Novel Multiplex Test for Lyme Disease

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Abstract

Lyme Disease (LD) is caused by spirochetal bacteria from the genus Borrelia. Borrelia burgdorferi is the predominant cause of Lyme Disease in the United States, whereas Borrelia afzelii and Borrelia garinii are predominantly implicated in most European incidences. The CDC reported 28,921 confirmed US cases of LD in 2008, presenting a 5% increase of confirmed cases in comparison to 2007. It is estimated that 2.5 million serological LD tests are performed annually. Recommendations by the CDC for the diagnosis of LD include a two-tiered approach: an initial ELISA-based screening test in conjunction with western blotting to establish the presence of anti-Borrelia antibodies. Currently available LD tests are not only time consuming, but also display a high probability of false negative and/or positive results. To address this problem, we cloned, expressed and purified 14 Borrelia-specific antigens for use in the development of a highly sensitive house-ovin-spike-down (POD) multiplex diagnostic device capable of detecting anti-Borrelia-specific IgM and IgG antibodies in human serum. These 14 antigens, expressed during different stages of the disease, are used to probe LD positive and negative human sera and to raise antigen-specific antibodies. Here we present the preliminary data in our efforts to develop a POC testing device for LD.

Introduction

The current methodology applied in the diagnosis of LD primarily relies on serological applications for the detection of anti-Borrelia antibodies. It is estimated that over 2.5 million LD serology tests are performed annually in the United States, in which a two-tiered approach by ELISA and western blotting confirms the presence of anti-Borrelia antibodies using a protein cell lysate of an in vitro cultured B. burgdorferi strain. The process is costly and time consuming in its entirety and is subjective in the visual and evaluation interpretation of individual test strips. Furthermore, an additional drawback of the current method is that protein cell lysates used for western blotting contain numerous highly conserved housekeeping proteins that can result in false positive results when detected by antibodies against other bacterial infections. In addition, as B. burgdorferi undergoes rapid adaptive gene expression in response to environmental signals encountered during the different stages of its life cycle in either the arthropod vector or the mammalian host, some proteins are only expressed in the host and not in cell cultures.

To improve upon and simplify the current LD diagnostic approach, we are developing a serological POC assay that may yield results within 10 to 15 minutes. The following are our specific aims:

1. Clone, express and purify 14 B. burgdorferi antigens known to be expressed at different stages throughout the infection.
2. Establish the reactivity of human sera from LD patients to define positive and negative assay criteria.
3. Establish lateral flow test development and independent evaluation thereof using defined clinical samples.

Cloning of B. burgdorferi genes into E. coli expression vectors

The following fourteen (14) proteins were selected as individual components in the flow test device panel: OspA, OspB, OspC, OspE, VlsE, Flagellin, CRASP-1, CRASP-2, DbpA, DbpB, Arp37, surface protein p27, OspB, OspC, and OspE. (Table 1) Gene-specific primers with 15bp extensions homologous to vector were used to amplify DNA sequences from the European B. burgdorferi sensu lato PBoe strain (GenBank Accession # CAH61549). Gene-specific primers with 15bp extensions homologous to vector are cloned into the corresponding linearized vector. Upon releasing the MBP-OspA protein from the MBP-TEV protease, the MBP-OspA protein is released with metal affinity chromatography prior to separation of the respective recombinant LD fusion protein mixture by either size exclusion or ion chromatography (Figure 3D).

Future Developments:

1. The evaluation of a greater number of well characterized LD positive and negative human sera in the preliminary format, as shown in Figure 4.
2. The selection of ten (10) LD indicative markers, including among others the VlsE and DbpA proteins.
3. The possible expression and characterization of additional Borrelia proteins.
4. The arrangement of recombinant bacterial antigens on a nitrocellulose membrane. LD positive human sera is capable of binding Borrelia-specific proteins, whereas LD negative human sera fails to bind 11 of the 14 antigens. This degree of specificity is sufficient for the further development of a POC lateral flow strip test as proposed.

Proof-of-Concept Studies

To determine the functionality of a strip-based diagnostic test for the detection of circulating LD-specific antibodies, 14 recombinant LD proteins were imprinted onto a nitrocellulose membrane using a slot blotter (immunoblot). In addition, secondary antibody-specific controls (IgG and IgA) as well as MBP were absorbed onto the membrane prior to blocking. Confirmed positive and negative LD human sera were used to bind recombinant proteins followed by the addition of peroxidase (HRP) conjugated anti-human IgG (Fcg) or anti-human IgM Fdc secondary antibodies (Figure 4).

References


References


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