The most sensitive method for the detection of Lyme disease and other tick-borne diseases (Babesia, Ehrlichia, Bartonella)

Immunosciences Lab., Inc. (ISL) now offers new assays for assessing Lyme disease (LD) that cover the measurements of antibodies to antigens of *Borrelia* grown in culture (the traditional method), as well as antibodies against antigens expressed *in vivo* during the invasion of the human immune system.

Prompt diagnosis and treatment of LD is the key to avoiding chronic Lyme borreliosis and its serious effect on the human system. Diagnosis can be difficult because symptoms of LD share commonalities with ALS, Alzheimer's, autism, chronic fatigue, fibromyalgia, lupus, Parkinson's and RA. Therefore, it is crucial to combine clinical symptomatology with the most sensitive technique available to diagnose Lyme disease.

**Multi-Peptide ELISA for Lyme**

The antigenic diversity of *Borrelia burgdorferi* in the host suggests that antigenic variation plays an important role in immune invasion. This antigenic variation is detected by a very new technique called *in vivo* induced antigen technology. This technique identifies pathogen antigens that are immunogenic and expressed *in vivo* during human infection.

Based on this novel technique, ISL uses particular peptides from various components of *Borrelia* during different cycles, including peptides from outer surface proteins A, C and E, leukocyte function associated (LFA) antigens, immunodominant antigens, variable major proteins, and peptides from decorin-binding proteins of *Borrelial* subspecies (*B. sensu stricto, B. afzelii, B. garinii*).

In addition to the measurement of antibodies against antigens expressed *in vivo* and *in vitro*, ISL's Lyme Profiles also assess antibodies against antigens of *Borrelia* subspecies and its co-infections, whose clinical manifestations may be similar to Lyme, but whose treatment differs from that of Lyme disease.

In structuring the Multi-Peptide ELISA assay, ISL tested 103 different specimens from patients with Lyme disease symptoms from two different clinics, one on the East Coast and the other on the West Coast.

Altogether, with this population of patients, sensitivity of Western Blot was 44.6%, while with Multi-Peptide ELISA sensitivity was 71%. Since *Borrelia*, Babesia, Ehrlichia and Bartonella are transmitted by the same tick species, serum samples were tested for the simultaneous elevation in IgG and IgM antibodies against multiple organisms. Between 35-64% of specimens, in addition to *Borrelia* antibodies, were also positive for Babesia, Ehrlichia or Bartonella.

The Multi-Peptide ELISA methodology has been tested on over 2,000 clinical specimens; while correlation between Western Blot and Multi-Peptide ELISA is good, the Multi-Peptide ELISA assay has greater advantages over the Western Blot method. These advantages are summarized on the back of this booklet.
# Immunoserology of Lyme Disease by Multi-Peptide ELISA

## Lyme-Specific Antibodies
- *B. burgdorferi* Antigens (IgG, IgM)
- OspA + OspC Peptides (IgG, IgM)
- OspE Peptide (IgG, IgM)
- Leukocyte Function Associated Antigen (IgG, IgM)
- Immunodominant Protein (IgG, IgM)
- Variable Major Protein (IgG, IgM)

## Borrelia Subspecies Antibodies
- *B. b. sensu stricto* (IgG, IgM)
- *B. garinii* (IgG, IgM)
- *B. afzelii* (IgG, IgM)

## Lyme Co-Infection
- Babesia (IgG, IgM)
- Ehrlichia (IgG, IgM)
- Bartonella (IgG, IgM)

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**Specimen requirement:**
2 mL Serum
Breaking the blood brain barrier by *Borrelia* activation of the fibrinolytic system, which allows invasion of the CNS, resulting in neuroborreliosis

After a tick bite, in addition to *Borrelia burgdorferi*, tick salivary proteins enter the host and exert an immunosuppressive effect (1). On one hand, tick salivary protein-15 (Salp-15) binds to the outer surface protein-C (OspC) of *B. burgdorferi*, protecting it from immune attack (2). On the other hand, Salp-15 binds to CD4 on helper cells and inhibits TCR ligation-induced-T-cell signaling and immunosuppression (3). This way the spirochete can move freely in the circulation and possibly in different tissues (4). Eventually, immune response to *Borrelia* and Salp-15 occurs, resulting in antibody as well as proinflammatory cytokine production (5). Simultaneously, a few spirochetes may make contact with the endothelial cells of the blood brain barrier, stimulating expression of plasminogen activators, plasminogen activator receptors and matrix metalloproteinase, all of which contribute to activation of the fibrinolytic system (6). This in turn results in focal and transient degradation of tight junction proteins, allowing *B. burgdorferi*, CD4+ and Th17 cells to invade the CNS (7). This invasion of the CNS may result in the destruction of neuronal cells, the release of neural cell antigens (8), and the production of antibodies against MBP, MOG, α-B-crystallin and other neural cell antigens (9).
IMMUNOSEROLOGY OF LYME DISEASE BY MPE & WESTERN BLOT

**Lyme-Specific Antibodies**
- *B. burgdorferi* Antigens (IgG, IgM)
- OspA + OspC Peptides (IgG, IgM)
- OspE Peptide (IgG, IgM)
- Leukocyte Function Associated Antigen (IgG, IgM)
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**Lyme Co-Infection**
- Babesia (IgG, IgM)
- Ehrlichia (IgG, IgM)
- Bartonella (IgG, IgM)

**WESTERN BLOT ASSAY**
- *B. burgdorferi* (IgG and IgM)

Specimen requirement: 2 mL Serum

FOR MORE INFORMATION PLEASE CONTACT:
IMMUNOSCIENCES LAB., INC.
822 S. ROBERTSON BLVD., STE. 312, LOS ANGELES, CA 90035
TEL: (310) 657-1077 • FAX: (310) 657-1053
E-MAIL: IMMUNSCI@IX.NETCOM.COM • WWW.IMMUNOSCIENCESLAB.COM

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CAM enhancement of NK cell activity, phagocytosis, and reduction in bacterial load by:  
- Antibiotics  
- Antioxidants  
- Mushroom B-Glucan  
- Astragalus  
- Other Immune Response Modifiers

CAM inhibition of pro-inflammatory cytokine production by:  
- Samento  
- Cumada  
- Curcumin  
- Boswellia  
- Capsaicin  
- Quercetin  
- Probiotic  
- EPA/DHA  
- Bee Propolis  
- Cat’s Claw and others.

CAM inhibition of fibrinolytic system by:  
- Hydrolytic Enzymes  
- Capsaicin  
- Vitamin E  
- COQ-10  
- EPA/DHA

CAM inhibition of tight junction degradation by:  
- Doxycycline  
- Minocycline  
- α-lipoic Acid  
- Vitamin D  
- Ginkgo Biloba  
- DHEA, Glutathione  
- N-acetylcysteine

CAM inhibition of tick binding to the skin by Lemon Eucalyptus Extract

CAM intervention resulting in brain tissue or cell remodeling

CAM inhibition of adhesion molecules and prevention of lymphocyte entry into the brain tissue by  
- Doxycycline  
- Minocycline  
- α-lipoic Acid  
- Vitamin D  
- Ginkgo Biloba

Using CAM treatment could prevent different processes ranging from the attachment of the tick to the skin, to inflammatory events, CNS invasion, and the induction of neuroborreliosis. CAM can act through the enhancement of natural killer cell activity, macrophage function, inhibition of pro-inflammatory cytokine production, inactivation of the fibrinolytic system and repair of blood brain barriers.
### Comparisons between Western Blot and Multi-Peptide ELISA

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<thead>
<tr>
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<th>Western Blot</th>
<th>Multi-Peptide ELISA</th>
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<tbody>
<tr>
<td>Detects antibodies against borrelial antigens prepared in culture</td>
<td>✓</td>
<td>✓</td>
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<td>Selects antigenic expressions of <em>Borrelia</em> during human infection</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Antigen used represents different life cycles of spirochete in infected tissue</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Detects antibodies against OspA and LFA to identify treatment-resistant arthritis</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>May provide predictive model for development of autoimmune inflammatory disease induced by <em>Borrelia</em></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Detects antibodies against <em>Borrelia</em> subspecies: <em>B. b. sensu stricto</em>, <em>B. afzelii</em>, <em>B. garinii</em></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Can detect antibodies against Babesia</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Can detect antibodies against Ehrlichia</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Can detect antibodies against Bartonella</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Method is sensitive</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Method is quantitative</td>
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<td>✓</td>
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<tr>
<td>Method is subjective</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Results can be used for follow-up treatment</td>
<td>✓</td>
<td>✓</td>
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Based on sera samples from the Centers for Disease Control and Prevention (CDC), 200 specimens from patients with Lyme disease, and more than 200 samples from healthy controls, the sensitivity of the Multi-Peptide ELISA assay was found to be more than 80%, with a specificity greater than 95%.