

# Laboratory diagnosis of tick-borne infections

by Real-Time PCR



## Tick-borne infections – a group of naturally occurring focal diseases, the causative agents of which are transmitted to humans through a tick bite

### Features of tick-borne infections

- Association with natural foci, heterogeneous occurrence in different regions
- Pronounced seasonality associated with the life cycle and periods of activity of vectors – ticks
- High frequency of mixed infections due to circulation of different pathogens in the same natural foci and vectors
- Difficulties in making the correct diagnosis in time due to the similarity of symptoms of various tick-borne infections and other diseases, a long incubation period and unclear, diverse clinical manifestations

### Distribution areas of Ixodidae ticks



The main reservoir of pathogens causing tick-borne infections in nature are ticks of the family *Ixodidae*, some of which are carriers of microorganisms pathogenic to humans. They are transmitted to humans by an infected tick of the genus *Ixodes*, in our latitudes by the tick *Ixodes ricinus*. Ticks are active from spring to late autumn and go through three stages of development: larva, nymph and adult imago.

Human disease is associated with direct invasion of the microorganism or secondary inflammatory changes. Reporting the number of infections is lagging behind, and the actual situation about the incidence of infections can only be assumed. The inaccuracy of data on the number of infections in individual areas and regions is also hampered by the diversity of late clinical manifestations.

## The most widespread tick-borne pathogens

Pathogen	Disease
Tick-borne encephalitis virus (TBEV)	Tick-borne encephalitis (TBE)
<i>Borrelia burgdorferi sensu lato</i> : <i>Borrelia burgdorferi sensu stricto</i> , <i>B. afzelii</i> , <i>B. garinii</i>	Lyme disease (borreliosis) (LD)
<i>Borrelia miyamotoi</i>	Disease similar to tickborne relapsing fever
<i>Rickettsia sibirica</i> , <i>R. heilongjiangensis</i> , <i>R. conorii</i>	Rickettsiosis
<i>Anaplasma phagocytophilum</i>	Human granulocytic anaplasmosis
<i>Ehrlichia chaffeensis</i> , <i>Ehrlichia muris</i>	Human monocytic ehrlichiosis
<i>Babesia divergens</i> , <i>B. microti</i> , <i>B. venatorum</i>	Babesiosis (piroplasmiasis)

## Prevention of tick-borne infections

- The risk of transmitting an infectious agent through a bite depends on the type of pathogen, the nature of the tick infestation and the duration of its bite.
- Infection without bite is possible when crushing an insect and getting its contents on damaged skin.

In order to timely and effectively implement specific preventive measures, it is possible to detect various pathogens in ticks using DNA or RNA detection methods.

The complex of preventive measures against tick-borne infections includes specific prevention measures (prophylaxis with a vaccine against the TBEV virus), emergency antibiotic prophylaxis and nonspecific prophylaxis, aimed at preventing a tick bite.

**The combination of serological and molecular-biological methods significantly increases the possibilities for laboratory diagnosis of tick-borne infections.**

Disease risk assessment

Deciding on preventive therapy

Diagnosis verification

Choosing the right treatment strategy to detect mixed infections

Diagnosis of acute and chronic forms of tick-borne infections

Diagnosis of tick-borne infections in the early stages, including during the incubation period

Blood screening prior to TBEV vaccination, assessment of vaccine effectiveness and post-vaccination period control

## Recommendations for transportation and storage of biological specimen for analysis



### Blood specimens:

- +2 to +8 °C max. 1 day
- 18 to -60 °C max. 1 month

### Cerebrospinal fluid specimens:

- +2 to +8 °C max. 1 day
- 18 to -60 °C max. 2 weeks

- **Only one freezing and thawing of specimens is allowed**



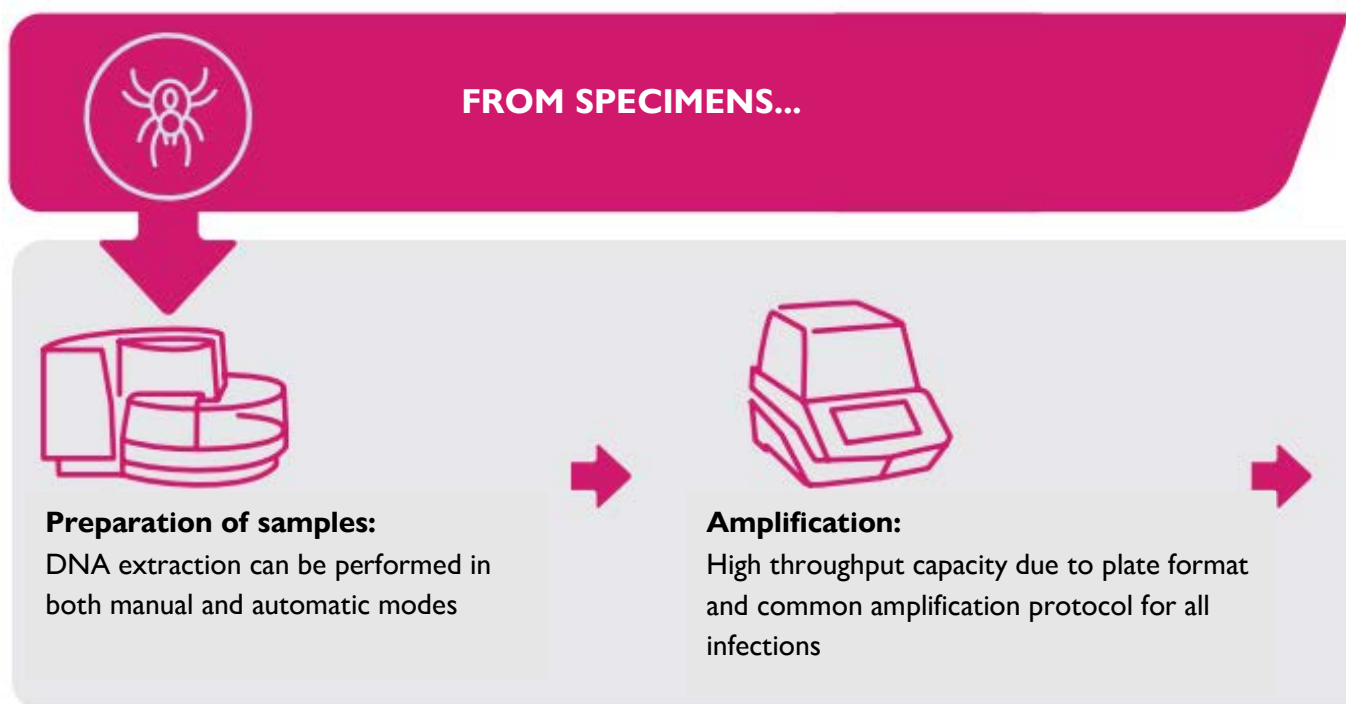
### Ticks:

- +2 to +8 °C max. 3 days (suspension 24 hours)
- 18 to -60 °C max. 2 weeks
- at temperature under -60 °C – long-term (up to 1 year)
- Whole tick samples can be used for analysis
- It is convenient to transport ticks and store them alive, maintaining a moist environment in tubes (wet gauze, cotton wool)

## Reagent kits for the diagnosis of tick-borne infections by real-time PCR method

- **Ready Master Mix for PCR:** just add extracted nucleic acid to the test tube and start the test
- **Multiplexity:** testing one sample for multiple infections
- **Universal amplification protocol**
- **Automation option:** improving the quality and quantity of testing
- **Wide range of biological materials:** solving any diagnostic problems
- **Easy to store and transport:** no freezing required

## RealBest® technology: solution for PCR diagnostics of tick-borne infections





**Interpretation of results:**  
performed in automatic mode



**...TO THE RESULT!**



### Kits for nucleic acids extraction

Pathogen	Biological material	Extraction kit
<b>Tick-borne encephalitis virus (TBEV)</b>	ticks, blood samples (leukocyte fraction of blood, plasma, whole blood), cerebrospinal fluid, swabs/biopsy samples from the latch site	<b>RealBest extraction 100</b>  <b>RealBest UniMag</b>  When working with whole blood, it is recommended to use a hemolytic <b>RealBest Hemolytic</b>
<b><i>Borrelia burgdorferi sensu lato, Borrelia miyamotoi</i></b>	ticks, blood samples (leukocyte fraction of blood, plasma, whole blood), liquor, synovial fluid, swabs/biopsy specimens from the latch site	
<b><i>Anaplasma phagocytophilum, Ehrlichia chaffeensis, Ehrlichia muris</i></b>	ticks, blood samples (whole blood, leukocyte fraction of blood)	
<b><i>Rickettsia</i></b>	ticks, blood samples (leukocyte fraction of blood, whole blood), swabs/biopsy samples	
<b><i>Babesia</i></b>	ticks, whole blood	

## Kits for the diagnostics of tick-borne diseases

### Diagnosis of tick-borne encephalitis

1198 €	RealBest RNA TBEV	48 tests
5396 €	RealBest DNA Borrelia burgdorferi s.l./RNA TBEV	48 tests

### Diagnosis of Lyme disease

1498 €	RealBest DNA Borrelia burgdorferi s.l	48 tests
1495 €	RealBest DNA Borrelia miyamotoi	48 tests
5396 €	RealBest DNA Borrelia burgdorferi s.l./RNA TBEV	48 tests

### Diagnosis of anaplasmosis and ehrlichiosis

5398 €	RealBest DNA Anaplasma phagocytophilum/Ehrlichia muris, Ehrlichia chaffeensis	48 tests
-----------	----------------------------------------------------------------------------------	----------

### Diagnosis of rickettsiosis

5391 €	RealBest DNA Rickettsia species	48 tests
5393	RealBest DNA Rickettsia sibirica/Rickettsia heilongjiangensis	48 tests

### Diagnosis of babesiosis

5389 €	RealBest DNA Babesia species	48 tests
-----------	------------------------------	----------

---

**Diagnostické centrum DNK, s.r.o.**

Brestová 14, 821 02 Bratislava

+421 911 299 324, +421 911 211 404

[dnk@pharma.sk](mailto:dnk@pharma.sk), [diagnostika@pharma.sk](mailto:diagnostika@pharma.sk)

[www.pcr.sk](http://www.pcr.sk)