Multiplex Bead Based Immunoassays For The Serodiagnosis of Lyme Borreliosis

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Abstract

Laboratory diagnosis of Lyme borreliosis is based on the detection of specific IgG and IgM antibodies against relevant antigens of Borrelia burgdorferi. Serological diagnosis usually is performed by a two-step procedure. Where immunoassays for antibody screening exhibit high sensitivity, second-line tests, e.g. Western-blot or line-blot assays, show high specificity, and are used for confirmation of positive screening results. In addition to serology, in certain cases Borrelia spp. can be detected directly, for instance in synovial fluid from patients with Lyme arthritis by PCR or bacterial culture. With the development of multiplex technology, e.g. bead-based immunoassays, multiple antibodies to distinct bacterial antigens can be detected in a single run. Detection systems used in this context, e.g. analysers based on flow cytometry, can be highly standardised and automated. Furthermore, these analysers can be connected bidirectionally to an order-entry based laboratory information system, with random access for high throughput analysis. Thus, multiplex bead assays may have the power to replace the current two-step procedure.

Keywords: Neuroborreliosis; Cerebrospinal fluid; Erythema chronicum migrans; Immunoblot; Lineblot

Introduction

In Europe, there are two common infectious diseases transmitted by ticks. Infection by FSME-virus leads to meningoencephalitis, while Lyme borreliosis is caused by the spirochetal bacterium, Borrelia burgdorferi. Other tick-borne diseases, e.g. relapsing fever caused by other Borrelia species (B. hermsii, B. duttoni, etc.), spotted fever (Rickettsia rickettsii, R. coroni, etc.), Ehrlichiosis (Anaplasma phagocytophilum, formerly named Ehrlichia chaffeensis), or Babesiosis (B. microti, B. divergens), are rare [1]. In the USA, almost only infection with B. burgdorferi sensu stricto results in development of Lyme borreliosis, while in Europe, borreliosis is commonly caused by infection with B. afzelii and B. garinii. Infections with B. spielmanii and B. bavariensis are rarely found in Europe, and in contrast to the USA, also in infections with B. burgdorferi sensu stricto. B. burgdorferi sensu stricto is mainly associated with arthritis (USA and Europe), where erythema migrans is most frequently caused by B. afzelii, and B. garinii most frequently causes neurological disorders [2].

Manifestations of Lyme disease are often classified into three stages, according to corresponding incubation periods from infection to clinically overt manifestation (Table 1). There is no strict discrimination of the stages and late manifestations may occur without early disease symptoms. Consequently, the updated European case definition does not imply this classification [2]. The main early manifestation is erythema chronicum migrans. This characteristic skin disease occurs 3 to 30 days after tick-bite. Benign lymphocytoma may occur within a few weeks after tick-bite. Some weeks to months after tick-bite, there is a hematogenous dissemination of the pathogens. Although various organs can become infected, neuronal symptoms are the prominent feature of systemic infection, for instance, meningitis, radiculitis, paralysis of cerebral nerves, especially facial palsy or rarely carditis. Late stage disease occurs months to years after primary infection. Especially in USA, arthritis (Lyme arthritis) is a common symptom of late stage borreliosis, but also encephalomyelitis and acrodermatitis chronica atrophicans may occur. B. burgdorferi infections can effectively be treated with antibiotics, but a few patients may have some long term sequelae, especially if the diagnosis is delayed. Currently, no Borrelia vaccine for human use is available [3].

Laboratory Diagnosis

Serological laboratory diagnosis of Lyme disease is usually done in a two-step procedure, beginning with a screening assay to detect IgG and IgM anti-borrelia antibodies. Screening assays should be highly sensitive to avoid missing Borrelia-infected patients. However, high sensitivity usually results in low specificity of laboratory test systems, especially for IgM antibody assays. Moreover, unspecific reactions are common in almost all assays for detection of IgM antibodies. Therefore, it is essential to confirm reactivity observed in screening assays by highly specific second-line test systems.

In screening assays to detect anti-Borrelia antibodies, whole cell lysates, a mixture of purified native or recombinant antigens, or
a combination of these are used. These antigen preparations have to fulfill sophisticated demands. On the one hand, antigens should cover a broad diversity of relevant antigens of the different pathogenic *Borrelia* species. On the other hand, antibodies targeting non-pathogenic *Borrelia* species or other bacteria, e.g. *Treponema pallidum*, should not bind to the antigen preparations. Second-line confirmatory tests often are Western-blot or line-blot. Initially, crude native homogenates of whole bacteria were used in screening and second-line tests. More recently, purified native or recombinant single antigens have been used for construction of test assays. However, the two-assay principle is currently being challenged, since recombinant proteins and peptides combine high specificity, while not loosing sensitivity [4,5].

Multiplex immunoassays are appropriate tools to analyse complex antibody response patterns. In parallel to the development of DNA-CHIPS, antigen-CHIPS have also been designed. Benefits result from the possibility to examine a large number of antibody reactions, while using a minimum amount of the patient’s serum. This is especially relevant for paediatricians, but also for veterinarians. A major drawback of classic CHIP-formats was the difficulty to standardize quantitative CHIP assays, and thus, generate reproducible results on glass slides containing spotted antigen preparations. In contrast, it is easier to standardize other multiplex formats generating quantitative results, for instance bead assays. However, very expensive analysers such as flow cytometers are necessary to evaluate antibody reactions. Fortunately, bench-top analysers like the Luminex flow cytometer and well standardized commercially available test kits have been developed, allowing the use of multiplex assays for routine diagnostics in the medical laboratory [6].

### Multiplex assays based on bead technology

The commercially available bead assays are distributed in Germany by MIKROGEN, Neuried (recomBead and Multimetrix *Borrelia* IgG/IgM); PROGEN, Heidelberg, (Multimetrix *Borrelia* IgG/IgM Test) and Virion/Serion, Würzburg (Multianalyt *Borrelia* IgG/IgM). All of these companies distribute second-line tests and Multimetrix, also a screening test for IgM and IgG. Antigens used in this screening assay for IgM antibody detection is a lysate from *B. afzelii*, recombinant OspC from *B. afzelii*, and recombinant OspC from *B. garinii*, while for the IgG test, only lysates from *B. garinii* and *B. afzelii* are used. Two second-line tests use *Borrelia* lysate, while one assay (recomBead) omits lysate [6,7]. Table 2 shows some of the antigens used in the bead assays in comparison to some blot tests. For some antigens, for instance p83/100, p39, OspC, p41 (flagellin) internal fragment, Osp17 and p58 improvement of test performance has been documented in these multi-antigen tests. For other antigens, the selection criteria and the specificity of individual antigens in control populations often are not well documented [5,7,8].

#### Borrelia specific antigens used in immunoassays

In Europe, primarily three species of the *Borrelia* genogroup, namely *B. afzelii*, *B. garinii* and *B. burgdorferi* sensu stricto, but also *B. spielmanii* and *B. bavariensis* are pathogenic for humans. These species exhibit proteins which are strongly conserved with high homology, but also proteins showing inter-species variability. To cover a maximum of relevant immune reactions, careful selection of antigens is essential. Ideally, antigens showing high homology between various *Borrelia* species should be used.

Consequently, usage of those antigens will allow detection of antibodies even when species causing infection differ from that providing antigens for test assay. A further aspect to consider in antigen selection is the fact that antibody production to certain antigens changes during the course of infection. Therefore, in addition to the presence of IgG and/or IgM antibodies, the reactivity against such antigens can help to differentiate between early and late infection. The determination of antibody avidity would be an additional way to assess the temporal course of *Borrelia* infection; however, to date no commercial assays are available to analyse antibody avidity of anti-*borreliosis* antibodies.

Some antigens of other bacteria-for instance flagella proteins of spirochetes and *Enterobacteriaceae*-show structural similarities to the corresponding *Borrelia* antigens. Therefore, usage of these epitopes could decrease specificity of test assays due to binding of cross-reacting antibodies. According to European recommendations [5], a clear positive reaction of at least two specific antigens in an immune blot is considered sufficient to interpret the assay result as "positive".

In addition to the differentiation of immune reactions against early and late antigens, and the presence of IgG and/or IgM antibodies, an increasing number of positive reactions is expected during an ongoing infection. While OspC and VlsE are the most sensitive antigens for an IgM or IgG response, respectively, most second-line assays, including the multiplex bead assays, use at least five and sometimes more than 10 different specific antigens [9-11].

In table 3, some relevant *Borrelia* antigens are listed. As already discussed, the use of recombinant or purified native antigens is preferable to whole cell lysates or classical Western-Blot assays, using electrophoretically separated antigens of whole cell lysates. Therefore, line-Blots and multiplex assays with recombinant antigens are now most commonly used in routine diagnosis. Another important improvement in the diagnosis concerning sensitivity and specificity of the immuno assays was the introduction of *in vivo*-induced antigens which are not expressed in cultured bacteria, but only in the infected host, and which are therefore, missing in whole cell lysates. The first such antigen to be introduced and still the most prominent is VlsE (vmp-variable major protein-like sequence expressed), which is now complemented with Dbp(A) (Decorin binding protein A) in some routine assays [10,12,13].

#### Interpretation of serological results

Guidelines from microbiological societies are available to

| Antigen         | VlsE | p100 | p83 | p68 | p43 | p41 | p39 | p30 | OspA | OspC (p22) | p21 | p19 | Osp 17 Dbp(A) | p14 | lysate |
|-----------------|------|------|-----|-----|-----|-----|-----|-----|------|------------|-----|-----|---------------|-----|--------|
| Line-Blot       | EUROLINE IgG | x    | x   | x   | x   | x   | x   | x   | x   | x          | x   | x   | x             | x   | x      |
|                 | EUROLINE IgM | x    |     |     |     |     |     |     |     |            |     |     |               |     |         |
| Viramed IgG     | x    | x    | x   | x   | x   | x   | x   | x   | x   |            | x   | x   |               | x   |         |
| Viramed IgM     | x    |     |     |     |     |     |     |     |     |            |     |     |               | x   |         |
| Bead            | recomBead IgG / IgM | x    | x   | x   | x   | x   | x   | x   | x   |            |     |     |               | x   |         |
| Multianalyt IgG/IgM | x    | x   |     |     |     |     |     |     |     |            | x   | x   |               |     |         |
| Multimetrix IgG | x    |     |     |     |     |     |     |     |     |            |     |     |               | x   |         |
| Multimetrix IgM | x    |     |     |     |     |     |     |     |     |            |     |     |               |     |         |

Table 2: Antigens used by different manufacturers in Bead- and Line-Blot-Assays (selection).
In up to 90% of neuroborreliosis patients, intrathecal IgG analysis of antibody titres in serum and cerebrospinal fluid should be performed to assess intrathecal production of antibodies targeting Borrelia infection. There is no recommendation for antibiotic therapy, if serological response early in infection (acute invasion) will be negative, following a tick bite, when the patient lacks symptoms of borreliosis. The diagnosis of infection probably consists of unspecific immunological reactions. Early local disease: In this stage of the disease, serological examination usually is not necessary if a typical erythema chronicum migrans is present. Erythema occurring within the first two days after infection probably consists of unspecific immunological reactions. However, in case of dubious symptoms, serological analysis is indicated. With up-to-date immunoassays, IgM antibodies may be detected in 50-70% of neuroborreliosis cases.

Table 3: Antibody response to specific antigens used in immunoassays.

| Molecular weight in kD | Antigen | Meaning for diagnosis | Antibody reactions |
|------------------------|---------|-----------------------|--------------------|
| p100/p83               | (p83 is degradation product of p100) | ***                 | Typically positive in late stages, but early antibody reaction possible |
| p88                    |         |                       | Often in stage 3    |
| p43/p45                |         |                       | Mostly in stage 3   |
| p41                    | Flagellin |                       | Very early reactive, cross reactions with some antigen preparations |
| p39                    | BmpA (Borrelia membrane protein A) | ***                 | Rather specific for late IgG-response; sometimes also early |
| p31                    | OspaA (Outer surface protein A) | ++                  | Late response |
| p30                    |         |                       | Not clearly defined |
| p22/p25                | OspC    | ***                   | Very early seen; mostly the first specific reaction of the early IgM response; species specific, especially the IgG response |
| p21                    |         | ++                    | Not clearly defined |
| p19                    | OspE    |                       | Not clearly defined |
| p17/p18                | Dib (A) Decorin binding protein A | ++                  | Surface protein, species specific response |
| p14                    | Internal protein |         | Mostly in early infection |
| VlsE                   | VlsE=Vmp (variable major protein) like sequence expressed, surface lipoprotein | ++++                | IgG in early and late infection, intra thecal response in neuroborreliosis |

facilitate interpretation of Western- or line-bLOTS results. However, recommendations are not available for interpretation of bead assay results [5]. Comparing these guidelines to the recommendations of the manufacturers of bead assays, it appears that manufacturers have adopted some recommendations for interpretation of test results generated when using bead assays. As the bead assays give quantitative results, the antibody titer is incorporated into the interpretation guidelines for some assays. Furthermore, the diagnostic relevance of certain antigens is used for classification. For example, according to the Progen protocol, one clear positive or two weak reactions should be interpreted as "Lyme borreliosis not reliably excluded." If more than one antigen shows a strong positive reaction, the overall interpretation is "strong suspicion of Lyme borreliosis." Another way to interpret results as "negative," "borderline" or "positive," respectively, is suggested for other assays.

Typical serological constellations are shown in table 4. The diagnosis of different disease stages according to serological results is only possible if the anamnestic and clinical situations are known. Even in this case, many results are not conclusive. Sometimes, the follow up of clinical signs but also serological results, can help to obtain a final diagnosis [1,3].

Tick bite: There is no indication to perform serological diagnostics following a tick bite, when the patient lacks symptoms of borreliosis. The serological response early in infection (acute invasion) will be negative, because formation of IgM antibodies usually starts only two weeks after Borrelia infection. There is no recommendation for antibiotic therapy, if seroconversion is noticed by chance when symptoms are lacking. More than 95% of infections are clinically inapparent [14].

Early local disease: In this stage of the disease, serological examination usually is not necessary if a typical erythema chronicum migrans is present. Erythema occurring within the first two days after infection probably consists of unspecific immunological reactions. However, in case of dubious symptoms, serological analysis is indicated. With up-to-date immunoassays, IgM antibodies may be detected in 50-90% and IgG in 10-50% of patients.

Early disseminated disease: Manifestations of early disseminated Borrelia infections are neuroborreliosis, accompanied by meningitis and/or neural paralysis, e.g. facial palsy. In these cases, serological tests are very useful, and if there is clinical suspicion of neuroborreliosis, analysis of antibody titres in serum and cerebrospinal fluid should be performed to assess intrathecal production of antibodies targeting Borrelia. In up to 90% of neuroborreliosis patients, intrathecal IgG production is present, and intrathecal IgM production is seen in 15-70% of neuroborreliosis cases.

Neuroborreliosis is proven if characteristic symptoms are present, and also pleocytosis in cerebrospinal fluid combined with intrathecal production of anti-Borrelia antibodies [15].

Late disease manifestations: Patients suffering from late stage diseases are always positive for IgG antibodies. Characteristically in the confirmatory assay, antibodies to many antigens are present. An active late stage disease with negative serology is an absolute rarity, or even an incorrect diagnosis. A positive IgM-result with negative IgG does not support the diagnosis of a stage 2 or 3 disease, but is rather a coincidence of early infection, a persistent IgM-Titer, or an unspecified IgM-reaction, together with an other disease mimicking borreliosis [1,3].

Critical points to consider in the interpretation serological test results

A negative serological result does not exclude an early infection. In unclear clinical situations, a follow up should be done in 3-6 weeks. If early antibiotic therapy has been initiated, antibody production may be blocked, e.g. no antibodies are induced at all or the switch of IgM to IgG antibody production does not occur, resulting in the persistence of IgM and lack of IgG antibodies for months, or even for years. Moreover, in some patients, no IgM is induced, even if later on in the course of infection specific IgG antibodies can be found. IgG usually persists over years or lifelong also in inapparent infections, and sometimes even with high titers with many positive bands in an immunoblot. Unspecific neurological symptoms, for instance, fatigue and depression, but also arthritis often coincide with IgG antibody finding, especially in endemic regions, or in persons with high risk for tick bites, e.g. forest workers. Unspecific antibody binding, especially in screening assays, might occur as a consequence of infections caused by other microorganisms, e.g. EBV, Treponema pallidium, but also as a consequence of autoimmune diseases [5,16].

Discussion

The basis of laboratory diagnosis of Lyme borreliosis is the detection of specific IgG and IgM antibodies against relevant antigens of pathogenic B. burgdorferi strains. Serological diagnosis is based on a two-step approach, starting with a screening assay (e.g. ELISA), and if reactive, a second-line test. Usually this confirmation assay is a test simultaneously detecting various antibodies to specific bacterial antigens, e.g. a Western- or line-blot. This two assay test strategy is based...
The diagnosis of borreliosis has the power to replace the classical two-step approach, and as a consequence, also assay formats like ELISA and Western- or line-blot. However, detailed studies with well-characterized patients suffering from different disease states to confirm sensitivity and specificity of the multiplex assays, are still necessary.

References
1. Hu LT (2012) In the clinic. Lyme disease. Ann Intern Med 157: 2-16.
2. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, et al. (2011) Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 17: 69-79.
3. Stanek G, Wormser GP, Gray J, Strie F (2012) Lyme borreliosis. Lancet 379: 461-473.
4. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjorseth DJ, et al. (2004) Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect 10: 1108-1132.
5. Wilks B, Fingerle V, Schulte-Spechtel U (2007) Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49: 13-21.
6. Gerritsen A, Brandt S (2012) Serodiagnosis of Lyme borreliosis with bead based immunoassays using multiplex technology. Methods 56: 477-483.
7. Porwancher RH, Hagergy CG, Fan J, Landsberg L, Johnson BJ, et al. (2011) Multiplex immunoassay for Lyme disease using VlsE-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. Clin Vaccine Immunol 18: 851-859.
8. Wilks B, Habermann C, Fingerle V, Hillenbrand B, Jauris-Heipke S, et al. (1999) An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. Med Microbiol Immunol 18: 139-144.
9. Ang CW, Notermans DW, Hommes M, Simonis-Smit AM, Herremans T (2011) Large differences between test strategies for the detection of anti-Borreli antibodies are revealed by comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 30: 1027-1032.
10. Goettner G, Schulte-Spechtel U, Hillemer R, Liegl G, Wilks B, et al. (2005) Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DcppA homologues. J Clin Microbiol 43: 3602-3609.
11. Hauser U, Lehntner L, Lobentanzer R, Wilks B (1997) Interpretation criteria for standardized Western blots for three European species of Borrelia burgdorferi sensu lato. J Clin Microbiol 35: 1433-1444.
12. Schulte-Spechtel U, Lehntner L, Liegl G, Fingerle V, Heimerl C, et al. (2003) Significant improvement of the recombinant Borrelia-specific immunoglobulin G immunoblot test by addition of VlsE and a DcppA homologue derived from Borrelia garinii for diagnosis of early neuroborreliosis. J Clin Microbiol 41: 1299-1303.
13. Coleman AS, Rossmann E, Yang X, Song H, Laminichane CM, et al. (2011) BBK07 immunodominant peptides as serodiagnostic markers of Lyme disease. Clin Vaccine Immunol 18: 406-413.
14. Fryland L, Wilhelmsson P, Lindgren PE, Nyman D, Ekberg E, et al. (2011) Low risk of developing Borrelia burgdorferi infection in the south-east of Sweden after being bitten by a Borrelia burgdorferi-infected tick. Int J Infect Dis 15: e174-e181.
15. Myland A, Ljostad U, Fingerle V, Ruppecht T, Schmutzhard E, et al. (2010) EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol 17: 8-16.
16. Hunfeld KP, Kraiczy P (2009) When is the best time to order a Western blot and how should it be interpreted? Curr Probl Dermatol 37: 167-177.
17. Schmidt C, Plate A, Angele B, Pfister HW, Wick M, et al. (2011) A prospective study on the role of CXCL13 in Lyme neuroborreliosis. Neurology 76: 1051-1058.

| IgM        | IgG        | Interpretation                                                                 |
|------------|------------|---------------------------------------------------------------------------------|
| Screening  | Confirmation| Screening  | Confirmation| No late Borreliosis. (CAVE: early infection with negative serology, depending on the clinical situation a control in 10-14 days should be recommended) |
| neg        | neg        | neg        | neg         | Unspecific reactions; the interpretation of the serological result has to be “negative” (CAVE: very early infection with positive screening test but negative confirmation test; depending on the clinical situation a control in 10-14 days should be recommended) |
| pos        | neg        | pos        | neg         | Early infection (CAVE persisting IgM from a former infection without IgG-production) |
| pos        | pos        | pos        | pos         | Early infection with IgG already positive (CAVE: former infection with persistent IgM) |
| neg        | neg        | pos        | pos         | Active infection stage 2 or 3 or persistent IgG after spontaneous healing or successful treatment |

Table 4: Interpretation of typical serological antibody constellations.

The possibility to obtain quantitative results when using multiplex assays alleviates calculations of antibody index, and therefore, improves determination of intrathecal antibody production.[6,15]

Immunoblots and bead based assays are quite expensive. This may be a reason why in the US it is discussed to use, for example, a VlsE based ELISA alone. The modern ELISA assays are already quite effective. However, studies are necessary to evaluate the performance of these ELISAs, in comparison to bead based assays and the utility of multiple antigens. The use of more antigens may even decrease specificity, and thus, diagnostic accuracy highly depends on good scoring algorithms. In conclusion, the introduction of multiplex assays for routine serologic diagnosis of borreliosis has the power to replace the classical two-step approach, and as a consequence, also assay formats like ELISA and Western- or line-blot. However, detailed studies with well-characterized patients suffering from different disease states to confirm sensitivity and specificity of the multiplex assays, are still necessary.

References
1. Hu LT (2012) In the clinic. Lyme disease. Ann Intern Med 157: 2-16.
2. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, et al. (2011) Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 17: 69-79.
3. Stanek G, Wormser GP, Gray J, Strie F (2012) Lyme borreliosis. Lancet 379: 461-473.
4. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjorseth D, et al. (2004) Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect 10: 1108-1132.
5. Wilks B, Fingerle V, Schulte-Spechtel U (2007) Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49: 13-21.
6. Gerritsen A, Brandt S (2012) Serodiagnosis of Lyme borreliosis with bead based immunoassays using multiplex technology. Methods 56: 477-483.
7. Porwancher RH, Hagergy CG, Fan J, Landsberg L, Johnson BJ, et al. (2011) Multiplex immunoassay for Lyme disease using VlsE-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. Clin Vaccine Immunol 18: 851-859.
8. Wilks B, Habermann C, Fingerle V, Hillenbrand B, Jauris-Heipke S, et al. (1999) An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. Med Microbiol Immunol 18: 139-144.
9. Ang CW, Notermans DW, Hommes M, Simonis-Smit AM, Herremans T (2011) Large differences between test strategies for the detection of anti-Borreli antibodies are revealed by comparing eight ELISAs and five immunoblots. Eur J Clin Microbiol Infect Dis 30: 1027-1032.
10. Goettner G, Schulte-Spechtel U, Hillemer R, Liegl G, Wilks B, et al. (2005) Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DcppA homologues. J Clin Microbiol 43: 3602-3609.
11. Hauser U, Lehntner L, Lobentanzer R, Wilks B (1997) Interpretation criteria for standardized Western blots for three European species of Borrelia burgdorferi sensu lato. J Clin Microbiol 35: 1433-1444.
12. Schulte-Spechtel U, Lehntner L, Liegl G, Fingerle V, Heimerl C, et al. (2003) Significant improvement of the recombinant Borrelia-specific immunoglobulin G immunoblot test by addition of VlsE and a DcppA homologue derived from Borrelia garinii for diagnosis of early neuroborreliosis. J Clin Microbiol 41: 1299-1303.
13. Coleman AS, Rossmann E, Yang X, Song H, Laminichane CM, et al. (2011) BBK07 immunodominant peptides as serodiagnostic markers of Lyme disease. Clin Vaccine Immunol 18: 406-413.
14. Fryland L, Wilhelmsson P, Lindgren PE, Nyman D, Ekberg E, et al. (2011) Low risk of developing Borrelia burgdorferi infection in the south-east of Sweden after being bitten by a Borrelia burgdorferi-infected tick. Int J Infect Dis 15: e174-e181.
15. Myland A, Ljostad U, Fingerle V, Ruppecht T, Schmutzhard E, et al. (2010) EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. Eur J Neurol 17: 8-16.
16. Hunfeld KP, Kraiczy P (2009) When is the best time to order a Western blot and how should it be interpreted? Curr Probl Dermatol 37: 167-177.
17. Schmidt C, Plate A, Angele B, Pfister HW, Wick M, et al. (2011) A prospective study on the role of CXCL13 in Lyme neuroborreliosis. Neurology 76: 1051-1058.