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Improved Immunoglobulin M Serodiagnosis in Lyme Borreliosis by Using a μ-Capture Enzyme-Linked Immunosorbent Assay with Biotinylated *Borrelia burgdorferi* Flagella

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A μ-capture enzyme-linked immunosorbent assay (ELISA) for detection of serum immunoglobulin M (IgM) antibodies to *Borrelia burgdorferi* by using biotinylated purified *B. burgdorferi* flagella was developed. The diagnostic performance of the μ-capture ELISA was compared with that of a conventional indirect ELISA. Sera from untreated patients with erythema migrans ($n = 50$), neuroborreliosis ($n = 100$), and acrodermatitis chronica atrophicans (ACA; $n = 48$) were investigated. The cutoff of the ELISAs was adjusted to a diagnostic specificity of 98% on the basis of examination of 200 serum specimens from healthy controls. The μ-capture ELISA increased the diagnostic sensitivity in patients with erythema migrans from 32 to 48% ($P < 0.01$) and in patients with neuroborreliosis from 37 to 57% ($P < 0.001$). Because of an increased signal/noise ratio, the μ-capture ELISA yielded a significantly better quantitative discrimination of individual positive measurements from the cutoff ($P < 0.001$). The increased signal/noise ratio was most likely a consequence of the elimination of IgG competition for the test antigen. This may also explain why 12% of patients with ACA showed significantly increased specific IgM levels only by the μ-capture ELISA. Of patients with ACA, 27% had IgM rheumatoid factor. The μ-capture principle with a directly labeled antigen showed no interference with IgM rheumatoid factor, in contrast to the indirect ELISA. The high diagnostic performance and ease of this three-step μ-capture ELISA make it suitable for routine anti-*B. burgdorferi* IgM serodiagnosis.

Lyme borreliosis is an increasingly recognized multisystemic infection caused by the recently discovered tick-borne spirochete *Borrelia burgdorferi* (4, 27). Since the direct demonstration of the organism in clinical specimens by cultivation is a low-yield procedure, confirmation of the clinical diagnosis is mainly achieved by detection of a specific antibody response. The diagnostic sensitivity of currently used serological assays, however, has been limited, especially in early disease (13, 14, 18, 25, 32), probably because of a low antigen load, a slow and late immune response, and also the different and often low specificities of the test antigens used. Recently, significantly improved diagnostic performance of immunoglobulin M (IgM) and IgG serodiagnosis has been reported by using either a flagellum-enriched protein fraction (7) or purified native *B. burgdorferi* flagella (13, 14, 18) instead of a sonic extract as a test antigen for an enzyme-linked immunosorbent assay (ELISA). Since the detection of specific IgM is of special interest in early disease and may be also an indicator of active or recent infection, further improvements of methods for specific IgM detection are essential. Two types of ELISAs are available for detecting antigen specific IgM: indirect and the μ-capture ELISAs (10, 31). In the indirect ELISA, the test antigen adsorbed to the solid phase binds specific antibodies of all isotypes in amounts proportional to their concentrations in the test sample. Bound specific IgM is subsequently detected with a second antibody. The main limitations of the indirect ELISA are false-positive results due to interference of IgM rheumatoid factor (RF) (6, 30) and false-negative or false-low IgM results due to competition with specific IgG for antigenic sites (6). Both of these limitations may be overcome by the μ-capture principle, as the selective binding of patient IgM eliminates IgG competition and furthermore, in combination with a directly labeled test antigen, avoids interference of IgM RF.

Here we describe a simple, sensitive, and specific three-step μ-capture ELISA for *B. burgdorferi*-specific IgM using biotinylated purified *B. burgdorferi* flagella. The diagnostic performance was compared with an indirect IgM flagellum ELISA by using 198 serum specimens from patients with erythema migrans (EM), neuroborreliosis (NB), and acrodermatitis chronica atrophicans (ACA).

MATERIALS AND METHODS

Serum specimens from patients. A total of 198 serum specimens from patients with active untreated Lyme borreliosis were used in this study. They were divided into three groups according to clinical manifestations.

(i) Sera from 50 Swedish patients with EM. The diagnoses of 50 Swedish patients with EM were based on clinical evidence and made by Eva Åsbrink (Department of Dermatology, Södersjukhuset, Stockholm, Sweden). These sera were collected from 1984 to 1988 from 9 males and 41 females between 6 and 71 years of age (median age, 45 years). The disease duration ranged from 1 to 12 weeks (median duration, 3 weeks). Furthermore, consecutive serum samples from six Danish patients with EM were included. These sera were taken up to 6 months after treatment.

(ii) Sera from 100 Danish patients with NB. One hundred

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Danish patients with NB were all hospitalized between 1984 and 1989 (61 males and 39 females between 5 and 74 years of age; median age, 47 years). For 91 patients the diagnosis was lymphocytic meningoradiculitis with typical painful radiculitis, mononeuritis multiplex (Bannwarth's syndrome), or both. Nine patients had chronic progressive encephalomyelitis with definite intrathecal antibody synthesis against *B. burgdorferi* (15). Before treatment all patients had lymphocytic pleocytosis in cerebrospinal fluid, with counts of 13×10^6 to 830×10^6 cells per liter (median cell count, 211×10^6 cells per liter). Of the 100 patients, 56 recalled a previous EM-like skin lesion.

The disease duration, defined as the time after onset of neurological symptoms, ranged from 4 days to 6 years (median duration, 26 days). Consecutive serum samples from six patients with lymphocytic meningoradiculitis followed up ≥4 months after treatment were also included in this study.

(iii) Sera from 48 Danish patients with ACA. Sera from 48 Danish patients with ACA were collected from 1985 to 1988 from 15 males and 33 females between 17 and 80 years of age (median age, 54 years). The diagnosis was in every case made by a dermatologist on the basis of the typical clinical appearance of ACA and a high IgG titer to *B. burgdorferi* in serum. The disease duration ranged from 0.5 to 20 years, with a median duration of 3 years.

Control serum specimens. Sera from 200 healthy controls (age range, 16 to 71 years; median age, 40 years) were used for determination of the 98%-specific cutoff level in the ELISAs. None of the 200 healthy control serum specimens contained anticardiolipin antibodies by the Wassermann reaction and rapid plasma reagin test. Additionally, we investigated sera from patients with active primary syphilis (n = 25) and secondary syphilis (n = 15). All sera from patients with syphilis showed a strong positive Wassermann reaction, a positive rapid plasma reagin test, and a reactivity ≥3+ in the fluorescent treponemal antibody absorption test. Furthermore, we included sera from patients with active and serologically proven leptospirosis (n = 22) and infectious mononucleosis (n = 15). *Leptospira* serological tests were performed by using a microscopic agglutination test (cutoff titer, 1:300). Of the 22 patients, 16 were seropositive for *Leptospira icterohaemorrhagiae* and 6 were seropositive for *Leptospira sejroe* (titer range, 1:1,000 to 1:10,000; median titer, 1:3,000). We also tested 15 serum samples from patients with an IgM RF level of ≥100 IU/ml of serum and sera from 259 forestry workers which were used in a recent serosurvey for anti-*B. burgdorferi* IgG (22).

Biotinylation of *B. burgdorferi* flagella. We used the 41-kDa native *B. burgdorferi* flagellar protein as the test antigen, which was purified from strain DK1 as described previously (14). The protein concentration of the antigen preparation was determined by the modified Bradford technique (11). Biotinyl *N*-hydroxysuccinimide (BNHS) with a spacer consisting of six aminocaproic acid residues (B2643; Sigma Chemical Co., St. Louis, Mo.) was used to covalently bind biotin to the flagellar protein (19). A ratio of 0.14 mg of BNHS to 1 mg of flagellar protein was the optimal binding ratio. BNHS (20 mM) dissolved in dimethylformamide (Sigma) was added to the flagellar protein in 0.125 M carbonate ($NaHCO_3$) buffer (pH 9.0). After incubation for 8 h at 20°C, the reaction mixture was dialyzed for at least 24 h against several changes of 0.05 M phosphate (KH_2PO_4) buffer–0.1 M NaCl (pH 7.3). Biotinylation of a 1-ml suspension of purified flagella (protein concentration, 1 mg/ml) yielded a sample of biotin-labelled flagella which could be diluted 1:500 for ELISA use.

μ-Capture ELISA. Flat-bottom microdilution plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with 100 μl of μ-chain-specific rabbit anti-human IgM (code A425; Dakopatts, Copenhagen, Denmark) diluted 1:1,000 in phosphate-buffered saline (PBS) (pH 7.2) and incubated for 2 h at 20°C on a rocker platform and overnight at 4°C. After the plates were washed, 100 μl of serum diluted 1:200 in PBS containing 0.5 M NaCl, 0.1% (vol/vol) Tween 20, and 1% (wt/vol) bovine serum albumin (pH 7.2) was added to the wells and incubated for 2 h at 20°C on a rocker platform. Biotinylated *B. burgdorferi* flagella and avidin peroxidase (code P347; Dakopatts) were mixed at dilutions of 1:500 and 1:5,000, respectively, in the same buffer that was used for dilution of sera. After the plates were washed, 100 μl of the solution containing the biotinylated *B. burgdorferi* flagellum-avidin peroxidase complex was added to the wells and incubated for 3 h at 20°C on a rocker platform. Unbound biotinylated flagella were removed by washing. Next, 200 μl of the substrate *o*-phenylenediamine (0.41 mg/ml; Sigma) in citrate buffer (pH 5.0) with 0.04% (vol/vol) H_2O_2 was added to each well. After 15 min protected from light, the enzymatic reaction was stopped by the addition of 50 μl of 3 M H_2SO_4. The optical density (OD) at 492 nm was read spectrophotometrically (Immuno Reader NJ2000; Nippon Inter Med, Tokyo, Japan). All washings were done three times with PBS containing 0.5 M NaCl and 0.1% (vol/vol) Tween 20 (pH 7.2). Samples were tested in duplicate and retested if the two OD values differed more than 10% from the mean. To limit interassay variation, three calibrator serum samples, one each with a low, median, and high OD value, were included on every plate for construction of a standard curve. The average OD obtained from every sample was adjusted to this standard curve.

Indirect ELISA for IgM and IgG antibodies to *B. burgdorferi* flagella. The indirect ELISA for IgM and IgG antibodies to *B. burgdorferi* flagella was performed as previously described (14). Briefly, microdilution plates (Immunoplates; code 2-69620; Nunc) were coated with purified *B. burgdorferi* DK1 flagellar protein. After being blocked with PBS containing 1% (wt/vol) bovine serum albumin, sera diluted 1:200 in PBS containing 0.1% (vol/vol) Tween 20 and 0.5% (wt/vol) bovine serum albumin (pH 7.2) were incubated for 2 h at 20°C. Bound antibody specific to *B. burgdorferi* was then detected with a peroxidase conjugate, either rabbit anti-human IgM (code P215; Dakopatts) or rabbit anti-human IgG (code P214; Dakopatts). The substrate reaction and reading of the test were performed as described above. The diagnostic cutoff OD was adjusted to be 98% specific on the basis of the examination of sera from 200 healthy controls and was 0.160 for the IgG assay and 0.375 for the IgM assay.

IgM RF detection and absorption. The 48 serum specimens from patients with ACA were investigated for the presence of IgM RF by an ELISA using human IgG, and the results were evaluated according to the 95%-specific upper limit of the assay, 8 IU/ml (16). IgM RF was absorbed by immunoprecipitation of IgG with a commercial kit (RF-Absorbans; Behringwerke, Marburg, Federal Republic of Germany).

Statistical analysis. The diagnostic sensitivities of the μ-capture and indirect ELISAs were compared by the nonparametric McNemar's test (1) by considering only the number of samples which disagreed in positivity or negativity in the two assays. These observations are assumed to follow a binomial distribution of paired data. Furthermore,

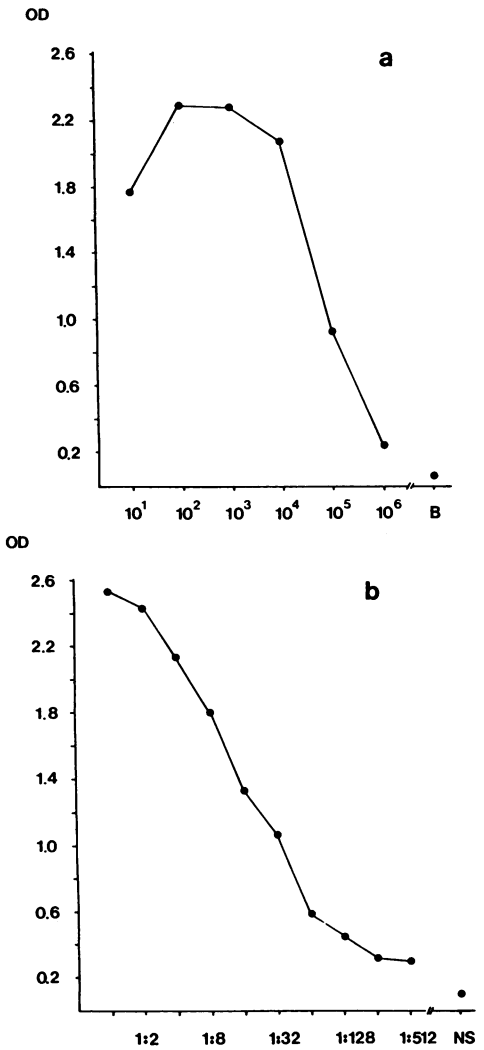

FIG. 1. Measurement of anti-*B. burgdorferi* IgM by the μ-capture ELISA in a 10-fold dilution series of a strongly IgM-positive serum in sample dilution buffer (B) only (a) and in a 2-fold dilution series of the same IgM-positive serum into negative serum (NS) before the final dilution of 1:200 in sample dilution buffer (b). The very different effects of dilution with serum and sample dilution buffer on the IgM signal demonstrate that the μ-capture ELISA measures not an absolute concentration of specific antibody but the relative amount of specific antibody versus total IgM. Addition of negative serum displaces the specific IgM fraction, thus reducing the signal.

the quantitative discrimination of the two assays between controls and seropositive patients was estimated. For each individual sample positive in both ELISAs, the difference between the achieved OD value and the cutoff level was calculated for both assays. These differences were then compared by the Wilcoxon rank sum test for paired data.

RESULTS

Figure 1 shows the IgM reactivity in the μ-capture ELISA of a serum sample strongly positive for anti-*B. burgdorferi* IgM diluted in either sample dilution buffer (Fig. 1a) or negative serum (Fig. 1b). According to Fig. 1a, the μ-capture layer was saturated with patient IgM, yielding an almost maximal specific IgM signal, until the sample was diluted $1:10^4$. On the other hand, if the positive serum was diluted in a negative serum, the specific IgM signal decreased rapidly (Fig. 1b). These curves demonstrate that the μ-capture ELISA measures the relative amount of specific IgM antibody versus total IgM and not the concentration of specific IgM.

TABLE 1. Diagnostic sensitivity of the *B. burgdorferi* flagellar μ-capture, indirect IgM, and indirect IgG ELISAs in EM and NB

Clinical manifestation and no. of wk after onset	No. of patients	% of patients positive by indicated ELISA		
		μ-Capture	Indirect IgM	Indirect IgG
EM				
<2	10	50	20	10
2–5	27	44	41	26
≥6	13	54	23	46
Total	50	48	32	28
NB				
<2	23	65	48	70
2–5	47	60	45	81
≥6	30	47	17	100
Total	100	57	37	84

The interassay variation of the μ-capture ELISA was determined by testing three serum specimens on 11 independent days. Serum specimen 1 had a mean OD of 0.535 (standard deviation, 0.0446) and a coefficient of variation of 8.33%; serum specimen 2 had a mean OD of 0.880 (standard deviation, 0.0769) and a coefficient of variation of 8.73%, and serum specimen 3 had a mean OD of 1.714 (standard deviation, 0.180) and a coefficient of variation of 5.86%. The 98%-specific diagnostic cutoff of the μ-capture assay was fixed at an OD of 0.300 (Fig. 2) on the basis of the examination of the same 200 healthy control serum samples used for cutoff determination in the indirect IgM and IgG ELISAs.

The overall diagnostic sensitivity of anti-*B. burgdorferi* IgM detection increased significantly from 32 to 48% ($P < 0.01$) when the μ-capture ELISA was used for patients with EM and from 37 to 57% ($P < 0.001$) when this assay was used for patients with NB (Fig. 2; Table 1). Furthermore, the μ-capture ELISA generally yielded much higher OD signals than the indirect ELISA, resulting in a significantly improved quantitative discrimination of individual seropositive samples from the cutoff (Fig. 3). Except for serum from one patient with NB which showed a slightly elevated IgM antibody level (OD, 0.470) in the indirect ELISA (Fig. 3b), all sera from patients with EM and NB that were reactive in the indirect assay were also reactive in the μ-capture ELISA (Fig. 3). Regarding the IgG and μ-capture ELISA results of the sera from 50 patients with EM, 6 were only IgG positive, 16 were only IgM positive, 30 were either IgG or IgM positive, and 8 were IgG and IgM positive. The corresponding figures for the 100 patients with NB were as follows: 38 were only IgG positive, 11 were only IgM positive, 95 were IgG or IgM positive, and 46 were IgG and IgM positive. None of the 200 serum specimens from healthy controls contained significant IgG and IgM anti-*B. burgdorferi* anti-

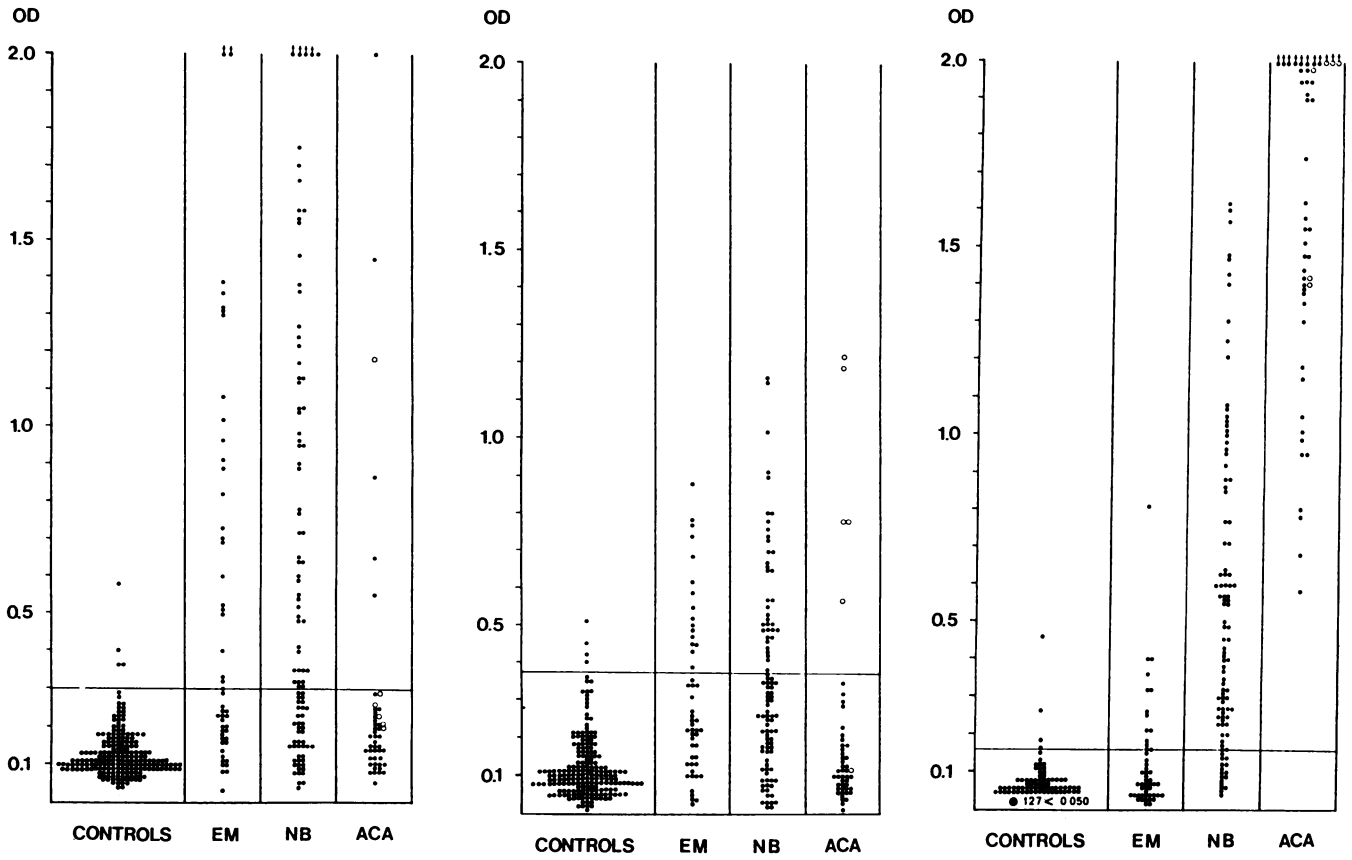

FIG. 2. Anti-*B. burgdorferi* IgM measured by μ-capture ELISA (left) and indirect ELISA (center) and anti-*B. burgdorferi* IgG measured by indirect ELISA (right) in sera of 200 healthy controls, 50 patients with EM, 100 patients with NB, and 48 patients with ACA. The horizontal lines mark the 98%-specific diagnostic cutoff levels in the indicated test. Open circles indicate patients with ACA who had an IgM RF level of >20 IU/ml.

body levels. Dividing the patients with EM and patients with NB into three groups according to disease duration (Table 1) demonstrates the relatively higher frequency of IgM-positive tests in early disease and the increasing frequency of detectable specific IgG with increasing disease duration. Only one of the nine patients with chronic NB was low-grade IgM positive in the μ-capture ELISA (OD, 0.410).

We also tested 48 serum specimens from patients with ACA. All had high IgG antibody levels to *B. burgdorferi*, whereas only six and five serum specimens were IgM positive in the μ-capture and indirect IgM ELISAs, respectively (Fig. 2). To rule out the possible influence of IgM RF on the outcome of the IgM detection, the 48 serum specimens were investigated for IgM RF. An IgM RF level of ≥8 IU/ml was found in 13 patients (27%), and in all but 6 patients this level was <20 IU/ml. The five IgM-positive samples in the indirect IgM ELISA all had a significantly increased concentration of IgM RF (range, 38 to 222 IU/ml; median, 55 IU/ml). Absorption of the IgM RF was performed on the six serum samples with IgM RF levels of >20 IU/ml and on eight serum samples with IgM RF levels of <8 IU/ml. Measurements of IgM antibodies by the indirect assay before and after RF absorption showed that the absorption also lowered the OD values in sera without a detectable RF (Fig. 4). The previously high IgM reactivity of five serum specimens in the indirect ELISA was reduced significantly in four and to a lesser degree in one serum specimen (Fig. 4). All four samples with significant reductions after RF absorption were negative in the μ-capture ELISA (Fig. 4). In the μ-capture assay, on the other hand, a total of six ACA serum specimens (12%) were clearly positive (Fig. 2). Only one of these contained IgM RF, and it was this serum that showed a decreased but not abolished IgM reactivity in the indirect assay after RF absorption. This may indicate that the IgM reactivity in this patient partly was due to IgM RF and partly was specific IgM reactivity. A further 15 serum samples from patients selected for IgM RF levels of ≥100 IU/ml were studied. All 15 serum samples were negative for IgG antibodies to *B. burgdorferi*. Only one of them had a low level of IgM reactivity in the indirect ELISA (OD, 0.410) and none was positive in the μ-capture ELISA.

The diagnostic specificity of the μ-capture ELISA was further evaluated with sera from patients with active primary and secondary syphilis, leptospirosis, and infectious mononucleosis. The results are summarized in Table 2. Only patients with infectious mononucleosis showed considerable anti-*B. burgdorferi* IgM reactivity in the μ-capture ELISA as well as in the indirect IgM ELISA (Table 2).

Figure 5 shows the investigation of consecutive serum samples from six patients with EM and six patients with NB. Sharp titer rises were detectable within 1 to 7 weeks in patients with EM. The specific IgM response in EM and NB, although declining, was still above the cutoff for at least 4 to 6 months.

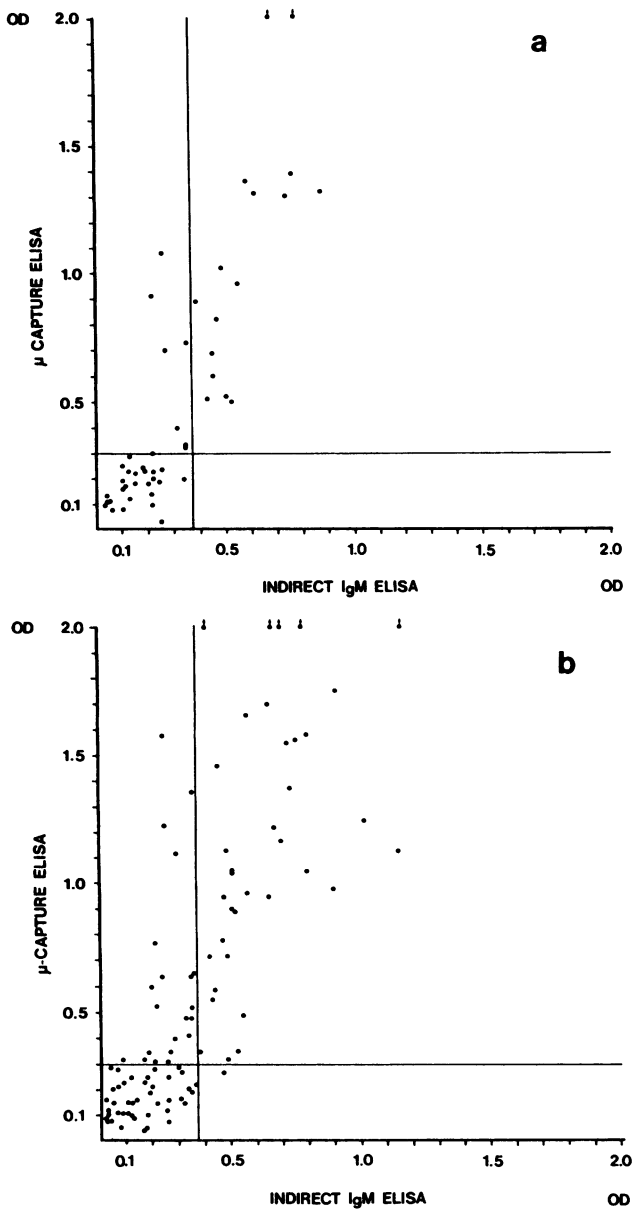

FIG. 3. Correlation of specific anti-*B. burgdorferi* IgM measurement in sera of 50 patients with EM (a) and 100 patients with NB (b) by the μ-capture and indirect IgM ELISAs. The vertical lines represent the cutoff of the indirect ELISA, and the horizontal lines mark the cutoff of the μ-capture ELISA. The μ-capture ELISA demonstrated a significantly improved signal/noise ratio, as estimated by comparing the differences between the achieved OD values and the cutoff level in each test by the Wilcoxon rank sum test for paired data. (a) $P < 0.001$; (b) $P < 0.001$.

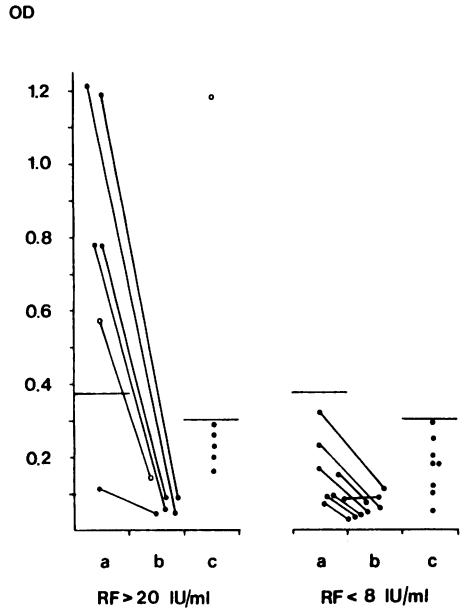

FIG. 4. Anti-*B. burgdorferi* IgM levels in six serum specimens from patients with ACA with high levels of IgM RF (38 to 222 IU/ml) and in eight serum specimens from patients with ACA with IgM RF levels of <8 IU/ml. The specific IgM was measured before (a) and after (b) absorption of IgM RF in the indirect IgM ELISA and without absorption in the μ-capture ELISA (c). Open circles indicate the only RF-positive patient who tested positive in the μ-capture ELISA (see text). The horizontal lines mark the cutoff levels in the indicated tests.

Sera from 259 forestry workers which in a previous serosurvey for anti-*B. burgdorferi* IgG (22) had shown a high prevalence (35%) of specific IgG antibodies were tested by the μ-capture ELISA. Only 4.6% of the samples were IgM positive (OD range, 0.310 to 1.520; median, 0.400).

DISCUSSION

Since the introduction of the μ-capture ELISA in 1978 (10), this technique has been shown to be very useful for the detection of antigen-specific IgM, especially in viral diseases but also in syphilis (23) and toxoplasmosis (26). Recently the μ-capture principle was applied for specific IgM detection in Lyme borreliosis by Berardi et al. (3) and Karlsson and Granström (17). Although both studies used a very similar assay design with five incubation steps and a sonic extract of *B. burgdorferi* as a test antigen, only Berardi et al. (3) reported significantly improved IgM detection. The concept of our μ-capture assay was different, with only three steps and a single, directly labeled purified *B. burgdorferi* antigen. This assay improved specific IgM detection significantly compared with an indirect ELISA (Table 1). The improvement consists of increased sensitivity due to a much higher signal/noise ratio and increased specificity due to elimination of IgM RF interference. There are two main explanations for the increased sensitivity. (i) The selective binding of IgM avoids competition with specific IgG for the test antigen, which may give false-low or false-negative IgM results in the indirect assay. This may explain the significantly increased signal/noise ratio (Fig. 3) and why only the μ-capture ELISA detected specific IgM in 12% of the patients with ACA, all with high specific IgG levels (Fig. 2). (ii) The binding of a representative part of the total IgM and subsequent detection of anti-*B. burgdorferi* specific IgM reflect the relative amount of specific versus total IgM (Fig. 1) and not the absolute concentration of specific IgM as measured by the indirect ELISA. An increase in the relative amount of specific IgM seemed to be a more sensitive parameter than an increase in the absolute concentration of specific IgM in serum measured by an indirect ELISA (26).

Comparing our assay with the recent attempts to measure *B. burgdorferi*-specific IgM with a μ-capture ELISA (3, 17),

TABLE 2. Diagnostic specificity of the *B. burgdorferi* flagellar μ-capture, indirect IgM, and indirect IgG ELISAs

Diagnosis (no. of patients)	No. of patients positive by indicated ELISA (OD range in positive samples)		
	μ-Capture	Indirect IgM	Indirect IgG
Primary syphilis (25)	1 (0.540)	0	9 (0.170–0.300)
Secondary syphilis (15)	0	0	4 (0.160–0.210)
Leptospirosis (22)	1 (0.700)	5 (0.390–0.870)	3 (0.190–0.300)
Infectious mononucleosis (20)	6 (0.690–1.400)	5 (0.650–1.370)	3 (0.170–0.520)

we feel that our assay provides several advantages. (i) Purified flagellar antigen, which is used in our ELISA, is the earliest and most constantly recognized *B. burgdorferi* antigen involved in the human immune response (7, 8). Access to a highly relevant immunodominant antigen is thus higher than if a sonic extract is used. (ii) The direct labeling of flagella with biotin and simultaneous incubation with avidin peroxidase reduces the number of sequential incubations from four to two and avoids the use of two secondary antibodies. This makes the three-step assay more simple, reduces the background, and eliminates the possibility of false-positive reactions due to IgM RF. Compared with the five-step μ-capture ELISA (3, 17) and the indirect ELISA, the design of our μ-capture ELISA reduces the number of washings after specific patient IgM has reacted with the test antigen from three to one or from two to one, respectively. This favors detection of low-avidity IgM antibodies. Since biotinylation has no concomitant effect on the antigenicity of the protein and since the biotin-avidin system additionally provides amplification, biotinylation of flagellar protein is preferable to direct enzyme conjugation. Before the use of biotinylated flagella, we worked with peroxidase-conjugated purified IgG of an anti-*B. burgdorferi* rabbit hyperimmune serum to detect bound flagellar antigen. This buildup gave a considerably lower signal/noise ratio, primarily because of a high and variable background.

The previous studies using a μ-capture technique for anti-*B. burgdorferi* IgM detection (3, 17) did not evaluate the still possible influence of IgM RF in their assay, in which RF bound to the catching antibody may bind secondary antibodies even in the absence of antigen. As in syphilis (5), IgM RF does occur in patients with Lyme borreliosis, especially in active secondary and tertiary manifestations (13, 20). In this study, IgM RF was found in Danish patients with ACA with a frequency (27%) similar to that previously reported for Swedish patients (36%) (13). Especially when associated with high levels of specific IgG, IgM RF is a well known cause of false-positive IgM results in indirect serological techniques such as immunofluorescence assays and ELISAs (6, 30) and thus also in Western immunoblotting. Therefore, measures against this problem should be taken by absorption of IgM RF (6, 30, 32) or by using a μ-capture ELISA with either a directly labeled antigen or only one secondary $F(ab')_2$ antibody.

The diagnostic specificity of the μ-capture flagellar ELISA was limited mainly in patients with acute infectious mononucleosis, for whom the results were equal to those of the indirect ELISA. This cross-reactivity is inevitable, since the well known polyclonal B-cell stimulation in infectious mononucleosis apparently also stimulates *B. burgdorferi*-specific B-cells. No assay will be able to avoid this phenomenon, which also may occur in other immunological and infectious disorders.

As in previous studies using either sonic extract or purified flagella, samples from patients with syphilis contained cross-reacting anti-*B. burgdorferi* antibodies primarily of the IgG isotype (14, 18, 24, 29). Regarding the general suitability of *B. burgdorferi* flagella as a diagnostic antigen, their superiority to a whole-cell sonic extract has previously been documented (13, 14, 18). Because of conserved epitopes, this antigen still does not allow serological discrimination between patients with syphilis and patients with Lyme borreliosis. However, two independent studies (13, 18) showed that the cross-reactivity that occurred when samples

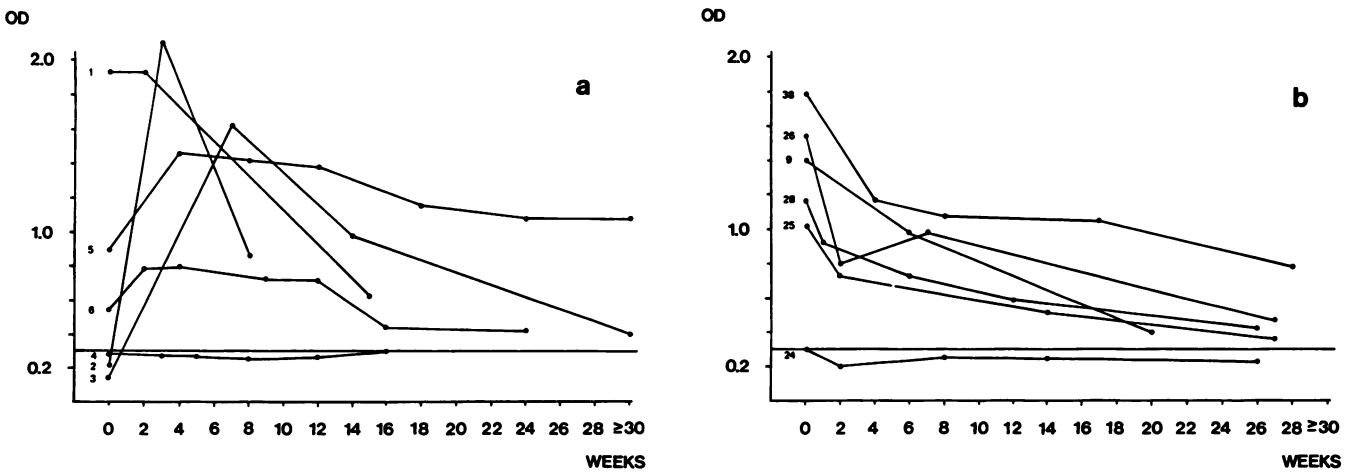

FIG. 5. Anti-*B. burgdorferi* IgM measured by the μ-capture ELISA in consecutive sera from six patients with EM (a) and 6 patients with NB (b) (numbers next to curves are patient numbers). All patients received antibiotic treatment immediately after the first sample was taken. Cultivable *B. burgdorferi* was isolated from skin biopsies from patients 4 to 6 (a). The horizontal lines mark the cutoff levels.

from patients with syphilis were tested was considerably reduced by using purified *B. burgdorferi* flagella. Thus, it may be assumed that the flagellar antigen is far from the only antigen responsible for cross-reactivity between *B. burgdorferi* and *Treponema pallidum*. The potential implications for the differential diagnosis between Lyme borreliosis and syphilis have previously been discussed in detail (13).

The μ-capture ELISA was suitable for monitoring the specific IgM level in consecutive samples (Fig. 5). Sharp titer rises within 2 to 7 weeks in patients with EM were clearly detected, as were declining IgM antibody levels. It should be noted that despite successful treatment, IgM levels in patients with EM or NB seldom dropped below the cutoff within a follow-up period of 4 to 6 months. A prolonged specific IgM response has previously been noticed (8, 9), although the mechanism is unclear. So far, no larger prospective studies of IgG and IgM titer kinetics with, e.g., 1 to 3 years of follow-up have been presented. An IgM response is not obligatory, as seen in two patients with culture-proven Lyme borreliosis (Fig. 5). However, the specific IgM response may still increase during the first weeks after treatment of early EM (12). Significant levels of specific IgM may still be detected in late Lyme borreliosis, as in ACA, if IgG competition is avoided. These results are in accordance with findings with late syphilis, in which specific IgM may also be detected in some patients if IgM-IgG separation is performed (2, 5, 21). It has been suggested that the presence of specific IgM in chronic disease could be an indication of the presence of viable spirochetes (8). However, the frequent isolation of *B. burgdorferi* from skin biopsies of IgM-negative patients with ACA shows that the presence of viable spirochetes is far from always accompanied by specific IgM. The observation of a significantly lower prevalence of IgM-positive individuals compared with the high prevalence of highly IgG-positive individuals in a population of forestry workers (22) may reflect either the low prevalence of active Lyme borreliosis in this group or merely a typical anamnestic T-cell-dependent immune response due to multiple exposures to *B. burgdorferi*. The duration of a specific IgM response after recovery from Lyme borreliosis is unknown. Thus, to make any conclusion about whether a positive IgM finding in itself indicates an active infection and thus is an indication of treatment is not yet justified.

A γ-capture ELISA for anti-*B. burgdorferi* IgG in serum was recently reported (3). Considering the very low ratio of antigen-specific IgG to total IgG in serum and our own experience with a γ-capture ELISA (15), we believe that this assay is not superior to a conventional indirect IgG ELISA for detection of specific IgG antibodies in serum.

Unlike the indirect ELISA (14, 29), a capture ELISA cannot be used for the examination of single cerebrospinal fluid specimens without simultaneous examination of a paired serum sample. Local antibody synthesis occurs only if the relative amount of specific antibody in cerebrospinal fluid is higher than in the corresponding serum (15, 28).

The presented μ-capture ELISA is now used routinely in our laboratory. Measurements are performed with a single dilution (1:200), and the results are expressed in arbitrary ELISA units (0 indicates negative and 1 to ≥14 indicates positive). One unit is defined as the OD interval representing twice the interassay variation in the respective OD range. This results in a logarithmic transformation of the OD scale. We consider a significant change in IgM antibody level not due to interassay variation to have occurred if consecutive samples run in independent assays differ by ≥3 ELISA units.

In conclusion, the μ-capture ELISA using biotinylated *B. burgdorferi* flagella significantly increases the diagnostic sensitivity and specificity of anti-*B. burgdorferi* IgM detection. The assay effectively avoids competition with IgG and interference by IgM RF without labor-intensive IgG-IgM separation and IgM RF absorption. The direct labeling of the test antigen and combined incubation with avidin peroxidase makes the assay easy to perform and suitable for IgM routine serological testing. The detection of specific IgM antibodies improves serological confirmation, especially of early Lyme borreliosis.

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