Limitations and Confusing Aspects of Diagnostic Testing for Neurologic Lyme Disease in the United States

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ABSTRACT In the United States, laboratories frequently offer multiple different assays for testing of cerebrospinal fluid (CSF) samples to provide laboratory support for the diagnosis of central nervous system Lyme disease (CNSLD). Often included among these diagnostic tests are the same enzyme immunoassays and immunoblots that are routinely used to detect the presence of antibodies to *Borrelia burgdorferi* in serum. However, performing these assays on CSF alone may yield positive results simply from passive diffusion of serum antibodies into the CSF. In addition, such tests are only U.S. Food and Drug Administration cleared and well validated for testing serum, not CSF. When performed using CSF, positive results from these assays do not establish the presence of intrathecal antibody production to *B. burgdorferi* and therefore should not be offered. The preferred test to detect intrathecal production of antibodies to *B. burgdorferi* is the antibody index assay, which corrects for passive diffusion of serum antibodies into CSF and requires testing of paired serum and CSF collected at approximately the same time. However, this assay also has limitations and should only be used to establish a diagnosis of CNSLD in conjunction with patient exposure history, clinical presentation, and other laboratory findings.

KEYWORDS cerebrospinal fluid, diagnostic tests, Lyme disease, neurologic Lyme disease

The Centers for Disease Control and Prevention (CDC) reported over 36,000 cases of confirmed or probable Lyme disease in the United States in 2016 (<https://www.cdc.gov/lyme/stats/tables.html>). When unreported cases are considered, however, the total number of cases occurring annually has been estimated to be between 240,000 and 444,000 (1). Lyme disease, caused by tick-borne transmission of pathogenic members of the *Borrelia burgdorferi sensu lato* complex (e.g., *B. burgdorferi sensu stricto* [*B. burgdorferi*], *Borrelia garinii*, *Borrelia afzelii*, *Borrelia bavariensis*, *Borrelia mayonii*, etc.), can involve multiple different organ systems, including the central nervous system (CNS). The principal manifestation of CNS involvement is a lymphocytic meningitis, but rarely, meningoencephalomyelitis may develop; here, these entities will be referred as CNS Lyme disease (CNSLD) (2). CNSLD is only a component of neurologic Lyme disease. The peripheral nervous system may be involved, with or without CNSLD, resulting in peripheral facial nerve palsy, radiculopathy, and other manifestations. Neurologic symptoms frequently develop 4 to 6 weeks following a tick bite, although ranges of 1 to 12 weeks postinfection have been reported (3).

Diagnosis of CNSLD can be challenging. First and foremost, the risk of infection, including exposure history (e.g., prior travel to a region where Lyme disease is endemic) and time of year (e.g., lack of tick activity during late winter months in areas where Lyme disease is endemic [except in California]) should be considered, and CNSLD

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lowered on the differential diagnosis for patients at low risk of infection. For patients with appropriate exposure history and symptoms consistent with CNSLD, examination of cerebrospinal fluid (CSF) is necessary to establish the diagnosis. The majority of studies evaluating patients with neurologic Lyme disease originate from northern Europe. Cases often arise there due to infection with *B. garinii* or *B. bavariensis*, which are neurotropic species of the *B. burgdorferi sensu lato* complex that do not exist in the United States (4, 5). Characteristic CSF abnormalities in patients with CNSLD include lymphocytic pleocytosis and mildly elevated protein levels. Such CSF findings, however, are nonspecific and do not distinguish CNSLD from viral meningoencephalitis or other infectious and noninfectious conditions. Targeted testing to confirm *Borrelia* spirochete infection is therefore required to establish the diagnosis. This commentary will focus on vetted diagnostic assays for CNSLD, including culture, detection of Lyme *Borrelia* DNA using nucleic acid amplification tests (NAATs), and serologic testing.

Culture of CSF for Lyme *Borrelia* is not recommended for multiple reasons, including the requirement for specialized culture media and procedures that are not routinely maintained in clinical or reference laboratories. Additionally, Lyme *Borrelia* species grow slowly in culture, thus, limiting the clinical utility of this method during the acute phase of disease. More importantly, however, CSF culture is associated with extremely low sensitivity, ranging from 10% to 30%, in European patients with CNSLD (Table 1) (3, 6, 7). Detection of Lyme *Borrelia* DNA is similarly insensitive and is also not routinely recommended (8). There are currently no U.S. Food and Drug Administration (FDA)-cleared NAATs for Lyme disease, making all available molecular tests for Lyme *Borrelia* laboratory-developed tests with variable performance characteristics. A recent review of 22 studies evaluating the performance characteristics of different NAATs for detection of Lyme *Borrelia* DNA in CSF from European and U.S. patients with presumed CNSLD showed that the median sensitivity was only 22.5% (range, 5% to 100%), although the specificity was high (>99%) (Table 1) (9). These findings indicate that reliance on NAATs for routine diagnosis of CNSLD should be avoided, and that such testing should be considered an adjunct methodology only. Additionally, detection of CXCL13, a B cell-attracting chemokine, in CSF has been proposed as a biomarker to support the diagnosis of CNSLD (10–12). However, although CXCL13 CSF levels are elevated in patients with CNSLD, the presence of this chemokine does not provide sufficient specificity to distinguish CNSLD from other neuroinflammatory syndromes, and levels can rapidly fall below the cutoff threshold if antibiotic therapy is initiated prior to CSF collection (10–13). Additionally, testing for CXCL13 in CSF is not routinely offered by clinical laboratories in the United States.

Currently, serologic testing for antibodies to Lyme *Borrelia* in serum is recommended to aid in the diagnosis of CNSLD and other extracutaneous manifestations of Lyme disease, using the standard two-tier testing algorithm (STTA) and preferably using FDA-cleared assays (14). This algorithm, however, might not establish a diagnosis of CNSLD for multiple reasons. First, up to 15% of patients with CNSLD, particularly those presenting soon after symptom onset, may not have detectable antibodies to *B. burgdorferi* in serum (15–17). Second, it is well established that reactivity by the STTA may simply reflect background antibody seropositivity, which can potentially exceed 9% in some populations in the United States, with even higher rates in parts of Europe (3, 11, 18). On the other hand, patients presenting acutely, but having just exceeded the 4-week cutoff for use of the IgM immunoblot, might be considered to have a negative serologic test result if only the IgM immunoblot were positive.

Testing of CSF alone by either the STTA, enzyme immunoassays (EIAs), and/or immunoblots for diagnosis of CNSLD is unequivocally not recommended. Nevertheless, an informal survey in 2017 of 7 commercial or hospital-based laboratories by one of us (P.V.A.) showed that 6 (86%) offered such testing. The challenges associated with serologic testing of only CSF include the lack of FDA-cleared assays for detection of antibodies to *B. burgdorferi* in CSF (serum is the only approved specimen source for these assays). Also, the interpretive criteria for the STTA, specifically for assessment of the supplemental immunoblots, were defined and validated for detection of antibodies

TABLE 1 Diagnostic testing of CSF for Lyme disease of the central nervous system in the United States

Test	Advantage(s)	Concerns or disadvantages
Culture	Proof of active infection.	Delay in time to positivity; false positive results have been reported; testing is very insensitive and not routinely offered by clinical laboratories.
NAAT	Proof of active infection.	False positives may occur and insufficient sensitivity to exclude the diagnosis.
CXCL13	Often elevated in patients with CNSLD.	Insufficient specificity to distinguish CNSLD from other neuroinflammatory syndromes; also, CXCL13 levels can rapidly fall below the cutoff threshold if antibiotic therapy is initiated prior to CSF collection; not routinely available through U.S. laboratories.
Enzyme immunoassay	None	May be positive due to passive antibody diffusion from blood.
Immunoblot	None	May be positive due to passive antibody diffusion from blood and lack of interpretive criteria for evaluation of CSF.
Intrathecal antibody detection	Often positive; positivity may precede serum antibody positivity.	Poorly standardized; positive results do not prove active infection.

in serum, not in CSF, where the targeted *B. burgdorferi* epitopes and development of the immune response may differ from what is observed in serum. Additionally, approximately 0.5% to 1.0% of peripheral blood IgG normally diffuses into the CSF, and this percentage increases in patients with a dysfunctional blood-brain barrier, making it challenging to determine whether anti-*B. burgdorferi* antibodies detected in CSF are present due to intrathecal antibody synthesis (and are therefore suggestive of CNSLD), or whether they are present due to passive antibody transfer (19). Factors that influence the CSF concentration of proteins from blood include the serum concentration, the age of the patient, the anatomic site where the CSF was obtained, the volume of CSF obtained, presence and extent of blood contamination of the specific CSF sample submitted for protein level determination, and the CSF flow rate (20). To reduce the potential contribution from serum proteins, the aliquot or tube of CSF submitted for analysis should not be the first out of the several customary tubes collected during the CSF tap procedure, since the first tube is most likely to have blood contamination. Unfortunately, attention to the tube order for testing might not always be considered and is not communicated when sent to the laboratory. In recognition of these interpretative challenges, published guidelines from the Infectious Diseases Society of America (IDSA), the American Academy of Neurology, and the European Federation of Neurologic Sciences instead recommend testing for intrathecal production of antibodies to *B. burgdorferi sensu lato* via determination of an antibody index (AI), using paired CSF and serum samples (Table 2) (3, 14, 21).

However, the AI is available through very few commercial and academic laboratories in the United States. Establishing an AI by an individual laboratory is complicated by the lack of FDA-cleared tests for this purpose, and therefore any Lyme AI assay offered through laboratories in the United States is considered a laboratory-developed test. Consequently, some commercial laboratories have validated their own workflow processes using previously defined algorithms for calculation of a Lyme AI, using patient populations with presumed CNSLD (since there is no gold standard for this entity) (20, 22, 23). Of note, some of these laboratories have validated Lyme AI methods using CSF and serum samples collected from European patients with CNSLD, which differs in its presentation and causative pathogen(s) compared to CNSLD occurring in the United States. While the Lyme AI has been studied extensively in European patient populations and is a part of the criteria for establishing definite CNSLD in Europe, the Lyme AI has not been well-validated in U.S. patients, where its performance characteristics may differ. We are unaware of a systematic comparison of the different AI testing procedures used in the United States for detection of intrathecal antibodies to *B. burgdorferi* performed on the same CSF and serum specimens. A comparison study of the different commercially available Lyme *Borrelia* AI assays would be desirable to ensure that results are consistent across performing laboratories in the United States.

At its core, the AI used to diagnose CNSLD is essentially a ratio comparing the level of anti-*B. burgdorferi* antibodies in CSF versus serum, normalized to total immunoglobulin levels in both specimen sources, in order to correct for blood-brain barrier permeability. While an AI can be determined for any antibody class, most Lyme *Borrelia* AI studies have evaluated IgG-based assays (24, 25). Determination of an AI requires collection of paired serum and CSF samples, preferably collected near the same time of each other in order to avoid variability in antibody levels that may be introduced if samples are collected days apart or that may be associated with normal CSF turnover, which occurs approximately once every six hours (26). CSF and serum samples are subsequently tested for anti-*B. burgdorferi*-specific IgG-class antibodies using a quantitative EIA, while total IgG and albumin (an analyte not produced in the CNS) levels are determined using standard laboratory assays. Although specific testing algorithms may vary between laboratories, the Lyme AI is determined using these values within the context of equations that take into account normal CSF flow and passive diffusion rates, upper limits of total IgG in CSF, and other parameters—a detailed discussion of which is beyond the scope of this article (17, 23, 27, 28). Briefly, however, the CSF-to-serum ratios for total IgG and albumin are established and compared. If the total IgG ratio is

TABLE 2 Advantages and limitations of the Lyme antibody index

Advantage(s)	Limitation(s)
Differentiates between intrathecal synthesis versus passive diffusion of specific anti- <i>Borrelia</i> antibodies in CSF. High sensitivity (>95%) in patients with ≥6 weeks of symptoms, according to studies performed in European patients with CNSLD.	Need for submission of CSF and serum samples collected around the same time. There are no FDA-cleared assays for determining a Lyme AI. Assays, methods and cutoffs for establishing the Lyme AI are not standardized and vary between laboratories in the United States. The performance characteristics of these assays in the U.S. patients with CNSLD are not described Available through very few laboratories in the United States, mostly reference commercial labs. Sensitivity ranges from 70% to 90% in patients with <6 weeks of symptoms. False-positive results may occur, particularly in patients with neurosyphilis. Remains positive for months to years following resolution of disease and therefore cannot be used as a standalone test to establish acute vs past infection or to establish cure. Lyme AI results cannot be relied upon solely to make a diagnosis of CNSLD

less than or equal to the albumin ratio, this indicates the absence of intrathecal total IgG synthesis, in which case, the patient's total IgG CSF-to-serum ratio is used to calculate the *Borrelia*-specific AI. Alternatively, if the total IgG CSF-to-serum ratio is greater than the albumin CSF-to-serum ratio, this suggests the presence of intrathecal total IgG synthesis (unrelated to CNSLD *per se*). In these situations, an upper discrimination limit for the total IgG CSF-to-serum ratio is calculated, and this corrected ratio, rather than the original total IgG CSF-to-serum value, is used to establish the *Borrelia*-specific AI. Based on these calculations, a Lyme AI of >1 indicates that the level of anti-*B. burgdorferi* antibodies detected in CSF is higher than would be expected solely due to passive diffusion of antibodies from blood (i.e., intrathecal antibody synthesis) (27). The precise AI cutoff levels interpreted as positive, negative, or equivocal vary among laboratories (24, 29).

As an alternative to this AI method, antibody capture EIAs may be utilized, in which the ratio of anti-*B. burgdorferi*-specific versus total antibody (of the same class) is determined separately for both CSF and serum (24, 30). Similarly to the indirect EIAs, a positive result is considered when the AI is >1 . The advantage of the antibody capture assay is that determination of total immunoglobulins in serum and CSF is not necessary, since class specific anti-*B. burgdorferi* antibodies will be captured in the assay. Disadvantages include the cumbersome nature of the assay, limited availability at reference laboratories, and failure to correct for intrathecal total IgG synthesis, as discussed above. Irrespective of the particular AI assay methodology utilized, an elevated AI value in a patient with appropriate tick exposure history, symptoms consistent with CNSLD, and CSF lymphocytic pleocytosis is strongly suggestive of CNSLD.

Finally, immunoblots have also been used to assess intrathecal antibody production by testing serum and CSF in parallel at equivalent immunoglobulin concentrations (or at concentrations after correction for intrathecal total IgG synthesis) (31). Intrathecal production of antibodies to *B. burgdorferi sensu lato* can be confirmed by detecting greater CSF reactivity, as determined by a higher number and/or intensity of bands compared to those of the corresponding serum immunoblot.

Performance characteristics of the AI to detect intrathecal synthesis of IgG-class antibodies to *B. burgdorferi sensu lato* are variable across studies, most of which were conducted in Europe, with reported sensitivity values ranging from 70% to 100% (16, 24, 25, 29, 32). This range in sensitivity can be attributed to multiple different factors, including the use of different assays and testing algorithms, the duration of patient symptoms or treatment prior to testing, and the specific Lyme *Borrelia* species causing the infection, among others. Generally, however, sensitivity of the AI is considered to range from 70% to 90% in patients with CNSLD with less than 6 weeks of symptoms, whereas it is 95% or higher in untreated patients with longer disease duration (11, 33). Although less frequently evaluated than IgG, the sensitivity of IgM- or IgA-based Lyme *Borrelia* AIs ranges from 42% to 74% across studies (16, 24, 25).

An important limitation of testing for intrathecal production of antibodies to *B. burgdorferi sensu lato*, particularly IgG-based assays, is that the AI may remain positive for years after successful antibiotic treatment and cannot be used as a "test of cure" (Table 2) (34). Additionally, given the widespread use of oral doxycycline for empirical treatment of possible Lyme disease and possible human granulocytic anaplasmosis (also transmitted by *Ixodes* species ticks), coupled with increasing evidence of the efficacy of oral doxycycline for all manifestations of neurologic Lyme disease, there is likely to be a background rate of intrathecal antibody positivity, just as there is for antibody positivity in serum. Furthermore, although the specificity of the AI has been reported to be high ($>95\%$), cross-reactivity and false-positive results can occur, particularly among patients with neurosyphilis (29). Finally, intrathecal antibody production may occur as part of a polyspecific immunologic response, especially in the setting of chronic inflammatory diseases that are completely unrelated to *B. burgdorferi* infection, and not all Lyme AI calculation methods currently used in the United States are able to correct for this phenomenon (20). It is therefore important to consider a

positive AI for intrathecal production of antibodies to *B. burgdorferi sensu lato* in the context of exposure history and other clinical and laboratory findings.

In conclusion, CNSLD in the United States may be misdiagnosed if incorrect laboratory testing on CSF is ordered. Inappropriate test utilization, and, as a result, increased cost, unneeded antibiotic therapy, and undue patient anxiety, can be minimized if laboratories eliminate the availability of inappropriate assays (e.g., EIAs or immunoblots) for evaluation of CSF in cases of suspected CNSLD. Ideally, laboratories offering testing for CNSLD in the United States would provide testing exclusively for intrathecal production of anti-*B. burgdorferi* antibodies, using a validated AI assay. Developing a Lyme *Borrelia* AI is challenging, however, due to multiple factors, including lack of access to paired CSF and serum samples from patients with confirmed CNSLD, the need for immunoglobulin class-specific EIAs and EIAs using a better source of antigens, such as recombinant antigens, coordinating testing of paired CSF/serum samples for multiple analytes (i.e., anti-*B. burgdorferi* antibodies, total IgG, and albumin) across different areas of the laboratory, the need for instruments that can measure immunoglobulins with the level of sensitivity needed for CSF, and establishing a simplified and controlled process to perform AI calculations for high-throughput testing. Importantly, results from Lyme *Borrelia* AI assays should not be used alone, but rather in conjunction with patient exposure history, clinical presentation, and other laboratory findings to establish a diagnosis of CNSLD. Finally, developing and validating an AI assay for FDA clearance is warranted.

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