Borrelia Antibody

IF Test

Indirect Immunofluorescence Test for Detection of Human Antibodies against Borrelia sp.

Cat. No.: PR79079
Kit Size: 100 Tests
Storage: 2-8°C

FOR RESEARCH USE ONLY!

Instruction sheet / Gebrauchsanweisung / Carnet d’instruction / instrucciones de uso / gebruiksaanwijzing / istruzioni per l’uso / σελίδα οδηγιών / bruksanvisning / modo de empleo: www.progen.de

1. Introduction

PROGENs Borrelia Antibody IF Test is an indirect immunofluorescence test for the detection of anti-Borrelia antibodies in human serum. Borrelia is the etiological agent of Lyme Disease. The bacterium is transmitted by ticks (Ixodes sp.) and infects humans as well as a number of domestic and wild animals. The disease appears to be endemic in certain geographical areas and shows seasonal prevalence, mainly in spring and summer.

Like other spirochetal infections, Lyme disease occurs in distinct stages. The initial manifestation after infection is a characteristic rapidly expanding erythematous rash (stage 1) - erythema chronicum migrans which, if diagnosed and treated early, shows a good prognosis. However, if it is not diagnosed and treated immediately, the patient may develop secondary and tertiary stage symptoms, i.e. neurologic and cardiac involvement or chronic arthritis.

Genetic analysis has revealed many different species of Borrelia: The most common Borrelia species in Europe and the USA is Borrelia burgdorferi with the genospecies B. burgdorferi sensu stricto present in Europe and predominant in the USA but absent from Russia and Asia; B. garinii, B. afzelii, the genospecies B. valaisiana, B. lusitaniae and B. spielmanii in Eurasia, and B. japonica restricted to Japan. B. burgdorferi sensu stricto, B. garinii and B. afzelii are commonly designated as B. burgdorferi sensu lato. B. burgdorferi sensu stricto is most often associated with arthritis, particularly in North America. B. garinii is associated with neurological symptoms and B. afzelii with acrodermatitis chronica atrophicans (ACA). Overlap between species in relation to clinical manifestations occurs and all can cause the pathognomonic symptom erythema migrans (EM), though there is evidence in Europe that this early sign occurs more frequently in B. afzelii infections than in those caused by B. garinii.

The distribution of the different genospecies within Europe is gradually emerging. Neurological symptoms seem to be the most common manifestation in Western Europe and B. garinii is most frequently associated with these cases. B. afzelii shows to be more often associated with the clinical symptom of ACA and is relatively common in Central Europe and in Scandinavia. B. burgdorferi sensu stricto does not seem to dominate in any European region and is apparently absent from Russia and Asia.

2. Principle of the Test

PROGENs Borrelia antibody IFT is an indirect immunofluorescence method.

In a first step patient sample is incubated on a multi-well-slide coated with Borrelia garinii (MMS strain) microorganisms. If specific antibodies are present in the sample they bind to the antigen. Unbound antibodies are removed by a washing step. The slide is then incubated with goat anti-human IgG or IgM conjugate. After a washing step, the slide is examined under the microscope. The immune complexes formed in situ appear as bright apple green fluorescence.

3. Materials and Reagents Required but Not Provided

Fluorescence microscope with filter system for FITC and Evans Blue
Glass cover slips (24 x 60 mm)
Automatic pipettes
Moist incubation chamber (e.g. Petri dish with moistened filter paper)
Distilled water
Slide baths (staining cuvettes)

4. Materials and Reagents Provided

GS, 10 individually sealed glass slides (10 wells per slide) with fixed Borrelia garinii (MMS strain). Although fixed, slides should be handled as potentially infectious.
POS*, 1 vial positive human control (lyophilized) contains a preservative. Reconstitute with 0.5 ml distilled water.
NEG* 1 vial negative human control (lyophilized) contains a preservative. Reconstitute with 0.5 ml distilled water.
CON G 1 vial Alexa 488 conjugated goat-anti-human IgG (lyophilized) with Evans Blue counterstain. Reconstitute with 2 ml distilled water.
CON M 1 vial Alexa 488 conjugated goat-anti-human IgM (lyophilized) with Evans Blue counterstain. Reconstitute with 2 ml sterile distilled water.
M 1 vial mounting medium (2 ml) contains a preservative. Store at 2-8°C.
PBS 2 vials with powdered phosphate buffered saline (PBS, 10 g each). Reconstitute with 1 l distilled water each.
* The sera have been tested for HIV I and II, Hepatitis B and C and found negative. However, all human material should be treated as potentially infectious.
Caution: All laboratory equipment should be decontaminated after use and biological waste disposed by autoclaving.

5. Stability

Store the test kit and components at 2-8°C. The unopened reagents are stable until the expiry date indicated.
**Stability after reconstitution at 2-8°C:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
<td>4 weeks</td>
</tr>
<tr>
<td>NEG</td>
<td></td>
</tr>
<tr>
<td>CON G/M</td>
<td>1 week</td>
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<tr>
<td>PBS</td>
<td>(For prolonged storage: add 0.05% (w/v) sodium azide)</td>
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</tbody>
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6. **Dilution of Patient Sera**

1. Add 20 µl patient serum to 140 µl PBS solution to obtain an initial dilution of 1:8.
2. Prepare a 1:16 dilution by adding 0.1 ml of the initial dilution to 0.1 ml PBS solution.
3. To determine the titer, use a 2-fold dilution scheme by mixing 0.1 ml of serum dilution with 0.1 ml PBS solution.
4. It is recommended to prepare 1:16 - 1:256 dilutions. For IgG antibodies dilutions of 1:64, 1:128 und 1:256 are used for testing, IgM antibodies are screened with 1:16, 1:32 and 1:64 dilutions. Sera with positive results should be titered out.
5. Separation of IgG is recommended before testing for IgM (IgG Absorb, PROGEN Cat. No.: PR715).

7. **Test Procedure**

1. Allow all reagents to reach room temperature (20-26°C).
2. Take out *Borrelia* slide and remove it carefully from its bag.
3. Transfer 20 µl of each dilution of patient serum on individual wells of the slide.
   - Place 20 µl of undiluted positive human control of predetermined IgG titer (showing 2+ fluorescence) into one well.
   - 20 µl of negative control is placed into another well. Include both controls each time the test is performed.
4. Place the slides in a moist chamber for 30 min at room temperature.
5. Aspirate serum dilutions and place the slide for 5 min in 2 consecutive PBS solution baths. Drain off excess liquid and air-dry.
6. Cover each well with 20 µl of IgG resp. IgM conjugate.
7. Place the slide in a moist chamber and incubate for 30 min (keep in the dark).
8. Aspirate and wash as described in step 5.
9. Add 3 drops of mounting fluid and cover the preparation with a cover slip.
10. Examine the slide with an appropriate fluorescence microscope (40x or 63x oil objective)

8. **Quality Control**

The negative control should reveal no fluorescence. The positive control should show a bright yellow-green staining pattern on the typically spiral-shaped, single laying and homogenously spread spirochete.

Note: *Borrelia*-specific IgM-antibodies usually show speckled fluorescence all along the spirochete.

9. **Results and Interpretation**

Using the reactive (2+) control well as the reading standard, record the intensity of fluorescence as follows:

- 4+  brilliant yellow-green fluorescence
- 3+  bright yellow-green fluorescence
- 2+  definite but dim fluorescence
- 1+  barely visible staining
- Ø  no fluorescence.

The endpoint titer is the reciprocal of highest serum dilution giving 1+ fluorescence.

10. **Limitations**

Antibiotic therapy, if administered in early stages following infection, may prevent the development of immunological response to *Borrelia*.

To eliminate non-specific reactions from sera, preabsorption with Treponema-ABS absorbent is required.

11. **Literature**

European Union Concerted Action on Lyme Borreliosis
http://vie.dis.strath.ac.uk/vie/LymeEU/

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