ILADS' Position Paper on the CDC's Statement Regarding Lyme Diagnosis

International Lyme & Associated Diseases Society
www.ilads.org

The Center for Disease Control's (CDC) position on diagnosing Lyme disease (LD) is an oversimplification of a complicated clinical condition.[1] The CDC's two-tiered approach—using an ELISA and confirming positives by both IgM and IgG Western blots—potentially misses more than 40% of the patients. One year after the tick bite, this percentage may be greater than 50%.

The two-tiered protocol was developed from studies using Lyme patients presenting with the Erythema Migrans (EM) rash and arthritis or neuro-borreliosis. However, not all Lyme patients have these symptoms. In one of the NIH-sponsored studies, blood was taken from Lyme-suspected patients every two weeks for a period of four months, and any positive event (defined by the presence of 5 of the 10 bands by IgG Western blot) qualified the patient.[2] In contrast, other NIH-sponsored research indicated that many defined Lyme patients did not meet the CDC Western blot criteria (the presence of 5 of the 10 bands), and that the IgM response was a useful predictor of infection at all stages of disease.[3, 4]

Lyme disease is a problematic diagnosis. The position adopted by the CDC makes it more complicated. Many patients do not elicit an antibody response great enough to be positive by currently available ELISA assays. In fact, studies conducted by the group responsible for Lyme Disease proficiency testing for the College of American Pathologists (CAP) concluded that the currently available ELISA assays for Lyme Disease do not have adequate sensitivity to be part of the two-tiered approach of the CDC/ASPHLD, where only ELISA-positive samples can be tested by Western blotting.[5]

Furthermore, if patients are treated early with antibiotics, their antibody response may be reduced or curtailed.[6] In addition, initial mild flu-like symptoms may be overlooked. Later, if the symptoms return, most of the antibody markers may have disappeared. Aguero-Rosenfeld *et al.* showed that only 70% of the documented Lyme patients in their study had a significant antibody response.[3, 4] They suggested that the degree of antibody response might be related to the length of time the EM rash persists. They also reported only a 64% rate of IgM to IgG seroconversion.

The reason that most ELISA assays are inadequate as screening tests is that they were not designed by the manufacturers to be sensitive at the 95% confidence level, the level typically required for screening.[5] In fact, Luger and Krause found up to a 56% false-negative rate (depending upon the commercial kit), when compared to their clinical diagnoses.[7] Golightly *et al.* observed a lack of sensitivity (over a 70% false-negative rate) with commercial kits in early Lyme disease and from 4 to 46% with late manifestations of Lyme disease.[8] Thus, independent of the ELISA results, using both IgM and IgG Western blots may improve laboratory detection of LD.

The immunoblot or Western blot is the most useful antibody test for *B. burgdorferi*, when performed in a quality laboratory by experienced testing personnel. It is necessary to evaluate both IgM and IgG antibodies to *B. burgdorferi*. Studies by Ma, *et al.* and others point out the large degree of antibody variability in patients with clinically confirmed Lyme disease, including patients with physician-diagnosed EMs.[9] Variability in the Western blot reflects the variability observed in the immune response of other diseases, including Hashimoto's thyroiditis, SLE, Sjögren's syndrome, and scleroderma.

Some studies show that it is common to miss patients if only the CDC serological criteria are used.[3, 4] Indeed, the CDC/ASPHLD criteria for a positive *B. burgdorferi* Western blot are very conservative.[1] Five of ten antibody bands are required for IgG positivity. This cut-off is based on the assumption that all Lyme patients, even those without arthritis and neuroborreliosis, have similar immune systems and responses.

The diversity of the immune response seen in other diseases is also disregarded. The CDC's studies were problematic in that they primarily focused on patients with early Lyme disease (usually within four months of an EM). They also collected blood in most patients every few weeks during this four-month period and counted any positive event (five out of ten bands) as LD, even if the same patient had a negative test at a different time of the study.[2]

Engstrom *et al.*[2] and Aguero-Rosenfeld *et al.*[3, 4] confirmed that almost one-third of all Lyme patients are IgG negative during the first year. Two years after a physician-diagnosed EM, 45% of the patients were negative by ELISA. In another study, Aguero-Rosenfeld *et al.* showed that the ELISA response declined much more rapidly than the Western blot response.[4]

Their study also demonstrated that the two-step protocol of the CDC/ASPHLD criteria would fail to confirm infection in some patients with culture-proven EM. Furthermore, although a majority (89%) of patients with the EM rash developed IgG antibodies by Western blot sometime during disease, only 22% were positive by the criteria of the CDC/ASPHLD.[4] The Engstrom *et al.* study did not use the IgG blot criteria of the CDC/ASPHLD. [2] They found that 2 of 5 bands gave them a specificity of 93 to 96% and a sensitivity of 100% in the 70% of patients that produced antibody. This could imply an even lower sensitivity would be obtained had the more stringent CDC/ASPHLD criteria been used as a guideline for laboratory screening.

The CDC/ASPHLD criteria for a positive IgM Western blot include the 23-25 kDa (OspC), the 39 kDa and the 41 kDa, but overlook the 31 kDa (OspA) and the 34 kDa (OspB).[1, 10] Yet the CDC reported a specificity of 95% for the IgM Western blot, based on several hundred negative controls. Engstrom *et al.* reported specificities of their IgM Western blot to be between 92 and 94%.[2] Some studies have suggested that the IFA and ELISA IgM assays may cross-react with ANA, EBV and other spirochetal infections,[11] while other studies did not observe this with either IFA or Western blot.[9,12]

A major disagreement with the CDC/ASPHLD group arises from its statement that the IgM Western blot should be used only during the first month after tick bite. They have seemingly overlooked their own reported excellent specificity of the IgM Western blot. Studies by IGeneX,[13] Steere's group,[14] and Jain *et al.*[15] emphasized the importance of the IgM Western blot in recurrent and/or persistent disease. Sivak *et al.* found that the IgM Western blot had a specificity of 96% if the patients surveyed had at least a 50% probability of having Lyme disease.[16]

It is important to note that a positive Western blot, to IgG and/or IgM antibodies, merely implies exposure to *B. burgdorferi*. The Western blot is only part of the test battery and is not, by itself, confirmatory for Lyme disease. One cannot conclude from Western blot results that a patient has Lyme disease, because that requires a clinical diagnosis. It must also be kept in mind that these antibody tests are not static but in fact change over time. Thus, a patient negative by the Western blot may seroconvert to a positive blot with treatment. Conversely, a patient positive for IgG response may develop another IgM response, suggestive of a recurrent infection.

A considerable body of literature demonstrates that some seronegative Lyme patients are positive for either the Lyme bacteria DNA or pieces of the unique *Borrelia* outer surface antigens.

Studies by Goodman *et al.* found that 30% of their patients with early Lyme disease were positive by PCR.[17] This percentage is comparable to blood culture data by others.[18] However, some studies could not obtain positive cultures or positive PCR from patients with acute Lyme disease.[19] Both of these methods are technique-dependent. Manak *et al.* were able to detect 33% of early Lyme and 50% of late stage Lyme disease in patients not on antibiotic therapy.[20] Most of their patients became PCR negative within two weeks of antibiotic therapy. They also found that during a relapse, patients might become PCR positive for a short period of time. On the other hand, using a combination of genomic and plasmid PCR, Bayer *et al.* found that 74% of patients with chronic (persistent) Lyme disease were PCR positive in urine samples.[21]

Persistent/recurrent (chronic) infection is a unique diagnostic problem because the IgG response may be absent in more than 50% of the patients.[2,3,4] Thus in addition to the IgG Western blot, an IgM Western blot should be used. Assays that focus on antigen detection or DNA may be particularly useful diagnostically during persistent/recurrent disease.[22] *B. burgdorferi* antigens in urine have been detected in animal models with Lyme disease.[23,24,25] Similarly, *B. burgdorferi* antigen in urine has been seen in humans and appears to be a useful diagnostic tool.[23,24,22]

Data extrapolated from vaccine studies and CDC lectures suggest that the number of patients with Lyme Disease may be ten-fold higher than what is being currently reported. In spite of this, the CDC seems to be more concerned with diagnostic criteria that prevent false positives, with little concern for false negatives. A system with better balance in regard to this issue is urgently needed for accurate statistics concerning the magnitude of the number of patients with Lyme disease.

References

1. Association of State and Territorial Public Health Laboratory Directors (ASTPHLD). "Proceedings of the second national conference on the serological diagnosis of Lyme disease." 27-29 October, 1994, Dearborn, MI. Washington DC: ASTPHLD, 1995.

2. Engstrom, SM, Shoop, E, and RC Johnson. "Immunoblot interpretation criteria for serodiagnosis of early Lyme disease." *Journal of Clinical Microbiology* 33 (1995): 419-427.

3. Aguero-Rosenfeld, ME, Nowakowski, J, McKenna, DF, Carbonaro, CA, and GP Wormser. "Serodiagnosis in early Lyme disease." *Journal of Clinical Microbiology* 31 (1993): 3090-3095.

4. Aguero-Rosenfeld, ME, Nowakowski, J, McKenna, DF, Carbonaro, CA, and GP Wormser. "Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans." *Journal of Clinical Microbiology* 34 (1996): 1-9.

5. Bakken, LL, Callister, SM, Wand, PJ, and RF Schell. "Interlaboratory comparison of test results for detection of Lyme disease by 516 participants in the Wisconsin State laboratory of hygiene/College of American Pathologists proficiency testing program." *Journal of Clinical Microbiology* 35 (1997): 537-543.

6. Steere, AC, Grodzidki, RL, Kornblatt, AN, *et al.* "The spirochetal etiology of Lyme disease." *New England Journal of Medicine* 308 (1983): 733-740.

7. Luger, SW, and E Krauss. "Serologic tests for Lyme disease: interlaboratory variability." *Archives of Internal Medicine* 15 (1990): 761-763.

8. Golightly, MG, Thomas, JA, and AL Viciana. "The laboratory diagnosis of Lyme Borreliosis." *Laboratory Medicine* 21 (1990): 299-304.

9. Ma, B, Christen, B, Leung, D, and C Vigo-Pelfry. "Serodiagnosis of Lyme Borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*." *Journal of Clinical Microbiology* 30 (1992): 370-376.

10. CDC. "Recommendations for test performance and interpretation from the second national conference on serologic diagnosis of Lyme disease." *MMWR* 44 (1995): 590-591.

11. Magnarelli, LA, Anderson, JF, and RC Johnson. "Cross-reactivity in serological tests for Lyme disease and other spirochetal infections." *Journal of Infectious Disease* 56 (1987): 183-188.

12. Mitchell, PD, Reed, KD, Aspeslet, TL, Vandermause, MF, and JW Melski. "Comparison of four immunoserologic assays for detection of antibodies to *Borrelia burgdorferi* in patients with culture-positive erythema migrans." *Journal of Clinical Microbiology* 32 (1994): 1958-1962.

13. Harris, NS, Harris, SJ, Joseph, JJ, and BG Stephens. "*Borrelia burgdorferi* antigen levels in urine and other fluids during the course of treatment for Lyme disease: a case study." Presented at the VII International Congress of Lyme Borreliosis, 16-21 June 1996, San Francisco, CA.

14. Craft, JE, Fischer, DK, Shimamoto, GT, and AC Steere. "Antigens of *Borrelia burgdorferi* recognized during Lyme disease: appearance of a new immunoglobulin M response and expansion of the immunoglobulin G response late in the illness." *Journal of Clinical Investigation* 78 (1997): 934-939.

15. Jain, VK, Hilton, E, Maytal, J, Dorante, G, Ilowite, NT, and SK Sood. "Immunoglobulin M immunoblot for diagnosis of *Borrelia burgdorferi* infection in patients with acute facial palsy." *Journal of Clinical Microbiology* 34 (1996): 2033-2035.

16. Sivak, SL, Aguero-Rosenfeld, ME, Nowakowski, J, *et al.* "Accuracy of IgM immunoblotting to confirm the clinical diagnosis of early Lyme disease." *Archives of Internal Medicine* 156 (1996): 2105-2109.

17. Goodman, JL, Bradley, JF, Ross, AE, *et al.* "Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using the polymerase chain reaction." *American Journal of Medicine* 9 (1995): 6-12.

18. Wormser, GP, Nowakowski, J, Nadelman, RB, *et al.* "Improving the yield of blood cultures for patients with early Lyme disease." *Journal of Clinical Microbiology* 36 (1998): 296-298.

19. Wallach, FR, Forni, AL, Hariprashad, J, *et al.* "Circulating *Borrelia burgdorferi* in patients with acute Lyme disease: results of blood cultures and serum DNA analysis." *Journal of Infectious Disease* 168 (1993): 1541-1543.

20. Manak, MM, Gonzalez-Villasenor, LI, Crush-Stanton, S, and RC Tilton. "Use of PCR to monitor the clearance of *Borrelia burgdorferi* DNA from blood following antibiotic therapy." *Journal of Spirochetal and Tick-Borne Disease* 4 (1997): 11-20.

21. Bayer, ME, Zhang, M, and MH Bayer. "*Borrelia burgdorferi* DNA in the urine of treated patients with chronic Lyme disease symptoms. A PCR study of 97 cases." *Infection* 24 (1996): 347-353.

22. Harris, NS, and BG Stephens. "Detection of *B. burgdorferi* antigen in urine from patients with Lyme Borreliosis." *Journal of Spirochetal and Tick-Borne Disease* 2 (1995): 37-41.

23. Hyde, FW, Johnson, RC, White, TJ, and CE Shelburne. "Detection of antigens in urine of mice and humans infected with *Borrelia burgdorferi*, etiologic agent of Lyme disease." *Journal of Clinical Microbiology* 27 (1989): 58-61.

24. Dorward, DW, Schwan, TG, and CF Garon. "Immune capture and detection of extracellular *B. burgdorferi* antigens in fluids or tissues of ticks, mice, dogs, and humans." *Journal of Clinical Microbiology* 29 (1991): 1162-1171.

25. Magnarelli, LA, Anderson, JF, and KC Stafford. "Detection of *Borrelia burgdorferi* in urine of *Peromyscus leucopus* by inhibition enzyme-linked immunosorbent assay." *Journal of Clinical Microbiology* 32 (1994): 777-782.

The International Lyme and Associated Diseases Society (ILADS) provides a forum for health science professionals to share their wealth of knowledge regarding the management of Lyme and associated diseases.

The ILADS website, www.ilads.org, provides the following information:

- The Society's position papers and practice guidelines
- The most recent diagnostic methods and treatments in the management of Lyme and associated diseases
- The Society's schedule of events