

The IGeneX Western Blot: Better by Design

The IGeneX Western Blot was designed to be effective in all stages of Lyme Disease, not just early Lyme. The current Western Blot, in most clinical laboratories was designed for early Lyme Disease. The publications referring to this generic blot studied patients with Lyme disease within the first few months after a tick bite and an EM rash.

IGeneX designed the blots differently, based upon the principles discussed in the publication by Ma et al. J Clin Microbiol., 1992, 30:3 70-79. (see graph below). Pedigreed samples were obtained from Yale, University of Connecticut (UCON), and a Hospital in Old Lyme, CT. These samples came from patients with a physician diagnosed EM rash and symptoms of active Lyme Disease. The difference from the CDC studies was that these patients were not just early Lyme Disease (<5 months), but also mid-Lyme (>5 months to 1 year) and late Lyme disease (>1 year).

In addition, instead of using just one strain of bacteria, two strains (B 31 and 297) are used to make IGeneX Western Blot strips. This allowed IGeneX to have all the Lyme antigens more or less equally represented.

IGeneX also uses a 12.5% polyacrylamide gel to get better separation of the important 31 kDa to 39 kDa antigens. Dressler et al. JID, 1993, 167:392-400 only used a 10% gel and had poor separation of the 31 to 39 kDa antigens. That publication was a cornerstone of the CDC surveillance criteria published in MMWR 1995, 44:590-591.

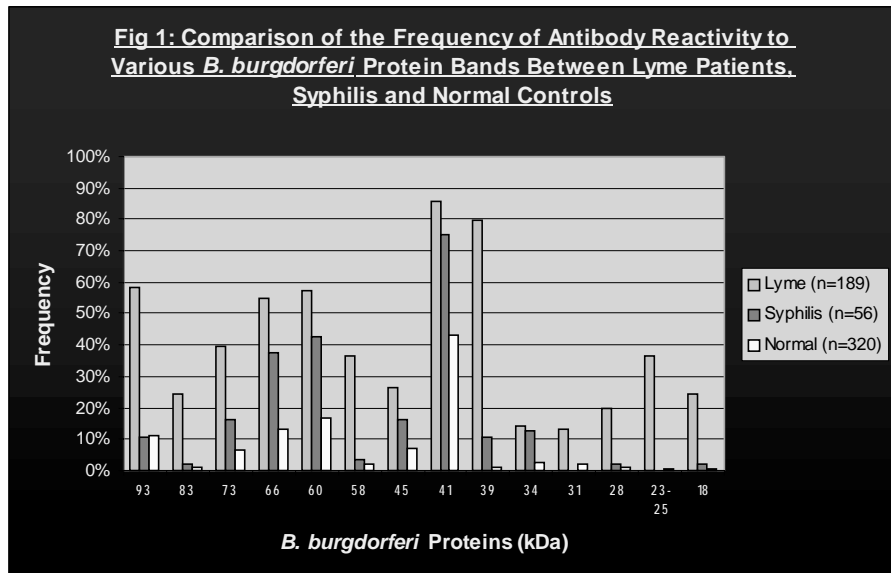
In summary the IGeneX Western Blot for Lyme Disease has:

- High sensitivity in all phases of disease.
- Ability to detect Lyme antibodies in all parts of the US.
- Ability to detect late Lyme due to the presence of 31kDa and 34 kDa antigens.
- Ability to detect immunization by European strains of Borrelia due to high uniform antigen concentration.

The IGX Western Blots can accomplish this due to the relatively equivalent amount of each unique antigen used in our strips. This is done by harvesting each cell line at an optimum phase.

The IGX Western Blots have been evaluated and have reactivity against various *B. burgdorferi* strains including: European strains *B. afzelli* and *B. gainii*; the Japanese strain of *B. japonica*; and the sub-strains of *B. burgdorferi* from Colorado, Missouri and Texas.

The high amount of antigen to OspA-31kDa and 34kDa gives the IGX Western Blots the ability to detect patients with persistent/recurrent (chronic) symptoms of Lyme Disease.



IGeneX PCR Tests

IGeneX currently offers PCR tests for:

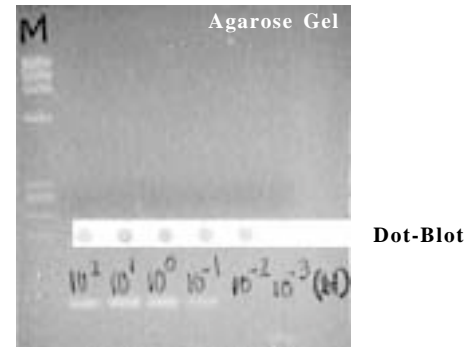
- *Borrelia burgdorferi*
- *Babesia microti*
- *Babesia WA-1*
- Ehrlichia (Monocytic and Granulocytic)
- Bartonella

We added a confirmation test by Southern Dot Blot for *B. burgdorferi* and *Babesia microti* and WA-1 in April 2003 and for Ehrlichia species confirmation, Human Granulocytic Ehrlichia (HGE) and Human Monocytic Ehrlichia (HME) in September 2003. We are currently working on developing PCR tests with a confirmation test by Southern Dot Blot for different species of Bartonella and Rickettsia.

IGeneX PCR tests have higher sensitivity and specificity than most standard PCR tests for the following reasons:

1. PCR targets of interest from clinical samples are concentrated and highly purified by using a proprietary hybrid select technology. This technology not only concentrates and purifies the target, but at the same time also removes more than 99% of the PCR inhibitors. Briefly, the DNA target of interest, from the clinical sample is hybridized to an oligomer probe labeled with a biotin. The complex is captured on to a streptavidin conjugated magnetic bead. The complex bound to the bead is washed several times to remove debris and other nucleic acids, proteins, PCR inhibitors, etc., present in the sample. The DNA target is disassociated from the complex and amplified with target specific primers.
2. The purified concentrated DNA is PCR amplified using specific primers. Note: Two PCRs are performed for Lyme on each sample to increase sensitivity. Following the PCR, the PCR products of the Multiplex PCR, Babesia PCR, HGE PCR and HME PCR are tested by Southern Dot Blot assay using highly specific probes. A positive signal appears if the clinical sample had the right PCR product.

Lyme Multiplex PCR - Limit of Detection Agarose Gel Analysis vs. Dot-Blot Assay



M = DNA Size Marker
B. burgdorferi DNA – 10^2 to 10^{-3} copies/ μ l
 Limit of Detection: Gel Analysis = 10^1 copies
 N = Negative PCR Control
 Dot-Blot Analysis = 10^2 copies

As shown in the example above, for Lyme, not only does the Southern Dot-Blot assay confirm the PCR product but also increases the sensitivity of the assay by one log.

Clinical Samples

Over the last 2 ½ months we have tested over a 1000 clinical samples by Lyme Multiplex PCR. The rate of positive samples by gel analysis was about 3.9% (39 samples). By Southern Dot Blot, the rate increased by 10% to 43 samples. This was expected since the limit of detection by Dot Blot is one log higher than by agarose gel analysis.

IMPORTANT UPDATE

In order for IGeneX to keep current with CLIA regulations, we now require the following for your patient's test requests:

- 1. Physician must sign or stamp Test Request Form with the tests indicated**

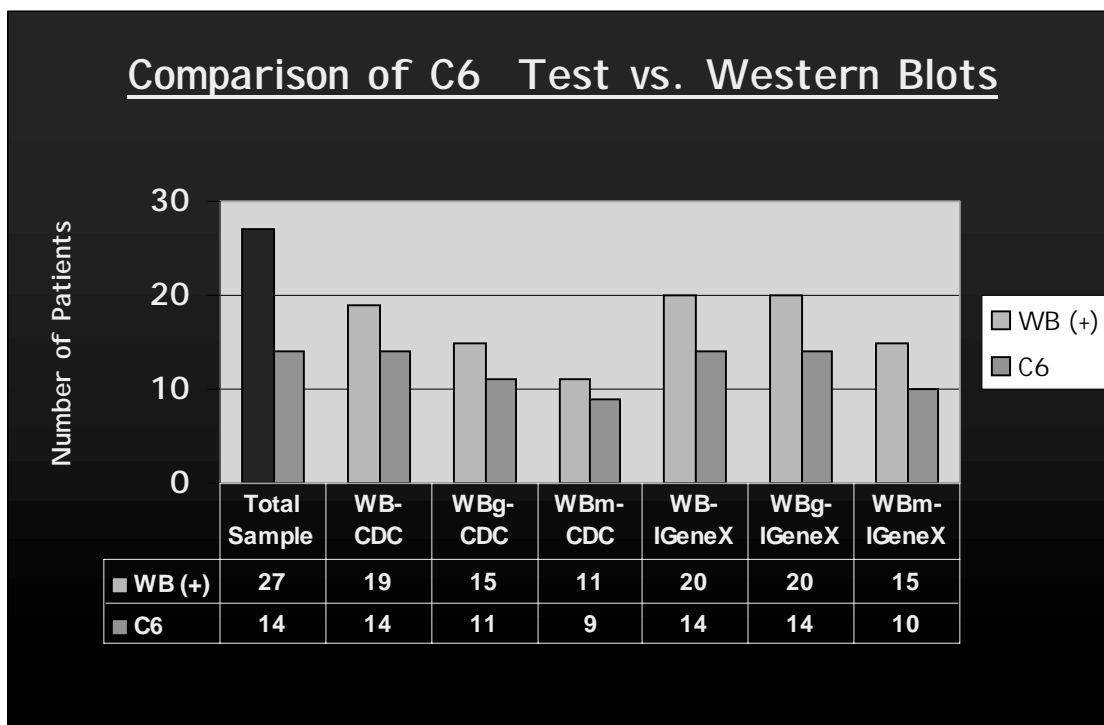
OR

- 2. A copy of the physicians prescription for the testing must accompany the Test Request Form.**

C6 Peptide Assay

C6 is a synthetic peptide (C6 Peptide) derived from the VISE protein which appears in early and late Lyme Disease. The assay looks for the presence of antibodies against this synthetic peptide. While not as sensitive as the IGeneX Western Blots, it has no demonstrated cross reactivity in patients who may have received the LymeRix® vaccine.

Starting October 2003, IGeneX is offering the FDA approved Immunitics C6 Assay. The C6 B. burgdorferi (Lyme) ELISA Kit assay is intended for use in the presumptive detection of IgG and IgM antibodies to B. burgdorferi in human serum. The assay is approved for use on samples from patients with clinical history, signs or symptoms consistent with B. burgdorferi infection, including individuals who have received the licensed recombinant OspA Lyme disease vaccine (LymeRix®).



A set of 27 patient sera suspected of having Lyme Disease were tested by the C6 assay and Lyme Western blot IgG and IgM assays. The Lyme IgG and IgM Western Blots, were scored positive or negative based on the IGeneX criteria (WB—IGeneX) and by the CDC criteria. 14 (52%) of the 27 patient sera were positive by the C6 assay. All 14 were positive by the Western Blot assay, by both IGeneX Criteria (WB—IGeneX) and CDC criteria (WB—CDC). In addition, 5 more were positive by the IGeneX Western Blot criteria. Of these, 4 were also positive by the CDC criteria.

As shown in the graph above, based on the study performed at IGeneX, the assay sensitivity of the C6 assay was between 70 and 74%, as compared to Western Blots. Thus, we continue to recommend that **all patients'** sera be tested by a Western Blot method. Positive Western Blot results provide evidence for exposure to or infection with B. burgdorferi. The diagnosis of Lyme disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to B. burgdorferi. Negative results should not be used to exclude Lyme disease.

Upcoming Events

1. LDA, Lyme Disease Association, 4th Medical Conference on Lyme and Other Tick-borne Diseases

Friday, November 14, 2003 8 am – 6 pm

Hyatt Hotel at Penns Landing
201 S. Christopher Columbus Blvd.
Philadelphia, Pennsylvania 19106
Tel: (215) 928-1234

For physicians and other health care providers. The public is also invited to register. Columbia is the co-sponsor providing CMEs for physicians. For more information, please contact the LDA at: 888-366-6611 or e-mail Lymeliter@aol.com

2. ILADS, International Lyme and Associated Diseases Society, 6th International Conference on Lyme and Associated Diseases

Friday, November 14
Saturday, November 15
Sunday, November 16

Board of Directors Dinner Meeting
Scientific Meeting 8 AM – 6 PM
Scientific Meeting 8 AM – 12 Noon

The conference is open to members of ILADS and to non-member health care professionals:

Location:

Hyatt Hotel at Penns Landing
201 S. Christopher Columbus Blvd.
Philadelphia, Pennsylvania 19106
Tel: (215) 928-1234

For more information, contact the INTERNATIONAL LYME AND ASSOCIATED DISEASE SOCIETY (ILADS) at (301) 263-1080 or e-mail: lymedocs@aol.com

ATTENTION PHYSICIANS: IGeneX continually receives requests from patients' insurance companies for diagnosis codes. In order to avoid calls from IGX to your office, please indicate the DX code on the requisition form or on the prescription. Please contact the office at (800) 832-3200 if you have any questions concerning this request. Thank you.



IGeneX Inc
797 San Antonio Rd
Palo Alto, CA 94303

www.igenex.com