Clinical Microbiology and Infection 31 (2025) 1307–1312

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative review

The choice of study designs of diagnostic accuracy using *Borrelia* specific IgG and IgM antibodies for the diagnosis of Lyme borreliosis

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ARTICLE INFO

Article history:
Received 26 January 2025
Received in revised form
20 March 2025
Accepted 2 April 2025
Available online 7 April 2025

Editor: S.J. Cutler

Keywords:
Case-control design
Cross-sectional design
Diagnostic accuracy
Diagnostic antibody tests
Diagnostic test accuracy
Lyme borreliosis
Research design
Serology
Systematic review

ABSTRACT

Background: Laboratory diagnosis of Lyme borreliosis (LB) is used in a variety of clinical settings where a range of other diagnoses may be considered. Therefore, it is essential that diagnostic accuracy studies and literature reviews consider information from different types of studies and choices of sample groups. The quality of patient selection is important to minimize the risk of misclassification. This narrative review was inspired by systematic reviews where nearly all studies on the diagnostic accuracy for LB tests were determined as biased and having low quality based solely on study design considerations—not the clinical relevance.

Objectives: To propose flexible design and interpretation of studies used to assess diagnostic accuracy in clinical microbiology.

Sources: Criteria for rating the quality of studies were discussed among the ESCMID study group for LB ESGBOR (The ESCMID study group for Lyme borreliosis). The literature was searched for similar methodological discussions.

Content: Knowledge of antibody reactivity in the background population across various clinical patient groups with and without infection should consider variations in clinical presentation and duration of disease. Case-control studies are the most frequently used design and were judged particularly instrumental in assessing serologic testing. However, clinical and epidemiological studies not specifically intended for diagnostic accuracy may also contribute estimates of sensitivity and specificity. Systematic reviews should focus on the application of the diagnostic assay for the individual patient in various

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https://doi.org/10.1016/j.cmi.2025.04.001

clinical settings, rather than seeking an unbiased average. Different LB sample groups and controls for test panels are discussed.
Implications: Case-control (two-gate design) studies, case series, and seroprevalence studies representing the range of LB in different populations are necessary to assess the diagnostic accuracy of serological tests for LB. A broader range of studies should be considered for inclusion in systematic reviews of diagnostic accuracy. **Ram Benny Dessau, Clin Microbiol Infect 2025;31:1307**

Introduction

The diagnosis of Lyme borreliosis (LB) should be based on the complete clinical evaluation with fitting clinical findings, but is supported by laboratory testing. Laboratory diagnosis of LB often rests on detecting of antibody responses because direct detection of the infecting organism is not feasible in most clinical situations [1]. However, this is a weakness for clinical diagnosis because serological response may be from previous infection or actual infection. Humoral response in early infection can be delayed and for early LB (erythema migrans) laboratory testing is not recommended. Thus, the diagnosis of LB should not rest on antibody testing alone.

As laboratory testing can play a pivotal role in the diagnosis of disseminated LB, test accuracy is a fundamental consideration. Therefore, the design of individual diagnostic test accuracy (DTA) studies for detecting *Borrelia burgdorferi* sensu lato-specific antibodies and the inclusion of the existing literature in systematic reviews are important to discuss.

The essential quality for a DTA study for LB is that it should give relevant information for the interpretation of the test result in the context of the individual clinical setting [1].

One approach to achieve this goal would be to use a case-control design choosing a spectrum of case groups and controls. Furthermore, studies highlighting various other aspects, such as treatment studies, case series, seroprevalence and epidemiological studies, may provide important information for individual clinical diagnosis. However, these approaches are not in line with the approaches by tools such as QUADAS (Quality Assessment of Diagnostic Accuracy Studies) [2]. QUADAS is a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews [2–4]. The QUADAS-2 tool for diagnostic accuracy studies emphasizes the risk of bias and states that diagnostic accuracy studies are often characterized by markedly heterogeneous results originating from differences in the design and conduct of included studies. A signalling question on design is: “Was a case-control design avoided?” implying that this type of study has poor quality [5]. Of consequence, in systematic reviews on the diagnostic accuracy of LB, the main critique has been risk of bias due to the prevalent use of case-control design instead of cross-sectional studies. In a systematic review of 78 evaluated studies, all were classified as having risk of bias in all four QUADAS-2 domains [6]. Another example was a diagnostic test review when the NICE (National Institute for Health and Care Excellence, in the UK) guidelines were formulated [7]. For example, all 123 included studies concerning initial test for LB were classified as having VERY LOW Quality, with some of the cross-sectional studies having just LOW quality [8,9]. Therefore, the question is whether all LB-DTA studies are inappropriate for their purpose or if the assessment criteria defined in the QUADAS framework need adaptation concerning the use of serology to support the diagnosis of infectious diseases like LB.

The aim of this narrative review is to discuss both the design of individual studies and systematic reviews and propose flexible design and interpretation of studies used to assess diagnostic accuracy in clinical microbiology and LB in particular.

Study design

Case-control (two-gate) study design offers several advantages. They are practical. feasible and cost-effective, as the number of samples may both be relatively easily to collect fulfilling statistical strength. The crucial issue lies in careful reporting of how samples were collected and cases and controls were defined. The two types of study design share the same selection method for cases with disease, but differ considerably concerning selection of controls and cases without the disease in question (Fig. 1).

The challenge with the cross-sectional design is that it depends on the organizational and referral patterns determining the clinical pathway and composition of the tested population. This preselection of samples may significantly impact the rate of samples with antibody reactivity in the controls without LB (selection bias) [10]. Even if this allows for predictive values to be calculated based on unbiased average disease prevalence, this will hardly be generalizable beyond the study population or a very similar study population. Moreover, average estimates will not be useful for diagnosis in the individual clinical case with a large variation in individual risk of tick exposure, disease duration, seasonal presentation and clinical signs. Negative and positive predictive value calculations are rather a feature of disease prevalence in the selection of patients than a measure of DTA [1,11].

Choice of control groups (specificity)

Rates of positive samples in persons without LB may vary with the criteria for selection. Examples are presented below and in Table S1.

Background seroprevalence

Many patients with suspected LB are from the general population in primary care, often consisting of individuals who are physically active, in contact with nature and generally in good health (Table S1). Thus, background seroprevalence in the healthy population that varies according to age, geographic location and social factors is useful information [12,13]. A German study, employing diagnostic methods for routine LB diagnosis, showed an overall IgG seroprevalence of 4.8% in a large cohort of children and showed that natural background immunity increases with age [14]. This study used an ELISA (Enzyme-linked immunosorbent assay) assay (Enzygnost Lyme link VlsE/IgG, Siemens Healthcare Diagnostics GmbH), which was used in many laboratories and was well described in the literature. This product has been discontinued, but the rate of IgG seropositivity was similar to other assays currently used in many laboratories [10]. The absolute seroprevalence could vary between assays, but the relative tendencies stratified by age and sex should theoretically show similar trends. Adults may have a higher seroprevalence increasing with age [15].

Similar data on background seropositivity from many countries may be extracted from epidemiological studies and included as

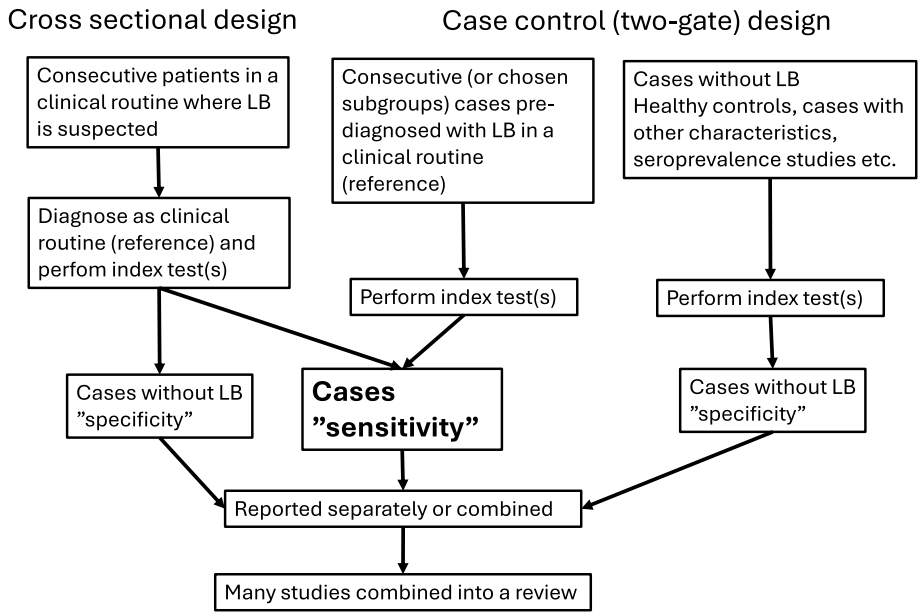

Figure 1. Flow diagram showing the design of a cross sectional study and case control. The "index test" designates the test being investigated whereas as "reference" is the diagnostic procedure used as a standard, if applicable.

control groups in DTA studies. Usually, such studies are excluded from systematic reviews of diagnostic accuracy, in spite of a very detailed reporting, representative recruitment and large sample numbers [13,15].

Diagnosing LB in focal areas with high endemicity or in patients with individual high risk of tick exposure requires local knowledge generally not found in the literature. The local laboratory or clinic may contribute information about antibody prevalence.

Challenge samples

The next choice is to challenge using samples from persons with different non-LB conditions (e.g. other spirochetal diseases and autoimmune conditions; see Table 1 and Table S1) known to stimulate antibody reactivity. This increases the risk of antibody reactivity unrelated to an infection with *B. burgdorferi* s.l.

Choice of LB cases (sensitivity)

Types of studies that may be considered for sensitivity are proposed in Tables 1 and S2. The cases should follow established clinical case definitions using appropriate interpretation of laboratory testing [1,16,17]. The reference standard for LB relies on both clinical judgement and laboratory results. The clinical process should be detailed, displaying what criteria the patients fulfilled and perhaps listed in a supplementary appendix. When considering Lyme neuroborreliosis (LNB) a table of the spinal fluid data could qualify the selection, especially with information on cerebrospinal fluid pleocytosis and intrathecal antibody synthesis. Cases without pleocytosis in the cerebrospinal fluid should be considered critically, even if they fulfil European Federation of the Neurological Societies criteria as possible cases [17,18]. Clinical information on the disease duration before sampling is important, as antibody

Table 1
Studies that can be used to assess the performance of diagnostic testing for Borrelia specific antibodies. See also appendix tables S1 and S2 for further details and discussion.

Various populations to assess specificity and background seroprevalence	
Type of control group	Diagnostic accuracy parameter
Population controls	Highest obtainable specificity
Blood donors/healthy controls	Highest obtainable specificity
Routine consecutive samples (winter season)	Specificity in the tested population, assuming negligible risk of LB in the winter season.
Samples from nonendemic population	True or analytical specificity
Challenge samples	Specificity in selected samples with antibody reactivity known to potentially cause cross reactivity.
Consecutive samples from patients without LB (clinically or laboratory selected) including convenience samples	Specificity in the clinical situation, with cases having other diseases.
Various populations to assess sensitivity	
Type of case group	Diagnostic accuracy parameter
Any clinical manifestation of LB	Sensitivity
Erythema migrans	Sensitivity in early disease
Lyme neuroborreliosis	Sensitivity in disseminated disease
Lyme arthritis	Sensitivity in disseminated disease
Acrodermatitis chronica atrophicans	Sensitivity in late disease
Other LB manifestations	Not feasible, few patients, reference definition is missing. Diagnosis is often uncertain.
Atypical cases	Difficult to interpret in context of diagnostic accuracy due to possible diagnostic misclassification

LB, Lyme borreliosis.

development is time dependent. It could also be considered to include an independent review process of the included cases, preferably by colleagues with clinical experience of LB. Serological testing is not recommended for clinical diagnosis of erythema migrans (EM). However, samples from EM cases are often used as differences in sensitivity are most likely to be found in early disease and the case definition is independent of serology.

Atypical or questionable cases

Patients may be treated for presumed LB without fulfilling case definitions, when another diagnosis has not yet been established [19]. This patient group is not relevant in a DTA, as they often are preselected for seropositivity and may include an unknown or even high proportion of misclassified patients without LB [10]. The size of this proportion will depend on referral patterns and the health care organization. Thus, estimates of sensitivity based on such patients are not meaningful DTA studies. Serology is generally of little use in these situations as the positive predictive value is very low [1]. If an individual patient has *Borrelia* specific antibodies, then knowledge of the background seroprevalence is important to consider. A Danish study showed that in patients with diffuse musculoskeletal and neurological complaints the rate of *Borrelia* specific IgG seropositivity was very low and at the same level as the healthy population at the initial clinical evaluation in general practice [20]. Other examples are the variations in the clinical presentation of dermatoborreliosis or uncharacteristic initial presentations of LNB [21,22]. If there is *B. burgdorferi* sensu lato infection in the tissue, the stochastic variation of antibody development should be similar regardless of the visual presentation of the skin lesion.

Comparing assays

Comparison of assays in DTA assessment should depend on the same proposals for the selection of samples. Retrospective, stored samples are instrumental (Table 1) as they are readily available and may be just as representative as prospective sample collection. However, the selection process should be described carefully to assess if these stored samples represent a subgroup of patients with some special characteristics, such as samples with low antibody levels, or if guided by the results of previous testing. Quantitative measurements and ROC (receiver operating characteristic) curve analysis are important and aid in comparing the performance of diagnostic assays. Differences between assays can be due to the choice of cut-offs, rather than assay performance per se [10,23]. Samples with low level-reactivity around the cut-off should not be excluded, and the term indeterminate should refer to interpretation and not the measurement itself. It is important to note that differences between assays in positive/negative interpretation often involve these low reactivity samples.

Examples of studies that are not designed for DTA

Case series

Clinical case series describing consecutive well-diagnosed cases of LB are important, as they may present details of clinical features and, though not being DTA studies, give useful information to assess sensitivity. An example of such a study is a clinical description of 187 consecutive LNB cases [24]. The study describes variations in clinical presentation, and the serological results are carefully documented, including seronegative and borrelial intrathecal antibody synthesis negative results with a motivated explanation for why these cases are considered LNB. Thus, the reference standard is carefully documented. The study was not considered for inclusion in a systematic review as controls were documented in another study [6,25]. A large cohort from a centre where the main focus is the long-term outcome of LNB is an excellent study to use for assessing sensitivity of serology as the whole spectrum of clinical presentations of LNB are included [26]. Further examples of high-quality clinical case series could be added here. For example, a study of EM combined with neurological symptoms delineates many details to considered in the individual patient and, at the same time, gives useful information about serological tests [27].

Treatment studies

Randomized controlled treatment studies (or clinical series) offer another sound opportunity to evaluate serological performance. These studies are also clinically based on case series, usually with clearly described inclusion criteria and attentively managed. They often also include the subgroup of possible cases and represent a cohort of consecutively treated cases, for example, in this Norwegian study 70% had intrathecal Bb antibody production, indicating the assay's sensitivity [28]. In patients with a short duration of clinical disease, antibody response has not yet developed.

Reporting standards

STARD (Standards for Reporting of Diagnostic Accuracy) is a reporting guideline for DTAs listing essential items for reporting diagnostic accuracy studies [29]. A commented version of the checklist is presented in appendix 2 to highlight important points to consider when writing the DTA manuscript.

Discussion

We propose that the interpretation of serology for LB needs to be based on findings in case and control groups from a range of clinical scenarios, including studies not primarily designed for testing of diagnostic accuracy, such as case series, treatment studies and seroprevalence studies. This proposal has implications for the planning of individual studies and subsequent reports and reviews summarizing the available evidence. These reports should focus on explaining heterogeneity in various studies rather than reflexively writing off heterogeneity as poor quality and unwanted bias. Such an approach aligns with the Cochrane Handbook stating that *"Systematic reviews of test accuracy should aim to address clinically relevant questions for which knowing a test's sensitivity and specificity, or other accuracy measures, is important"* [30]. It is also stated that "*Test accuracy is not a fixed property of a test: accuracy describes the performance of a test in specific circumstances. The accuracy of a test may therefore vary with the intended use…*" In addition, "*a relevant objective may therefore be to investigate potential sources of heterogeneity,*" which implies considering subgroups of cases and controls. Whether a meta-analysis is relevant and how to design such a study are matters of an interesting discussion [31]. A meta-analysis in many cases does not fit into this scenario. A previous review and meta-analysis for LB serology showed heterogeneity between studies [6,32]. A restriction to be discussed is exclusion of high-quality controlled trials and case series without a non-LB control group, where the primary outcome is not diagnostic accuracy.

Another issue about the detection of antibodies is that there are two levels. Level one is the analytical, where modern methods appear quite accurate for detecting specific antibodies - if they are present in the sample. Indeed, the analytical variation between laboratories and assays is expected to be small [10,33]. The other level is clinical, and case selection probably explains most of the heterogeneity concerning sensitivity and specificity. Sensitivity will

depend on disease presentation, especially the duration of the clinical disease.

In general, investigators have used a variation of designs for diagnostic accuracy studies [34]. Developing criteria for the literature search and inclusion of studies is considered by the Cochrane collaboration [30,35]. The statement of 'bias' should require the unbiased situation to be described and defined [5]. If the unbiased setting does not exist, the concept of bias has no meaning.

This narrative review has focused on serology, but for direct detection of *Borrelia* (e.g. PCR) case-control design is also applicable. Recommendations such as using a statistically sufficient number of samples has been proposed by WHO that sample size should be at least 100 sera, but this may not be possible if occurrence is rare [36].

Similar considerations may be relevant for a broad range of infectious diseases with diagnostic uncertainty.

Conclusions

In routine practice of clinical infectious disease (including LB), each patient is diagnosed and treated according to individual considerations. Case-control studies, case series and seroprevalence studies representing different populations are proposed as choices of study design for diagnostic accuracy studies and for inclusion in systematic reviews. Cross-sectional studies are only relevant, if an unbiased representative clinical context can be defined and may be generalized.

Ethical statement

The manuscript is a narrative review citing published studies.

Author contributions

Discussion and opinion paper by the ESGBOR (The ESCMID study group for Lyme borreliosis www.escmid.org/esgbor). R.B.D. has prepared and drafted the manuscript and collected references. All authors have contributed to sections of the manuscript, critically read and commented extensively on draft versions of the manuscript and approved the final version. Conceptualization was a topic at ESGBOR meetings, also discussed with colleagues outside the author group.

Transparency declaration

Potential conflict of interest

R.B.D. has participated in Pfizer advisory board meetings. A.R. has participated in manuscript writing for Elsevier and support for congress participation from ELIVIE and MSD. V.F.—consultant activities Pfizer and Global Lyme Alliance. A.J.H.—research projects with Abbott diagnostics, Reagena Oy, and Pfizer. B.J.—Elsevier manuscript writing and travel expenses ESCMID teaching course. M.M.—presentations with Diasorin, Mikrogen, and Biomed Austria. The remaining authors have declared no conflict of interest.

Acknowledgements

No specific external funding was received. ESGBOR (The escmid study group for Lyme borreliosis) has received support from ESCMID.

Appendix. A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.04.001.

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