# Limitations of Antibody-Based Diagnostic Tests for Lyme Disease

Most diagnostic tests are based on the detection of specific antibodies in the blood of patients suspected of having a given infection.  Such tests, especially those that have survived years of rigorous scrutiny and have been validated and approved for clinical use by the Food and Drug Administration (FDA) and the Centers for Disease Prevention and Control (CDC), have been invaluable in the control, diagnosis, and treatment of many infectious diseases. Notwithstanding, they have limitations that must be considered in evaluating the results obtained. In this presentation, these limitations will be considered with respect to the diagnosis of Lyme disease which historically has been defined as a tick-borne infectious disease caused by the spirochete, *Borrelia burgdorferi* (1).

Although sensitivity and specificity are decisive factors in judging the value of any antibody-based diagnostic test, several variables influence the assessment of each of these parameters.  Of primary concern is the minimal amount of antibody that must be present, in a reasonable volume of blood or some other body fluid, to give a positive test result. Here, an understanding of the concept of "antigen load" with reference to the least amount of antigen required to stimulate the immune system to produce a detectable antibody response, is useful.

The term "antigen" refers to any substance that stimulates the immune system to make antibody that is specific for that antigen.  In the case of *B. burgdorferi*, this consists of an array of several well-characterized molecules that are present in extremely small amounts on its cell surface.  Most are proteins or lipoproteins that are characteristic of *B. burgdorferi* and play a role in pathogenesis (2); consequently, many have diagnostic significance, although some (e.g., the flagellar antigens) may be shared by unrelated nonpathogenic bacteria. Antibodies against shared or cross-reactive antigens such as flagella certainly are produced during Lyme disease; however, they are not diagnostically relevant since they are not specific for *B. burgdorferi*, the causative agent of Lyme disease.

There is abundant evidence to indicate that the magnitude of an antibody response is dependent upon the amount of antigen available to stimulate the immune system. As the amount (dose) of antigen is increased (antigen load), more antibody is produced until a peak level is attained. It is relatively easy to

determine the optimal dose required to generate a detectable antibody response against an isolated purified antigen or a vaccine; however, it is much more difficult to do so when antigen is delivered in the form of live bacterial cells and the total amount delivered is dependent upon the extent to which bacterial cells multiply (replicate) during the course of infection.

In contrast to rapidly growing bacteria such as *Escherichia coli* or *Staphylococcus aureus* that replicate by cell division once every 17-30 minutes (3), *B. burgdorferi* replicates much more slowly -- once every 12-24 hours (4). Although detectable amounts of antibody may be present in the blood within a few days after infection with rapidly growing pathogens, it may take several weeks before detectable amounts of antibody appear in the blood of patients infected with slow-growing *B. burgdorferi*. This influences the sensitivity of a diagnostic test and is a major limitation as to <u>when</u>, during the course of an infection, a given antibody based tests can be used for diagnostic purposes, regardless of specificity.

The "bull's eye "or erythema migrans rash (EM) is considered to be sufficiently diagnostic for Lyme disease to justify antibiotic therapy without the need for a positive serological test result (5). It develops at the site of a tick bite, within 7-14 days after an infected tick has taken a blood meal and detached (6, 7). Usually, patients with EM are seronegative at the time of presentation; however, the probability of becoming seropositive increases with the duration of EM and as infection becomes more disseminated, i.e., as antigen load increases, and the amount of antibody produced reaches detectable levels in the blood (8, 9, 10). Although only 25-50% of patients with EM are ELISA positive during the early or acute phase of their infection, 80-90% of treated EM patients are seropositive as the duration of the EM is increased and by convalescence. This occurs, despite the fact that such patients were treated with an antibiotic that may have reduced "antigen load" to some degree, thereby lessening the magnitude of the resultant antibody response (10, 11).

 So, as one would anticipate and has been elegantly demonstrated by several investigators, the sensitivity of an antibody-based diagnostic test for Lyme disease increases progressively with the duration of EM (10), and in patients with: (a) acute neurologic or cardiac abnormalities; and, with (b) arthritis or chronic

neurologic abnormalities (11). Under such circumstances, and as infection becomes more disseminated, both sensitivity and specificity range from 85% to 99% (11). Similar results were obtained when the <u>same</u> specimens were assayed in parallel by another highly specific antibody-based procedure, the Vlse C6 peptide ELISA (10, 11).

Because of the acknowledged low sensitivity of ELISA tests during the early acute EM phase, neither the CDC nor the Infectious Diseases Society of America (IDSA) advocate serological testing of such patients. Rather, they consider it appropriate to treat such patients with antibiotics, and then do follow-up serological testing during convalescence, when detectable amounts of antibody are likely to be present in blood (4). Thus, the early or acute EM phase of Lyme disease is the <u>only</u> time during active infection when the sensitivity of two-tier testing is low.

Despite the above mentioned well-documented observations, some continue to discount the validity of two-tier testing as a diagnostic for Lyme disease by saying that its sensitivity is "no better than that of a coin toss" (12). However, the author of such a general statement ignores the fact that most -- if not all -- of the test results referenced were derived from patients with EM, i.e., from patients with early acute Lyme disease when the immune response is just beginning and antibody levels are very low (12). Under such circumstances, low sensitivity is to be expected as has been reported by other investigators (8, 9, 10); this is why testing is not recommended under such circumstances (5). Specificity is not an issue since whenever antibody is detected -- albeit in small amounts -- it is found to be 99-100% specific for *B. burgdorferi* (12).

The dissemination of such misleading information on the validity of two-tier testing (12) at best reflects ignorance of both the disease process and limitations associated with antibody-based tests when used for the diagnosis of Lyme disease. At worse, it is an inept attempt to selectively report only those observations that serve to discredit the validity of two-tiered testing. Neither is in the best interest of the public health or helpful to patients who suspect that they have Lyme disease.

There is abundant evidence indicating that two-tier testing has performed well when applied under conditions when the probable risk of contracting Lyme disease is high (13, 14 ). Those competent and experienced in the use of

diagnostic tests understand that the results obtained with antibody-based diagnostic tests are valid <u>only</u> when such tests are used when detectable amounts of antibody are likely to be present. Even the most sensitive and specific diagnostic test one can imagine is not going to show that a patient has Lyme disease, if that patient doesn't have it. Alternatively, if a patient has long-standing non-specific symptoms of a type often associated with Lyme disease but is seronegative, it is not prudent to treat such patients with an extended course of antibiotics for an infection that may not even exist. It makes more sense to consider other causes for their symptoms.

Certainly, continual efforts should be made to improve existing technology so that diagnostic tests are able to detect small amounts of antibody early during the course of an infection; this would ensure that curative antibiotic therapy can be commenced as early as possible. This is now being done. The recent establishment of a reference serum repository in which the results of newly developed diagnostic tests can be compared to those derived from existing procedures -- using the same well-characterized panel of reference specimens in both cases-- will greatly accelerate progress in that regard (15).

The ELISA format, with a sensitivity estimated to range from 0.01 to 0.1 nanograms of antibody per milliliter (16), seems to be ideally suited for diagnostic testing. Its remarkable sensitivity is due largely to the ability of an enzyme conjugated to a second antibody to <u>amplify</u> the reaction between a single specific antibody molecule and its relevant ligand (or antigen), by a factor of 10,000-fold or more. The replacement of bacterial cell lysates often used in conventional ELISAs with well-defined peptides associated with specific antigens produced early in infection (17) reduces variability and greatly facilitates comparisons of results from different laboratories. The Vlse C6 peptide ELISA is but one example of just such an application. Parallel testing has shown that it gives results comparable to those obtained with the conventional ELISA used in the standard two-tier test procedure. However, it should be noted, that there have been no reports of patients who are seropositive for Lyme disease by the Vlse C6 peptide ELISA, who are not also seropositive by the conventional ELISA (10, 11). Although sensitivity appears to be equivalent since the results obtained have been comparable in all instances (10, 11), additional comparative studies are needed before this single diagnostic test can replace two-tier testing.

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