Interpretation criteria in Western blot diagnosis of Lyme borreliosis

S. MAVIN, S. McDONAGH, R. EVANS, R. M. MILNER, J. M. W. CHATTERTON and D. O. HO-YEN
National Lyme Borreliosis Testing Laboratory, Microbiology Department, Raigmore Hospital, Old Perth Road, Inverness IV2 3UJ, UK

Accepted: 27 August 2010

ABSTRACT

This study reviews the Lyme borreliosis Western blot interpretation process, including what bands are classed as specific, the number of bands needed for a positive result, the role of band intensity and the use of clinical information. In 2008, 3688 patients (4223 serum samples) were tested by enzyme immunoassay (EIA), with 832 patients tested by confirmatory in-house IgG Western blot: 272 patients were Western blot-positive, 170 were weak positive, 156 were equivocal and 234 were negative. These results were assessed, and a review of interpretation criteria from both the USA and Europe was carried out. New interpretation criteria and a testing algorithm were developed. The revised criteria changed the results in 109/3688 (3%) patients and produced significantly more Western blot-positive and weak-positive patients than with the current criteria (485 *vs*. 442, $P<0.0001$). In total, 76 patients who were negative/equivocal became positive, which may have led to a change in their management. Conversely, 33 patients who were weak-positive became equivocal but their management may not have been affected. The authors believe that the revised criteria have simplified blot interpretation and improved the sensitivity and robustness of their Western blot method. Using a protocol tailored to patients that incorporates clinical characteristics means that the entire process will be easier and will aid the management of patients.

KEY WORDS: Borrelia burgdorferi.
Lyme borreliosis.
Western blot.

Introduction

Since it was first described in the 1970s, the laboratory diagnosis of Lyme borreliosis (LB) has been dependent mainly on serology,[1] with the Western blot test used for confirmation.[2] The interpretation criteria of a Western blot are crucial as these affect its sensitivity and specificity.[3] Although criteria are standardised in the USA,[4] a consensus for the interpretation of Western blots to detect *Borrelia burgdorferi* infection in Europe has been difficult to obtain. The result is that individual countries and laboratories utilise different strains and interpretation criteria.[2,3,5]

In 1999 two pathogenic species from the *B. burgdorferi* sensu lato complex, *B. burgdorferi* sensu stricto and *B. afzelii*, were isolated in Scotland.[6] The third pathogenic species that has previously been isolated in Europe, *B. garinii*, was not found. We now use a mix of these local *B. burgdorferi* sensu stricto and *B. afzelii* antigens in a single Western blot, as this increases sensitivity, but the interpretation criteria (based on *B. burgdorferi* sensu stricto) is modified to permit the inclusion of the *B. afzelii* antigen.[7]

While there has been focus on the sensitivity and specificity of the Western blot, it is extremely important that the clinical features are considered in the interpretation of results. To ensure the best use of the Western blot in a routine diagnostic laboratory it is essential that the entire interpretation process remains as simple as possible. Therefore, this study reviews our interpretation process, what bands are classed as specific, the number of bands needed for a positive result, the role of band intensity and the use of clinical information.

Materials and methods

All serum samples referred to the National Lyme Borreliosis Testing Service Laboratory, Raigmore Hospital, Inverness, from laboratories throughout Scotland during 2008 were included in the study. In accordance with Centers for Disease Control and Prevention (CDC) guidelines,[4] all sera were screened by commercial *B. burgdorferi* IgM/IgG enzyme immunoassay (EIA) utilising *B. burgdorferi* (B31 strain) antigen (Zeus, Scientific, NJ, USA). All EIA-positive/equivocal samples and negative samples with a high clinical suspicion of Lyme borreliosis were then tested by a confirmatory in-house IgG Western blot which uses a local *B. burgdorferi* sensu stricto and *B. afzelii* antigen (50:50) mix.[8] The 50:50 local antigen mix previously has performed better than individual *B. burgdorferi* sensu stricto and *B. afzelii* antigen blots at their optimum and 40:60/60:40 antigen mixes.[8] The Western blot results were interpreted according to current published criteria.[7]

For a Western blot-positive result, these criteria required at least four bands in total, including the 41 kDa band and a further two specific bands with strong intensity. The molecular weights of the eleven bands currently considered to be specific are 18, 22, 26, 30, 32, 34, 39, 43, 46, 58 and 92 kDa. The accurate identification of band molecular weight is routinely determined using polyclonal sera (Abcam, Cambridge, UK) and well-categorised Western blot-positive sera. For the purpose of this study, only results from the first

Correspondence to Sally Mavin
Email: nhshighland.microbiology@nhs.net

Table 1. Review of *B. burgdorferi* IgG Western blot interpretation criteria. Publications are listed chronologically (except 22–24 which are the authors'criteria).

Publication	*Borrelia* species	Country	Specific bands included in criteria	
			Total number	Bands similar to Mavin *et al.* 2009
1 Grodzicki 1988[9]	Bbss	USA	–	–
2 Fister 1989[10]	Bbss	USA	8	58, 41, 34, 31, 25, 17
3 Karlsson 1990[11]	NS	Sweden	4	41, 18, 21.5
4 Rose 1990[12]	Bbss	USA	2	41
5 Zoller 1993[13]	NS	Germany	5	94, 39, 31, 30, 21
6 Sood 1993[14]	Bbss	USA	7	93, 43, 28, 21, 18
7 Dressler 1993[15]	Bbss	USA	10	93, 58, 45, 41, 39, 30, 28, 21, 18
8 Cutler 1993[16]	Bbss	UK	11	93, 31, 30, 20 (56-58, 48, 39, 34) 10 points specific, (5 points semi), no 41 minus 10 points
9 Seppala 1994[17]	*B. afzelii*	Finland	4	83, 48, 41, 34, 31
10 Kowal 1994[18]	Bbss	USA	14	83, 45, 41, 39, 34, 31, 29, 25, 21, 18
11 Engstrom 1995[19]	Bbss	USA	5	88, 39, (22), 20
12 CDC 1995[4]	Bbss	USA	10	93, 58, 45, 41, 39, 30, 28, 21(OspC), 18
13 Norman 1996[20]	All	Europe/USA	11	93, 41, 39, 34, 31, 27, 23(OspC), 21, 18
14 Hilton 1996[21]	Bbss	USA	12	93, 58, 45, 41, 39, 34, 31, 30, 28, 24(OspC), 18
15 Ryffel 1998[22]	*B. garinii*	Switzerland	5	93, 41, 39, 32.5, 22
16 Hauser 1997[5]	Bbss	Germany	6	83/100, 58, OspC, 21, 17
17 Hauser 1999[23]	*B. afzelii*	Europe	9	83/100, 58, 43, 39, 30, OspC, 21, 17
18 Robertson 2000[3]	All	Europe	6	83/100, 58, 41, 39, OspC, 17
19 Schulte-Spechtel 2003[24]	All	Europe	7	83/100, 58, 41i, 39, OspC, Osp17(DbpA) recombinant
20 Hernandez-Novoa 2003[25]	All	Spain	5	100, BmpA, OspA, OspC, 18 recombinant
21 Branda 2010[26]	Bbss	USA	11	93, 58, 45, 41, 39, 30, 28, 23, 18 recombinant
22 Davidson 1996[27]	Bbss	Scotland	10	92, 46, 42, 39, 34, 31, 29, 26, 21, 19
23 Evans 2005[28]	Bbss	Scotland	10	92, 58, 46, 39, 34, 32, 30, 26, 22, 18
24 Mavin 2009[7]	Bbss/*B. afzelii*	Scotland	11	92, 58, 46, 43, 39, 34, 32, 30, 26, 22, 18

NS: not stated, Bbss: *B. burgdorferi* sensu stricto, Osp: outer surface protein, VlsE: variable major protein-like sequence expressed.

sample received during the study period from each patient was considered. Statistical analysis was carried out when appropriate using the McNemar test.

A review of interpretation criteria used in the USA and Europe was carried out and comparisons were made. The results were assessed and revised interpretation criteria and a testing protocol algorithm were developed.

Results

During 2008, 4223 serum samples from 3688 patients were tested for LB by EIA. Of these, 1014 samples from 832 patients were tested by in-house IgG Western blot: 272 patients were Western blot-positive, 170 were weak-positive, 156 were equivocal and 234 patients were negative according to current interpretation criteria.

A total of 21 interpretation criteria from the USA and Europe were examined, with our publications at the end (refs 22–24) (Table 1[3–28]). The number of specific bands recognised by each group ranged from two[12] to 14.[18] Groups in the USA recognised more specific bands than those in Europe; for example, CDC recommendations recognised 10 specific bands, whereas Hauser *et al*. recognised six for *B. burgdorferi* sensu stricto and nine for *B. afzelii*. The number of specific bands required for a positive result ranged from one[17,25] to five.[4,15] The CDC recommendations required five specific bands, whereas Hauser *et al*. required two for *B. afzelii* and one for *B. burgdorferi* sensu stricto (although MIQ 2000 guidelines based on this study recommended two).[29] Twelve groups specified that they recorded band intensity, although it was only used in interpretation criteria in 7/21 (33%). Only five groups required a total number of bands for a positive result.[9,12–14,17]

Revised interpretation criteria were developed (Table 2). When the revised criteria were applied to the Western blots of the 832 patients tested, there were significantly more positives and weak positives than with the current criteria (485 *vs*. 442; $P<0.0001$) (Table 3). In total, 76 patients previously negative or equivocal became positive or weak-positive. Seventeen (7.3%) negative patients became weak-positive ($n=13$) or positive ($n=4$) with the revised criteria,

For each group studied the species, country, specific bands, total bands and band intensity (if used) are stated.

			Total number bands (Specific + non-specific)		Band intensity	
	Bands different from Mavin *et al.* 2009	Number for positive result	Required	Number	Measured	Used in criteria
	–	–	Yes	4	No	No
	66, 55	4	No	–	NS	No
	23	2 (41 + 1 other)	No	–	NS	No
	60	2	Yes	4	NS	No
	–	Early = 1 (21) Late = 5	Yes (early)	2 (incl. strong 41)	Yes	Yes
	73, 60	2	Yes	5	NS	No
	66	5	No	–	Yes	Discounted weak bands
	(82, 52, 50)	3 (29 points)	No	–	No	–
	37	1	Yes	2 (incl. strong 41)	Yes	Yes
	75, 66, 60, 15	>4 (low intensity), 2 (moderate intensity)	No	–	Yes	Yes
	35, 24 (strong) 66	2	No	–	Yes	Yes
		5	NS	–	No	–
	75, 66	4	No	–	Yes	No
	66	5	No	–	NS	–
	–	3 (6 points)	No	–	Yes	Yes
	–	1 (2)	No	–	Yes	No
	14	2	No	–	Yes	No
	–	2/3	No	–	Yes	N
	VlsE	2	No	–	NS	–
	–	1	No	–	Yes	Yes
	66, VlsE	(Early = 1, VlsE) 5	No	–	Yes	No
		2	Yes	4 (incl. 41)	Yes	Yes
		2	Yes	4 (incl. 41)	Yes	Yes
		2	Yes	4 (incl. 41)	Yes	Yes

and 14 (82.3%) had symptoms of LB. Likewise, 59 (37.8%) equivocal patients became weak-positive, 37 (62.7%) of whom had symptoms of LB. Conversely, 33 (19.4%) patients previously weak-positive became equivocal. Twenty-one (63.6%) of these patients had symptoms of LB, five of whom had erythema migrans. There was an overall change in 109 (3.0%) of the 3688 patients. For patient management, results must be interpreted with clinical characteristics (Fig. 1).

Discussion

The interpretation criteria review of *B. burgdorferi* Western blots showed that different specific bands are recognised, and different numbers of specific and non-specific bands of specified and unspecified intensity are required for a positive result (Table 1). It is not surprising that the bands classed as specific by each group varied as it is well recognised that the use of different strains and species as antigen leads to variations in expression of immunogenic proteins.[3,5]

Different Western blot protocols, band resolution, visual scoring and subjective interpretation also compound the problem.[1,15,19] However, the review and the analysis of the band patterns of the 832 patients tested by Western blot highlighted the need to address the bands in the criteria that are classed as specific. The 41 kDa band has always been required for a positive result using our current criteria,[7,27,28] as is required by three other groups.[11,13,17] Interestingly, 14/21 (66.7%) groups include the 41 kDa as a *Borrelia*-specific band in their criteria but not as a requirement (Table 1). We adopted this approach in our revised criteria (Table 2). Likewise, the 20, 28 and 48 kDa bands were included as specific bands in the revised criteria. These bands were found in significantly more Western blot-positive patients than negative patients.[30] All three bands, in addition to the bands already classed as specific in our criteria, are recognised as specific by at least one other group of workers.

Our revised criteria recognise 15 specific bands, which is more than other groups studied. However, this is not unexpected as the Inverness group is the only one that uses a mixed antigen whole cell lysate blot incorporating antigen

Table 2. Revised interpretation criteria for mixed *B. burgdorferi* sensu stricto/*B. afzelii* IgG Western blots. The criteria are based on the number and intensity of specific bands. However, if the criteria for an equivocal/weak-positive result are not met but a sample has 5 or ≥6 bands in total (intensity 2–4), the result will be equivocal or weak-positive, respectively. The interpretation is dependent on the Western blot result and the clinical information available.

Result	No. of specific* bands	Intensity† of specific bands (1–4)	Total no. of bands	Interpretation
Negative	≤1	1–4	≤4	No evidence of *B. burgdorferi* infection
Equivocal	2	2–4	5	Requires second sample Two equivocal results, at least two weeks apart, with symptoms, unlikely to be *B. burgdorferi* infection
Weak positive	≥3	2–4	≥6 (if specific band requirement not fulfilled)	Past infection or early infection
Positive	≥4	3–4	–	Results of current *B. burgdorferi* infection

*Specific bands: 18, 20, 22, 26, 28, 30, 32, 34, 39, 41, 43, 46, 48, 58 and 92 kDa.
† Intensity: 1= +/– (faint), 2= + (moderate), 3= ++ (strong), 4= +++ (very strong).

both from *B. burgdorferi* sensu stricto and *B. afzelii* strains. Some of the antigens detected will be common to both strains but some antigens will be unique to each strain. From the criteria review (Table 1), seven of our bands appear to be common to both strains (92, 58, 39, 30, 22 and 18 kDa).[4,5,23] Two bands (43 and 48 kDa) may be *B. afzelii* as they are only recognised in Europe, whereas a further six bands (46, 34, 32, 28, 26 and 20 kDa) may be *B. burgdorferi* sensu stricto. The revised criteria require three and four specific bands for a weak-positive and positive result, respectively (Table 2). This is more specific than the criteria adopted by 11 groups (all European, which require fewer bands), less specific than those adopted by five groups (all in the USA, which require more bands) and similar to four groups.[10,16,20,22]

The difficulty of Western blot interpretation is compounded by the problem of band intensity.[19] Some groups discount weak bands[15] but determination of band intensity is often subjective as it is usually determined visually.[3,19] Although band intensity was a criterion adopted by seven groups, we felt that keeping band intensity in the revised criteria was important, with four strong (3–4 intensity) specific bands required for a positive result, and weaker bands for a weak-positive result (Table 2).

Cutler *et al.* stated that they did not use band intensity due to blot variability, but this is precisely the reason it should be included. With slight variations in blot intensity, some bands may not be detected in repeat runs. Therefore, it is important to require strong bands that withstand slight blot variations when repeated to ensure robust, reproducible results. In our laboratory, samples are routinely tested with previous samples to identify any changes in band profile that may indicate current or past infection.

Where our revised criteria differ from all groups (Table 1) is in the recognition of patients with five or more bands in total, who do not have the required number of specific bands for an equivocal, weak-positive or positive result. This change in criteria meant that 14 patients (four previously negative, 10 equivocal) became weak-positive as they had six bands in total, 10 of whom had symptoms of LB. Likewise, a further three patients previously negative became equivocal. Although some groups consider that the inclusion of non-specific bands in the criteria is not beneficial,[13,19,23] we feel that it is important. These sera are reactive but, due to technical constraints of the test, we are unable to say that these bands are related to *B. burgdorferi* infection.

Although every attempt is made to ensure band measurements are accurate, blots are assessed by eye and some bands can be diffuse and difficult to read accurately. Therefore, it is essential that potential diagnostic bands are not overlooked. This approach is validated by the fact that three bands previously classed as non-specific (20, 28 and 48) are now classed as specific[30] in the revised criteria, and there may be even more non-specific bands that are actually specific.

The increased sensitivity obtained using the revised criteria is extremely important as it may have led to a change in the management of 76 patients who had weak-positive or positive Western blots with the revised criteria but negative/equivocal with the current criteria. Further support for our revised criteria is demonstrated by the fact that 51 (76.1%) of these patients had symptoms of LB. However, patient management may not be greatly affected in the 33 patients whose results changed from weak-positive to equivocal according to the revised criteria (Table 3); a second sample is required from equivocal patients before a clinical interpretation can be made. Five of these patients had erythema migrans, which is diagnostic of LB and does not warrant further serological testing.

Table 3. *B. burgdorferi* IgG Western blot results for 832 patients. The number of patients with positive, weak-positive, equivocal and negative results and totals with current and revised criteria are stated.

Current criteria[7]	Revised criteria				
	Positive	Weak positive	Equivocal	Negative	Total
Positive	151	121	0	0	272
Weak positive	1	136	33	0	170
Equivocal	0	59	97	0	156
Negative	4	13	34	183	234
Total	156	329	164	183	832

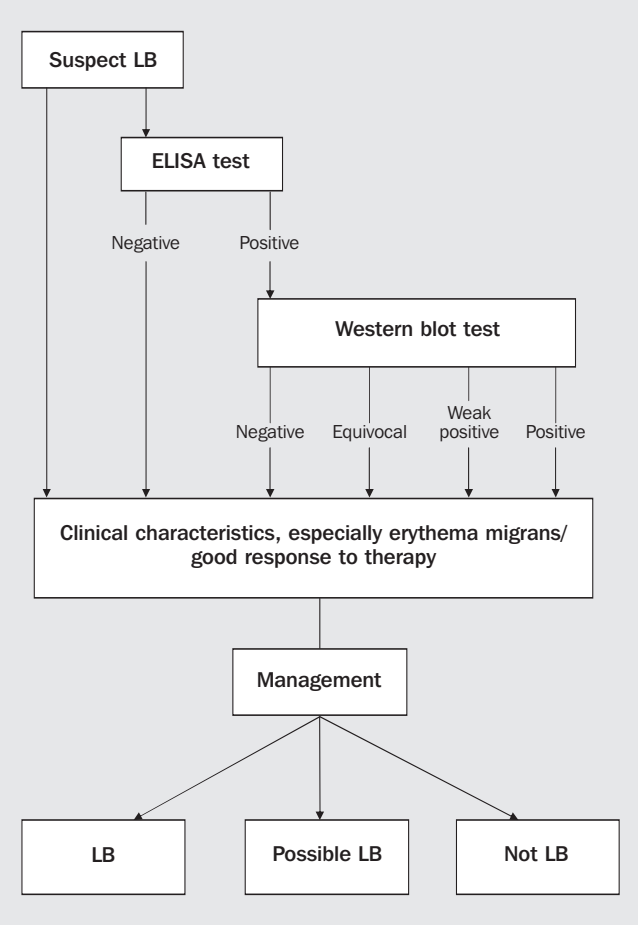

Fig. 1. Lyme borreliosis (LB) testing protocol algorithm. The algorithm demonstrates the importance of the test results and the clinical characteristics in patient management.

It is interesting that no other group had a weak-positive category (Table 1) and only four (19%) included an equivocal category.[12,16,17,23] This may be because most studies were based on well-characterised LB sera. However, our study was on a heterogeneous patient population with a large clinical spectrum, many of whom had non-specific symptoms. Previously we have reported that 62% of our samples tested were from patients with symptoms suggestive of late Lyme borreliosis, and only 57% of our seropositive patients had erythema migrans.[31,32] In addition, most infections in Scotland are in the 60-65 age group,[32] many of whom have pre-existing conditions. In these patients, the use of the equivocal and weak-positive categories can be beneficial to clinical interpretation and management.

When results are interpreted in Scotland, an endemic area of *B. burgdorferi* infection, from many different symptomatic groups, it is important to use an algorithm (Fig.1). This emphasises the fact that the clinical interpretation of all results depends both on the test result and the clinical characteristics of the patient.

The present study attempted to address some of the problems of ambiguity in the literature. While we do not suggest that our revised criteria should be adopted in Europe, this work highlights the need for all researchers to evaluate their own systems continually. We believe that the revised criteria have simplified blot interpretation and improved the sensitivity and robustness of LB diagnosis using Western blotting. Use of a protocol tailored to a patient population which incorporates clinical characteristics means that the entire process will be simplified and will assist in the management of patients. □

The authors thank all the users of the National Lyme Borreliosis Testing Laboratory for their help and support, staff at the Highland Health Sciences Library for providing expert literature search facilities, Edwina Kehoe and Gill Brown for technical assistance, and Laura Smith for invaluable help with data entry.

References

1 Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. *Clin Microbiol Rev* 2005; **18**: 484–509.
2 Wilske B. Diagnosis of Lyme borreliosis in Europe. *Vector Borne Zoonotic Dis* 2003; **3**: 215–27.
3 Robertson J, Guy E, Andrews N *et al*. A European multicentre study of immunoblotting in serodiagnosis of Lyme borreliosis. *J Clin Microbiol* 2000; **38**: 2097–102.
4 Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the second national conference on serologic diagnosis of Lyme disease. *MMWR Mob Mortal Wkly Rep* 1995; **44**: 590–1.
5 Hauser U, Lehnert G, Lobentanzer R, Wilske B. Interpretation criteria for standardised Western blots for three European species of *Borrelia burgdorferi* sensu lato. *J Clin Microbiol* 1997; **35**: 1433–44.
6 Davidson M, Evans R, Ling CL, Wiseman AD, Joss AWL, Ho-Yen DO. Isolation of *Borrelia burgdorferi* from ticks in the Highlands of Scotland. *J Med Microbiol* 1999; **48**: 59–65.
7 Mavin S, Evans R, Milner RM, Chatterton JMW, Ho-Yen DO. Local *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* strains in a single mixed antigen improves Western blot sensitivity. *J Clin Pathol* 2009; **62**: 552–4.
8 Mavin S, Milner RM, Evans R, Chatterton JMW, Joss AWL, Ho-Yen DO. The use of local isolates in Western blots improves serological diagnosis of Lyme disease in Scotland. *J Med Microbiol* 2007; **56**: 47–51.
9 Grodzicki RL, Steere AC. Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosis of early Lyme disease. *J Infect Dis* 1988; **157**: 790–7.
10 Fister RD, Weymouth LA, McLaughlin JC, Ryan RW, Tilton RC. Comparative evaluation of three products for the detection of *Borrelia burgdorferi* antibody in human serum. *J Clin Microbiol* 1989; **27**: 2834–7.
11 Karlsson M. Western immunoblot and flagellum enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. *J Clin Microbiol* 1990; **28**: 2148–50.
12 Rose CD, Dubbs SB, Gibney KN, Doughty RA, Fawcett PT. Interpretation of Western blot (WB) serology for Lyme disease (LD): towards the establishment of minimal criteria for positivity. *Arthritis Rheum* 1990; **33**: S143.
13 Zoller L, Cremer J, Faulde M. Western blot as a tool in the diagnosis of Lyme borreliosis. *Electrophoresis* 1993; **14**: 937–44.
14 Sood SK, Rubin LG, Blader ME, Ilowite NT. Positive serology for Lyme borreliosis in patients with juvenile rheumatoid arthritis in a Lyme borreliosis endemic area: analysis by immunoblot. *J Rheumatol* 1993; **20**: 739–41.

15 Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993; **167**: 392–400.
16 Cutler SJ, Wright DJM, Luckhurst VH. Simplified method for the interpretation of immunoblots for Lyme borreliosis. *FEMS Immunol Med Microbiol* 1993; **6**: 281–6.
17 Seppala IJT, Kroneld R, Schauman K, Forsen KO, Lassenius R. Diagnosis of Lyme borreliosis: non-specific serological reactions with *Borrelia burgdorferi* sonicate antigen caused by IgG2 antibodies. *J Med Microbiol* 1994; **40**: 293–302.
18 Kowal K, Weinstein A. Western blot band intensity analysis. *Arthritis Rheum* 1994; **37**: 1206–11.
19 Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J Clin Microbiol* 1995; **33**: 419–27.
20 Norman GL, Antig JM, Bigaignon G, Hogrefe WR. Serodiagnosis of Lyme *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* Western blots (immunoblots). *J Clin Microbiol* 1996; **34**: 1732–8.
21 Hilton E, Devoti J, Sood S. Recommendation to include OspA and OspB in the new immunoblotting criteria for serodiagnosis of Lyme disease. *J Clin Microbiol* 1996; **34**: 1353–4.
22 Ryffel K, Peter O, Binet L, Dayer E. Interpretation of immunoblots for Lyme borreliosis using a semi-quantitative approach. *Clin Microbiol Infect* 1998; **4**: 205–11.
23 Hauser U, Lehnert G, Wilske B. Validity of interpretation criteria for standardised Western blots (immunoblots) for serodiagnosis of Lyme borreliosis based on sera collected throughout Europe. *J Clin Microbiol* 1999; **37**: 2241–7.
24 Schulte-Spechtel U, Lehnert G, Liegl G *et al*. Significant improvement of the recombinant *Borrelia*-specific immunoglobulin G immunblot test by addition of VIsE and DbpA homologue derived from *Borrelia garinii* for diagnosis of early neuroborreliosis. *J Clin Microbiol* 2003; **41**: 1299–301.
25 Hernandez-Novoa B, Orduna A, Bratos MA *et al*. Utility of a commercial immunoblot kit (BAG-Borrelia blot) in the diagnosis of the preliminary stages of Lyme disease. *Diagn Microbiol Infect Dis* 2003; **47**: 321–9.
26 Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJB, Wormser GP, Steere AC. 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VIsE band as the second-tier test. *Clin Infect Dis* 2010; **50**: 20–6.
27 Davidson MM, Chisholm SM, Wiseman AD, Joss AWL, Ho-Yen DO. Improved serodiagnosis of Lyme disease. *Clin Mol Pathol* 1996; **49**: M80–4.
28 Evans R, Mavin S, Ho-Yen DO. Audit of the laboratory diagnosis of Lyme disease in Scotland. *J Med Microbiol* 2005; **54**: 1139–41.
29 Wilske B, Zoller L, Brade V *et al.* MIQ 12 Lyme-borreliose. In: Mauch H, Lutticken R eds. *Qualitatsstandards in der mikrobiologisch infektiologischen Diagnostik*. Munich: Urban & Fischer Verlag, 2000.
30 Evans R, Mavin S, McDonagh S, Chatterton JMW, Milner RMM, Ho-Yen DO. More specific bands in the IgG Western blot in sera from Scottish patients with suspected Lyme borreliosis. *J Clin Pathol* 2010; **63**: 719–21.
31 Ho-Yen DO, Mavin S, Hopkins PC *et al.* Lyme borreliosis: differences between the patients who are tested and those with the infection. *HPS Weekly Report* 2008; **42**: 441–3 (www.hps.scot.nhs.uk/giz/wrdetail.aspx?id=39903&wrtype=9).
32 Milner RM, Mavin S, Ho-Yen DO. Lyme borreliosis in Scotland is different. *J Infect* 2009; **59**: 146–7.