Laboratory Diagnosis of Lyme Disease

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Conflicts: Dr. Porwancher owns two patents on the laboratory diagnosis of Lyme disease using bioinformatics.
The laboratory diagnosis of Lyme disease

Unfortunately, the diagnosis of Lyme disease (LD) has become highly politicized (1, 2). This monograph will provide a practical approach toward diagnosis with a modicum of laboratory input. Well before the bacterial cause of Lyme disease (*Borrelia burgdorferi*) was known, researchers recognized the characteristic rash, erythema migrans (EM), and subsequently identified complications such as facial palsy, aseptic meningitis, and arthritis among untreated patients (3, 4). Traditionally the clinical presentation has been divided into three stages: Stage I (solitary EM), Stage 2 (disseminated disease with multiple EM and/or early neurological or cardiac disease) and Stage 3 (late neurological disease and arthritis). However, the duration of illness is the most reliable guide for assessing the immune response (5).

Early Lyme disease is principally a clinical diagnosis. Laboratory tests are not recommended for patients presenting with EM (6) because they are not sensitive in the early phase of the disease. Based on a prospective study of over 10,000 individuals living in an endemic area, over 70% of all patients with Lyme disease will present with EM (7). Most EM is solitary, although 8% to 25% may have multiple skin lesions (8, 9). Unlike routine insect bites, erythema migrans is larger (> 5cm), less pruritic, and often associated with systemic symptoms such as fever, arthralgias, and headache. Fewer than half of patients will recall a tick-bite. About 18% of patients will present with an influenza-like illness without rash and the remaining 5% to 10% will demonstrate later manifestations of disease (7); diagnosing the former requires a high degree of clinical suspicion. For
patients living in or visiting endemic areas, developing fever, myalgias, and arthralgias, without rash, respiratory, or gastrointestinal manifestations, may represent early manifestations of Lyme disease (10); infectious agents such as viruses, anaplasma, and babesia must also be considered (11).

The Centers for Disease Control and Prevention (CDC) advocate a two-step diagnostic procedure, whereby serum is first tested for antibody to *B. burgdorferi* using a whole-cell enzyme-linked immunoassay (EIA); Western blotting is then used to confirm test results that are positive or equivocal by the first step (12). During the first month after disease onset, evidence of either immunoglobulin G (IgG) or immunoglobulin M (IgM) production to key antigens is sufficient for diagnosis. Any 2 of 3 IgM Western blot bands at the 23-, 39-, and 41-kDa positions, or any 5 of 10 IgG Western blot bands at the 18-, 23-, 28-, 30-, 39-, 41-, 45-, 58-, 66-, and 93-kDa positions are considered diagnostic. More than one month after disease onset only positive EIA assays confirmed by IgG Western blot are accepted for diagnosis; an isolated IgM Western blot at that stage is very likely a false-positive result (13, 14). Acute and convalescent sera are recommended when diagnosis is critical and initial serology is negative or equivocal. Although alternative criteria for interpretation of Western blots have been proposed, they often fail to consider the high rate of false-positive serology attendant with more liberal standards, sometimes as high as 40% (2).

Initially only 30% to 40% of patients with solitary EM will demonstrate positive two-step serology (5, 15). Specific antibody production becomes more pronounced when multiple
EM are seen and when the patient has been ill for at least 2 weeks (5). With close follow-up, specific serum antibody is measurable about 60% to 70% of the time, even in treated early disease (5, 15). Given the significant fraction of patients with false-negative tests during the early phase of the illness, a negative result is not reliable and therefore a physician must use their best clinical judgment concerning treatment. This caveat concerning early diagnosis should not be applied to late disease, where over 95% of sera are positive using the two-step method (15). On occasion patients with early neurological disease (e.g. facial palsy and aseptic meningitis) may become negative by the two-step method following antibiotic treatment (16); seronegative Lyme arthritis is rare, even in treated patients (15, 16). Serology is most important for the diagnosis of those without a typical rash and individuals with Stages 2 and 3 Lyme disease.

The two drivers of test accuracy are the intrinsic ability of the test to distinguish individuals with Lyme disease from healthy controls and the prevalence of Lyme disease among those tested. The former is best described by the test’s sensitivity and specificity. Test sensitivity refers to the number of patients with positive results out of the total number with disease. Test specificity refers to the total number of negative test results out of those individuals without Lyme disease. Ideally we want a test that is both highly sensitive and highly specific. However, there is always a trade-off between these two goals; the less stringent the criteria to label a test positive, the more healthy people will have falsely-positive results. Usually included among the controls are individuals who live in endemic areas in the Northeast or Midwest; some will demonstrate positive serology related to past exposure to *B. burgdorferi*, which can be confused with current
disease. A good test must also be able to distinguish cross-reacting conditions, such as syphilis, from Lyme disease.

The second driver of accuracy, disease prevalence, can be understood through the following example: suppose that the risk of Lyme disease in a given situation is 1% and you employ a test that is 99% sensitive and 99% specific. In the above case, the test will very likely identify the 1 in 100 patients with Lyme disease; at the same time, about 1 in 100 patients without disease will be falsely-positive; therefore only 1 of every 2 people with a positive test (50% in this scenario) will actually have Lyme disease. Let’s take a different example: suppose that the risk of Lyme disease in a specific population is 50% and we apply the same test as before. In this second example, the test will detect about 49 out of every 50 patients with disease and about 1 in 50 without disease; therefore about 49 of every 50 people with a positive test (98%) will have Lyme disease. These examples demonstrate that one cannot divorce the interpretation of a test from the clinical setting to which it is applied. Another way of looking at the above scenarios is that you can make even a very good test look bad if it is misapplied (e.g., when used in areas where LD is either rare or non-existent). There exists a limited amount of good clinical research that aids physicians in determining the pretest risk of disease (17, 18).

There are two main reasons for a false-positive test: one is intrinsic to a patient’s own serum and the other related to test equipment and antibody detection systems. A typical EIA test measures serum antibody through production of a colored reagent which is then detected using a spectrophotometer. Other tests generate an immunofluorescent signal
detected by a light sensor. Some of the reagents used above adsorb to the surface of the polystyrene test wells that hold the patients serum, thereby causing excess signal (e.g. colored reagent) detected by the measuring equipment. Most manufacturers are well aware of the technical modifications needed to minimize this extra signal in their detection systems.

It is more difficult to minimize reactions due to the patient’s own serum. Cross-reacting medical conditions and prior host exposure to similar organisms from the environment can lead to false-positive serology. The immune system has an extensive memory of previous exposure to microbial agents and toxins. Many proteins involved in basic cellular function, such as heat-shock proteins, are present in a broad variety of bacteria. Previous infection with spirochetes such as leptospirosis, syphilis, and tick-borne relapsing fever can all produce cross-reacting antibodies (15). Even infection with Epstein-Barr virus can result in false-positive serology to *B. burgdorferi* (19). Connective tissue disorders, such as Lupus erythematosis, can also cause antibodies that can be confused with *B. burgdorferi* infection. For unknown reasons immunoglobulins from some sera will adsorb in a non-specific way to the plastic wells holding the patient’s sample; this phenomena can also cause falsely-positive serology.

Patients with Lyme disease can usually be distinguished from those with cross-reacting conditions by both the number of antibodies that they share in common and by the specific type of antibodies produced (20). The CDC has identified endemic areas where 5% to 10% of patients demonstrate serologic evidence of prior exposure to *B. burgdorferi*
At least 20% of patients followed for 1 year after treatment for EM will have persistent IgG antibody (19) and, in some cases, persistent IgM antibody too (22, 23). Most laboratory tests cannot distinguish between active and inactive disease (24); while declining levels of C6 IgG antibody after treatment suggest a favorable prognosis (25, 26), persistent levels of antibody are commonly seen in patients following successful antibiotic therapy. These observations underscore the need for both better diagnostic tests and for more standardized clinical assessment.

Newer diagnostic approaches include the detection of B. burgdorferi DNA by polymerase chain reaction (PCR) in body fluids or tissue (27), enhanced culture techniques from plasma (28), and recombinant or peptide-antigen EIA techniques (15). The latter appear to hold the greatest promise, particularly antibody to the highly conserved C6 peptide (26); the most significant advantage of these newer antigen assays is improved specificity compared to older EIA methods that use whole-cell preparations. Some studies have used combinations of antibodies to improve sensitivity, while taking advantage of their improved specificity (15, 29, 30). The C6 IgG EIA is also able to detect antibody to B. garinii and B. afzelli, the predominant strains of B. burgdorferi seen in Europe. Serum borreliacidal antibody detected by flow cytometry and immune complex assays appear promising but are technically demanding and not widely available (31). Antibody-capture techniques have demonstrated their greatest utility in measuring the production of antibodies specific to B. burgdorferi in spinal fluid (32).
Except for Lyme arthritis, the ability to detect *B. burgdorferi* DNA by PCR in body fluids has been poor (24). The sensitivity of PCR for *B. burgdorferi* DNA in plasma, urine, and cerebrospinal fluid is less than 50%. In most cases, *B. burgdorferi* DNA is detectable only in very early disease, either before or shortly after receiving antibiotic treatment. It is very uncommon to observe positive PCR tests beyond 4 to 6 weeks after providing appropriate antibiotic therapy (27). A recent study by Hodzic et al. (33) suggested that mice treated with one month of ceftriaxone for *B. burgdorferi* infection can develop persistent but attenuated spirochetal infection in connective tissue, as detected by PCR for *B. burgdorferi* DNA; this claim must be tempered by the fact that the mice were treated with a low dose of ceftriaxone, resulting in undetectable antibiotic levels for at least 16 hours daily. The latter study also attempted to transmit infection to uninfected mice by having ticks first feed on the treated mice and then feed on the uninfected group. Using the PCR technique, *B. burgdorferi* DNA could then be detected in multiple tissues of the previously uninfected mice; however, all tissue cultures and pathology were negative and no serological evidence was presented to support the authors’ claim. This contradictory evidence raises the possibility that the PCR results for *B. burgdorferi* DNA were falsely-positive.

The practice of repeatedly ordering serum, plasma, or urine samples for *B. burgdorferi* DNA by PCR after treatment is much more likely to detect false-positive assays than persistent infection. PCR assays are highly susceptible to cross-contamination from other specimens, either during initial processing or subsequent DNA amplification (31, 34). Laboratories that frequently detect *B. burgdorferi* DNA by PCR in serum, plasma, or
urine should consider genetic sequencing of positive specimens to look for evidence of cross-contamination.

Early antibiotic treatment with amoxicillin or doxycycline hastens recovery and prevents late complications, such as arthritis; a few patients (less than 5%) have some residual symptoms lasting for months or years (9). In most instances, residual symptoms are not debilitating, but for reasons yet unknown, some will experience significant residual fatigue, joint pain, or memory loss (35, 36). Residual symptoms are more often seen in patients with disseminated disease who received either late or partial treatment (23, 35). Although rare, progressive arthritis has also been reported; in these cases cell-mediated immune mechanisms may be responsible for persistent joint damage, long after the infection has been cured (11). There is no convincing evidence that prolonged antibiotic therapy leads to resolution of these symptoms (37); transient improvement in fatigue has been noted in some studies, but not others (37, 38). Some community-based studies of antibiotic therapy have employed vague clinical and laboratory diagnostic criteria, making interpretation of treatment response suspect (39, 40). Tetracyclines are commonly used for treatment of Lyme disease but also have anti-inflammatory properties (41); these properties must be counterbalanced by concern about creating highly resistant bacteria and antibiotic-associated colitis. While most research has focused on early laboratory diagnosis, the clinical ability to distinguish late manifestations of Lyme disease from other etiologies has become increasingly important. Greater understanding of the immunology of Lyme disease is also a high research priority.
In summary, the diagnosis of early Lyme disease should be based on clinical assessment, with laboratory testing reserved for atypical presentations of EM, neurological disease, carditis, and arthritis. Serology remains the most important diagnostic tool. The current 2-tier approach is adequate for diagnosis of late Lyme disease, but often misses early cases; therefore acute and convalescent serology remains an option. Newer antibody assays, such as the C6 IgG EIA, represent an alternative to the 2-tier for late disease, but are not sensitive enough to rule out early Lyme disease. Because the C6 polypeptide is highly conserved among different *B. burgdorferi* strains, it has become a valuable diagnostic tool for European Lyme disease. Assays for *B. burgdorferi* DNA by PCR should be reserved for follow-up of Lyme arthritis; repeated testing of serum, urine, or plasma for *B. burgdorferi* DNA should be discouraged.
REFERENCES


Figure 1.

**Erythema Migrans- confluent**