Consistency of CLIA method and Immunoblot method

Mgr. Barbora Hamáková, Czech Republic

BORRELIA BURGDORFERI SENSU LATO

Borrelia burgdorferi sensu lato (hereinafter referred to as *Borrelia*) was discovered in 1982. *Borrelia* belongs to the family of *Spirochetes*. *Borrelia* causes a disease called Lyme disease or Lyme borreliosis. This multiorgan disease is transmitted by various types of ticks of genus *Ixodes*.
Borrelia survives and persists in circulation of infected patients even after the creation of a clear immune response.

LYME BORRELIOSIS

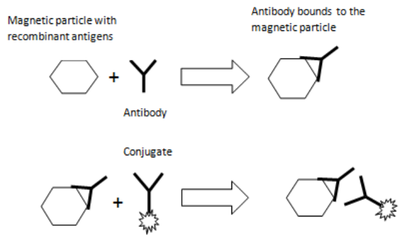
The clinical manifestations of Lyme disease can be divided into three stages. The first stage, early localised infection, is typically characterised by erythema migrans (EM). In the second stage, early disseminated infection, the most frequently diagnosed symptoms are neurological disorders. The third stage, late disseminated infection, lasts for months or years. The most typically diagnosed immunopathological change is Acrodermatitis chronica atrophicans.
The disease can be asymptomatic to the late stages.

LIAISON® KITS

Kits LIAISON® Borrelia IgM II (hereinafter referred to as Borrelia IgM II) and LIAISON® Borrelia IgG II (hereinafter referred to as Borrelia IgG II) contain specific recombinant antigens which originate from *Escherichia coli*. These antigens raise accuracy of assessment of diagnosis of Lyme borreliosis. Solid phase of Borrelia IgM II kit is coated with a specific surface protein OspC and antigen VlsE. OspC is dominant for an early immune response. There is only antigen VlsE in Borrelia IgG II kit. VlsE is a lipoprotein typically associated with an immune response against *Borrelia*.

TEST PRINCIPLE

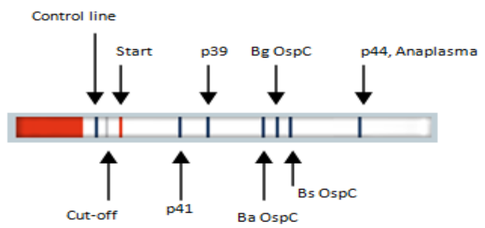
Antibodies are determined by an indirect chemiluminescent immunoassay method (hereinafter referred to as CLIA method). Magnetic particles (solid phase) are coated by recombinant antigens which are specific for *Borrelia*. Mouse monoclonal antibodies are marked by a derivate of isoluminol (conjugate).

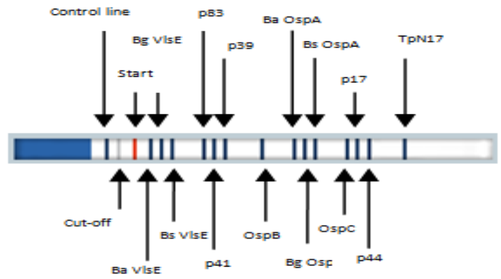


Antibodies against *Borrelia* are bound to the solid phase during the first incubation. In the second incubation, conjugate reacts with antibodies. The reaction mixture is washed after each incubation to remove unbound antibodies.

BLOT-LINE Borrelia / HGA IgM and BLOT-LINE Borrelia / HGA IgG

Immunoblot is considered to be a very precise confirmation test. BLOT kits use a combination of parts of specific antigens of *Borrelia sp*., for example VlsE *Borrelia sp*., OspB, OspC.
The presence of IgM antibodies against *Anaplasma* (p44 antigen) and IgG antibodies against *Anaplasma* (p44 antigen) and





Treponema (TpN17) can be also determined.
BL strips (nitrocellulose membrane) are coated by these recombinant antigens. In case of presence of antibodies against *Borrelia* in tested sera, antibodies bind to the antigens. Subsequently, the bound antibodies interact with conjugate and are detected by colour reaction with the substrate.

EXPERIMENTAL

LIAISON® Borrelia IgM II, LIAISON® Borrelia IgG II (DiaSorin S.p.A.), BLOT-LINE Borrelia / HGA IgM and BLOT-LINE Borrelia / HGA IgG (TestLine Clinical Diagnostics Ltd.).

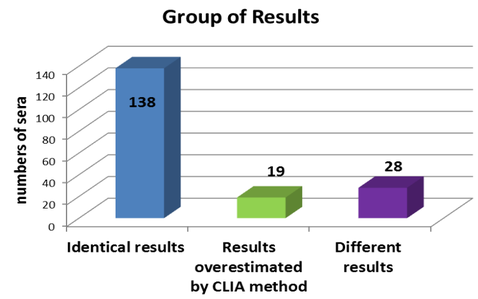
STUDY

Study was carried out from July to October 2014 in the Czech private laboratory MeDiLa spol. s.r.o.
Data were collected from 185 patients. At first, all these sera were tested for the presence of antibodies against *Borrelia*. Presence of antibodies was tested by CLIA method. CLIA method is considered to be a screening method. If IgM or IgG antibodies against *Borrelia* were present in a serum, it was further tested using Immunoblot method.
All repeatedly borderline results obtained by CLIA method or by Immunoblot method were considered as positive.
The obtained data were evaluated and divided into three groups according to the results (see the table and the graph below).
Samples in the first group provided identical results in both tests. This group contained 138 samples. The second group of 19 samples is characterised by more sensitive CLIA detection than Immunoblot detection. The presence of antibodies was detected by CLIA method but not proved by Immunoblot. In the last group of 28 samples, the results obtained by CLIA and Immunoblot were different.
These discrepancies could be caused by nonspecific reactions.

Results	Number of samples	%
Identical results	138	74.59
Results overestimated by CLIA method	19	10.27
Different results	28	15.14

CONCLUSION

CLIA method from DiaSorin SpA is a reliable screening method for detecting the presence of the IgM and the IgG antibodies against *Borrelia*. CLIA method is performed on a fully automated high throughput random access analyser Liaison XL hence the testing for the presence of antibodies against *Borrelia* is not time-consuming.



ACKNOWLEDGEMENT

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Leaflet DiaSorin S.p.A. LIAISON® Borrelia IgM II
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Leaflet TestLine Clinical Diagnostics Ltd. BLOT-LINE Borrelia /HGA IgM and BLOT-LINE Borrelia / HGA IgG

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