iSpot Lyme™: A New Generation of Lyme Disease Testing

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\section*{ABSTRACT}

Lyme disease is the most reported vectorborne illness in the United States. The incidence may be vastly under-reported due to limitations of current testing methodologies; the need for more sensitive and specific tests is apparent. The iSpot Lyme™ assay is a new generation of \textit{in vitro} diagnostic test for the detection of antigen-specific effector/memory T cells that respond to stimulation by \textit{Borrelia burgdorferi} antigens. The highly sensitive T cell-based enzyme-linked immunospot (ELISpot) methodology enumerates the \textit{Borrelia burgdorferi} activated effector/memory T cells. This assay is intended for clinical use as a laboratory aid in the diagnosis of \textit{B. burgdorferi} infection. The iSpot Lyme™ test represents a breakthrough in diagnostic accuracy with improved sensitivity and specificity over current Lyme disease testing methodologies, and can increase the speed of diagnosis and treatment.

\section*{Introduction}

Lyme disease is the most prevalent tick-borne disease in the United States. The Centers for Disease Control and Prevention (CDC) reported nearly 32,500 new cases\textsuperscript{1} in 2011, though it is estimated that the actual number could be up to 10-fold higher\textsuperscript{2}, making Lyme disease an epidemic larger than AIDS, West Nile Virus, and Avian Flu combined\textsuperscript{3}. Unfortunately, only a fraction of these cases are being treated due to unclear clinical manifestations, inaccurate tests and underreporting\textsuperscript{2}. Patients not receiving adequate treatment may develop chronic infection or late-stage Lyme diseases such as chronic Lyme arthritis or chronic Lyme neuroborreliosis, which can be devastating in some cases\textsuperscript{4}.

Lyme disease is caused by \textit{B. burgdorferi}, a bacterium of the spirochete class. Lyme disease is a zoonotic, vector-borne disease transmitted by the \textit{Ixodes} (blacklegged) tick. Symptoms generally include a subset of the following symptoms:

- Observed tick bite
- Erythema Migrans (Bullseye rash)
- Flu-like symptoms
- Lymphadenopathy
- Joint pain
- Neurological symptoms
- Heart palpitations
- Severe fatigue

The current CDC recommended evaluation for diagnosis is a two-tier test including ELISA and Western Blot (WB) analyses. These tests are serological assays which detect antibodies to \textit{B. burgdorferi}. The low sensitivity of the two-tier tests (about 30% in early Lyme disease and 50% in Late Lyme disease) and the significant seronegativity of Lyme patients (as many as 30% to 50% of cases) suggests that more sensitive T cell-based laboratory tests should also be developed\textsuperscript{5}. The ELISPOT assay is an effective method for assessment of the magnitude and the quality of T cell immunity by measuring stimulated antigen-specific T cells\textsuperscript{6,7}.

A thorough evaluation for Lyme requires testing for both a humoral and a cellular immune response (Figure 1). This is done by measuring both antibodies (humoral/WB) and T cell activity (cell-mediated/ELISpot).

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Inflammatory immune response: Individuals who have been infected with \textit{B. burgdorferi} harbor \textit{B. burgdorferi}-specific immune cells (T cells) in their bloodstream. Typically, these T cells can be detected before an antibody response.}
\end{figure}

\section*{Clinical Relevance}

The ELISpot method utilized in iSpot Lyme™ is the most sensitive technique available for detecting immune cells that secrete signature proteins (such as a given cytokine). It is the only technology that accurately detects, measures, and performs functional analysis of low-frequency immune cells. The sensitivity of ELISpot is much higher than that of ELISA and flow...
The advantage of the ELISPOT test is its ability to detect a cellular immune response against Lyme antigens, which appears much earlier in disease than the antibody response detected by the traditional Western Blot test\textsuperscript{11}. More importantly, Lyme ELISPOT can detect antigen-specific T cell responses in seronegative patients\textsuperscript{12}. Therefore, the Lyme ELISPOT test can be used to provide information regarding the current immune status of a Lyme disease patient.

**iSpot Lyme™ Test**

The iSpot Lyme™ test detects *B. burgdorferi*-specific T cell responses in patients who have been exposed to a *B. burgdorferi* spirochete. Individuals who have been infected harbor *B. burgdorferi*-specific immune cells (T cells) in their bloodstream. Typically, these T cells can be detected before an antibody response. The T cell response to Lyme infection is detectable about 2 weeks after the tick bite, and lasts approximately 2-3 months after the acute phase of infection. *B. burgdorferi*-specific memory T cells develop and may last for years.

This test measures frequency of antigen-specific T-cells by identifying T cells that are specific for Lyme antigens. This is indicative of exposure to Lyme. A single result is reported that is obtained by measuring interferon gamma (IFN-γ) secreted by T cells in response to stimulation by the *B. burgdorferi* antigens DbpA, OspC, p100, and VlsE-1.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Description</th>
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<tr>
<td>DbpA</td>
<td>Early stage antigen: Contributes to spirochete dissemination within the host</td>
</tr>
<tr>
<td>OspC</td>
<td>Early stage antigen: Appears shortly after tick bite and transfer of the spirochete</td>
</tr>
<tr>
<td>VlsE-1</td>
<td>Marker of acute Lyme Disease: Upregulated in the host particularly during immune response</td>
</tr>
<tr>
<td>p100</td>
<td>Late stage antigen</td>
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*iSpot Lyme™* is a highly reliable and accurate test with a sensitivity of 84% and specificity of 94%. Researchers at Pharmasan Labs conducted a thorough literature research and experiment design, setting up and optimizing the ELISPOT assay, fully validating it following the guidelines of the Clinical Laboratory Improvement Amendments (CLIA) program, conducting a study with approximately 500 samples from diagnosed Lyme patients as well as healthy controls, and completing data analysis using the standard method Receiver Operating characteristic (ROC) curve for assessing diagnostic tests.

**Methodology**

The immune response to infection with *B. burgdorferi* includes both B cell and T cell activation\textsuperscript{13,14}. T cells are sensitized to *B. burgdorferi* antigens and activated effecter T cells produce the cytokine IFN-γ upon stimulation. The iSpot Lyme™ test uses the ELISPOT technique to count *B. burgdorferi*-sensitized T cells by capturing IFN-γ secreted by these T cells. More specifically, when IFN-γ is released a “spot” of insoluble precipitate is formed at the site of the reaction (Figure 2). Evaluating the number of spot forming units (SFUs) provides a measurement of *B. burgdorferi* sensitive effector/memory T cells in the peripheral blood. The SFU count correlates to a patient’s T cell reaction to *B. burgdorferi* (see Figure 2).

**Figure 2:** When peripheral blood mononuclear cells (PBMCs) from a *B. burgdorferi*-infected patient are exposed to *B. burgdorferi* protein antigens (A), *B. burgdorferi*-specific T cells are activated and secrete small proteins called cytokines (B). T cells that are not specific for *B. burgdorferi* do not become activated. **iSpot Lyme™** measures the cytokine IFN-γ secreted by the patient’s T cells. Cytokine proteins (IFN-γ) are captured near the cells that secreted them, and are then detected using a color reagent (C).

Sources of inflammation other than Lyme disease may also cause IFN-γ spots to form. This is taken into account and compensated for by size-gating. Size-gating is a way to screen out other sources of inflammation that appear as smaller spots. These are considered to be non-specific indicators of inflammation, and are considered as background interference that is not counted as part of the Lyme-specific SFU determination. The results of iSpot Lyme™ are analyzed using the CTL S6 Ultimate-V Analyzer/BioSpot 5.0 Software (Cellular Technology Limited, OH).

**Interpretation**

Diagnosing or excluding Lyme disease requires a combination of epidemiological, historical, medical and diagnostic findings when interpreting the iSpot Lyme™ test. The iSpot Lyme™ assay should be used and interpreted only within the context of the entire clinical presentation.
A “Positive” result indicates that the sample contains effector or memory T cells reactive to *B. burgdorferi*. With the traditional western blot testing alone the potential for false positives is as high as approximately 30%\(^{13}\), especially if a co-infection is present. Due to the high specificity of the iSpot method, there is very little chance for false positive results even with concurrent co-infections. An important consideration is that a positive result does not distinguish currently active infection from previous infection. A “Negative” result indicates that the sample likely does not contain effector T cells reactive to *B. burgdorferi*. It is also possible that a previously infected patient that has undergone successful treatment could eventually test negative due to low numbers of circulating memory T cells (<0.001%\(^{14}\)). Patients with recent exposure to Lyme disease (such as tick bite, skin lesion) exhibiting a negative iSpot Lyme™ test should be considered for retesting within 6 weeks or if other relevant clinical symptoms indicate possible infection.

**Conclusion**

Lyme disease is an increasingly common condition that can have debilitating effects if not diagnosed and treated appropriately. A comprehensive approach to diagnosis will lead to the most positive outcomes and healthcare savings; however, testing options thus far have the potential for false negative results, making diagnosis difficult. NeuroScience’s testing options (iSpot Lyme™ including Western Blot analysis) can aid in the diagnosis of *B. burgdorferi* infection and allow for early detection leading to earlier treatment. iSpot Lyme™ is a highly sensitive T cell-based enzyme-linked immunospot (ELISPOT) method which enumerates the *B. burgdorferi* -specific activated effector/memory T cells. This test represents a breakthrough in diagnostic accuracy, and can increase the speed of diagnosis and treatment leading to improved clinical outcomes.