Comparison of five different immunoassays for the detection of *Borrelia burgdorferi* IgM and IgG antibodies

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ABSTRACT

The performances of five commercially available enzyme immunoassays were compared for the detection of *Borrelia burgdorferi* IgM and IgG antibodies. Sensitivity was assessed with European serum samples collected from 45 patients with clinically defined Lyme disease in conjunction with a positive immunoblot ($n = 44$) or other serological test ($n = 1$). Sensitivities for the detection of IgM and IgG with each test were: Dako IgM 64%; Dako IgG 53%; Serion IgM 89%; and Serion IgG 88%. The Immunetics assay makes no distinction between IgM and IgG antibodies and had a sensitivity of 91%. Specificity was calculated by testing a control group comprising 40 patients with acute Epstein–Barr virus infection, cytomegalovirus infection, syphilis or rheumatoid factor positivity. The specificities achieved for each test were: Dako IgM 78%; Dako IgG 100%; Serion IgM 52%; Serion IgG 92%; and Immunetics 92%. The discriminatory power between control and patient samples appeared highest for the Immunetics assay. Between-run variation was comparable for the five tests and did not exceed 13%. When the Immunetics assay was used as an initial screening test, with low-titre positive results confirmed by an immunoblot, a sensitivity of 91% and a specificity of 100% were achieved. To attain maximal sensitivity, the Serion IgM and IgG tests were also performed on samples with negative Immunetics results. All positive Serion IgM and IgG results were also confirmed by immunoblot. In conclusion, the Immunetics assay, based on a synthetic C6 peptide, can be used reliably as an initial screening test for the serodiagnosis of Lyme disease.

Keywords *Borrelia burgdorferi*, diagnosis, enzyme immunoassays, immunoblot, sensitivity, serodiagnosis

INTRODUCTION

Lyme borreliosis is an infectious disease with multi-organ involvement, including skin, nervous system, joints and heart [1–7]. The illness is caused by a tick-transmitted spirochaete, *Borrelia burgdorferi sensu lato*, with three subspecies that are known to be pathogenic to humans: *B. burgdorferi sensu stricto*, *Borrelia afzelii* and *Borrelia garinii* [8,9]. Three stages of the disease can be distinguished: early localised, early disseminated, and late persistent. Diagnosis of Lyme borreliosis on the basis of clinical symptoms is difficult, as

this disease can mimic a wide range of disorders [7]. Laboratory tests, such as culture, genomic amplification and serology, are therefore frequently necessary. Culture is only of value with biopsy samples of skin lesions, and is seldom positive with cerebrospinal fluid and plasma samples [10], while molecular techniques lack sensitivity, and are only superior to culture for joint fluid analysis [10]. Thus, laboratory diagnosis relies mostly on detecting a serological response [11–13].

Accurate tests with a high predictive value are of great importance during the early phases of the disease, as a short course of antibiotics administered at that time may be curative [7,14]. A delay in the diagnosis increases the risk of protracted symptoms, with an uncertain outcome, even after prolonged courses of antibiotics [7]. Current guidelines for Lyme serological testing in the

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USA and Europe rely on a two-tiered approach in which sera are first screened with a sensitive enzyme immunoassay (EIA), with positive results confirmed by western immunoblotting [11,13]. This approach improves specificity, but is expensive and technically difficult, and the final interpretation requires previous experience [10,15]. For these reasons, replacement of the two-tiered protocol with a sensitive, specific, objective and less expensive test is warranted.

In recent years, a substantial effort has been made to develop new serological tests using synthetic and recombinant antigens [16–18]. However, tests that have optimal predictive values in the USA appear to be of limited use in Europe, since only *B. burgdorferi sensu stricto* is present in the USA, whereas all three *Borrelia* subspecies are encountered in Europe [19–21]. However, new serological tests that could also be of value in Europe have now been made available [22]. The aim of the present study was to assess the diagnostic performance of new commercially available assays for the detection of IgM and IgG antibodies against *B. burgdorferi* in samples from European cases of early localised and early disseminated Lyme borreliosis. Clinically defined criteria were used, in combination with a positive test result in a recombinant immunoblot, as the reference standard to calculate sensitivity [23]. Specificity was determined by analysing sera from patients with infections mimicking Lyme disease, or with conditions that are known to interfere with the assay. The study also evaluated whether a single test or a combination of tests could replace the current two-tiered approach.

MATERIALS AND METHODS

Patient samples

Human serum samples were obtained from a panel of 23 patients with early localised cutaneous Lyme disease, comprising 22 patients with erythema migrans and one with a lymphocytoma, and a second panel of 22 patients with early disseminated Lyme disease, comprising patients with arthritis ($n = 2$), cranial neuritis ($n = 9$), radiculoneuropathy ($n = 3$), erythema migrans with clinical signs of dissemination ($n = 7$) and polyneuropathy ($n = 1$). These patients were admitted to the Maastricht University Hospital between 1 January 2002 and 31 December 2004. Patients from the first panel were all positive in the IgM blot assay, and 12 were also positive in the IgG blot assay. Other causes of symptoms were excluded for patients belonging to the second panel; all samples were IgG-positive and 13 were also IgM-positive in the blot assays, except that from a patient with a bilateral facial palsy, for whom the blot was both IgM- and IgG-negative, but who had a documented cerebrospinal fluid pleocytosis and intrathecal antibodies that were detected with an IgG EIA (Dako, Glostrup, Denmark).

Specificity was calculated with a patient control group comprising 40 patients with disorders known to interfere with *Borrelia* serology and/or to resemble Lyme disease clinically: ten patients with acute Epstein–Barr virus (EBV) infection, ten with acute cytomegalovirus (CMV) infection, ten with syphilis, and ten with rheumatoid factor positivity.

IgM and IgG immunoassays

Detection of IgM and IgG antibodies was compared using five commercially available enzyme immunoassays: (1) QuickELISA C6 Borrelia kit (Immunetics, Boston, MA, USA); (2) IDEIA *B. burgdorferi* IgM; (3) IDEIA *B. burgdorferi* IgG (Dako); (4) *B. burgdorferi* second-generation IgM; and (5) *B. burgdorferi* second-generation IgG (Serion, Wurzburg, Germany). All kits were used according to the instructions of their respective manufacturers. Assay characteristics are listed in Table 1. Borderline results were considered to be positive for the calculation of sensitivity and specificity.

Western immunoblot

The *Borrelia* recomBlot IgM and IgG assay (Mikrogen, Martinsried, Germany) is an immunoblot assay that uses recombinant proteins. The antigens used are OspA, OspC, p100, p39, p18 (decorin-binding protein A), p41 (flagellin), and a specific internal part of the p41 antigen. The blot was performed according to the instructions of the manufacturer.

Statistics

Sensitivity was defined as true-positives/(true-positives plus false-negatives), and specificity was defined as true-negatives/(true-negatives + false-positives). Graph Pad Prism v.4.0 for Windows was used for statistical analysis.

Table 1. Characteristics of the enzyme immunoassays for the detection of IgM and/or IgG antibodies against *Borrelia burdorferi*

Test	EIA	*B. burgdoferi* subspecies	Preabsorption[a]	Antigen	Incubation time (min)
Dako IgM	μ Capture	*B. afzelii*	No	Purified p41	130
Dako IgG	Indirect	*B. afzelii*	No	Purified p41	130
Serion IgM	Indirect	*B. afzelii B. garinii*	Yes	Whole-cell lysate rec p41 and p100	120
Serion IgG	Indirect	*B. afzelii B. garinii*	Yes	Whole-cell lysate rec p41 and p100	120
Immunetics IgM + IgG	μ Capture	*B. afzelii B. garinii B. burgdorferi*	No	Synthetic peptide C6	30

[a]Preabsorption with *Treponema phagedenis*.
EIA, enzyme immunoassay.

Table 2. Sensitivity and specificity of three enzyme immunoassays for the detection of *Borrelia burgdorferi* IgM antibodies in early localised and disseminated Lyme disease

Assay	Sensitivity % (95% CI)			Specificity % (95% CI)				
	Localised ($n = 23$)	Disseminated ($n = 13$)	Total ($n = 36$)	EBV ($n = 10$)	CMV ($n = 10$)	RF ($n = 10$)	TP ($n = 10$)	Total ($n = 40$)
Serion IgM	91 (87–96)	85 (81–89)	89 (85–93)	20 (18–22)	60 (56–63)	90 (86–94)	40 (37–43)	52 (49–56)
Dako IgM	61 (57–64)	69 (66–63)	64 (60–67)	40 (37–43)	90 (96–100)	100 (96–100)	80 (76–84)	78 (74–81)
Immunetics	91 (87–96)	85 (81–89)	89 (85–93)	80 (76–84)	100 (96–100)	100 (96–100)	90 (86–94)	92 (88–97)

EBV, positive for Epstein–Barr virus; CMV, positive for cytomegalovirus; RF, positive for rheumatoid factor; TP, positive for syphilis.

RESULTS

IgM enzyme immunoassay

The sensitivities of the Serion, Dako and Immunetics EIAs were evaluated for the detection of *Borrelia* IgM in all serum samples with IgM reactivity in the blot (Table 2). This included the 23 patients with localised Lyme disease and 13 patients with the disseminated form of the disease. The symptoms of these latter 13 patients were heterogeneous and consisted of cranial neuritis ($n = 5$), arthritis ($n = 2$), erythema migrans with signs of dissemination ($n = 3$) and radiculopathy ($n = 3$). In the localised and the disseminated phase, sensitivities of 91% and 85%, respectively, for the Serion EIA, and 91% and 85%, respectively, for the Immunetics assay, were calculated. These were significantly higher than the corresponding Dako EIA sensitivities of 61% and 69%, respectively.

The specificity for each assay was determined by analysing 40 sera from patients with an acute EBV or CMV infection, or who were positive for syphilis or rheumatoid factor, each of which are conditions that are well-known to cause cross-reactivity. Specificity was lowest for the Serion assay, compared with the Dako and Immunetics assays, mainly because of positive results with EBV, CMV and syphilis, with specificities of 20%, 60% and 40%, respectively, whereas the Serion assay maintained a specificity of 90% with rheumatoid factor positivity. In the Dako EIA, only EBV, CMV and syphilis caused false-positive results, with specificities of 40%, 90% and 80%, respectively. The Immunetics EIA showed a specificity of 90–100% for all other conditions, except for acute EBV infection, which decreased the specificity to 80%.

Data were expressed as the test value divided by the manufacturers' defined assay cut-offs in order to assess the discriminatory power of the three evaluated IgM EIAs between the control group ($n = 40$) and all IgM-positive patient samples ($n = 36$) (Fig. 1A). The geometric mean values of these ratios for the Serion, Dako and Immunetics assay determined in the control group were 0.92 (95% CI 0.64–1.32), 0.94 (95% CI 0.69–1.28) and 0.25 (95% CI 0.21–0.31), respectively, compared with 2.33 (95% CI 1.72–3.16), 2.36 (95% CI 0.41–3.93) and 6.65 (95% CI 4.17–10.61), respectively, in the patient
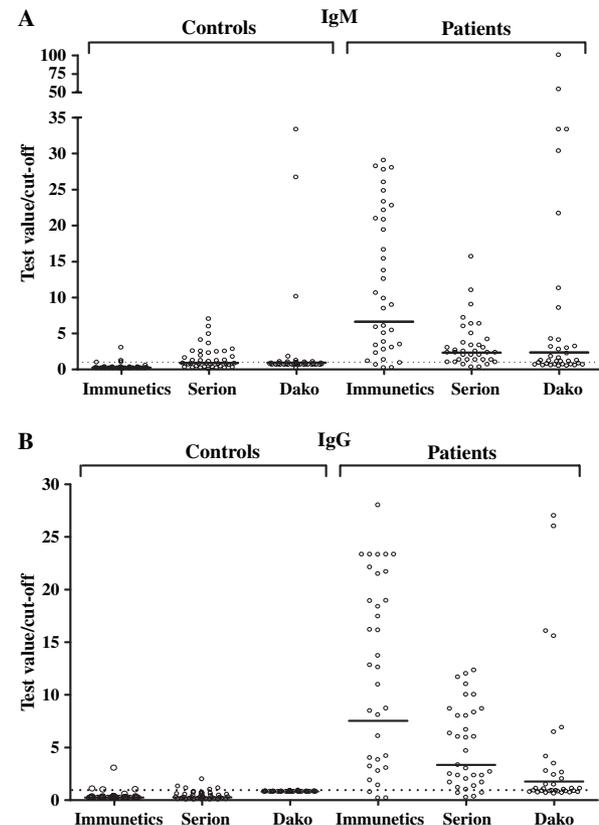

Fig. 1. Results of the *Borrelia burgdorferi* IgM enzyme immunoassays (EIAs) (A) and IgG EIAs (B) in sera from controls ($n = 40$) and Lyme disease patients ($n = 36$ for IgM results, $n = 34$ for IgG results). The levels of positivity for test values/cut-off values ≥ 1 are shown by a broken line. Geometric means are depicted for each assay by a solid line.

group. Relative to the control samples, the ratios of the patient samples were 2.53-, 2.51- and 26.6-fold higher using the Serion, Dako and Immunetics assays, respectively. The inter-assay coefficients of variation were determined for the Dako and Serion IgM assays by analysing the positive control samples delivered with the kit, and were found to be 9% ($n = 23$) and 7% ($n = 21$), respectively.

IgG immunoassay

The sensitivities of the Serion IgG, Dako IgG and Immunetics EIAs for detecting *Borrelia* IgG were evaluated with sera collected from 12 patients with early localised Lyme disease, and from 22 patients with the disseminated form of the disease (Table 3). IgG positivity was confirmed for each sample with the immunoblot, except for one patient with neuroborreliosis, who nevertheless fulfilled the diagnostic criteria of the disease. At the localised stage, a sensitivity of 92% was obtained with the Immunetics EIA, which was superior to the sensitivities of 83% and 42% obtained with the Serion and Dako EIAs, respectively. The Immunetics and Serion EIAs showed an equivalent high sensitivity of 91% during the disseminated phase of Lyme disease, whereas the Dako EIA reached a sensitivity of only 59%. The Immunetics assay failed to detect IgG in two patients. The first patient had a radiculopathy and a history of a non-treated erythema migrans, and showed strong and weak reactivity in the Serion and Dako assays, respectively. The second patient, presenting with a bilateral facial palsy, showed no reactivity in the Serion and Immunetics assays, but showed IgM with the Serion and Dako assays. The Serion assay also failed to detect IgG in a child presenting with a bilateral facial palsy, pleiocytosis in the cerebrospinal fluid, intrathecal IgG antibody synthesis (detected by the Dako assay), and a strong serum

reactivity with the Immunetics assay. Nevertheless, a high IgM antibody titre was detected in this patient by the Serion assay. The Dako assay failed to detect IgG in nine patients presenting with arthritis ($n = 1$), cranial neuritis ($n = 4$), erythema migrans with clinical evidence of dissemination ($n = 3$), and polyneuropathy ($n = 1$). All nine patients showed reactivity in the Immunetics and Serion IgG assays, but the Dako IgM assay remained negative for two of these patients.

Specificity was assessed by measuring reactivity in 40 samples from patients with an acute CMV and EBV infection, or who were positive for syphilis or rheumatoid factor (Table 3). A specificity of 100% was achieved by the Dako assay. The Serion assay had a high specificity of 90–100%, except for patients with syphilis, which caused a drop in the overall specificity to 80%. The Immunetics EIA also had a specificity of 90–100%, except for patients with an acute EBV infection, which decreased the overall specificity to 80%.

To assess the discriminatory ability of the different EIAs (Fig. 1B), the geometric mean of the test value was divided by the cut-off of each assay for the patient ($n = 34$) and the control group ($n = 40$). The geometric mean values of these ratios with the Serion, Dako and Immunetics assays for the control group were 0.26 (95% CI 0.18–0.38), 0.81 (95% CI 0.80–0.82) and 0.25 (95% CI 0.21–0.31), respectively, compared with 3.36 (95% CI 2.32–4.88), 1.77 (95% CI 1.2–2.6) and 7.53 (95% CI 4.74–11.95), respectively, for the patient group. Relative to the control samples, the ratios of the patient samples were 12.9-, 2.2- and 30.1-fold higher for the Serion, Dako and Immunetics EIAs, respectively. The inter-assay coefficients of variation for the Serion IgG, Dako IgG and Immunetics assays, obtained by analysing the positive control samples delivered with the kit, were 12% ($n = 21$), 13% ($n = 23$) and 13% ($n = 15$), respectively.

Table 3. Sensitivity and specificity of enzyme immunoassays for the detection of *Borrelia burgdorferi* IgG antibodies in early localised and disseminated Lyme disease

| Assay | Sensitivity % (95% CI) | | | Specificity % (95% CI) | | | | |
	Localised ($n = 12$)	Disseminated ($n = 22$)	Total ($n = 34$)	EBV ($n = 10$)	CMV ($n = 10$)	RF ($n = 10$)	TP ($n = 10$)	Total ($n = 40$)
Serion IgG	83 (79–87)	91 (87–95)	88 (84–92)	90 (86–94)	100 (96–100)	100 (96–100)	80 (76–84)	92 (88–97)
Dako IgG	42 (39–44)	59 (56–62)	53 (50–56)	100 (96–100)	100 (96–100)	100 (96–100)	100 (96–100)	100 (96–100)
Immunetics	92 (87–96)	91 (87–95)	91 (87–95)	80 (76–84)	100 (96–100)	100 (96–100)	90 (86–94)	92 (88–97)

EBV, positive for Epstein–Barr virus; CMV, positive for cytomegalovirus; RF, positive for rheumatoid factor; TP, positive for syphilis.

Combined IgM and IgG antibody testing

Overall, a combination of the IgG and IgM assays showed increased sensitivity compared with the corresponding IgM and IgG tests considered in isolation. However, the improved sensitivity was at the expense of specificity (Table 4). The best combination of specificity and sensitivity was achieved by the Immunetics assay, with a sensitivity of 91% and a specificity of 92% in samples with either IgM or IgG positivity or both (Table 4). Analysis of the receiver operating characteristic curve for the Immunetics assay indicated that increasing the cut-off level from 0.12 to 0.38 (data not shown) decreased the sensitivity to 80% while achieving a specificity of 100% (Table 4).

A sequential test protocol to restrict the number of confirmatory immunoblots

Based on the above data, a model of sequential testing was constructed in which the Immunetics assay was used as a screening test. Referring to the specificity data, a positive Immunetics assay with an OD of 0.12–0.38 is confirmed with an immunoblot, whereas an OD exceeding 0.38 is considered to be true-positive for the presence of antibodies to *B. burgdorferi* (Fig. 2). If the Immunetics assay is negative, testing of a follow-up serum or the Serion IgG and IgM tests should be performed (Fig. 2). This approach should reduce the total number of blots from 100% to 20% in a patient population with localised or disseminated

Table 4. Overall sensitivity and specificity for the detection of *Borrelia burgdorferi* IgM and/or IgG antibodies in early localised and disseminated Lyme disease

Assay	Sensitivity % (95% CI)			Specificity % (95% CI)				
	Localised (*n* = 23)	Disseminated (*n* = 22)	Total (*n* = 45)	EBV (*n* = 10)	CMV (*n* = 10)	RF (*n* = 10)	TP (*n* = 10)	Total (*n* = 40)
Serion IgG + Serion IgM	100 (96–100)	100 (96–100)	100 (96–100)	20 (18–22)	60 (56–63)	90 (86–94)	30 (28–32)	50 (47–53)
Dako IgG + Dako IgM	78 (74–82)	91 (87–95)	84 (80–88)	40 (37–43)	90 (96–100)	100 (96–100)	80 (76–84)	78 (74–81)
Immunetics	91 (87–96)	91 (87–95)	91 (87–95)	80 (76–84)	100 (96–100)	100 (96–100)	90 (86–94)	92 (88–97)
Immunetics results with OD > 0.38	78 (74–82)	82 (78–86)	80 (76–84)	100 (96–100)	100 (96–100)	100 (96–100)	100 (96–100)	100 (96–100)

EBV, positive for Epstein–Barr virus; CMV, positive for cytomegalovirus; RF, positive for rheumatoid factor; TP, positive for syphilis; OD, optical density.
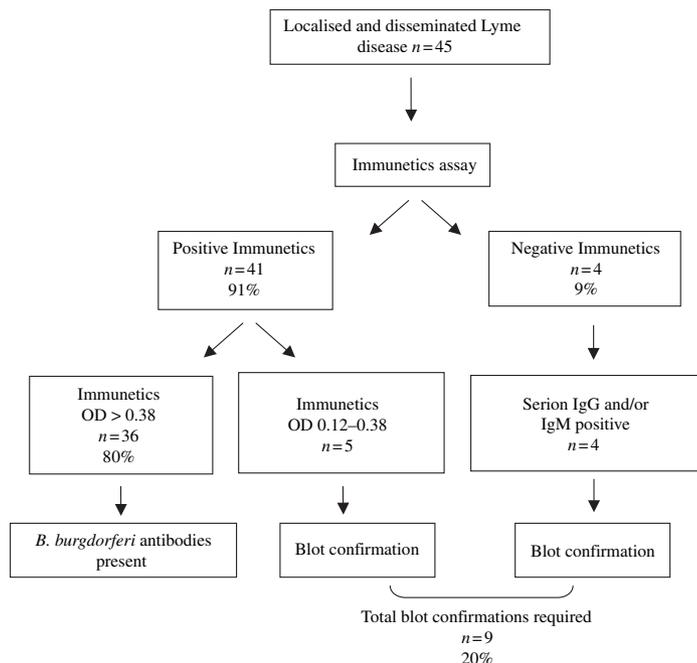

Fig. 2. *Borrelia burgdorferi* antibody testing in patients with localised and disseminated Lyme disease: results for serial testing reaching 100% sensitivity and 100% specificity in comparison with use of the immunoblot as the reference standard.

Lyme disease, while preserving 100% sensitivity and specificity.

DISCUSSION

Unlike Europe, where the three pathogenic sub-species of *B. burgdorferi* that cause Lyme disease can be found, the only organism associated with Lyme disease in the USA is *B. burgdorferi senso stricto* [8,9,21]. This makes laboratory diagnosis in Europe more complicated than in the USA, and serodiagnostic tests should therefore include either antigens of the different subspecies or shared antigens. The Dako assay is the test used most widely in The Netherlands, and is based on purified p41 flagellin, an antigen that shows little variation among the different *B. burgdorferi* subspecies. The Serion assay uses recombinant and purified whole-cell lysate proteins that, theoretically, detect all *Borrelia* spp. The Immunetics assay relies on a synthetic peptide called C6. The structure of this peptide is based on the 26-mer conserved region (IR 6) of the variable surface antigen of *B. burgdorferi* VlsE, which is conserved among all pathogenic subspecies of *B. burgdorferi* [17,24]. The Immunetics assay makes no distinction between IgM and IgG antibodies. Accordingly, data obtained by the other two EIA assays with respect to IgM or IgG were compared with those from the Immunetics assay for each antibody subclass separately. In addition, the combined IgM and IgG results of the Immunetics assay were compared with the combined IgM and IgG results of the Serion and Dako assays.

First, the sensitivity of the three assays was assessed for the detection of IgM and/or IgG to *B. burgdorferi* in well-defined sera from patients at the early localised and early disseminated stages of the disease, using the immunoblot as reference. The selection criteria used may have excluded samples with low antibody titre, as the immunoblot has a lower sensitivity [25]. However, this selection bias affected all tests equally, and would preclude their improved performance only in comparison with the immunoblot. The Immunetics EIA showed the highest sensitivity (> 90%) for the detection of IgM and/or IgG at the localised stage of infection. This finding is consistent with data from monkeys infected experimentally, which indicated an IgG response against the C6 peptide in 70% and 100% of the monkeys at 3 and 6 weeks after inoculation, respectively [24]. In

addition, a sensitivity of 83% in European patients with erythema migrans was reported with a home-made C6 immunoassay [22].

In recent Finnish [26] and Italian [27] studies, the Immunetics C6 assay had sensitivities of only 64% and 62%, respectively. The first study attempted only to validate the methodological performance of the assay without defining the clinical criteria of borreliosis. The absence of clinical information defining the activity of the disease may explain the discrepancy with the present results. In addition, there is an ongoing debate concerning the validity of the C6 assay as a marker for the outcome of therapy in Lyme disease patients [28,29]. In the second study [27], the difference in sensitivity can be attributed to either a different reference standard for Lyme diagnosis, or to the composition of the patient group, which was limited to cases with erythema migrans. The Serion IgM and IgG EIAs both contain antigens, such as OspC and p41, which are able to elicit an early and intense antibody response [30–32]; as such, both assays yielded acceptable sensitivities in the present patient group of 91% and 83%, respectively, at the cutaneous stage. Surprisingly, the Dako IgM and IgG EIAs showed sensitivities of only 61% and 42% during the localised stage. Comparison of these data with a previous study involving European samples showed similar sensitivities for the Dako IgM assay, whereas the present study revealed 20% lower sensitivity for the Dako IgG test [25]. At the disseminated stage, similar results were obtained with the Serion and Immunetics EIA for the detection of both IgM and IgG (sensitivities of 88–91%). In this patient group, the Dako IgG EIA had a sensitivity of only 59%.

The specificity of an assay is another important factor in the serodiagnosis of Lyme disease. An intention to increase the sensitivity by inclusion of multiple antigens may lead to overdiagnosis because of potential similarity with other non-*Borrelia* proteins. Typical examples are the 41-kDa flagellin, an antigen that has shown cross-reactivity with other spirochaetes [33], and the 66-, 68-, 71- and 73-kDa antigens, which are homologues to bacterial heat-shock proteins [34]. Therefore, US and European guidelines still recommend confirmation of a positive or an equivocal EIA result by western blotting [11,13,35]. However, the C6 antigen has a unique protein sequence [17], and home-made C6 EIAs also have a high

specificity [24]. Goossens *et al.* [25] recommended assessing specificity by using sera from patients with infections mimicking Lyme disease instead of using healthy control samples. Accordingly, the present study used a patient control group with disorders that are prone to interfere with *Borrelia* antibody testing, including recent EBV and CMV infections, rheumatoid arthritis and syphilis. The specificity for the three IgG EIAs was comparable (92–100%), but the Dako IgM assay had a specificity of 78%, and the Serion IgM EIA had an even lower specificity of 50%. Cross-reactive IgM responses with the 41-kDa flagellin included in the Serion and Dako EIAs are known to reduce specificity; the same is true for the OspC antigen, which is also present in the Serion assay [36]. The highest discriminatory power between Lyme patients and control samples was achieved with the Immunetics assay for both IgM and IgG determination. Between-run coefficients of variation were comparable and acceptable for all the assays investigated, with a maximum variation of 13%.

The use of an EIA–western blot two-test protocol certainly improves the specificity obtained with the EIA alone, but may result in a loss of sensitivity [25]. In addition, this two-tiered approach is costly, time-consuming and requires technical experience. Thus, a screening strategy that omits the confirmatory immunoblot in as many cases as possible, while attaining comparable levels of sensitivity and specificity, is highly desirable. Based on the results obtained in the present study, first-step screening with the Immunetics EIA is recommended. Only low-titre positive Immunetics results (OD between 0.12 and 0.38) require an immunoblot to distinguish false-positives, whereas results with an OD > 0.38 have 100% specificity, thus obviating any need for confirmation. If the Immunetics assay is negative, a follow-up serum can be requested, or the Serion IgM and IgG assay can be performed. This approach improves cost-effectiveness and circumvents the high inter-laboratory variation that occurs as a consequence of differences in the methodology and interpretation of immunoblotting [37]. A similar method of combined analysis for the detection of *B. burgdorferi* IgG in a Finnish population of Lyme disease patients and healthy blood donors, using the Recomwell Borrelia IgG assay (Mikrogen, Martinsried, Germany) and the C6 Immunetics assay, resulted in a 44% decrease

in workload, while preserving a sensitivity of 91% and a specificity of 99% [26].

In conclusion, the Immunetics assay, used as a single test, gave the best performance of the five assays evaluated for the diagnosis of European Lyme disease patients. The assay requires no pre-treatment, can be fully automated, and offers high sensitivity and specificity for detection of early Lyme disease, combined with low variation and a high signal-to-noise ratio. The use of a synthetic peptide shows promise for quality assurance. These advantages make the Immunetics assay suitable for use as a first-tier test for *B. burgdorferi* antibody detection. Prospective studies are underway to validate these findings.

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