Comparison of Seven Commercial Kits for Detection of Antibodies to *Borrelia burgdorferi*

J.L. Schmitz, C.S. Powell, J.D. Folds*

Five enzyme immunoassay (EIA) and two Western blot (WB) commercial kits were compared for their ability to detect antibodies to *Borrelia burgdorferi*. The panel of 53 test sera consisted of 25 sera positive for antibodies to *Borrelia burgdorferi*, 15 sera negative for such antibodies, 5 sera reactive in serologic tests for syphilis, and 8 sera containing antinuclear antibodies and/or rheumatoid factor. The rate of agreement with reference results was 93 %, 90 %, 90 % and 88 % for EIA kits from Diamedix, Cambridge Biotech, Mardx and Sigma respectively. The sensitivity and specificity was 84 % and 100 % respectively for Cambridge Biotech, 76 % and 94 % for Diamedix, 68 % and 83 % for Mardx, and 68 % and 83 % for Sigma. The three confirmatory tests, Cambridge Biotech WB, General Biometrics P39 EIA and Mardx WB, demonstrated 75 %, 60 % and 63 % agreement respectively. The sensitivity and specificity was 52 % and 100 % respectively for Cambridge Biotech WB, 24 % and 100 % for General Biometrics P39 EIA, and 44 % and 100 % for Mardx WB. The results demonstrate the variable performance of commercial serologic kits for detection of antibodies to *Borrelia burgdorferi*. WB appears to be a better confirmatory test than the single protein EIA.

Lyme disease is a multi-system infection caused by the spirochete *Borrelia burgdorferi* (1, 2). Because of the variability of signs and symptoms, establishing a clinical diagnosis of Lyme disease can be problematic. Thus, laboratory methods are frequently used for the diagnosis of Lyme disease. While culture provides a definitive diagnosis, it is not routinely used because of the lack of sensitivity, the cost and the time required for recovery of organisms (3, 4). Antigen detection (5, 6) and the polymerase chain reaction (7–9) have also been used to detect *Borrelia burgdorferi* in clinical specimens. Their routine use in the clinical laboratory is limited at the present time, however. Currently, serologic tests are the laboratory methods most frequently used in the diagnosis of Lyme disease.

A variety of serologic tests have been developed to detect antibodies to *Borrelia burgdorferi*. Indirect immunofluorescent assay (IFA), enzyme immunoassay (EIA) and Western blot (WB) are frequently used techniques (10). IFA was first used to demonstrate the reactivity of serum from patients with Lyme disease to *Ixodes dammini* spirochetes (11). This assay has subsequently been used clinically as a serologic test. EIAs developed for serological diagnosis of Lyme disease have been found in some studies to be superior to IFA in sensitivity (12–16). Modifications of standard indirect EIAs have shown even greater sensitivity during early Lyme disease (17–20).

Because of the potential for false positive results in serologic assays to detect Lyme disease due to the presence of other bacterial and viral infections (2, 21, 22), alternate tests, including WB (10, 23–25) and a recombinant protein EIA (26), are available to confirm the specificity of results of standard EIA and IFA. While these assays help increase the specificity of serologic testing for Lyme disease (24, 26), the problem of sensitivity during early disease remains. The development of such a variety of serologic tests for diagnosis of Lyme disease has resulted in a variety of commercial kits to choose from when implementing a serologic testing program for Lyme disease.

In this study we evaluated the performance of seven commercial Lyme disease serology kits using a panel of sera previously assayed for the

Clinical Immunology Laboratory, University of North Carolina Hospitals, Chapel Hill, North Carolina 27514, USA.

presence of antibodies to *Borrelia burgdorferi*. A total of four EIA kits utilizing spirochetal lysates as antigen were tested. Three additional kits, two WB kits and an EIA utilizing a recombinant 39 kDa protein as antigen, were also evaluated as confirmatory tests.

Materials and Methods

Serologic Kits. The suppliers of the diagnostic kits are listed in Table 1. Four EIA and both WB kits utilized *Borrelia burgdorferi* strain B31 as antigen. The General Biometrics P39 EIA utilized a recombinant 39 kDa protein from *Borrelia burgdorferi* strain B31 as the antigen. The serum diluent in the following kits contained absorbents: General Biometrics (*Escherichia coli* proteins), Sigma and Diamedix (nature of absorbent not disclosed). Kits from Mardx and Cambridge Biotech did not contain absorbents in the serum diluent. In all EIAs a 1:100 final dilution of serum was used, except in the General Biometrics EIA in which a 1:400 final dilution and goat anti-human IgG and IgM conjugates at dilutions specified by the manufacturer were used. Kits from Mardx, General Biometrics and Cambridge Biotech used peroxidase as the enzyme label while kits from Sigma and Diamedix used alkaline phosphatase. The substrates, stopping reagents and incubation times varied in each kit. All assays were performed according to the manufacturers' instructions provided with the kits. Western blot kits were provided with nitrocellulose strips containing *Borrelia burgdorferi* strain B31 as the antigen. Diluted sera (1:100) and controls were incubated with strips for the appropriate times (2 hours for Cambridge Biotech; 30 min for Mardx) followed by three washes. Alkaline phosphatase conjugated goat anti-human IgG at dilutions specified in the manufacturers' insert was then added and the strips incubated for specified times (1 hour for Cambridge Biotech; 15 min for Mardx). Strips were then washed three times and substrate added. After an appropriate incubation period specified by the manufacturer, the strips were rinsed in distilled water and air-dried.

Table 1: Suppliers and assay method of the seven diagnostic kits tested.

Supplier	Method
Cambridge Biotech, USA	EIA[a]/WB[b]
Diamedix, USA	EIA[a]
General Biometrics, USA	P39 EIA[c]
Mardx, USA	EIA[a]/WB[b]
Sigma, USA	EIA[a]

[a] All EIAs except the P39 EIA utilized whole cell lysates of *Borrelia burgdorferi* strain B31 and polyvalent conjugates (anti-human IgG and IgM).
[b] The Western blot kits used in this study were not FDA approved.
[c] The P39 EIA kit utilized in this study was not FDA approved.

Sera. A total of 53 sera were used in the analysis. Thirty sera, 20 positive and 10 negative for antibodies to *Borrelia burgdorferi*, were obtained from the Centers for Disease Control (Dr. Thomas Quan). Twenty-five of these were from patients with clinical symptoms of Lyme disease (stage of disease unknown by us) and five were from normal donors. Twelve of the 25 patient sera were collected 43 or fewer days after the onset of disease, the other 13 were collected 60 or more days after onset of disease. The diagnoses were made by academic clinical experts in Lyme disease based on the CDC case definition (personal communication, Dr. Robert Craven, CDC, Fort Collins, Colorado). Ten additional sera, five with significant levels and five with non-significant levels of antibodies to *Borrelia burgdorferi* were obtained from the Gundersen Medical Foundation, La Crosse, Wisconsin, USA (Dr. Steven Callister). No clinical information was available for these specimens.

In addition to the Lyme disease serum panel, five sera positive in the Venereal Disease Research Laboratory/ florescent treponemal antibody absorption test (VDRL/ FTA-ABS), and eight antinuclear antibody (ANA) and/or rheumatoid factor (RF) positive sere were obtained from the Clinical Immunology Laboratory at University of North Carolina Hospitals.

Interpretation of Results. All EIA results were interpreted as positive, negative or borderline according to instructions provided by the manufacturer. WB results were interpreted as positive, negative or equivocal based on the manufacturer's criteria. A positive Cambridge Biotech WB required reactivity to the 41 kDa antigen and any two of the antigens of 30, 31, 34, 39 or 66 kDa molecular weight. Sera not meeting these criteria were considered non-reactive. A positive Mardx WB required reactivity to the 41 or 39 kDa antigens plus reactivity to either the 31 or 34 kDa antigen. Reactivity to both the 31 and 34 kDa antigens was also considered positive. The presence of bands between the 41 kDa antigen up to and including the 66 kDa antigen or a complete lack of bands was considered negative. All other band patterns were considered equivocal.

Analysis of Data. Results obtained with the diagnostic kits were compared to reference results provided by the serum panel suppliers (CDC and Gundersen Medical Foundation) and expressed as percentage of agreement. Specificity of the kits was determined, using the CDC serum panel (25 Lyme disease patients and 5 normal subjects), the five VDRL/FTA-ABS positive sera and the eight RF/ANA positive sera. Sensitivity of the kits was calculated for the 30 CDC sera. The sensitivity for sera collected < 43 days after onset anf for sera collected > 60 days after onset was calculated separately. The ten sera from the Gundersen Medical Foundation were not included in calculations of sensitivity and specificity.

Results

Results with Four Enzyme Immunoassays. The rate of agreement with reference results was 88 % for Sigma, 90 % for Cambridge Biotech and Mardx, 93 % for Diamedix (Table 2). The sen-

Table 2: Performance of four commercial EIA kits for diagnosis of Lyme disease related to results for reference sera.

Diagnostic kit	Agreement	Sensitivity[a] (early/late)	Specificity[b]
Cambridge Biotech	90 %	92 % / 77 %	100 %
Diamedix	93 %	75 % / 77 %	94 %
Mardx	90 %	58 % / 77 %	83 %
Sigma	88 %	58 % / 77 %	83 %

[a] Sensitivity for sera collected ≤ 43 days after onset of symptoms / sensitivity for sera collected ≥ 60 days after onset of symptoms.
[b] Specificity was calculated with results from normal, VDRL/FTA-ABS reactive and ANA/RF reactive sera.

Table 3: Analysis of five syphilitic sera (FTA-ABS reactive) with seven commercial kits for diagnosis of Lyme disease. Results are expressed as negative (–), positive (+) or borderline (B) for antibodies to *Borrelia burgdorferi*.

Diagnostic kit	Syphilitic serum (VDRL titer)				
	Serum 1 (1:2)	Serum 2 (1:4)	Serum 3 (1:64)	Serum 4 (1:128)	Serum 5 (1:128)
Cambridge Biotech EIA	–	–	–	–	–
Diamedix EIA	–	–	–	–	+
Mardx EIA	–	–	+	+	+
Sigma EIA	–	B	+	–	+
Cambridge Biotech WB	–	–	–	–	–
General Biometrics P39 EIA	–	B	–	B	B
Mardx WB	–	B	B	B	–

sitivity (sera collected early after onset/later after onset) was 92/77 %, 75/77 %, 58/77 % and 58/77 % for the Cambridge Biotech, Diamedix, Mardx and Sigma kits respectively. The specificity was 100 %, 94 %, 83 % and 83 % respectively for the Cambridge Biotech, Diamedix, Mardx, and Sigma kits.

Analysis of syphilitic sera with each of the kits demonstrated significant variation in results (Table 3). The kit from Cambridge Biotech showed no positive results with any of the syphilitic sera. One of five sera was positive with the Diamedix kit, two of five sera with the Sigma kit and three of five sera with the Mardx kit. None of the kits gave positive results with sera containing RF or ANA (data not shown).

Results with Three Confirmatory Tests. The rate of agreement with reference results was lower for the confirmatory tests (Table 4, Figure 1). The General Biometrics P39 EIA and the Mardx WB demonstrated 60 % and 63 % agreement respectively, while the Cambridge Biotech WB demonstrated 75 % agreement. The sensitivity (sera collected early after onset/collected later

after onset) was 58/77 % for the Cambridge Biotech WB, 8/39 % for the General Biometrics P39 EIA and 50/77 % for the Mardx WB. A specificity of 100 % was achieved with all three kits.

Discussion

The diagnosis of Lyme disease by culture has been problematic due to low sensitivity (3, 4), although a recent report demonstrated significantly improved sensitivity (27). Antigen detection (5, 6) and the polymerase chain reaction (7–9) show promise as diagnostic tools, however they are not routinely used in the clinical laboratory at present. Serological tests are the most frequently used laboratory method for diagnosis of Lyme disease but because of a slow developing antibody response to *Borrelia burgdorferi* lack sensitivity during early disease (2, 23). Three to six weeks may pass before significant levels of antibody can be detected. In addition to a lack of sensitivity, serologic tests for Lyme disease also lack

Table 4: Performance of three confirmatory commercial kits for diagnosis of Lyme disease related to results for reference sera.

Diagnostic kit	Agreement	Sensitivity[a] (early/late)	Specificity[b]
Cambridge Biotech (WB)	75 %	58 % / 77 %	100 %
General Biometrics (P39 EIA)	60 %	8 % / 39 %	100 %
Mardx (WB)	63 %	50 % / 77 %	100 %

[a] Sensitivity for sera collected ≤ 43 days after onset of symptoms / sensitivity for sera collected ≥ 60 days after onset of symptoms.
[b] Specificity was calculated with results from normal and VDRL/FTA-ABS reactive sera.
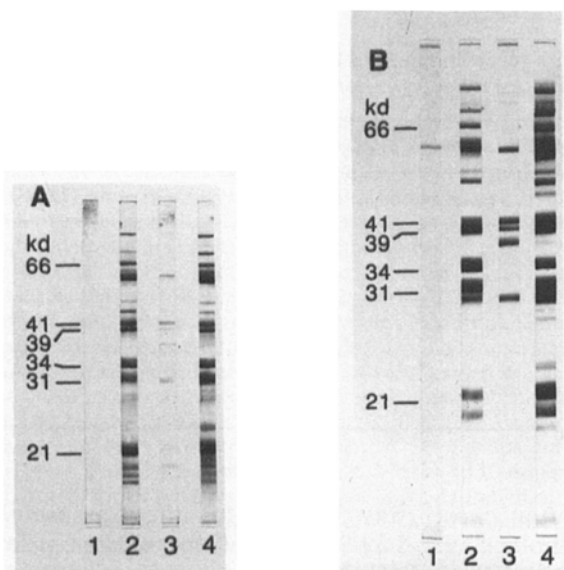
Figure 1: Appearance of the Mardx IgG Western blot (panel A) and the Cambridge Biotech IgG Western blot (panel B). In panels A and B, lane 1: negative control; lane 2: positive control; lane 3: weakly positive serum; lane 4: strongly positive serum.

specificity. False positive results resulting from the presence of other bacterial, viral or rheumatic diseases are well known to occur (2, 21–23).

Serologic tests utilizing alternative techniques to the standard indirect EIA, such as IgM antibody capture (17, 20), purified or recombinant antigen based EIAs (18, 20, 22, 26) and WB (10, 23, 24), have been developed to improve the sensitivity and specificity. In addition to the standard IFA and EIAs many of these assays are commercially available.

Results of our comparison of commercial EIA kits demonstrated variability in sensitivity and specificity. Using the CDC sera, VDRL positive sera and ANA/RF positive sera, specificity of the

various kits ranged from 83 % to 100 %. The sensitivity varied from 24 % to 84 %. However, as seen in Table 2, sensitivity was dependent on the time after onset at which sera were collected. The sere collected ≤ 43 days after onset in our study may have been primarily from cases of early disease, although not exclusively as some patients may not develop the early symptoms of Lyme disease. Analysis of sera collected later (≥ 60 days after onset) demonstrated more consistent results with the various kits. This probably reflects the additional duration of infection, resulting in more time to develop detectable levels of antibodies to *Borrelia burgdorferi*. Three sera collected > 60 days after onset yielded negative results with all kits. This could be due to effective antibiotic treatment or misdiagnosis. Since we did not have access to data on the clinical symptoms of these patients, analysis by time after disease onset was the only means to evaluate sensitivity. These variable results are not unexpected as the kits varied with respect to reagents used and incubation times. Other investigators have also demonstrated variability in the performance of serologic kits for detection of Lyme disease (28, 29).

There was surprising variability in the results of tests with the five VDRL reactive sera. The kits from Cambridge Biotech and Diamedix showed the best results with these sera, while the kits from Mardx and Sigma demonstrated several false positive results. Some kits included an absorbent in the sample diluent to decrease non-specific reactivity, however our results suggest that the absorbent(s) in some of the kits does not effectively remove treponemal antibodies cross-reactive with *Borrelia burgdorferi*. Ironically, one of the kits with no absorbents (Cambridge Biotech) generated the best results with the syphilitic sera.

The sensitivity of the WB kits was lower than expected on the basis of published reports (24–26). However, we evaluated only IgG WB kits and it

has been shown previously that the use of an IgM in addition to an IgG conjugate may help increase sensitivity during early disease (24, 25). Since the stage of disease of the patients in our serum panel was not known, it is possible that sera from cases of early Lyme disease (especially in the group collected ≤ 43 days after onset) containing predominantly IgM antibodies may have been included which would result in decreased sensitivity. Otherwise, modification of the criteria for a positive WB result might help increase the sensitivity (but possibly reduce the specificity) of WB, especially the Cambridge WB kit.

The poor sensitivity of the P39 EIA kit may also be due to the possible inclusion of sera from cases of early Lyme disease when reactivity to P39 may not be as consistent as in later disease (26, 30). However, we observed two sera with reactivity to P39 on WB which were negative in the P39 EIA, and five sera with a reactive p39 band on WB and borderline p39 EIA results. This suggests that the WB may be a more sensitive method than EIA in detecting reactivity to this protein. A potential drawback to the identification of P39 on WB is the presence of a heat shock protein of similar molecular weight (31). To our knowledge, it is not known if this heat shock protein is immunogenic in humans and if it would thus pose problem(s) in interpretation of reactivity to P39 by WB. While others have demonstrated good reactivity to P39 in larger panels of serum using WB (26, 30), our data do not support the sole use of this antigen in the current EIA format as serodiagnostic test, although the benefits of its high specificity are clear. Modification of the P39 assay might enhance its sensitivity however.

The testing of VDRL reactive sera with the EIAs and the WBs demonstrated their usefulness in determining the specificity of the kits. In WB, reactivity to the 41 kDa flagellin protein was frequently detected (not shown) with both kits, as has been reported by others (22). This is probably due to shared amino acid sequences in the flagellin proteins of *Treponema pallidum* and *Borrelia burgdorferi* (32). Three sera showed borderline reactivity in the P39 EIA. This was an unexpected result, however other investigators have demonstrated reactivity to P39 in WB using VDRL reactive sera (30).

In conclusion, our results demonstrate variable performance of serologic kits for detection of IgG antibody to *Borrelia burgdorferi*. In addition, Western blot appears to be a better means of confirmatory testing than single protein EIAs. How-ever, serum panels from culturally proven cases of Lyme disease are needed to evaluate further the performance of commercial kits.

References

1. **Steere AC:** Medical progress: Lyme disease. New England Journal of Medicine 1989, 321: 586–596.
2. **Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE:** The spirochetal etiology of Lyme disease. New England Journal of Medicine 1983, 308: 733–740.
3. **Benach JL, Bosler EM, Hanrahan JP, Coleman JL, Bast TE, Habicht GS, Cameron DJ, Ziegler JL, Burgdorfer W, Barbour AG, Edelman R, Kaslow RA:** Spirochetes isolated from the blood of two patients with Lyme disease. New England Journal of Medicine 1983, 308: 740–742.
4. **Steere AC, Grodzicki RL, Craft JE, Shrestra M, Kornblatt AN, Malawista SE:** Recovery of Lyme disease spirochetes from patients. Yale Journal of Biology and Medicine 1984, 57: 557–560.
5. **Dorward DW, Schwan TG, Garon CF:** Immune capture and detection of *Borrelia burgdorferi* antigens in urine, blood, or tissues from infected ticks, mice, dogs, and humans. Journal of Clinical Microbiology 1991, 29: 1162–1170.
6. **Hyde FW, Johnson RC, White TJ, Shelburne CE:** Detection of antigens in urine of mice and humans infected with *Borrelia burgdorferi*. Journal of Clinical Microbiology 1989, 27: 58–61.
7. **Debue M, Gautier P, Hackel C, Van Elsen A, Herzog A, Bigaignon A, Bollen A:** Detection of *Borrelia burgdorferi* in biological samples using the polymerase chain reaction. Research in Microbiology 1991, 142: 565–572.
8. **Goodman JL, Jurkovich P, Kramber JM, Johnson RC:** Molecular detection of persistent *Borrelia burgdorferi* in the urine of infected patients with active Lyme disease. Infection and Immunity 1991, 59: 269–278.
9. **Melchers W, Meis J, Rosa P, Claas E, Nohlmans L, Koopman R, Horrevorts A, Galama J:** Amplification of *Borrelia burgdorferi* DNA in skin biopsies from patients with Lyme disease. Journal of Clinical Microbiology 1991, 29: 2401–2406.
10. **Barbour AG:** Laboratory aspects of Lyme borreliosis. Clinical Microbiology Reviews 1988, 1: 399–414.
11. **Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP:** Lyme disease – a tick-borne spirochetosis? Science 1982, 216: 1317–1319.
12. **Craft JE, Grodzicki RL, Shrestha M, Fischer DK, Garcia-Blanco M, Steere AC:** The antibody response in Lyme disease. Yale Journal of Biology and Medicine 1984, 57: 561–565.
13. **Craft JE, Grodzicki RL, Steere AC:** Antibody response in Lyme disease: evaluation of diagnostic tests. Journal of Infectious Diseases 1984, 149: 789–795.
14. **Cutler SJ, Wright DJM:** Comparison of immunofluorescence and enzyme linked immunosorbent assays for diagnosing Lyme disease. Journal of Clinical Pathology 1989, 42: 869–871.

15. **Magnarelli LA, Meegan JM, Anderson JF, Chappell WA:** Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. Journal of Clinical Microbiology 1984, 20: 181–184.

16. **Wilkinson H:** Immunodiagnostic tests for Lyme disease. Yale Journal of Biology and Medicine 1984, 57: 567–572.

17. **Berardi VP, Weeks KG, Steere AC:** Serodiagnosis of early Lyme disease: analysis of IgM and IgG antibody responses by using an antibody-capture enzyme immunoassay. Journal of Infectious Diseases 1988, 158: 754–760.

18. **Coleman JL, Benach JL:** Isolation of antigenic components from the Lyme disease spirochete: their role in early diagnosis. Journal of Infectious Diseases 1987, 155: 756–765.

19. **Hansen K, Hindersson P, Pedersen NS:** Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis of Lyme disease. Journal of Clinical Microbiology 1988, 26: 338–346.

20. **Hansen K, Pii K, Lebech A:** Improved immunoglobulin M serodiagnosis in Lyme borreliosis by using a μ-capture enzyme-linked immunosorbent assay with biotinylated *Borrelia burgdorferi* flagella. Journal of Clinical Microbiology 1991, 29: 166–173.

21. **Feder HM, Gerber MA, Luger SW, Ryan RW:** False positive serologic tests for Lyme disease after varicella infection. New England Journal of Medicine 1991, 325: 1886–1887.

22. **Magnarelli LA, Anderson JF, Johnson RC:** Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. Journal of Infectious Diseases 1987, 156: 183–188.

23. **Craft JE, Fischer DK, Shimamoto GT, Steere AC:** Antigens of *Borrelia burgdorferi* recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G response late in the illness. Journal of Clinical Investigation 1986, 78: 934–939.

24. **Grodzicki RL, Steere AC:** Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. Journal of Infectious Diseases 1988, 157: 790–797.

25. **Karlsson M, Mollegard I, Stiernstedt G, Wretlind B:** Comparison of Western blot and enzyme-linked immunosorbent assay for diagnosis of Lyme borreliosis. European Journal of Clinical Microbiology & Infectious Diseases 1989, 8: 871–877.

26. **Simpson WJ, Schrumpf ME, Schwan TG:** Reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to *Borrelia burgdorferi*. Journal of Clinical Microbiology 1990, 28: 1329–1337.

27. **Berger BW, Johnson RC, Kodner C, Coleman L:** Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. Journal of Clinical Microbiology 1992, 30: 359–361.

28. **Christenson VD, White DH:** Evaluation of four commercially available EIA assays for the serologic diagnosis of Lyme disease. Journal of Clinical Laboratory Analysis 1991, 5: 340–343.

29. **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. Journal of Clinical Microbiology 1989, 27: 2834–2837.

30. **Ma B, Christen B, Leung D, Vigo-Pelfrey C:** Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. Journal of Clinical Microbiology 1992, 30: 370–376.

31. **Cluss RG, Boothby JT:** Thermoregulation of protein synthesis in *Borrelia burgdorferi*. Infection and Immunity 1990, 58: 1038–1042.

32. **Gassmann GS, Duetzman R, Vogt A, Goebel UB:** N-terminal amino acid sequence of the *Borrelia burgdorferi* flagellum. FEMS Microbiology Letters 1989, 51: 101–105.