic microorganisms not only in their carriers, ixodic ticks, but also in small rodents, which serve as a natural reservoir for the infection.

An important step in testing the RealLine DNA *Borrelia miyamotoi* assay kit was its application for laboratory study of blood samples of patients admitted to Municipal Clinical Hospital for Infectious Diseases No. 1, Novosibirsk, with suspected TBI. The medical history of all 125 patients 7 to 14 days prior to the onset of the disease mentioned a tick bite and absence of erythema, but, when admitted to the hospital, they mentioned feverish condition (a temperature of 38 °C and above), hyperaemia of the face, diffuse headache, and meningeal symptoms and signs. Based on these data, they were preliminary diagnosed with tick-borne encephalitis.

The results of the study of 125 patients using a complex of serological and general laboratory methods is described in a separate publication. In this work, we report data on the analysis of blood of these patients by real-time PCR, using assay kits to detect the DNA of *B. miyamotoi*, as well as the NA of TBE, ITB, HGA, and HME pathogens.

An analysis of the samples performed using the "RealLine TBEV" assay kit yielded positive results only for two of 125 (1.6 %) patients with the preliminary diagnosis of tick-borne encephalitis. The DNA of *E. muris*, *A. phagocytophilum*, or the DNA of the *B. burgdorferi* s.l. complex were not found in blood of any of the studied patients. At the same time, the analysis performed using the developed kit detected *the B. miyamotoi* DNA in

15 (12 %) patients, with negative results obtained for the TBE RNA test. The concentration of the *B. miyamotoi* DNA in their blood varied from 200 to 250 thousand copies/ml, which supports the data published earlier by researchers of the Central Research Institute of Epidemiology (Russia) on high level of this pathogen in patient's blood in the early stages of the infection [23].

In general, the results of testing of the "RealLine *Borrelia miyamotoi*" assay kit have demonstrated that the erroneous preliminary diagnosis of tick-borne encephalitis could have been excluded, and the actual TBRF pathogen could have been detected in 12% of the patients. This is extremely important, since the methods and measures employed in TBE treatment are not effective for TBRF therapy. Another equally important result of this part of our work is the fact that the analysis of clinical samples of 15 patients, who had the *B. miyamotoi* DNA in their blood, with the "RealLine *Borrelia burgdorferi* s.l." assay kit produced negative results in 100% of the cases, which confirms the high diagnostic specificity of this kit. This allows differentiating *B. miyamotoi* and *Borrelia burgdorferi* s.l. using the RealLine kits in the ticks that have attacked people and in patient's blood at an early stage of the tick-borne infection. The obtained data can be used to predict the development of TBRF or ITB, and to take the appropriate preventive or therapeutic measures.

For the purpose of additional confirmation of the diagnostic specificity of the RealLine DNA *Borrelia miyamotoi* assay kit, the samples obtained from the blood of patients and rodents (as well as ticks), which were found positive when tested using the kit, were sequenced over two regions of the glpQ and 23S rRNA genes. The results of this work confirmed the presence of a *B. miyamotoi* DNA in the test samples.

Thus, based on the studies performed, the "RealLine *Borrelia miyamotoi*" assay kit for real-time PCR detection of the DNA of *B. miyamotoi*, the pathogen for insufficiently studied tick-borne relapsing fever, was developed. The kit was demonstrated to have a high sensitivity, specificity, and can be successfully used to study ticks and samples obtained from small rodents (tick feeders), as well as for differential diagnostics of tick-borne infections in patients with no pathognomonic symptoms (e.g., erythema or primary affect). To this end, probably, the most efficient application of this kit will be in combination with the diagnostic other tests, namely: "RealLine TBEV", "RealLine *Borrelia burgdorferi* s.l.", and "RealLine *Anaplasma phagocytophilum* / *Ehrlichia muris*, *Ehrlichia chaffeensis*". When using this line of kits for complex laboratory express testing of ticks that have attacked humans, the resulting detection of nucleic acids of certain pathogens allows us to provide the appropriate preventive treatment to the patients who have suffered from a bite of an infected tick, and prevent the development of a whole spectrum of serious diseases.

A wide application of the developed kit by diagnostic laboratories will promote correct diagnosis of fever patients admitted to healthcare facilities with suspected tick-borne infections. A feverish condition in certain patients with suspected TBE, absence of erythema, and subsequent detection of antibodies reacting with antigens of ITB pathogens in their blood lead to ambiguities and possible errors in diagnosis in the instance of the disease being actually caused by *B. miyamotoi*. A specific detection of this pathogen using real-time PCR is possible even in the early stages of the disease, and can be extremely useful for diagnosis specification.

Furthermore, the use of the "RealLine *Borrelia miyamotoi*" assay kit will promote acceptance of the *Borrelia miyamotoi* spirochete by the medical community as a widely-spread pathogen causing a transmissible, infectious disease, and inclusion of the latter into the official list of nosological entities.

References

1. Korotkov Yu.S., Kislenko G.S., Burenkova V.A. et al. // Parasitologiya. 2008. V. 42. Issue 6. P. 441–451 (In Russian).
2. Kolyasnikova N.M., Fedorova M.V., Gerasimov S.G. et al. // Molekulyarnaya Diagnostika. 2010. V. 2. P. 232–234 (In Russian).
3. Fomenko N.V., Livanova N.N., Bogoyarkov V.Yu. et al. // Parasitologiya. 2010. V. 44. Issue 3. P. 201–210 (In Russian).
4. Fukunaga M., Takahashi Y., Tsuruta Y. et al. // Int. J. Syst. Bacteriol. 1995. V. 45. P. 804–810.
5. Scoles G.A., Papero M., Beati L., Fish D. // Vect. Borne. Zoonotic. Dis. 2001. V. 1. N. 1. P. 21–34.
6. Fraenkel C., Garpmo U., Berglund J. // J. Clin. Microbiol. 2002. V. 40. N. 9. P. 3308–3312.

7. Richter D., Schlee D., Matuschka F. // Emerg. Infect. Dis. 2003. V. 9. N. 6. P. 697–701.
8. Mun J., Eisen R.J., Eisen L. et al. // J. Med. Entomol. 2006. V. 43. N. 1. P. 120–123.
9. Platonov A.E., Karan L.S., Kolyasnikova N.M. et al. // Emerg. Infect. Dis. 2011. V. 17. N. 10. P. 1816–1823.
10. Barbour A.G., Bunikis J., Travinsky B. et al. // Am. J. Trop. Med. Hyg. 2009. V. 81. N. 6. P. 1120–1131.
11. Bodarenko E.I., Timofeev D.I., Fomenko N.V. et al. // Sibirskiy Med. Zhurnal. 2012. V. 111. Is 4. P. 33–36 (In Russian).
12. Bogoyarkov V.Yu., Fomenko N.V., Panov V.V. et al. // Parasitologiya. 2010. V. 44. Issue 6. P. 543–555 (In Russian).
13. Travina N.S., Skrynnik S.M., Karan L.S. // Natsionalnye Prioritety Rossii. Special Issue. 2011. Issue 2 (5). P. 74–75 (In Russian).
14. Platonov A.E., Karan L.S., Garanina S.B. et al. // Epidemiol. i Infekts. Bolezni. 2009. Issue 2. P. 30–35 (In Russian).
15. Fomenko N.V., Yepikhina T.P., Chernousova N.Ya. // Molekulyarnaya Medicina. 2010. Issue 3. P. 28–31 (In Russian).
16. Caimano M. // The Prokaryotes / Ed. M. Dworkin et al. New York: Springer, 2006. V. 7. P. 236–293.
17. Pokrovsky V.I., Loban K.M. / Rukovodsvo po Infektsionnym Boleznyam. Moscow: MSU, 1986. P. 169–184 (In Russian).
18. Sarksyan D.S., Platonov A.E., Karan L.S. et al. // Terapevticheskiy Arkhiv. V. 84. Issue 11. P. 34–41 (In Russian).
19. Gugliotta J.L., Goethert H.K., Berardi V.P. et al. // N. Engl. J. Med. 2013. V. 368. N. 3. P. 240–245.
20. Hasin T., Davidovitch N., Cohen R. et al. // N. Engl. J. Med. 2006. V. 355. P. 148–155.
21. Krause P.J., Narasimhan S., Wormser G.P. et al. // N. Engl. J. Med. 2013. V. 368. N. 3. P. 291–293.
22. Halperin T., Orr N., Cohen R. et al. // Acta. Trop. 2006. V. 98. P. 189–195.
23. Karan L.S., Kalyasnikova N.M., Fedorova M.V. et al. VII Vseross. Conf. “Molekulyarnaya Diagnostika 2010”. Moscow, 2010 (In Russian).
24. Ivanov M.K., Poryvaev V.D., Trukhina A.V. et al. // Novosti Vector-Best. 2008. Issue 4 (50). P. 16–19 (In Russian).
25. Bondarenko E.I., Ivanov M.K., Yakimenko V.V. et al. // Klinicheskaya Laboratornaya Diagnostika. 2012. Issue 11. P. 54–57 (In Russian).
26. Bondarenko E.I., Titov S.E., Ivanov M.K. // Novosti Vector-Best. 2012. Is.1(63). P. 2–8 (In Russian).
27. Tamura K., Dudley J., Nei M. et al. // Mol. Biol. Evol. 2007. V. 24. N. 8. P. 1596–1599.
28. Timofeev D.I., Fomenko N.V., Ivanov M.K. // Sibirskiy Med. Zhurn. 2012. V. 111. Issue 4. P. 45–48 (In Russian).
29. Goncharova E.P. Investigation of the Possibilities of Intranasal Immunisation Against Tick-Borne Encephalitis: Cand. Biol. Sci. Thesis, Koltsovo, 2003. 131 p. (In Russian).
30. Lobzin Yu.V., Rakhmanova A.G., Antonov V.S. et al. // Epidemiology, Aetiology, Clinics, Diagnostics, Treatment, and Prevention of Ixodes Tick-Borne Borreliosis. (Guidelines for Medical Practitioners). St. Petersburg., 2000 (In Russian). http://www.epid.ru/docs/infdoc5.html
31. Fomenko N.V. Genetic Heterogeneity of Borrelia spp. of Western Siberia: Cand. Biol. Sci. Thesis, Novosibirsk, 2008. 147 p. (In Russian).

These results are represented by

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Rev01_1118

Portfolio for Detection Kits for Tick-associated diseases:

- **Borrelia burgdorferi s.l. complex:**
  B. afzelii, B. garinii, B. burgdorferi sensu strictu, B. spielmannii, B. bavariensis, B. bisettii, B. lusitania, B. valaisiana
- **Borrelia miyamotoi**
- **Anaplasma phagocytophilum**
- **Ehrlichia chaffeensis**
- **Ehrlichia muris**
- **Rickettsia sibirica**
- **Rickettsia heilongjiangensis**
- **Babesia species:**
  B. microti, B. venatorum, B. divergens, B.duncani, B. canisis
- **Tick Borne Encephalitis Virus**

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