

Test instructions

LYME DISEASE

There are over 800 species of ticks in the world. Ixodes ricinus (castor bean tick) is the most important species in the epidemiology of tick-borne diseases of humans and animals. Lyme disease is the biggest epidemiological problem among tick-borne diseases. Lyme disease is an infectious, animal-borne disease caused by an infection with the spirochaetes of *Borrelia burgdorferi sensu stricto*, but also other closely related species, including *B. afzelii*, *B. garinii*, *B. Spielmani*, referred to as genospecies.

Borrelia bacteria live in a tick's digestive tract through which they enter the host's body. Borrelia bacteria are excreted approximately two hours after the bite and reach a peak level after 72 hours. The likelihood of getting infected with Borrelia bacterium spirochaetes increases as ticks move through the stages of their lifecycle. We can notice the first symptoms of the disease about 10-14 days after the bite.

The symptoms of Lyme disease differ. One characteristic symptom of Lyme disease is erythema migrans.

If diagnosed early, it can reduce or prevent serious complications.

PURPOSE OF THE TEST

#checkthetick™ is a plate test which directly and quickly detects Borrelia bacteria in ticks. The quick **#checkthetick™** test detects B. garinii, B. afzelii, B. burgdorferi sensu stricto and B. spielmanii bacteria.

HOW DOES THE TEST WORK?

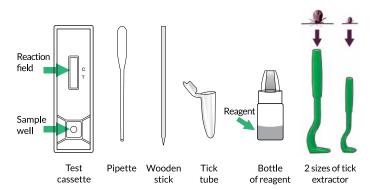
Comparison System: Polymerase chain reaction (PCR) (2009 and 2013/2014)

checkthetick™ TEST EFFICIENCY

	Sensitivity	Specificity	PPV/NPV	Ttp
checkthetick™	92.86 %	95.83 %	86.67 %/97.87 %	95.16 %

TEST KIT ELEMENTS

- 1. Foil packaging with 1 plate test.
- 2. Foil packaging with 1 pipette, a bottle with a reagent, a wooden stick and a test tube and 2 sizes of tick extractor.
- 3. Instructions.



WHAT DO YOU NEED?

You will need a watch or stopwatch to perform the test.

PRECAUTIONS

- 1. The kit should only be used to test ticks.
- 2. The test can only be used once.
- 3. Do not use the test past its expiration date.
- 4. Do not perform the test if the foil packaging with the plate test is broken or if it is not tightly closed.
- 5. Use only original test elements included in the kit.
- 6. Use the test cassette within 60 minutes after opening.
- 7. Crash a tick well with the wooden stick Borrelia bacteria are located in a tick's colon.
- 8. While testing, place the cassette horizontally on a smooth surface.
- 9. The wooden stick can only be used to crash a tick in the test tube.
- 10. Keep away from children.

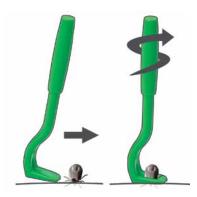
STORAGE AND STABILITY

- 1. Store at a temperature between 2 and 30°C
- 2. Keep away from direct sunlight.
- 3. Do not freeze.
- 4. Open the bag with the test plate before performing the test.

HOW TO REMOVE THE TICK

The kit contains 2 sizes of tick extractor, which are used to extract both small and large ticks.

- 1. Choose an extractor depending on the tick size.
- 2. Insert it from the side of the tick, as close to the skin as possible.
- 3. Turn the hook (either way) until the tick comes out (1-3 turns).
- 4. The injection site and hook should be disinfected.



TEST INSTRUCTIONS

To perform the test take the it out of the foil packaging by tearing the seal. Prepare other necessary elements: the pipette, the bottle with the reagent, the wooden stick and the test tube.

Collection of samples:

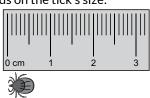
You should test a tick immediately after removing it from the skin. Crashing of a tick is an important element of the testing procedure.

Remove the tick carefully and make sure you remove the whole tick. The best way to remove a tick is to use tweezers (or special tick nipper or hook), grasping a tick as close to the head as possible and pull it counter to the direction it entered the skin. Disinfect the bite area after removing the tick.

Testing procedure

Optimum testing procedure depends on the tick's size:

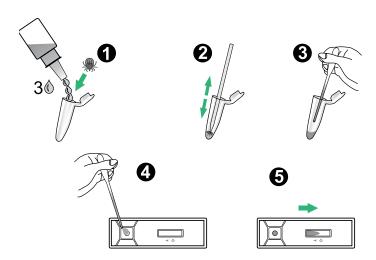
- ticks smaller than 8 mm see the procedure for "small and medium ticks";
- ticks bigger than 8 mm see the procedure for "large ticks".



Medium and small ticks (< 8 mm)

1. Place the tick in the test tube and add 3 drops of the reagent (the bottle with the reagent).

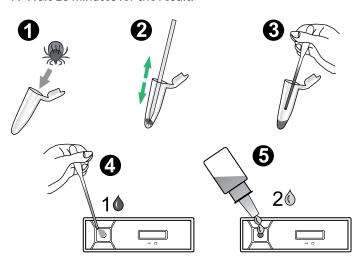
- 2. Crash the tick using the wooden stick until the liquid becomes discoloured. Use the sharp side of the wooden stick to crash the tick. Remove the stick.
- 3. Collect the liquid from the test tube using the pipette. Make sure you do not collect any part of the tick's body.
- 4. Measure 3 drops of the liquid collected from the test tube into a plate test hole. Make sure you do not collect any parts of the tick's body, as it may clog the test box.
- 5. The liquid starts running through the test strip. Wait 10 minutes for the result.



Application tips (when the liquid does not run through the strip): If parts of the tick's body get inside a test hole, you can add one more drop of the liquid.

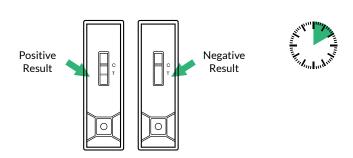
Large ticks

- 1. Place the tick in the test tube.
- 2. Crash the tick using the wooden stick (the sharp side).
- Collect the liquid obtained after crushing the tick with the pipette. Make sure you do not collect any part of the tick's body.
- 4. Add a drop of the liquid obtained after crushing the tick to a test hole and leave it for a few seconds.
- 5. Then add 2 drops of the reagent (the bottle with the reagent) directly to the hole.
- 6. The liquid starts running through the test strip.
- 7. Wait 10 minutes for the result.



Test result

The result is positive if two red lines appear in the reaction box of the test cassette. A red line in the T area (test) shows a positive



result – the tick is a carrier of Borrelia bacteria (a Borrelia antigen was detected) The less Borrelia antigens are present, the weaker the T line. A weak test line is also considered as a positive result. The second red line in the C area (check) shows a control line which confirms the correct test efficiency. The C line is not a reference line and its intensity is different from the T line.

Interpretation of test result:

A positive test result means that a tick is a carrier of one of 4 Borrelia spirochaetes genotypes.

If you have been bitten by a tick, contact your primary physician immediately, as the risk of infection with Borrelia spirochaetes is high.

A negative test result means that a tick does not carry Borrelia bacteria. Even if the result is negative, you should remember that ticks transmit many other diseases. If you have symptoms, inform your doctor about the bite.

Incorrect result

If no control line appears, it means that the test is incorrect. It means that most likely the test was performed incorrectly or is past the expiration date.

If the background colouring is strong after 10 minutes, you can rinse the test cassette with cold running water to wash off the background colour. The test result will remain visible.

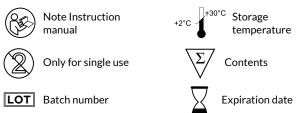
Place all elements of a used test in the original packaging and dispose of it as regular waste. Do not reuse any elements of the test kit.

BIBLIOGRAPHY

J. Eckert, K. T. Friedhoff, H. Zahner, P. Deplazes: "Lehrbuch Der Parasitologie für die Tiermedizin", Enke Verlag, 2.vollst. überarb. Aufl. 2008

W. Luttmann, K. Bratke, M. Küpper, D. Myrtek: "Der Experimentator Immunologie", Spektrum Akademischer Verlag, S.110 f., 3. Auflage 2009







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