Serologic Testing in Lyme Disease

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Topic Overview: How should a clinician employ serologic tests in a Lyme disease evaluation?
Before ordering an ELISA or Western blot the clinician should develop a clinical impression of the patient, taking into account the exposure history, symptoms and exam findings. If Lyme disease is judged to be a diagnostic possibility then testing is reasonable. Neither ELISA nor Western blot testing is sensitive enough to rule out Lyme disease. Because positive ELISA results require confirmation with a Western blot, one can make the case for eliminating the test altogether. Positive Western blots can confirm the clinical diagnosis of Lyme but, in and of themselves, they do not necessarily indicate that a patient is actively infected. In summary, serologic testing does not offer a reliable laboratory "shortcut" to the diagnosis of Lyme disease; Lyme disease remains a clinical diagnosis.

Supporting Evidence
Lyme disease is easier to treat when diagnosed early. Missing the diagnosis can lead to significant morbidity, therefore it is important that all potential Lyme patients be identified; doing this requires a highly sensitive test. Yet the inappropriate use of antibiotics can also be harmful so it is important to treat only patients who have Lyme disease; this requires a highly specific test. Thus, Lyme disease is an illness where sequential testing (beginning with a sensitive initial test followed by a specific test), such as the CDC's two-tier protocol, is appropriate.

Unfortunately, in clinical settings, the true sensitivity of the 2-tier test system using standard commercial testing has been estimated to be 44%–56%.[1-3] Both the FDA and the National Institute of Allergies and Infectious Diseases have cautioned that negative results on serologic testing do not rule out the infection.[4,5] Due to the limitations of the two-tier algorithm, new testing formats were sought. The C6 ELISA was considered a potential replacement but it also yields inadequate sensitivity.[6]

Serologic testing can be negative for reasons not related to the tests themselves. Antibodies may be bound to borrelial antigens in immune complexes, leaving no free antibodies to react with a test's reagent. A process for breaking the immune complexes apart exists but it is not used outside of research studies.[7] The administration of inadequate doses of antibiotics early in the infection can also lead to cases of seronegative Lyme disease. Dattwyler et al first reported this finding in 1988.[8] Seronegative Lyme may result when antibiotics remove enough of the bacteria to prevent a full immune response to the organism yet leave sufficient numbers behind to cause ongoing disease. Several authors have subsequently reported this phenomenon.[9,10]

Given that testing is inadequate, missing 44 – 56% of patients tested, Lyme disease is best diagnosed on clinical grounds. If testing is desired in a patient whose history and exposure are consistent with Lyme disease, then proceeding directly to the IgM and IgG Western blots is logical. This is true because a negative ELISA is not clinically useful and, in the 2-step algorithm, a positive ELISA requires a confirmatory Western blot. It should be noted that as of 1/1/08, the CDC definition for a surveillance case has changed; a positive single-tier IgG immunoblot, interpreted using standard criteria, is now considered adequate lab evidence for infection. [11]

The sensitivity of Western blots is partially dependent on the source of the test. There are pre-made kits with variable sensitivity and there are some specialized labs which create their own. Either way, the sensitivity remains low, generally no more than 70%.

Western blots are labor intensive tests. Most labs use FDA licensed kits; doing so reduces the work associated with the test but lab technicians still need to "read" the bands and grade them based on the intensity of the antibody signal. This is a subjective measure and it is reasonable to assume that techs who read blots frequently are better at it than those who rarely read them. There are dozens of separate bands to a Western blot and

multiple interpretation schemes, including the IgM and IgG criteria adopted by the CDC for surveillance purposes. When using a kit, lab techs can only comment on the 10 CDC designated bands. Even if a Borrelia burgdorferi-specific band were present, such as 31 or 34, the physician would not routinely be notified of the occurrence. Labs which develop their own Western blots are free to report on any identified band; the additional information may be crucial to the ordering physician.

To create their own in-house Western blot, a lab must satisfy several regulatory requirements to prove that their tests are valid. Labs specializing in testing for tick-borne illnesses, especially IGeneX, have had their results questioned by many. This particular lab developed a urine test for Lyme which performed badly in one study (the reason for the poor performance is under dispute), leading many physicians to wrongly assume that all of its testing is suspect. This is not the case. IGeneX is fully accredited by multiple regulatory agencies to perform Western blots and other high complexity testing. In the New York State Proficiency Testing Program from 2000 - 2007 the lab averaged 99% on Western blot and ELISA testing. IGeneX reports tests as positive or negative by the CDC criteria and by an interpretation developed in-house. The latter may be controversial but the actual reading of the bands is correct. I have no financial ties to this company and only address this issue because misinformed physicians have disregarded results from this lab to the detriment of many patients.

As stated above, Western blots interpreted via the CDC criteria are insufficiently sensitive but they are highly specific, usually 95% or greater. Therefore a positive result truly does confirm a clinical diagnosis. Complicating the picture is the fact that IgM antibodies may remain high in patients who have been ill for months or even years;[12,13] this is very different from the antibody response to other infections where IgM levels diminish after a month or so and are replaced by the IgG response. Additionally, the IgM response re-emerges in some patients without evidence of another tick bite. Clinicians must keep in mind that Western blots may remain positive after treatment. In asymptomatic patients you would expect them to eventually be clear of antibodies; when that event occurs in an individual patient is unknown. In symptomatic patients, it would seem prudent to consider a positive Western blot as a sign of ongoing infection. Furthermore, recent studies have documented that Western blots become negative following treatment whether or not treatment was successful.[14] Negative Western blots, like negative ELISAs, do not rule out Lyme disease.

The sensitivity and specificity of Western blots can be manipulated by changing the band criteria for what constitutes a positive test. The criteria adopted by the CDC at the Dearborn conference in 1994 were chosen on the basis of specificity.[15] Recall that these were supposed to be criteria for establishing a surveillance group of Lyme patients. Surveillance groups are very select; every effort is made to exclude from the group all who do not have the illness. Epidemiologists desire to follow an illness over time, by geographic region and by treatment outcome; surveillance groups make this possible. The CDC has repeatedly stated that the surveillance criteria were not intended to be used for clinical diagnosis.

A bit of background information on the Dearborn conference is necessary to put the CDC Western blot criteria in perspective. During the early 1990s multiple researchers had developed various criteria for the evaluation of Lyme Western blots; a positive test at one center might be considered negative at another. This created a good deal of confusion particularly because the range of symptoms and clinical presentations had expanded significantly from the early days of the illness when it was known as “Lyme arthritis”. The goal of the conference was to set uniform criteria for lab confirmation of the clinical diagnosis. There was debate around which bands should be included and whether Borrelia burgdorferi-specific bands should be weighted more than nonspecific bands. The bands considered specific for Borrelia burgdorferi (meaning that positive bands are not the result of cross reactivity with other bacteria) are**:** 18, 22, 23-25, 28, 31, 34, 35, 37, 39, 47, 50, 83, 93, and 94. Ultimately, IgG criteria advanced by Dressler and Engstrom’s IgM criteria, which are very specific and less sensitive, were adopted.[16,17] The restrictive nature of the criteria was discussed and participants were reminded that these were surveillance criteria for epidemiologic purposes and that Lyme disease should remain a clinical diagnosis.

The timing of the Dearborn conference was critical. Lyme disease vaccines were in the pipeline. Without establishing uniform criteria for the diagnosis of Lyme disease, it would be impossible for the manufacturers to determine the vaccines' efficacy. Lacking efficacy data, the FDA would be unable to approve the vaccines for use in humans. Lyme disease cases were increasing significantly so it made sense for the CDC to help move the vaccine development process along; this was the underlying reason for the Dearborn meeting. The vaccines being studied were based on OspA, a highly antigenic Borrelia burgdorferi protein. Antibodies to OspA form a band at the 31kd antigen site. Patients who had been successfully immunized would be positive at band 31, which is one reason for excluding it from the list of CDC bands. However, a positive result at band 31 in patients who have never been vaccinated is highly indicative of exposure to B. burgdorferi and should not be ignored.

Many patients seek medical evaluations for symptoms which are not easily assigned to a specific illness. Distinguishing those with Lyme disease from those who do not have the infection is a challenging, time-consuming clinical problem. Having reliable lab tests to clarify and speed the diagnostic process along would be very helpful but currently available commercial tests are insufficiently sensitive to rule out Lyme disease. Physicians should heed the cautions from the CDC, FDA and others; Lyme disease remains a clinical diagnosis.

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