PreventID® **Lyme Borreliosis**

Manual (FOR PROFESSIONAL USE)

→REF KSTILY402G10 | KSTILY402GP

Intended Use

PreventID® Lyme Borreliosis rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to Borrelia in human whole blood, serum or plasma specimen.

Introduction

Lyme disease, also known as Lyme borreliosis, is an infectious disease caused by bacteria of the *Borrelia sp.* which is spread by ticks.[2] The most common sign of infection is an expanding area of redness on the skin, known as erythema migrans, that begins at the site of a tick bite about a week after it has occurred.[1] The rash is typically neither itchy nor painful. Approximately 25–50% of infected people do not develop a rash.[1] Other early symptoms may include fever, headache and feeling tired.[1] If untreated, symptoms may include loss of the ability to move one or both sides of the face, joint pains, severe headaches with neck stiffness, heart palpitations or sollen lymph nodes and joints.[1] Months to years later, repeated episodes of joint pain and swelling may occur.[1] Occasionally, people develop shooting pains or tingling in their arms and legs.[1] Despite appropriate treatment, about 10 to 20% of people develop joint pains, memory problems, and feel tired for at least six months.[1, 4]

Lyme disease is transmitted to humans by the bite of infected ticks of the genus Ixodes.[5] Usually, the tick must be attached for 36 to 48 hours before the bacteria can spread.[6] In North America, *Borrelia burgdorferi* and *Borrelia mayonii* are the causes.[2, 7] In Europe and Asia, the bacteria *Borrelia afzelii* and *Borrelia garinii* are also causes of the disease.[2] The disease does not appear to be transmissible between people, by other animals, or through food.[6] Diagnosis is based upon a combination of symptoms, history of tick exposure, and possibly testing for specific antibodies in the blood.[3, 8] Blood tests are often negative in the early stages of the disease.[2] Testing of individual ticks is not typically useful.[9]

Test Principle

The **PreventID® Lyme Borreliosis** rapid test is a qualitative membrane-based immunoassay for the detection of IgG and IgM antibodies to Borrelia in whole blood, serum or plasma specimens. This test consists of two components, an IgG component and an IgM component. In the IgG component, anti-human IgG is coated in IgG test line region. During testing, the specimen reacts with Borrelia antigen-coated particles in the test cassette. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with the anti-human IgG in IgG test line region, if the specimen contains IgG antibodies to Borrelia. A coloured line will appear in IgG test line region as a result of this. Similarly, anti-human IgM is coated in IgM test line region and if specimen contains IgM antibodies to Borrelia, the conjugate-specimen complex reacts with anti-human IgM. A coloured line will appear in IgM test line region as a result.

Therefore, if the specimen contains anti-Borrelia IgG antibodies, a coloured line will appear in IgG test line region. If the specimen contains anti-Borrelia IgM antibodies, a coloured line will appear in IgM test line region. If the specimen does not contain anti-Borrelia antibodies, no coloured line will appear in either of the test line regions, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.The test contains anti-human IgM and anti-human IgG as the capture reagent, Borrelia antigen as the detection reagent. A goat anti-human IgG is employed in the control line system.

Materials

Materials provided

- test devices, individually packed TEST
- buffer BUF
- droppers
- manual

Materials Required but not Provided: timer or stop watch, blood specimen collection containers; lancets for fingertip whole blood, pipette and disposable tips (optional); centrifuge for serum or plasma

Storage and Stability

Store the test between 2 °C and 30 °C; **do not freeze**. The test device is sensitive to humidity as well as to heat. Perform the test immediately after removing the test device from the pouch. Do not use it beyond the expiry date.

Precautions

1. For in vitro diagnostic use only.
2. Do not eat or smoke while handling specimen.
3. Wear protective gloves and wash hands thoroughly after performing the test.
4. Avoid splashing or aerosol formation while handling specimen and performing the test.
5. All samples and materials used should be treated as potentially infectious and disposed in a biohazard container. Clean all contaminated objects and surfaces carefully. The used test should be discarded according to local regulations.
6. Do not use test if the pouch is torn or if the membrane of the test device is visibly damaged. Note the expiry date.
7. Read the instruction carefully before performing the test.
8. Do not mix reagents from different lots.
9. If you have any questions please contact Preventis GmbH

Note: The same lancet should only be used for one person and should not be shared with another person, because the used lancet is biohazard.

Sample Collection and Sample Preparation

PreventID® Lyme Borreliosis can be performed using whole blood (from venipuncture or fingertip), serum or plasma.

Fingertip whole blood specimens:

- Wash the patient's hand with soap and warm water and clean with an alcohol swab. Allow to dry.
- Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
- Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.

→ Add the fingertip whole blood sample to the test device by using a **capillary tube:**

- Touch the end of the capillary tube to the blood until filled to approximately 10 µL. Avoid air bubbles.
- Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the sample application window of the test device.

Note: Do not freeze whole blood specimens. Whole blood collected by fingertip should be tested immediately.

Serum and plasma specimens:

- Separate serum or plasma from blood as soon as possible to avoid haemolysis. Only clear, non-haemolysed specimens can be used.

Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 3 days, for long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8 °C if the test is to be run within 2 days of collection.

Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.

If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiological agents.

EDTA K2, heparin sodium, citrate sodium and potassium oxalate can be used as the anticoagulant for collecting the specimen.

Test Procedure

Allow the test, sample, buffer and/or controls to reach room temperature (15–30 °C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface.

For serum or plasma samples:

Use a pipette: Transfer **5 µL of serum/ plasma to the sample application window (S)**, then add **3 drops of buffer** (approximately 120 µL) **to the buffer application window (B)** (*Fig. 1a*).

Use a dropper: Hold the dropper vertically, draw the specimen up to the upper end of the nozzle as shown in *Fig. 1b* (**approximately 5 µL**). Transfer the specimen **to the sample application window (S)**, then add **3 drops of buffer** (approximately 120 µL) **to the buffer application window (B)**, and start the timer.

For whole blood samples:

- Use a pipette: Transfer **10 µL of whole blood to the sample application window (S)**, then add **3 drops of buffer** (approximately 120 µL) **to the buffer application window (B)** (*Fig. 1c*).
- Use a dropper: Hold the dropper vertically, draw the sample about 1 cm above the upper end of the nozzle and transfer **1 full drop (approx. 10 µL)** of specimen **to the sample application window (S)**. Then add **3 drops of buffer** (approx. 120 µL) **to the buffer application window (B)**, and start the timer (*Fig. 1d*).

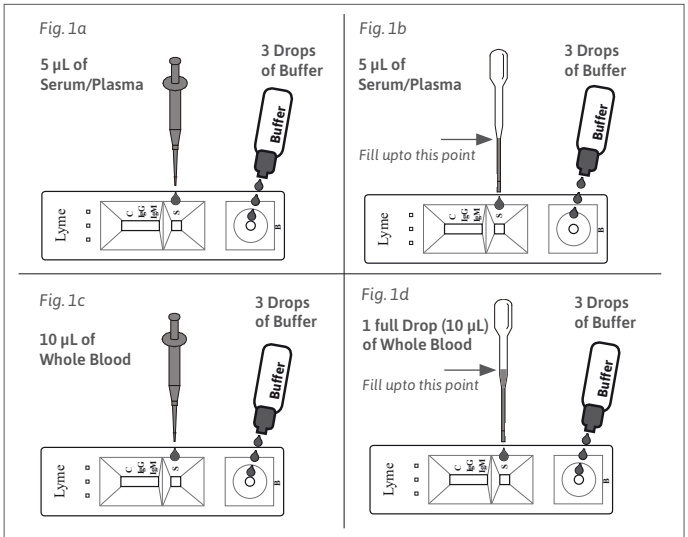


Fig. 1: Sample application

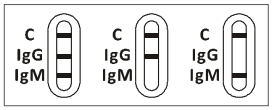
3. Wait for the coloured line(s) to appear. Read results at 10 minutes. Do not interpret the result after 20 minutes.

Note: It is suggested not to use the buffer beyond 3 months after opening the bottle with buffer solution.

Test Interpretation

IgG Positive: Two coloured lines appear. One coloured line should always appear in the control line region (C) and another coloured line should be in the IgG line region.

IgM Positive: Two coloured lines appear. One coloured line should always appear in the control line region (C) and another line should be in the IgM line region.

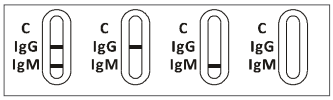


IgG and IgM Positive: Three coloured lines appear. One coloured line should always appear in the control line region (C) and two test lines should be in the IgG line region and IgM line region.

Note: The intensity of the colour in the test line regions may vary depending on the concentration of anti-Lyme antibodies present in the specimen. Therefore, any shade of colour in the test line region should be considered positive.

Negative: One coloured line appears in the control line region (C). No line appears in the IgG line region and IgM line region

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact Preventis.



Note: In order to prevent incorrect results the test should not be interpreted later than after 20 minutes.

Test Characteristics

Expected Values

The **PreventID® Lyme Borreliosis** rapid test has been compared with a leading commercial ELISA Lyme IgG test and Lyme IgM test. The correlation between these two systems is over 98%.

Sensitivity and Specificity

The **PreventID® Lyme Borreliosis** rapid test was compared with a leading commercial ELISA Lyme IgG test and ELISA Lyme IgM test; the results show that **PreventID® Lyme Borreliosis** rapid test has a high sensitivity and specificity.

IgG Results:		ELISA		
PreventID® Lyme Borreliosis	Results	Postive	Negative	**Total Result**
	Positive	21	1	22
	Negative	1	89	90
Total Result		22	90	112

Relative sensitivity: 95.5% (95% CI*: 87.3% – 100%)
Relative specificity: 98.9% (95% CI*: 97.1% – 99.8%)
Accuracy: 98.2% (95%CI*: 93.7% – 99.8%) *Confidence Intervals

IgM Results:		ELISA		
PreventID® Lyme Borreliosis	Results	Postive	Negative	**Total Result**
	Positive	17	1	18
	Negative	1	89	90
Total Result		18	90	108

Relative sensitivity: 94.4% (95% CI*: 72.7% – 99.9%)
Relative specificity: 98.9% (95% CI*: 96.7% – 100%)
Accuracy: 98.1% (95%CI*: 93.5% – 99.8%) *Confidence Intervals

Reproducibility

Intra-Assay:
Within-run precision has been determined by using 3 replicates of five specimens: negative, IgG low positive, IgG high positive, IgM low positive, IgM high positive. The negative, low positive, and high positive values were correctly identified >99% of the time.

Inter-Assay:
Between-run precision has been determined by 3 independent assays on the same specimens: negative, IgG low positive, IgG high positive, IgM low positive, IgM high. Three different lots of the **PreventID® Lyme Borreliosis** rapid test have been tested over a 3-days period using negative, low positive, and high positive specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The **PreventID® Lyme Borreliosis** rapid test has been tested for anti-HAV IgM, HBsAg, anti-HCV IgG, anti-HIV IgG, anti-RF IgG, anti-Syphilis IgG, anti-H. pylori IgG, anti-Rubella IgG, anti-Toxo IgG, anti-HSV 1 IgG, anti-HSV 2 IgG, anti-CMV IgG, anti-Rubella IgM, anti-Toxo IgM, anti-HSV 1 IgM, anti-HSV 2 IgM and anti-CMV IgM positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following compounds have been tested using the **PreventID® Lyme Borreliosis** rapid test and no interference was observed:

Acetaminophen: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL
Ascorbic Acid: 2 g/dL
Creatin: 200 mg/dL
Bilirubin: 1 g/dL
Caffeine: 20 mg/dL
Gentisic Acid: 20 mg/dL
Albumin: 2 g/dL
Hemoglobin 1000 mg/dL
Oxalic Acid: 60 mg/dL

None of the substances at the concentration tested interfered in the assay.

Quality Control

Internal procedural controls are included in the test. A coloured line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

Test Limitations

1. The **PreventID® Lyme Borreliosis** test is for in vitro diagnostic use only. This test should be used for detection of IgG and IgM antibodies to Borrelia in whole blood, serum or plasma specimens. Neither the quantitative value nor the rate of increase in the concentration of IgG or IgM antibodies to Borrelia can be determined by this qualitative test.
2. The **PreventID® Lyme Borreliosis** test will only indicate the presence of IgG and IgM antibodies to Borrelia in the specimen and should not be used as the sole criteria for the diagnosis of Lyme infections.
3. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Borrelia infection.
5. The haematocrit level of the whole blood can affect the test results.
6. Haematocrit level needs to be between 25% and 65% for accurate results.

References:
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5. Johnson RC (1996). „Borrelia". In Baron S; et al. Baron's Medical Microbiology (4th ed.). Univ of Texas Medical Branch. ISBN0-9631172-1-1. PMID21413339. Archived from the original on 7 February 2009.
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US: all products: Research Use Only. Not for use in diagnostic procedures.

Temperature limitation	Manufacturer
IVD *In vitro* diagnostic device	LOT Lot number
REF Catalogue number	Expiry date
→REF To be used with	Do not reuse
Read user instructions	Σ n Contains sufficient for <n> tests
Keep away from sunlight	

CE

Status: 2019-12-04

Distributed by:
Preventis GmbH
Stubenwald-Allee 8a
64625 Bensheim, Germany
Phone: +49 6251 70711-0
Fax:+49 6251 70711-299
info@preventis.com
www.preventis.com

Immundiagnostik AG
Stubenwald-Allee 8a
64625 Bensheim, Germany

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