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Review

To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis

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ABSTRACT

Background: Lyme borreliosis (LB) is a tick-borne infection caused by *Borrelia burgdorferi sensu lato*. The most frequent clinical manifestations are erythema migrans and Lyme neuroborreliosis. Currently, a large volume of diagnostic testing for LB is reported, whereas the incidence of clinically relevant disease manifestations is low. This indicates overuse of diagnostic testing for LB with implications for patient care and cost-effective health management.
Aim: The recommendations provided in this review are intended to support both the clinical diagnosis and initiatives for a more rational use of laboratory testing in patients with clinically suspected LB.
Sources: This is a narrative review combining various aspects of the clinical and laboratory diagnosis with an educational purpose. The literature search was based on existing systematic reviews, national and international guidelines and supplemented with specific citations.
Implications: The main recommendations according to current European case definitions for LB are as follows. Typical erythema migrans should be diagnosed clinically and does not require laboratory testing. The diagnosis of Lyme neuroborreliosis requires laboratory investigation of the spinal fluid including intrathecal antibody production, and the remaining disease manifestations require testing for serum antibodies to *B. burgdorferi*. Testing individuals with non-specific subjective symptoms is not recommended, because of a low positive predictive value. **R.B. Dessau, Clin Microbiol Infect 2018;24:118**

Introduction

Lyme borreliosis (LB) is a tick-borne disease caused by spirochaetes of the *Borrelia burgdorferi sensu lato* complex, hereafter

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referred to as *B. burgdorferi*. Case definitions for Europe with descriptions of the various clinical manifestations of LB have been published to establish guidelines for clinical diagnosis [1,2]. Despite these definitions, laboratory testing for *Borrelia*-specific antibodies continues to be frequently used in many clinical situations where testing is not recommended by current guidelines [3–8]. For example in the Netherlands only 9% of the patients tested had clinical symptoms designated in the guidelines [5]. In Denmark, 43% of samples from general practice originated from patients with suspected erythema migrans [4]. Unnecessary testing may delay proper diagnosis and treatment and increases healthcare costs. In Germany, the annual cost of laboratory testing for LB in the outpatient sector was estimated to be €51 million, a substantial portion of which obviously resulted from over-testing [3]. This study based on insurance reimbursements also documents a high rate of antibiotic treatments. The consequences of over-testing for Lyme borreliosis, have not been documented directly in clinical studies. The main concerns are delay of other relevant diagnoses, adverse effects and the development of antibacterial resistance. In an effort to address these issues a position paper on when to order a laboratory test for LB is timely and relevant.

Knowledge of the clinical presentation, pathology and immunology of LB is a prerequisite for the appropriate use of testing. There is no convincing evidence that *B. burgdorferi* produces any toxin, and it has been well established that the pathology is elicited by the host's innate and specific immune responses [9,10]. Typical mechanisms of immune evasion include complement regulation, antigenic variation, down-regulation of immunogenic surface proteins and invasion of protective niches in the connective tissue. This enables the pathogen to persist in the host and occasionally protracted disease such as chronic Lyme neuroborreliosis or acrodermatitis chronica atrophicans may result. The molecular detection of *Borrelia* DNA in human specimens varies greatly in sensitivity between studies, depending on tissue type and the duration of clinical disease [11,12]. The number of spirochaetes in a biopsy from erythema migrans ranges from 1 to 10^4 organisms per μg of tissue [13]. The sensitivity of PCR testing on skin biopsies declines from around 80% in the first weeks after onset of disease to 20% in patients with duration of months before the skin biopsy was performed [12]. For cerebrospinal fluid (CSF) the sensitivity is lower and is estimated, at around 40% [14]. Therefore, detection of antibodies to *B. burgdorferi* is currently the laboratory method of choice in a routine clinical setting. Infection with *B. burgdorferi* induces an immune response with clinical findings, such as skin lesions, neurological signs, cardiac involvement (e.g. atrioventricular block), or arthritis involving the large joints [9,15]. Although clinical presentations may vary, the diagnosis of LB requires the presence of objective signs of disease as described in the current European case definitions [1]. The key points for diagnosis and choice of diagnostic testing are presented in Box 1 and Table 1.

Evidence background for the review

The intention of the present review is to promote a reflective understanding of how the laboratory methods may support the clinical diagnosis of LB. The format of narrative review was chosen to present an educational paper considering broadly the available evidence and presenting the expert synthesis of this evidence. Members of the author group have been involved in both national and international guideline development, and the scientific evidence and strength of recommendations have been considered in these publications. Notably, concerning diagnostic accuracy of serological testing, two systematic reviews have recently been published [16,17].

Box 1
Key points for diagnosis and choice of diagnostic testing

Key points

- Diagnosis of Lyme borreliosis is based on a complete diagnostic workup, including medical history with compatible clinical symptoms, objective signs, possible exposure to tick bites, and exclusion of other diseases, but not laboratory testing alone.
- Patients with a typical erythema migrans should be diagnosed clinically and treated promptly without serological testing, which is insensitive at this stage of disease.
- Pathology is elicited by the host immune response. Detection of antibodies to *Borrelia burgdorferi* is necessary to support the clinical diagnosis of Lyme borreliosis manifestations other than erythema migrans.
- In patients with suspected Lyme neuroborreliosis examination of cerebrospinal fluid is strongly recommended.
- The use of *Borrelia* serology in patients with non-specific subjective symptoms is discouraged.
- In patients with disease duration >6 weeks a specific IgG response is a prerequisite, but an isolated IgM response is of no diagnostic relevance.
- Detection of antibodies to *B. burgdorferi* cannot discriminate between active, latent or past infection.

Types of laboratory tests for Lyme borreliosis

- *Serology*: Detection of specific IgG and IgM antibodies is recommended for routine laboratory testing for Lyme borreliosis. This is because direct detection of the pathogen in clinical samples has a lower sensitivity.
- *DNA*: Molecular detection of pathogen DNA is recommended as a supplementary diagnostic method for special indications such as further diagnostic confirmation in cases with atypical cutaneous manifestations or suspected Lyme arthritis.
- Other test principles cannot be recommended for general routine use or are discouraged.

When to order detection of *B. burgdorferi* antibodies?

The clinical manifestations and choice of testing for *B. burgdorferi* serum antibodies are listed in Table 1. As the diagnosis of typical erythema migrans is clinical and does not require serological confirmation, treatment may start promptly. Approximately 50% of patients with erythema migrans remain seronegative. If the skin lesion is not typical for erythema migrans, further differential diagnostic evaluation is necessary. Laboratory testing for antibodies to *B. burgdorferi* in serum is necessary for diagnosing suspected manifestations of LB such as Lyme carditis, borrelial lymphocytoma, Lyme arthritis, acrodermatitis chronica atrophicans and possibly other rare LB manifestations [1,2].

Ordering any serological testing should follow a consideration of the current European case definitions to achieve the highest possible pre-test probabilities and clinical predictive values [1,2]. As a general rule, antibodies are expected to develop in almost all patients (>99%) (Table 1) within 6–8 weeks [1,18–24]. Concerning clinical specificity, the natural background or cross reactivity of

Table 1
Testing for antibodies to *Borrelia burgdorferi* to support the diagnosis of suspected Lyme borreliosis.

Clinical suspicion of	Detection of antibodies to *B. burgdorferi*	Expected sensitivity[c]	Incidence per 100 000 population[d]
Tick bite	Not relevant	Not relevant	High
Erythema migrans	Not recommended	50% (40–61)	10–100
Lyme neuroborreliosis Clinical duration <6 weeks	Specific CSF/serum antibody index	77% (67–85)	<10
Lyme neuroborreliosis Clinical duration >6 weeks	Specific CSF/serum antibody index	>99%	
Long-lasting Lyme neuroborreliosis >6 months	Specific CSF/serum antibody index	>99%	<1
Lyme carditis	Serum IgG and/or IgM	>80%	<1
Borrelial lymphocytoma	Serum IgG and/or IgM	>80%	<1
Lyme arthritis	Serum IgG	96% (93–100)	<1
Acrodermatitis chronica atrophicans[a]	Serum IgG	98% (84–100)	1
Ocular manifestations[b]	Serum IgG	?	Rare

[a] Including acrodermatitis chronica atrophicans-associated peripheral neuropathy.
[b] Specialist care only, also consider other diagnoses.
[c] Sensitivities from the systematic review of European studies are indicated with 95% CI for erythema migrans and Lyme neuroborreliosis and with median and range for Lyme arthritis and acrodermatitis chronica atrophicans [65].
[d] Incidences are approximate magnitude and vary in different parts of Europe.

Borrelia-specific antibodies in otherwise healthy individuals remains relatively low, but not negligible, in the majority of the European population with a modern urbanized lifestyle. In a nationwide German survey, 9.4% of the general population were found to have IgG antibodies to *B. burgdorferi* [25]. However, background immunity above 20% has been described in selected populations with outdoor activities such as forest workers, hunters and residents in geographical hotspots [26–30]. Therefore, physicians should be aware of the local seroprevalence relevant to the diagnostic tests used in a given geographical region.

Importantly, the negative predictive value of negative test results is high because of the generally low disease incidence in the population and a low pre-test probability. This is why a negative test may effectively rule out infection with *B. burgdorferi* if symptoms persist for longer than 6 weeks. When LB is considered unlikely and the objective symptoms do not meet the case definition criteria, laboratory testing should not be performed at all to avoid irrelevant or possibly false-positive test reports, and other differential diagnoses should be taken into account or ruled out [31,32]. There are reports of immunocompromised LB patients without a detectable antibody response [33–35]. However, this may be coincidental, and a larger series of samples would be required to establish if a lower test sensitivity would apply in these special groups of patients.

Detection of antibodies to B. burgdorferi in CSF

When Lyme neuroborreliosis is suspected, the CSF should be examined for signs of inflammation and intrathecal antibody production (antibody index, AI) to *B. burgdorferi* determined by analysing paired serum and CSF samples obtained on the same day. Determination of the AI is more specific than detection of antibody reactivity in either serum or CSF alone [1,2,22,36–39]. However, an elevated AI may persist for several years post-infection even after successful treatment. Hence, confirmation of active Lyme neuroborreliosis requires the presence of additional signs of CSF inflammation such as pleocytosis. The diagnostic sensitivity of the AI is about 80% in patients with shorter duration (<6–8 weeks) of clinical disease and nearly 100% when the disease duration has been longer [2,19,20,22,37–40].

IgM antibodies

IgM antibodies to *B. burgdorferi* are relevant for detecting early infection, but their detection does not contribute to the serodiagnosis of late LB [1]. In some individuals, an IgM antibody response can persist for months or even years after treatment or past infection, although this phenomenon is not associated with a (persistent) infection with *B. burgdorferi* [41–44]. Diagnostic use of stand alone IgG assays may be sufficient if highly sensitive screening tests including VlsE antigen are used. In this instance, IgM detection appears to have no significant advantage over IgG testing in the recognition of early LB and may actually reduce the specificity of diagnostic testing, although this may depend on the antigen mix applied in the assay [45–47]. In patients with long-lasting disease manifestations, such as chronic Lyme neuroborreliosis, acrodermatitis chronica atrophicans, or Lyme arthritis, only the detection of IgG antibodies to *B. burgdorferi* should be considered diagnostic. For these LB manifestations the presence of an IgG response is a must in the diagnosis according to the current case definitions [1]. An isolated persisting IgM response clearly speaks against a long-lasting infection or late manifestation of LB according to the case definitions [1].

Two-tier testing

A combination of tests may perform better than one test alone [48]. The stepwise diagnostic approach uses an ELISA to screen for antibodies and, if positive, an immunoblot serves to support the findings of the first tier test. The immunoblot technique separates the different *Borrelia* antigens and typically, reactivity against at least two or more antigens is required for a positive interpretation of the assay concerning IgG, but for IgM OspC alone may be sufficient [49,50]. The characteristic spectrum of bands, particularly in the IgG immunoblot, also provides evidence to divide the immune response into an early and a late stage so that a better correlation can be made between the laboratory findings and the clinical symptoms. Hence a narrow spectrum of bands with antibodies against early-phase antigens (e.g. VlsE, OspC, p41) is typically compatible with an early manifestation (e.g. facial palsy), or a brief latent infection. However, it does not point to persistent clinical symptoms [49–52]. In contrast, a wide spectrum of bands, including late-phase antigens (e.g. p100, p17/p18) fits well with late manifestations (e.g. arthritis, acrodermatitis chronica atrophicans) [51]. Both patterns of antibody responses are also consistent with asymptomatic persistence of antibodies (past infection). Re-infections are difficult to diagnose based solely on serological findings without additional clinical information and can only be detected based on a verifiable increase of IgG antibodies in a

parallel approach, or significant changes in the immunoblot banding pattern in serum samples that have been tested in parallel. Although under debate, in the USA two-tier testing for LB using a screening assay and immunoblot as the second tier is an official requirement based on a consensus conference in 1995 [23,53,54]. This principle is also part of many current guidelines in laboratory testing for LB in Europe [19,46,49,55–59]. The use of an immunoblot as a first line or stand-alone test is not recommended. Choice of antigens and interpretative rules may vary among different guidelines and between manufacturers of such assays. Comparable interpretation of such tests in clinical practice has been difficult [59,60]. The two-tier approach may suffer from a loss of sensitivity depending on the individual sensitivities and specificities of the applied test combinations. This is especially true for localized and early phases of LB with a less pronounced immune response directed against a narrower spectrum of specific and non-specific *Borrelia* antigens. It is also unclear which combinations of ELISA and immunoblots are optimal [61], and standardization is difficult [3,51,62]. A recent systematic review of diagnostic accuracy evaluating two-tier testing found that the studies fulfilling the inclusion criteria were few in number and the specificity was not found to be improved concerning serum from patients with erythema migrans and Lyme neuroborreliosis [16]. Diagnostic studies for two-tier algorithms for the late-stage manifestations of LB such as Lyme arthritis and acrodermatitis chronica atrophicans were not included in the systematic review. The second tier of testing is often described as "confirmatory". This, however, applies to the specificity in the analytical detection of *Borrelia* antibodies, but not to the specificity of the correct clinical diagnosis of *Borrelia* infection. It is important to recognize for clinical interpretation that patients may remain immunoblot-positive for years following past infection. Supplementary immunoblot of CSF is not required and is not documented for the diagnosis of Lyme neuroborreliosis. Two-tier testing is not used routinely in all European countries and local preferences and recommendations should be followed concerning two-tier testing.

Indeterminate results

There is no available standard on how to define or how to interpret indeterminate results. There is no reason to repeat the test with the same technique, as reproducibility of modern automated ELISA is high with only small random variations in measurement results. Reproducibility of immunoblot pass rates for proficiency testing have been reported to give the same intended result in 82% of 239 participating laboratories [3]. Depending on the clinical manifestation and duration, an indeterminate or negative test result could be further evaluated by a follow up.

Does it make sense to repeat the serological test?

As explained above, disease development depends on the immune response, and most patients will already have detectable antibodies at the time of clinical presentation, except in patients with clinical disease of short duration. Therefore, in practice, retesting only rarely detects a change. In seronegative patients with a short duration of Lyme neuroborreliosis or borrelial lymphocytoma serological follow up may be helpful to detect seroconversion, which can further substantiate the diagnosis of LB, but cases of erythema migrans often remain seronegative after treatment. Moreover, as outlined above, in some patients IgM antibodies may persist for months or even years after a *Borrelia* infection, and the IgG response may even persist for life [41]. After therapy, the production of IgG and IgM antibodies may vary individually and the individual immune response cannot be correlated with the clinical course of the disease or the success of antibiotic treatment [63,64]. However, a three-fold change in antibody reactivity level may be considered beyond the measurement error of a typical ELISA [65]. As a general rule, a statement on the significance of changes in serological findings can only be made if initial and follow-up samples have been analysed in the same laboratory [49].

How to interpret diagnostic serology results?

Before using diagnostic tests in clinical practice, theoretical considerations on positive and negative predictive values (PPV and NPV) need to be taken into account. The NPV is the ability of a diagnostic test to rule out disease [66]. An important question is whether a test can rule out LB even if the sensitivity is not 100%? The answer is 'yes', with a large degree of certainty as justified by reasonable assumptions in test statistics. If the sensitivity of a diagnostic ELISA is >95% then <5% of cases might be overlooked. However, since the prevalence (pre-test probability) of LB among patients with clinical signs of arthritis could be estimated at about 2% [4], the risk of overlooking a true case of Lyme arthritis is very small ($0.05 \times 0.02 = 0.001$). Consequently, the NPV would be around 99% or higher even at pre-test probabilities up to 10%–20% (Fig. 1).

Another example is the serological diagnosis of late Lyme neuroborreliosis defined with a duration of disease >6 months. In a study of 187 consecutive patients with Lyme neuroborreliosis 6% were found to have late disease [39]. Conservatively assuming a sensitivity of 98% of the AI in cases of late Lyme neuroborreliosis, the NPV is 99.9%. Given the fact that approximately 180 cases of Lyme neuroborreliosis are reported in Denmark each year [67], the extrapolation of these incidence data suggests that only one patient will be overlooked every 5 years. However, the clinical diagnosis of Lyme neuroborreliosis is not solely based on AI determination but also on the clinical symptoms according to the case definitions [1,2].

In conclusion, the NPVs of ELISA are high due to a combination of exceptional sensitivity for disease of >6 weeks duration and low pre-test prevalence. Therefore, a negative serological result in a case of long-lasting disease has a high certainty to be correct and should not be repeated or 'substantiated' by other methods.

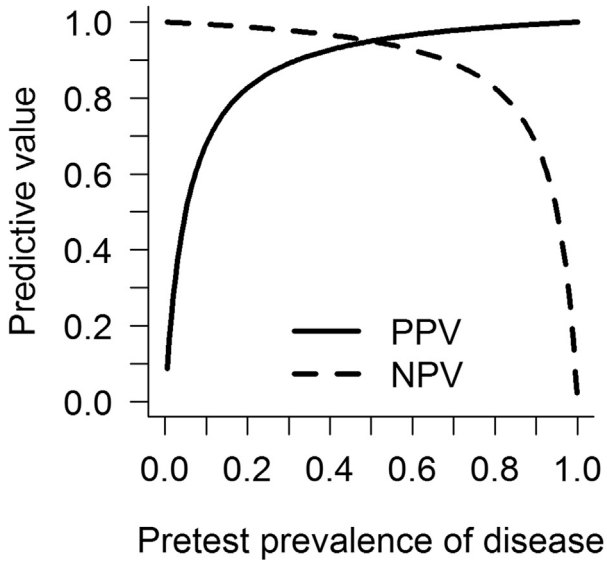

Fig. 1. The theoretical relationship of pre-test prevalence of disease to the positive and negative predictive values (PPV/NPV) in a test with 95% sensitivity and 95% specificity. Computation based on Bayes theorem [66].

The PPV is the ability of a diagnostic test to rule in the diagnosis of a possible disease. What is the contribution of a positive test result for the diagnosis of LB, even if sensitivity is not quite 100%? As a rule of thumb, the PPV is <5% when the pre-test probability (prevalence of disease in the tested population) remains <5%. The pre-test probability (disease prevalence) should approach 10% (Fig. 1) before ordering a diagnostic assay to reach a reasonable PPV of 50%–80% [46,68]. For example, given the clinical and diagnostic findings in patients with a combination of positive AI and CSF pleocytosis, the PPV for a diagnosis of Lyme neuroborreliosis can be estimated to be >90% [24,39,69]. In contrast, when the pre-test probability is <5%, the PPV will also be <5% and a false-positive test result is highly probable. As a consequence of these considerations, clinicians are advised to avoid serological testing whenever the clinical symptoms are not indicative of LB according to the case definitions [1,2]. This will avoid positive test results that have no clinical meaning in patients with non-specific symptoms and a low pre-test probability for LB.

Direct detection of *Borrelia burgdorferi*

The detection of *Borrelia* by culture or by molecular methods has a variable and suboptimal sensitivity (Table 2) due, for example, to the scarcity of the bacterium in clinical samples, heterogeneity of the species and problems with sampling procedures. However, detection of *Borrelia* DNA by PCR is provided at many centres as a complementary test to antibody detection methods because it may provide diagnostic support in atypical disease manifestations. PCR may be used to analyse skin biopsies, synovial fluid/tissue or CSF but not blood or urine samples [14,19,49,70,71]. In clinical samples (Table 2) the sensitivity of PCR is best for skin biopsies of erythema migrans with a median of 69% but lower for other types of clinical samples like CSF with medians of 40%. Concerning detection of *Borrelia* DNA in CSF, blood and urine, the variation in sensitivity of the published studies is 0%–100%. Hence, the clinical sensitivity must be considered unknown and there is concern about methodological issues in the published studies, especially concerning urine samples [14]. The low sensitivity has been further substantiated by low rates of positivity in 23 777 consecutive routine samples [72]. The diagnostic value of molecular diagnostics in patients with LB therefore remains uncertain and should be restricted to expert laboratories [1]. If DNA is detected in a seropositive patient with a compatible clinical manifestation then the PPV is expected to be high. However, the clinical decision to diagnose and treat LB should not be based on PCR alone, as false-negative results, but also false-positive results, may occur. For example, a positive PCR in a seronegative patient with long-lasting non-specific symptoms appears highly implausible. Furthermore, direct detection of *Borrelia* DNA cannot unequivocally establish whether or not there is an active infection [73]. Even after successful treatment, *Borrelia* DNA may persist for months in the skin or in synovial fluid, although negative cultures and absence of detectable RNA indicate that viable organisms are absent [73]. Guidelines consider molecular methods for the detection of *Borrelia* as supplementary diagnostic methods for special indications, such as further diagnostic confirmation in cases with atypical cutaneous manifestations or suspected Lyme arthritis [1].

Table 2
Sensitivities and specificities of PCR based on the references reviewed by AP van Dam [14]

Sample	Number of studies (groups)	Sensitivity (%)	Specificity (%)
Skin biopsy EM	11	69 (35–81)	100
Skin biopsy ACA[a]	3	16, 61, 92	100
CSF	17	40 (5–100)	100 (93–100)
Synovial fluid[a]	3	36, 85, 85	100
Serum or plasma	5 (6)	30 (15–100)	100 (95–100)
Urine	14 (9)	42 (0–92)	97 (94–100)

Abbreviations: ACA, acrodermatitis chronica athrophicans; EM, erythema migrans. Median and range were calculated from Tables 1–5 in reference [14]. Specificities are from fewer studies, as not all studies had a control group using clinical samples. Patients with Lyme borreliosis tested with serum, plasma or urine have a variation of clinical manifestations and may have more than one patient group per study.
[a] Three reported sensitivities only.

CXCL13

CXCL13 is a B lymphocyte attractant chemokine that is detectable in high concentrations in the CSF of patients with definite Lyme neuroborreliosis, but rapidly declines during antibiotic therapy [69,74,75]. However, CXCL13 may also be detected in some other diseases, such as neurosyphilis, human immunodeficiency virus infection and lymphoma [76,77]. Diagnoses in most cases of Lyme neuroborreliosis are perfectly supported by AI and pleocytosis, but CXCL13 can be of use in suspected very early cases (<2 weeks), if the AI is not yet positive, and to differentiate an active from a subsided disease (activity marker) provided that testing is done before antibiotic treatment [75]. This novel biomarker needs special expertise and a clear cut-off has not been defined so far [2,78].

Other types of diagnostic tests

Currently there are no other reliable and clinically relevant tests available to diagnose LB. Cellular tests such as lymphocyte transformation or activation tests should not be used until they have been adequately assessed [2,79–81]. The available data do not support a high diagnostic accuracy. Tests such as CD57 cell count and dark-field or phase-contrast microscopy have no rationale and have been found to be non-specific [82–84].

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* Key references for suggested further reading are 1, 2, 14, 15, 16, 19 and 39.

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