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A Concise Critical Analysis of Serologic Testing for the Diagnosis of Lyme Disease

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Abstract Diagnostic testing for Lyme disease in the clinical setting primarily relies on assessment of serologic responses to infection, with the exception of the early localized phase of disease, in which the diagnosis must be made clinically, due to the recognized insensitivity of serologic testing at this phase of disease. For the diagnosis of early disseminated and late disease, the Centers for Disease Control and Prevention (CDC) recommends a two-tiered approach to testing consisting of initial IgM and IgG quantitative enzyme-linked immunosorbent assay (ELISA), followed by confirmation of all indeterminate or positive ELISA tests with separate IgG and IgM Western blots. This critical analysis addresses the sensitivity, specificity, and predictive value of serologic testing for Lyme disease in early localized, early disseminated, and late disease. Other testing modalities currently under evaluation are also discussed, including IgG vlsE C6 peptide ELISA, other two-tiered testing strategies, rapid diagnostics, and PCR. An understanding of the strengths and limitations of currently available testing for Lyme disease is critical for appropriate diagnosis.

Keywords Lyme disease · Serology · Western blot · Diagnostics

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Introduction

Lyme disease is an infectious/inflammatory disorder that results in the USA from infection with the bacterial spirochete *Borrelia burgdorferi*. Infection in humans results from transmission of the organism by *Ixodes* species deer ticks, which serve as the primary vector. Transmission of disease has been reported in the majority of states in the USA; however, the bulk of reported cases occur in 10 states that are recognized as hyperendemic areas, located in New England, the eastern Mid-Atlantic, and the Upper Mid-West. Lyme disease may manifest as a variety of potential clinical manifestations, which occur at three different stages of infection. These include early localized disease, primarily presenting as erythema migrans rash; early disseminated disease, primarily presenting as multiple erythema migrans rash, cranial nerve palsies, meningitis, and/or carditis; and late disease, primarily presenting as monoarticular arthritis, and in very rare cases, encephalopathy or polyneuropathy [1•, 2, 3]. Asymptomatic infection is uncommon in the USA [4] Table 1.

Description of the Test

Diagnostic testing for Lyme disease in the clinical setting is primarily achieved by assessing serum serologic responses to infection, with the exception of the early localized phase of disease, in which the diagnosis must be made clinically, due to the recognized insensitivity of serologic testing at this phase of disease. For the diagnosis of early disseminated and late disease, the Centers for Disease Control and Prevention (CDC) recommends a two-tiered approach to testing consisting of initial IgM and IgG enzyme-linked immunosorbent assay (ELISA) (quantitative result), followed by confirmation of all indeterminate or positive ELISA tests with separate IgG and IgM Western blots (WB) [5]. For IgM results

Table 1 Potential clinical manifestations occurring at three different stages of Lyme disease

Clinical manifestation	Stage of Lyme disease	Recommended testing	Relative sensitivity of serologic testing	Additional commerically available testing to be considered
Nonspecific symptoms or isolated fatigue	Not consistent with Lyme disease	Lyme testing not recommended	N/A	
Erythema migrans rash	Early localized	Clinical diagnosis only	Insensitive	
Multiple erythema migrans Flu-like illness	Early disseminated	2 tiered serologic testing (quantitative screen, followed by Western blot only for indeterminate or positive screen)	Moderately sensitive	
Aseptic meningitis	Early disseminated		Highly sensitive	CSF PCR (specific, but unknown sensitivity)
Facial palsy				
Carditis				
Large joint arthritis	Late		Highly sensitive	Synovial fluid PCR (specific, but unknown sensitivity)

to be considered positive, WB must demonstrate 2 of 3 possible specific IgM bands. Furthermore, IgM WB testing and interpretation is only recommended for the diagnosis of early Lyme disease evaluated within the first 4 weeks of symptoms; IgM should not be used for the diagnosis of late Lyme disease. The criterion for a positive IgG WB is the presence of a minimum 5 of 10 possible specific IgG bands. IgG WB can be evaluated within any timeframe of infection, although IgG bands may not be detectable until 4 weeks after the onset of infection. Incorrect use and interpretation of IgM WB testing lead to much confusion in the diagnosis of Lyme disease, particularly in patients with prolonged and nonspecific symptoms. A positive IgM WB with a negative IgG WB in a patient with symptoms beyond a 4-week duration is very likely to reflect a false-positive rather than true infection [2, 6].

Recently, an additional IgG serologic test, IgG vlsE C6 peptide ELISA, has been become commercially available, with some reports of added sensitivity for detection of US and European strains [7••]. To add to the complexity of diagnostic testing, additional modalities, with less data to support standard interpretation, include cerebrospinal fluid antibodies (IgM and IgG), PCR from skin biopsy, CSF, and intraarticular joint fluid specimens [8–10]. A "gold standard" for the diagnosis of Lyme disease is not universally accepted, leading to confusion and controversy within the literature regarding the validity of serologic testing and reliability in the clinical setting.

Sensitivity/Specificity

Many attempts have been made to evaluate serologic testing as a diagnostic modality in various phases of Lyme disease, including retrospective and prospective determinations of sensitivity and specificity. For even this basic measure of test validity, there is marked controversy in the medical literature. For example, a recent meta-analysis of six separate studies assessing sensitivity/specificity of Lyme serologic testing yielded sensitivities in the range of 29–68 % and specificities of 96–100 %; mean sensitivity was 56 % and specificity 99 % [11]. These authors concluded that the sensitivity of the test was comparable to a simple toss of a coin and, thus, ineffective in the clinical setting. However, this study did not address phase of disease in conjunction with test performance. In a compelling recent prospective study that included patients with various manifestations of Lyme, patients with other diseases with or without a prior history of Lyme, healthy subjects from areas of high endemicity, and areas in which infection was not endemic, Steere et al. determined sensitivity and specificity of the two-tiered methodology in different stages of disease [7••]. The study corroborated that the sensitivity of serologic testing is poor in early localized disease; IgM or IgG sensitivity 29 % in the setting of acute-phase Erythema Migrans (EM), 64 % in the convalescent phase. Sensitivity was improved, but remained low in the setting of early disseminated disease presenting as multiple EM, with combined IgM or IgG sensitivity of 43 % in the acute phase and 75 % in convalescence. However, sensitivity was markedly increased in the setting of early disseminated disease presenting as neurologic or cardiac manifestations (combined IgM or IgG sensitivity of 100 %) or late disease presenting as arthritis or chronic neurologic abnormalities (combined IgM or IgG sensitivity of 100 %). Specificity was uniformly excellent in all disease settings (99 %) [7••].

This paper also assessed the sensitivity/specificity of the newer IgG vlsE C6 peptide ELISA by direct comparison to standard two-tiered testing. In the setting of early disseminated phase acute neurologic or cardiac disease, C6 IgG performed equally well to combined standard IgM/IgG testing (100 % sensitivity, 96 % specificity), but demonstrated increased sensitivity (100 %) compared to use of the standard IgG alone (85 % sensitivity). Sensitivity of C6 testing remained poor (comparable to standard testing) in the setting of early localized disease (29 % in acute-phase EM; 56 % in convalescent-phase EM). Regarding serologic responses from

other biological compartments (cerebrospinal fluid, joint fluid), there are no accepted standards for sensitivity/specificity, leaving interpretation of results dependant upon the practitioner.

As added food for thought, it should be pointed out that much of the existing conflicting literature results from publications from two different "worldviews" of Lyme disease diagnosis. Some studies are sponsored by Lyme advocacy groups, in which the concern for potential underdiagnosis/undertreatment is emphasized, whereas other studies are sponsored by the medical establishment, in which prevention of overdiagnosis/overtreatment is emphasized. These sometimes competing interests have given rise to the so-called "Lyme wars". As stated succinctly by one author, "With the ever-broadening clinical criteria used by some to define Lyme disease, the sensitivity of testing could approach zero, where no objective criterion would be helpful and a diagnosis could be supported by any subjective finding deemed suitable" [12].

Additional Comments on Specificity

Using the two-tiered methodology, specificity of testing is excellent (96–99 %) at all phases of disease. However, false positives are well known to occur, particularly when only the first step screening ELISA is utilized. The ELISA method may produce false-positive results due to cross-reactive antibodies from patients with other spirochetal infections (e.g., syphilis, leptospirosis, or relapsing fever), with viral infections (e.g., varicella, EBV), certain autoimmune diseases (e.g., systemic lupus erythematosus), or even due to cross-reactivity with antigens for spirochetes that are part of the normal oral flora [1•]. Additionally, the use of only the "second tier" Western blot also can lead to false positives since this test is qualitative, not quantitative; faint cross-reactive bands can easily (and erroneously) be interpreted as positive. For this reason, WB should only be performed in patients in whom the quantitative ELISA "first tier" criterion has been met.

Positive and Negative Predictive Value

The predictive value of Lyme serologic testing is influenced not only by the patient's stage of disease (primarily due to the sensitivity issues noted above) but also the prevalence of disease within the community in which they reside and the likelihood of exposure to the tick vector. As in other diagnostic tests, use of testing in patients with a low pre-test probability of illness (low prevalence area, low exposure to ticks) will result in a high rate of false-positive tests. Of even more potential importance, even in areas with a high prevalence of Lyme disease, patients with only nonspecific signs and symptoms, such as fatigue, headache, and arthralgia, are not likely to have Lyme disease, and the vast majority of positive serological tests for such patients will be false-positive results. Thus, positive predictive value will be dramatically reduced in both scenarios. On the other hand, in areas of low prevalence, negative predictive value will remain high. The Infectious Diseases Society of America published guidelines in 2000 and 2006, which stress that positive and negative predictive values can be maximized by applying established clinical criteria in conjunction with laboratory techniques [1•, 13].

To make matters more confusing, prevalence data in the USA are limited by the reliance on passive reporting systems and the likely high frequency of misdiagnosis of Lyme disease; both over- and underdiagnosis. Although incidence and prevalence are not interchangeable in this disease with low case-fatality rate, it is worth examining incidence data to gain an appreciation of the variability within the USA. In areas of endemicity, the reported annual incidence ranges from 20 to slightly more than 100 cases per 100,000 people, but may be as high as 1000 cases per 100,000 people in areas of hyperendemicity such as Lyme, Connecticut. In addition, children aged 5–10 years have an incidence rate which is almost twice as high as the incidence among older children and adults [1•]. This must be taken into consideration when assessing positive and negative predictive value of serologic testing.

Accuracy, Reproducibility

Currently, there are over 70 approved commercial laboratories that perform serologic testing for Lyme disease. Reproducibility between laboratories is not universal. In addition, serologic testing is also available from unapproved sources, in which accuracy/reproducibility has not been assessed. This has lead to even more confusion in the literature and among practitioners and patients regarding the interpretation of positive tests. Among infectious diseases consultants, it is well known that certain unregulated laboratories utilize techniques that are prone to result in higher rates of positive testing than when compared to approved laboratories. Unfortunately, these laboratories may be utilized by some groups who aim to demonstrate that Lyme is vastly underdiagnosed and requires treatment in patients who test negative by standard methodologies.

Conclusions

In summary, taking all the above factors into consideration, the *strengths* of Lyme serologic testing methodology include the following:

1) High sensitivity and specificity IF a two-tiered approach is utilized in the correct clinical setting (early disseminated

disease, particularly in setting of aseptic meningitis, facial palsy or carditis, or late disease).

2) High positive and negative predictive value IF used in the correct clinical setting AND the test is used in a high prevalence population.
3) Wide availability (over 70 FDA-approved commercial labs), relatively inexpensive, and relatively fast turnaround (usually several days).

However, there are a multitude of potential *weaknesses*:

1) Mass concern regarding Lyme disease (perpetuated by the media) has led to an increase in inappropriate testing for nonspecific symptoms, resulting in higher rates of false-positive testing and lower positive predictive value. The end effects of overdiagnosis include inappropriate antimicrobial therapy, inappropriate "labeling" of patients with disease with concomitant psychological effects, and the risk of missing alternative diagnoses that may be the true underlying etiology of patients with nonspecific symptomatology.
2) In lower prevalence areas, even if the test is applied in the appropriate clinical setting or stage of disease, the positive predictive value will be low, although high negative predictive value will be retained.
3) The complexities of applying serologic testing correctly and interpreting results lead to both over- and underdiagnosis. Clinicians must utilize serologic testing at only certain clinical stages of disease (not early localized disease), using the correct methodology (two-tiered testing), and then also interpret results correctly (IgM versus IgG time limits, appropriate number of WB bands). There are multiple steps where error may occur. Consultants in hyperendemic areas of disease, who are frequently asked to interpret serologic testing results, are witness to almost every permutation of testing utilized in practice: Some patients only undergo ELISA testing with no confirmatory WB (overly sensitive, nonspecific, poor positive predictive value). Some patients undergo only WB testing (potentially nonspecific, also poor positive predictive value). Both scenarios have important implications for interpretation and resultant recommendations for treatment.
4) Some physicians/patients perceive Lyme serologic testing to be an effective screening test, applied to a healthy population to detect disease, whereas serology was designed and evaluated as a diagnostic test, for the detection of disease in a diseased population. When used as a screening test, overdiagnosis is to be expected and will lead to the ill effects summarized above, including waste of precious health-care resources.
5) To make matters more confusing, both Lyme IgM and G can remain positive for years, and in some patients, indefinitely, leading to even further difficulty in interpretation of results. For example, CDC guidelines clearly state that IgM positivity beyond 4 weeks of initial infection, or at most 6 weeks based on the latest prospective data, cannot be used to make the diagnosis of acute infection. However, many clinicians utilize IgM testing months into nonspecific symptoms as evidence of current active disease.
6) In addition, some clinicians utilize serial serologic testing following standard antimicrobial therapy in an attempt to document "test of cure," which is clearly inappropriate, since like any other serologic response, are expected to persist as a marker of immune response, not a marker of active disease. This misconception often leads to increased anxiety, prolonged treatments, and a multitude of risks related to invasive (often intravenous) therapy for "refractory disease."
7) The lack of an accepted gold standard by which to gain agreement on sensitivity/specificity will continue to perpetuate the Lyme wars. Identification of the organism by culture or PCR is not widely available nor positive in all types/stages of disease, thus does the identification of "true positives" continue to be an area of controversy. For example, PCR is positive only "often" in the setting of Lyme arthritis [14], and a study comparing serum PCR to serologic testing showed no increased sensitivity over serologic testing [15].

The Future The research arena is ripe for the development of better diagnostic technologies. One strategy under evaluation is the utilization of different combinations of tests, still relying on a two-tiered approach. For example, one group evaluated a combined standard IgG Western blot with the addition of a VlsE band as the second-tier test [16]. The same group more recently evaluated the use of two enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay [17]. Another strategy has been the quest for a single test that does not require a two-tiered approach. For example, one group recently demonstrated that a comparison of a newer multiplex assay for VlsE1-IgG and pepC10-IgM antibodies compared to Western blot was equally specific (95.6 %) but over 20 % more sensitive for early-convalescent-phase disease (89.0 % versus 68.3 %), performing as well as or better as a second-tier test [18]. The performance of rapid diagnostic tests for Lyme has not been promising to date [19]. Very recently, the CDC has published the establishment of a serum repository to facilitate Lyme disease diagnostic test development and evaluation [20].

Ultimately, a single tier, more easily interpretable diagnostic modality, combined with better education of the public and physicians is needed to optimize the diagnosis of Lyme disease.

Compliance with Ethics Guidelines

Conflict of Interest Roberta DeBiasi has no disclosures relevant to this work.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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