Better Laboratory Testing for Lyme Disease: No More Western Blot

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(See the Major Article by Branda et al on pages 333–40.)

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It seems unlikely, in routine clinical practice, that direct methods, including culture or polymerase chain reaction, will ever become the primary tests for the laboratory diagnosis of Lyme disease. Indirect, serologic tests will continue to be used to support this diagnosis [1]. Can serologic testing for Lyme disease be improved? The Centers for Disease Control and Prevention (CDC) currently recommends a 2-tiered algorithm for serodiagnosis, using an enzyme immunoassay or immunofluorescent assay first, followed by immunoglobulin M (IgM) and immunoglobulin G (IgG) Western blots when the first test is positive or equivocal [2]. Sonicates of whole *Borrelia burgdorferi* are usually used as the antigen preparation in Western blots.

This approach works well for the diagnosis of late-stage Lyme disease [1], as almost all these patients have robust IgG antibody responses to many spirochetal antigens [3, 4], but not so well in early disease, because the early IgM response may be both insensitive and non-specific [5]. Current CDC guidelines compound the problem by limiting the application of IgM criteria for early diagnosis to the first 4 weeks of illness [2]. But not all patients demonstrate IgM antibodies as early as 4 weeks, reducing the sensitivity of the test [5], and not all clinicians follow the 4-week IgM guideline, leading sometimes, among other problems, to the misdiagnosis of late-stage “chronic Lyme disease” on the basis of a false-positive early-stage IgM Western blot [1, 6, 7].

Western blot testing not only results in false-positive IgM testing [7], but it is costly [8], has a slow turn-around time [8], and is subject to intra- and interlaboratory variation [9].

Could a new Lyme disease serologic test, without the Western blot, perform as well as the conventional CDC 2-tiered testing (enzyme-linked immunosorbent assay [ELISA] followed by immunoblot) using US assays compared to analogous European tests for the evaluation for Lyme borreliosis acquired in Europe? Their study assessed the sensitivity and specificity of CDC conventional 2-tiered testing (enzyme-linked immunosorbent assay [ELISA] followed by immunoblot) using US assays compared to analogous European tests for infection acquired in Europe. Sixty-four Lyme borreliosis patient samples were obtained from a single European site (Ljubljana, Slovenia). Various clinical manifestations of Lyme disease were well represented in these patients: 20 erythema migrans, 15 Lyme neuroborreliosis, 15 Lyme arthritis, and 14 acrodermatitis chronica atrophicans. One hundred healthy subjects from a nonendemic area (New Zealand) provided control samples to assess specificity.

This study confirmed the inferior performance of standard 2-tiered serologic testing using assays developed for use in the United States compared to European assays for the evaluation for Lyme borreliosis acquired in Europe [12].
North America is caused by a single bor- relial species, *Borrelia burgdorferi sensu stricto* (s.s.) [13]. In Europe, there is greater species heterogeneity. Infection can be caused by *Borrelia afzelii*, *Borrelia garinii*, and, less commonly, *B. burgdor feri* s.s. [14]. The resulting borreli surface antigenic diversity impairs serodiagnostic performance [15], especially by immuno blot [16]. European immunoblots are prepared from specific spirochet lysates and/or purified antigens and interpreted by criteria specific for the location of infection [2]. European tests would be expected to have better predictive value than US tests for European-acquired infection.

For the 64 European Lyme borreliosis patients, the conventional 2-tiered US assay was 52% sensitive compared to 81% for European assays (P < 0.0007). The greatest differences in sensitivity were seen in early-stage disease (20% vs 55% sensitivity for erythema migrans; US vs European; P = 0.05) and (40% vs 87% for Lyme neuroborreliosis; US vs European; P = 0.02). Both assays had high sensitivity for Lyme arthritis and acrodermatitis chronica atrophicans and both had excellent specificity.

To establish a valid Lyme disease serologic test, no matter where infection is acquired, the authors then tested newer-generation assays, now available in the United States, in patients who acquired infection in Europe. These assays incorporate the Vmp-like sequence, expressed (VlsE) protein [17], or an immunodominant, largely conserved 26-mer oligopeptide (C6 peptide) corresponding to the sixth invariant region within VlsE [18]. They compared the performance of European assays to a US C6 ELISA assay, either as a stand-alone test or combined in a 2-tiered algorithm using a standard US ELISA, followed by a C6 ELISA. They demonstrated that the sensitivity of both these new approaches equals that of conventional 2-tiered European assays. Specificity was also 100%. There are 2 reasons for the improved performance of these newer-generation assays. Sensitivity in early disease is improved because the C6 peptide ELISA relies on an early IgG antibody response that persists through late-stage infection [12]. Specificity is maintained because the IR 6 region of VlsE is antigenically conserved across Lyme-related genospecies [19].

So the C6 ELISA, used as a stand-alone test or as the second test in a 2-tiered algorithm following a standard US ELISA, can be recommended for the diagnosis of Lyme disease acquired in North America and Europe and probably elsewhere. But the C6 ELISA as part of the 2-tiered algorithm is a better test than the stand-alone test, because it is more specific [10].

In a larger, previous study, the authors studied the specificity of their same 2-tiered algorithm and found it to be 99.5% compared to 98.4% for their stand-alone C6 ELISA [10]. In the United States, where at least 3.4 million Lyme disease tests are performed annually [10–12], this difference would lead to an additional 37,000 false-positive test results per year, more than the reported incidence of Lyme disease in the United States (>35,000 cases annually) [20]. The excellent specificity of the 2-tier approach is a critical advantage [12].

The report by Branda et al demonstrates that a standard Lyme disease ELISA, followed by a C6 peptide ELISA, in a 2-step algorithm is widely geographically applicable [12]. This newer approach has been previously demonstrated to be more sensitive in early Lyme disease than conventional, CDC-recommended testing and equally specific for all stages of the illness [10]. There is no requirement for cumbersome immunoblots, including IgM Western blotting, a test that has not fared well in real-world settings. Both steps in this algorithm, the initial standard ELISA and the C6 peptide ELISA, are already commercially available. It may be time to incorporate this newer 2-tiered algorithm into clinical practice.

### References


