days of antibiotic therapy, which possibly is related to the release of spirochetal antigens into body fluids as spirochetes are killed by antibiotic therapy.[187]

IMMUNOFLUORESCENT ASSAY

Immunofluorescent assay (IFA) uses fluorescein-tagged antihuman immunoglobulin to detect serum, CSF, or synovial fluid IgG, IgM, or IgA antibody binding specifically to whole *B. burgdorferi* fixed on a slide.[1, 10]

The range of sensitivity of the polyvalent IFA is 13 to 100% in early Lyme disease, and 64 to 100% in late Lyme disease. With IFA, IgM antibody is detectable earlier in infection than IgG antibody; thus, it is more sensitive in the diagnosis of early Lyme disease, and it generally disappears by convalescence except in patients with persistently active Lyme disease. Under ideal circumstances, IFA may detect *B. burgdorferi*–specific IgM antibody in 100% of patients with early culture-positive erythema migrans on the day of presentation.[756] The specificity of Lyme IFA is low in patients with other spirochetal infections because of cross-reactivity between *B. burgdorferi* and other spirochetes, especially syphilis, but it is good in rapid plasma reagin (RPR)-negative patients (see Table 11–16). Major disadvantages of the IFA—subjective test reading, the need for highly trained personnel for test performance, lack of test automation, and unsuitability for high volume use—have resulted in its replacement by the ELISA in most laboratories.[250, 252, 750]

ENZYME-LINKED IMMUNOSORBENT ASSAY

The standard indirect enzyme-linked immunosorbent assay (ELISA) uses enzyme-tagged antihuman immunoglobulin to detect serum, CSF, or synovial fluid IgG, IgM, or IgA antibody binding specifically to either whole disrupted (sonicated) *B. burgdorferi* or specific *B. burgdorferi* components (antigens) bound to multi-well ELISA plates.[224, 252]

The ELISA is 13 to 92% sensitive in early Lyme disease and 89 to 100% sensitive in late Lyme disease. IgM antibody is detectable earlier in infection than IgG, and it generally decreases during convalescence except in patients with persistently active infection, although ELISA IgM antibody positivity has been found even in some successfully treated patients.[244]

The ELISA is more efficient and reproducible than the IFA.[224, 252] Comparisons of IFA and ELISA generally have shown that ELISA is also more sensitive and specific than IFA,[98, 224] although some reports have found them to be comparable.[250, 252] In RPR-negative patients, the sensitivity of IFA and ELISA is high for detection of late Lyme disease, when *B. burgdorferi* antibody levels are high, but lower for detection of early Lyme disease, when there is a high false-negative rate because of the combination of low *B. burgdorferi* antibody in the first few weeks and high background as a result of cross-reactive antibody.[222]

Cross-reactivity in both ELISA and IFA assays occurs between *B. burgdorferi* and other spirochetes (see Table 11–16). Because of the high cross-reactivity with syphilis, it is essential that an RPR test be performed on all Lyme-positive sera to exclude syphilis (as RPR does not cross react and should be negative in Lyme disease.[252] Only a low rate of cross-reactivity occurs with *Leptospira*[252, 746, 758] and *Rickettsia*.[252, 758] Cross-reactivity with *B. coriacea* may lead to confusion, as this infection is endemic in *Ornithodoros coriaceus* ticks in California's Mendocino County, and humans are an occasional host for this tick.

Other causes of false-positive Lyme ELISA or IFA results are normal spirochetal oral flora,[245] viral infections such as varicella-zoster virus (VZV), Epstein-Barr virus (EBV), or parvovirus,[18, 74, 224, 746] other bacterial infections such as subacute bacterial endocarditis,[745] and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Reiter's disease.[224, 252, 743] Lyme Western blot appears to be more useful than ELISA for evaluation of Lyme serologic status in patients with certain of the illnesses listed here. The significant problem of false Lyme seropositivity by ELISA or IFA testing should serve as a reminder that serologic data should be judiciously interpreted in the context of both the clinical illness and epidemiologic data.

The sensitivity and specificity of the standard whole-cell ELISA sometimes have been increased by using either purified or recombinant components of the organism such as outer surface membrane lipoproteins,[124, 239, 241, 245] the 41-kd flagellar antigen[241, 242, 746] combinations of recombinant Osp C with a recombinant flagellin fragment,[125] or synthetic, non–cross-reactive, immunodominant peptide sequences of the flagellar antigen as antigens. Some Lyme disease patient sera may react with individual antigens present in whole-cell antigen preparations but not with recombinant preparations of the same antigens, possibly because the immunogenic epitopes are presented differently in the recombinant antigens.[108, 124] *B. burgdorferi* loses Osp C during repeated passage in culture; these strains are often used to produce antigens for ELISA assays, which do not detect the early IgM antibody response that is mainly directed toward Osp C. The use of either recombinant Osp C or Osp C–positive strains in ELISA assays has increased the sensitivity of detection of specific IgM antibody in early Lyme disease.[239]

ANTIBODY CAPTURE ELISA, IMMUNE-COMPLEX ELISA, AND ANTIBODY CAPTURE IMMUNE-COMPLEX BIOTINYLATED ELISA

The antibody capture ELISA (ELISA-AC),[757] which reduces competition between IgG and IgM for the same antigenic sites in the assay and false positivity due to rheumatoid factor, increased the sensitivity of detection of IgM and IgG in early Lyme disease so that diagnosis could be confirmed in 67% of acute patient sera and in 93% of convalescent sera. The IgM ELISA-AC was particularly useful in disseminated disease, where the rate of positivity was 71 to 100% and 93 to 100%, respectively, of acute and convalescent sera, compared with 25% and 75%, respectively, in localized Lyme disease. The ELISA-AC assay with flagellar antigen, using the biotin–avidin peroxidase method for demonstration

of IgM, increased the sensitivity and specificity of detection of IgM antibody compared with the standard indirect IgM ELISA with flagellar antigen.[746]

The sensitivity of the ELISA for detection of IgG antibody in early Lyme disease was increased by using a polyethylene glycol (PEG) precipitation method to dissociate the antibody sequestered in circulating immune complexes (IC) before performing the ELISA.[278] This PEG ELISA-IC assay detected IgG antibody in 100% of patients with histories of recent erythema migrans who were seronegative by the standard ELISA assay, and 95% of patients with seropositive Lyme disease; the false positivity rate was zero.

The antibody capture immune-complex biotinylated ELISA (EMIBA)[756] was able to detect specific IgM seropositivity in culture-positive EM patients, as early as the day of lesion biopsy in 100% of disseminated EM patients and in 73% of localized EM patients, including some who were IgM-seronegative by standard IgM or polyvalent ELISA, IgM Western blot, IgM IFA, and IgM ELISA-AC. For localized EM patients, the rate of IgM seropositivity was 36% by the ELISA-AC assay for free serum antibody, 55% by the EMIBA assay with whole *B. burgdorferi* antigen, and 73% with the addition of the flagellar peptide. The false positivity rate was zero. The EMIBA assay is specific and sensitive, particularly for serologic confirmation of early disseminated EM. It is less cumbersome than the lymphocyte proliferative assay and may be useful in the evaluation of seronegative Lyme patients, but it is not widely available.

WESTERN BLOT (IMMUNOBLOT)

This method detects serum, CSF, or synovial fluid IgG or IgM antibodies to many of the over 30 individual *B. burgdorferi* protein antigens,[120, 223, 232, 241, 249, 742] including major outer surface proteins, flagellar antigen, and heat shock proteins. The pattern of antibody to these specific *B. burgdorferi* antigens, demonstrated by the pattern of bands seen in the Western blot assay, is characteristic of Lyme disease and shows temporal evolution with initial expansion of the antibody repertoire and increasing disease duration.[222, 232–234]

The Western blot test is currently recommended as the second test in a two-step serologic testing algorithm, in which ELISA or IFA is used as the highly sensitive initial test, and positive or equivocal ELISA or IFA results are then evaluated by a highly specific Western blot test to exclude false positivity.[236, 478, 748] The Western blot is generally considered more sensitive and more specific than ELISA or IFA, but because of its complexity, it has been plagued by lack of standardization. Efforts are under way to reduce interlaboratory variability in the definition of Western blot positivity.[225, 254, 741, 749, 766]

CDC criteria for positive Western blots[238] are based on studies by Dressler and associates[234] and Engstrom and colleagues.[233] CDC criteria for a positive IgM Western immunoblot are the presence of two of the following three bands in early disease: 24-kd Osp C, 39-kd Bmp A, and 41-kd Fla. For a positive IgG Western immunoblot, five of the ten most common bands must be present after the first few weeks of disease: 18-kd, 21-kd Osp C, 28-kd, 30-kd, 39-kd Bmp A, 41-kd Fla, 45-kd, 58-kd, 66-kd, and 93-kd bands. A dissenting opinion[759] regarding the CDC criteria[238] for Western blot positivity recommends inclusion of IgG antibody to 31-kd Osp A and 34-kd Osp B in the criteria because of concern that serologic confirmation in some patients would not be possible without these inclusions.

Although European criteria for Western blot positivity have not been officially standardized,[738] in 1997, Hauser and co-workers[237] proposed such criteria, based on extensive studies of over 500 European sera (from patients with early and late Lyme borreliosis and controls) tested in IgG and IgM Western blots prepared with antigens from the three major European genospecies: *B. burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii*. The proposed criteria for positivity, with over 96% specificities, are: for *sensu stricto* IgG, at least one band of molecular weight 83/100, 58, 56, Osp C, 21, and 17a, and for IgM, at least one of 39, Osp C, and 17a or a strong 41; for *afzelii* IgG, at least two bands of 83/100, 58, 43, 39, 30, Osp C, 21, 17, and 14, and for IgM, at least one of 39, Osp C, and 17 or a strong 41; and for *garinii* IgG, at least one of 83/100, 39, Osp C, 21, and 17b, and for IgM, at least one of 39 and Osp C or a strong 41-kd band.

Because of *B. burgdorferi* strain variability, both between genospecies and within genospecies, the molecular weights of several *B. burgdorferi* protein antigens may vary, and some antigens may be variably expressed, resulting in apparent differences in antibody patterns if different strains are used as antigen sources in preparation of Western blots[108, 529]; use of monoclonal antibodies for identification of the protein bands is important for Western blot standardization to allow comparisons between different laboratories.[232, 233, 237]

The Western blot reactivity pattern differs slightly in sera of patients infected with different strains and genospecies of *B. burgdorferi sensu lato* from the United States, Europe, and Asia,[237, 760, 761] and, because of antigenic heterogeneity, differs slightly to moderately in sera reacting in Western blots prepared with different strains and genospecies.[108, 231, 257, 529, 760, 761] Lyme borreliosis patient sera are usually more reactive in Western blot assays prepared with strains homologous to the infecting strains or from the same endemic area.[760, 761] In Europe, where the three major *B. burgdorferi* genospecies all cause Lyme borreliosis, because of the more frequent association of certain clinical manifestations with some genospecies than with others, sera of patients with different clinical manifestations may show different reactivity patterns in Western blots prepared with the different genospecies.[257]

In early Lyme disease, when ELISA and IFA antibody responses are low, Western blot may be more sensitive and specific, particularly during the first 4 weeks of illness.[223, 232, 233, 236, 757] Sera from early Lyme disease of less than 1 week's duration were positive in 79% by Western blot, compared with 71% by the two-step test using ELISA as the initial test followed by Western blot testing only of positives by ELISA.[236] Sensitivity of the polyvalent Western blot is 53 to 92% in early Lyme disease and 100% in late chronic Lyme disease. Use

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TABLE 11–17
Laboratory Diagnosis of Lyme Borreliosis (LB)

ASSAY FOR *B. BURGDORFERI* (*Bb*)	*B. BURGDORFERI* COMPONENT DETECTED BY OR USED IN ASSAY	TIME COURSE OF POSITIVE RESULT (% PATIENTS WITH POSITIVE ASSAY RESULT DURING DIFFERENT STAGES OF LB)				ASSAY SPECIFICITY FOR *B. BURGDORFERI*[a]	ASSAY COMMERCIALLY AVAILABLE
		Early Localized LB		Early Disseminated LB	Late LB		
Culture of biopsy (bx) or fluid	Whole *Bb*, live	46–71% ac	2–92% EM[b] bx low % BL[b] bx 1–6% whole blood 5% plasma ac	71–88% ac	10–26% ACA[c] bx low % Snv[e] bx low % CSF[f]	+ + + +	no
		20% ac	25% serum ac 6–100% CSF	50% ac			
Silver stain or FA stain[g] of biopsy	Whole spirochetes		29–100% EM bx 100% BL bx		low % ACA bx 25–100% Snv bx	+ + + +	no
PCR[h]	*Bb* DNA sequences	25% ac	25–80% EM bx ac 7% whole blood ac	79% ac	16–71% ACA bx 80–85% Snv fluid[i]	+ + + +	no
		9% ac	18% plasma ac 40–59% serum ac 45–90% urine ac	30% ac	100% Snv bx[i]		
IFA[j] IgG	Whole *Bb*		25–58% ac 58–100% cv[k]		80–100%	+ + +	yes
IFA IgM	Whole *Bb*	42–55% ac	20–94% ac 0–14% cv	100% ac	64–80%	+ + +	yes
IFA Polyvalent (IgG + IgM)	Whole *Bb*		13–100% ac 53–100% cv		94–100%	+ + +	yes
ELISA[k] IgG	Whole *Bb*	19% ac 28–50% cv	0–35% ac 8–100% cv 8% late cv	34–79% ac 60–62% cv	41–100% Ar[l] 90–92% NB[m] 90% ACA	+ + +	yes
	p83/100		14%	37%	86–100% Ar, NB, ACA	+ + + +	no
	Flagellin	21% ac	0–31% ac 26–88% cv 37–46% late cv	55–70% ac 81% cv 63–100% late cv	68–100% Ar 95% ACA	+ + +	no
	Osp A[n]				42% Ar	+ + + +	no
	Osp B[o]				42% Ar	+ + + +	no
	Osp C[p]	31% ac 44% cv	33% ac 49% cv	34% ac 51% cv 65% NB	84% Ar 36% NB	+ + +	no
ELISA IgM	Whole *Bb*	9–58% ac 21–67% cv	9–92% ac 23–100% cv 12% late cv	34–89% ac 35–44% cv	36–64% Ar 10% ACA	+ + +	yes
	p83/100		7%	0%	12% Ar 100% NB 5% ACA	+ + + +	no
	Flagellin	38% ac	18–50% ac 41–100% cv 23–44% late cv	43% ac 45% cv 17–53% late cv	68% 5% ACA	+ + +	no
	Osp C[r]	25% ac 50% cv	40–64% ac 67–85% cv	61% ac 72–80% cv	20–45% Ar, NB	+ + + +	no

ELISA Polyvalent (IgG + IgM)	Whole *Bb*	12–27% ac 52–60% cv	13–56% ac 19–98% cv	29–100% 77–96% cv	89–100% Ar, NB, ACA	+ + +	yes
	Flagellin	13–44% ac 53–54% cv	44% ac 53–65% cv	21–100% ac 83–100% cv	89–100% Ar, NB, ACA	+ + +	no
ELISA-AC[3] IgG	Whole *Bb*	0% ac 13% cv	13% ac 30% cv	0–50% ac 21–63% cv		+ + +	no
ELISA-AC IgM	Whole *Bb*	25–36% ac 75% cv	46–67% ac 90% cv	50–100% ac 60–100% cv	47%	+ + + +	no
	Flagellin		50% ac 44% cv 54% late cv	65% ac 60% cv 47% late cv	12%	+ + +	no
ELISA-AC Polyvalent (IgG + IgM)	Whole *Bb*	25% ac	67% ac 93% cv	71% ac 93–100% cv		+ + +	no
ELISA-IC[7] IgG	Whole *Bb* Osp A		33–100% 36% ac		95–100%	+ + +	no
ELISA-IC IgM	Osp A		73% ac				
ELISA-AC/IC (EMIBA) IgM	Whole *Bb*	55% ac	70% ac	89% ac		+ + + +	no
	Whole *Bb* + Flagellin	73% ac	85% ac	100% ac		+ + + +	no
Western blot[4] IgG	Whole *Bb*	0–6% ac 13–16% cv	0–48% ac 16–80% cv 49–50% late cv	17–72% ac 25–71% cv	70–100% Ar, NB, ACA	+ + + +	yes
	83–100 kd		23% ac 13–35% cv	28–29% ac 83% cv	76–100% Ar, NB, ACA		
	66 kd HSP		7% cv	56–83% cv	72–92% Ar, NB		
	58–60 kd HSP		0–54% ac 19–70% cv	84%	92–100% Ar, NB		
	41 kd Flagellin	0% ac 25% cv	57–85% ac 87–100% cv	92–100%	83–100% Ar, NB, ACA		
	31–33-kd Osp A	0% late cv	0–20% ac 5–7% cv	0–4%	40–100% Ar, NB 0–15% ACA		
	34–35 kd Osp B		0–5% ac 0–8% cv	0%	36–100% Ar, NB		
	20–25 kd Osp C		47–77% ac 19–83% cv	10–28%	48–95% Ar, NB 0–19% ACA		
	28–30 kd ?Osp D[8] 17–19 kd ?Osp E[9] 25–27 kd ?Osp F[9]		0% 0–5%	28–44% 84% 50% cv	72–100% Ar, NB 80–100% Ar, NB 49% Ar		
	45–46 kd		100% ac 70% cv	80%	46% Ar 72–84% Ar, NB		
	39 kd		85% ac 14–91% cv	20%	88–97% Ar, NB		
	37 kd 35 kd		46% ac 57% cv	4%	44% Ar, NB		
Western blot IgM	Whole *Bb*	24–36% ac 44–60% cv	23–87% ac 27–86% cv	31–76% ac 36–96% cv		+ + + +	yes
	83–100 kd	33% cv	8–42% ac 5–49% cv	7–25% ac 7–10% cv	14% ACA		
	66 kd HSP		4–68% ac 8–80% cv	24%			
	58–60 kd HSP		28–58% ac 22–87% cv	36%			

Table continued on following page

TABLE 11–17
Laboratory Diagnosis of Lyme Borreliosis (LB) *Continued*

ASSAY FOR *B. BURGDORFERI* (*Bb*)	*B. BURGDORFERI* COMPONENT DETECTED BY OR USED IN ASSAY	TIME COURSE OF POSITIVE RESULT (% PATIENTS WITH POSITIVE ASSAY RESULT DURING DIFFERENT STAGES OF LB)				ASSAY SPECIFICITY FOR *B. BURGDORFERI*[a]	ASSAY COMMERCIALLY AVAILABLE
		Early Localized LB		Early Disseminated LB	Late LB		
	41 kd Flagellin	33–65% ac 73–90% cv	14–68% ac 36–77% cv	39–100% ac	33–40% Ar, NB 19% ACA		
	31–33 kd Osp A		0% ac 5–11% cv	0–12% ac	0–4% ACA		
	34–35 kd Osp B	0% cv 0% cv	0–5% ac 0–4% cv	0–12% ac 0%	20–100% Ar		
	20–25 kd Osp C		44–84% ac 52–91% cv	37–68% ac	19% ACA		
	45–46 kd		32–74% ac 20–83% cv	28%			
	39 kd		4–84% ac 8–94% cv	0% ac			
	37 kd		24–53% ac 32–80% cv	28% ac			
	35 kd		47% ac 13–63% cv				
Western blot Polyvalent (IgG + IgM)	Whole *Bb*		25–55% ac 14–86% cv	83–100% ac	100% Ar 100% NB	+ + + +	yes
	Flagellin		75% ac 100% cv		83–100% Ar		
	Osp A		0% ac 0% cv		50% Ar		
	Osp B		0% av 0% cv		50% Ar		
LPA[a]	Whole *Bb*, killed or sonicated		0–36%		45–100%	+ + +	no
	Whole *Bb*, live		50–91%		82–100%	+ + +	no
	66 kd HSP				36%		no
	58 kd HSP				14%		no
	Flagellin		82%		21–68%	+ + +	no
	Osp A		82%		29–76%	+ + + +	no
	Osp B				14%	+ + + +	no
	Osp C				29%		no
PCR	*Bb* DNA sequence			25–100% CSF		+ + + +	no
IFA	Whole *Bb*			92% CSF		+ + +	yes
Antigen-capture ELISA	Whole *Bb*			49% CSF		+ + + +	no
	Osp A			24–100% CSF			
	Osp B			27% CSF			
	Flagellin			30% CSF			

ELISA IgG	Whole *Bb* or Flagellin	10–100% CSF		yes
ELISA IgM	Whole *Bb* or Flagellin	19–100% CSF		yes
	Osp A	20–100% CSF		
	Osp B	20–100% CSF		
	Osp C	50–100% CSF		
ELISA Polyvalent	Whole *Bb*	48–100% CSF		yes
ELISA-AC	Whole *Bb*			no
Polyvalent		42–100% CSF		
IgM		40–100% CSF		
ELISA-IC	Whole *Bb*			no
Polyvalent		38–43% CSF		
IgG		47% CSF		
IgM		64% CSF		
Western blot	Whole *Bb*	92% CSF	+++	yes
LPA	Whole *Bb*, sonicated	100% CSF	+++	no
	Whole *Bb*	100% CSF	+++	no

Culture data obtained from references 18–20, 22, 79, 80, 95, 183, 184, 260, 267, 280, 301, 302, 374, 434, 436, 439, 591, 595, 621, and 751.
Stain data obtained from references 82, 177, 596, 618, 621, and 695.
PCR data obtained from references 143, 280, 282, 287, 312, 313, 315–317, 436, 439, 595, 753, 754, and 755.
IFA data obtained from references 1, 18, 20, 39, 209, 235, 250–252, 466, 600, 621, 638, 644, 756, and 869.
ELISA data obtained from references 107, 124, 223, 224, 226, 232–236, 239–242, 246, 247, 251, 252, 267, 278, 621, 746, 756, and 757.
Western blot data obtained from references 108, 124, 222, 223, 225, 226, 229, 232–237, 239–241, 247, 249, 621, and 756.
LPA data obtained from references 208–210, 212, 213, 218, 219, 221, 267, 621, and 765.
CSF data obtained from references 214, 259, 260, 267, 282, 283, 285–287, 293, 309, 621, 654, 661, and 755.
[a]Estimation of specificity is given for RPR-negative sera (see Table 11–16).
[b]EM = erythema migrans skin lesion.
[c]ACA = acrodermatitis chronicam atrophicans skin lesion.
[d]BL = borrelial lymphocytoma skin lesion.
[e]Snv = synovial.
[f]CSF = cerebrospinal fluid.
[g]Warthin-Starry, Bosma-Steiner, or Dieterle silver stains, or *B. burgdorferi*–specific polyclonal or monoclonal FA (fluorescent antibody) stains.
[h]PCR = polymerase chain reaction.
[i]Excluding patients with antibiotic-resistant chronic arthritis, in whom a 96% polymerase chain reaction–positivity rate is found.
[j]IFA = immunofluorescence assay.
[k]ELISA = enzyme-linked immunosorbent assay.
[l]Ar = arthritis.
[m]NB = neuroborreliosis.
[n]Osp A = outer surface protein A.
[o]Osp B = outer surface protein B.
[p]Osp C = outer surface protein C.
[q]ELISA-AC = ELISA–antibody capture.
[r]ELISA-IC = ELISA–immune complex.
[s]Western blot: kd-kilodalton size of individual *B. burgdorferi* antigens.
[t]Osp D = outer surface protein D.
[u]Osp E = outer surface protein E.
[v]Osp F = outer surface protein F.
[w]LPA = lymphocyte proliferative assay.

of both immune-complex dissociation techniques and biotin-avidin Western blots has increased the sensitivity of detection of IgM and IgG Osp A antibody early in infection, when it may be located in immune complexes.[246]

Cross-reactivity with other spirochetes and the presence of low levels of positivity in control sera from endemic areas make it difficult to estimate the true incidence of false-positive Western blot assays, but it is considered to be low.

Patients with a strong clinical history of objective symptoms of Lyme disease and seropositivity by both ELISA and Western blot[279, 742, 763, 764] usually improve clinically with antibiotic therapy[742]; however, patients with only subjective symptoms, with either negative ELISA and negative Western blot or positive ELISA but negative Western blot, usually have some other inflammatory or rheumatologic disease instead of Lyme disease[279, 742, 763, 764] and do not improve with antibiotic treatment. Most patients with late Lyme borreliosis, such as arthritis, chronic neuroborreliosis, and ACA, who are seropositive by ELISA are also seropositive by Western blot.[223, 234–236]

LYMPHOCYTE PROLIFERATIVE ASSAY

Lymphocyte proliferative assay (LPA) determines specific reactivity of viable peripheral blood, CSF, or synovial fluid lymphocytes to whole *B. burgdorferi*, whole disrupted (sonicated) *B. burgdorferi*, or individual *B. burgdorferi* antigens incubated with these lymphocytes in vitro.[208, 209, 213, 218–221, 765] Some assays use peripheral blood mononuclear cells.

The development of the T cell response to Lyme disease precedes the antibody response, and the LPA may be positive in IFA- and ELISA-seronegative patients with early Lyme disease.[208, 219] After successful antibiotic therapy of Lyme disease, there may be some decrease in the level of LPA positivity.[213, 221]

The LPA may be positive in other patients who are IFA- and ELISA-seronegative as a result of prompt antibiotic therapy of early Lyme disease. The LPA was positive in all of 40 chronic Lyme disease patients in six studies, who were IFA- and ELISA-seronegative because of early antibiotic therapy.[208–210, 218]

In some patients, CSF[214, 220] and synovial fluid[221] lymphocytes are more reactive in the LPA than are peripheral blood lymphocytes; therefore, sensitivity may be increased by using these fluids.

The sensitivity of LPA is 50 to 91% in early Lyme disease, and 82 to 100% in late Lyme disease. Cross-reactions occur with other spirochetes, and the LPA positivity rate in healthy controls is 0 to 5%; in patients with non-Lyme inflammatory diseases, it is 5 to 11%.[210, 214, 218]

Although the LPA is more sensitive than antibody assays in certain patients, it requires use of live lymphocytes and whole *B. burgdorferi* or *B. burgdorferi* antigens, and is available only in research laboratories. The LPA therefore should be reserved for the diagnosis of Lyme disease in seronegative patients with good clinical objective evidence of Lyme disease, or for babies with potential congenital Lyme disease; it is not considered useful in following patient immune responses sequentially.

ANTIGEN CAPTURE ELISA ASSAY

The antigen capture ELISA, using either antibody against whole, sonicated *B. burgdorferi* or monoclonal antibodies against individual antigens such as recombinant outer surface proteins and flagellin, has been used to detect specific *B. burgdorferi* antigens in CSF.[283] In very early Lyme disease with neurologic involvement, this method detected specific antigen in CSF even before the development of specific CSF antibody. The Western blot method has also been used to determine the presence of specific antigens in CSF.[283] However, neither of these methods is commercially available.

Laboratory Variability and Efforts at Serodiagnostic Standardization

One of the major problems with laboratory diagnosis of Lyme disease is the wide intra- and interlaboratory variability of results both in the United States[739, 740, 750] and in Europe.[529, 738] Several comparisons in which standard Lyme disease case or control sera were sent simultaneously to different commercial, hospital, state, and national reference or research laboratories for *B. burgdorferi* antibody testing, usually ELISA or IFA, with or without Western blot testing, demonstrated that the percentage of laboratories that reported concordant results ranged from 10 to 93%, and the reproducibility of results within the same laboratory ranged from 27 to 96%. Agreement among laboratories was greatest for sera with high positive titers to *B. burgdorferi*, and least for sera with low positive titers.

There has been concern that *B. burgdorferi* strain heterogeneity in Europe may be responsible for variability in serologic results, but this may be more significant in Western blot assays than in ELISA assays.[257, 529]

Because of the problem of interlaboratory variability, proficiency testing programs have been recommended[478] for laboratories that perform *B. burgdorferi* testing, and these have been implemented in several areas.[739, 740] A study of a proficiency testing program in New York State, implemented in 1989 for clinical laboratories applying for *B. burgdorferi* antibody testing permits, found that performance improved during the study, partially because laboratories that initially used poorly performing test kits tended to change to better-performing kits, and overall sensitivity of ELISA, IFA, or solid-phase IFA assays was 95.4%; overall specificity 98.9%.[740]

Variability in results may therefore be due to differences in strains of *B. burgdorferi* used for preparation of the diagnostic kits, differences in the methods of kit preparation, use of different assays by different laboratories, differences in definitions of negative and positive results, geographic differences in the incidence of background Lyme seropositivity, and differences in quality control within individual laboratories. Better standardization of commercially available assays for Lyme disease is needed because the more specific, sensitive, and repro-

ducible research laboratory tests generally are not available.

Avoidance of Over- or Underdiagnosis

Establishment of a correct diagnosis of Lyme disease, with avoidance of over- or underdiagnosis,[24, 279, 763, 767, 768] allows selection of antibiotic therapy that is adequate and appropriate for the specific clinical presentation, as well as avoidance of over- or undertreatment, which is important for achieving maximal therapeutic efficacy with minimal adverse effects, both in the individual patient and in the population as a whole.

Between 38 and 79% of patients referred to Lyme disease or rheumatology specialty clinics for Lyme disease in endemic areas had been overdiagnosed and did not have active Lyme disease, and between 38 and 57% had alternate diagnoses made. Patients with only vague persisting symptoms, such as fatigue, headache, myalgia, and arthralgia, rarely had active Lyme disease and usually had fibromyalgia or fatigue syndrome (with or without previous Lyme disease); improvement in these patients did not correlate with antibiotic therapy.[279, 715, 763, 764, 767, 768, 770]

Although long-term persistence of active *B. burgdorferi* infection has been confirmed by culture,[200, 304, 306–308] and suggested by PCR,[312, 314] it is rare, particularly after adequate antibiotic therapy. Demonstration of objective evidence of persistent infection, preferably by culture positivity, but at least by PCR or diagnostic changes in specific *B. burgdorferi* antibody, is important because retreatment of true persistent infection is usually successful.[312, 314] Failure of a patient with a diagnosis of Lyme disease to respond to appropriate antibiotic therapy should raise the possibility that the initial diagnosis of Lyme disease may be incorrect, or that symptoms are not due to active *B. burgdorferi* infection.[24, 279, 767]

Alternately, underdiagnosis is also a potential problem, particularly after acquisition of the infection during travel to an endemic area and presentation with the clinical illness after return to a nonendemic area where the diagnosis may not be initially, or ever, considered. (See section Lyme Borreliosis in Travelers to Endemic Areas.)

Recommendations for Diagnostic Testing for Evaluation of Nongestational, Gestational, and Congenital Lyme Borreliosis

Because of wide variability in clinical case definitions used in the diagnosis of Lyme borreliosis, as well as in performance and interpretation of supportive *B. burgdorferi* diagnostic tests, there have been ongoing efforts in North America[238, 254, 478, 633, 741, 748] and Europe[8, 502, 738] to standardize clinical case definitions, as well as laboratory testing guidelines. The European Union Concerted Action on Lyme Borreliosis (EUCALB) has developed standardized European clinical case definitions[8, 502] (see Table 11–11), but serodiagnostic guidelines are not yet available.[738] Current CDC clinical case definitions of Lyme disease[633] (see Table 11–10) are intended for use in combination with CDC and FDA laboratory diagnostic test guidelines in the United States[238, 254, 633]; although they were initially designed for epidemiologic surveillance purposes, they have found widespread acceptance as a way to standardize the diagnosis of Lyme disease.

Several studies, including predictive statistical models[734–736] as well as prospective[767] and retrospective[279, 763, 768] clinical laboratory studies, have evaluated various approaches to the problem of how, when, and in whom to do diagnostic testing for Lyme disease. The American College of Physicians recently developed guidelines describing indications for diagnostic evaluation for Lyme disease.[478, 748]

The diagnosis of Lyme borreliosis in the nonpregnant patient should be a clinical diagnosis, made according to accepted case definitions, and supported by appropriate laboratory confirmation when needed, interpreted according to accepted criteria. According to the CDC case definition of Lyme disease (see Table 11–10), classic physician-diagnosed erythema migrans with endemic area exposure does not require laboratory confirmation, but other acute, early disseminated manifestations, including acute neuroborreliosis and carditis, or late manifestations require either positive culture or serologic confirmation by diagnostic CSF or serum levels of *B. burgdorferi* IgM or IgG antibody, according to the two-step method (by ELISA or IFA), followed by retesting of all positive or equivocal results by Western blot.[238, 254, 633] The EUCALB case definitions of Lyme borreliosis (see Table 11–11), similarly, do not require laboratory confirmation for classic erythema migrans, but do for other acute early manifestations, including borrelial lymphocytoma, acute neuroborreliosis, and carditis, and for late manifestations.[8, 502] There has been no standardization of diagnostic CSF *B. burgdorferi* antibody levels required for confirmation of neuroborreliosis,[478] but CSF IgM or IgG *B. burgdorferi* antibody levels exceeding serum levels are usually considered to indicate intrathecal antibody production.

The ELISA test, either polyvalent or IgM and IgG, is the most widely available assay for serodiagnosis of Lyme borreliosis; it is often preferred over the IFA for use as the initial test because of its suitability for large-volume testing. Antibody capture ELISA may have increased sensitivity and specificity but is not as widely available. Using Western blot retesting of sera that are positive or equivocal by initial ELISA or IFA tests increases specificity. Both IgM and IgG testing is recommended for evaluation during the first 4 weeks of early Lyme disease. Because negative serologic results during the first 2 weeks of illness are not sufficient to exclude Lyme disease, if early Lyme disease is strongly suspected and the first sample is negative, testing a 2- to 4-week convalescent sample is recommended. Although current CDC guidelines recommend only Western blot testing of ELISA-positive or equivocal sera, the Western blot may be useful in initial evaluation of early Lyme disease because its increased sensitivity may allow detection of more cases than ELISA during the first and second weeks of illness.[232, 233, 236] The Western blot may also

provide additional information regarding the time course of the infection.[233] Criteria for Western blot positivity are stringently defined in the CDC recommendations.[233, 234, 238] Use of IgM testing alone after the first 4 weeks of illness is not recommended, as a diagnosis of active Lyme disease should not be made on this basis alone. Use of IgM testing alone even for evaluation of early infection is not recommended, because some patients who have been reinfected with *B. burgdorferi* may have only an IgG response, which would be missed if IgG testing were not done.[233]

The lymphocyte proliferative assay is useful for diagnosis of seronegative patients with suspected Lyme borreliosis but is not generally available commercially. Biopsies of involved tissues for histopathology, culture, PCR, or silver or IFA staining are usually best reserved for special clinical circumstances, when serologic diagnosis is insufficient; culture of more readily accessible sites such as the CSF, blood, synovial fluid, or skin may be useful, but yields are low, except from erythema migrans lesions, and culture is not widely available. PCR is usually reserved for research purposes, but culture or PCR is useful when identification of the *B. burgdorferi* strain is needed. Although PCR provides evidence for the presence of *B. burgdorferi* DNA, it does not indicate the presence of viable spirochetes; culture of *B. burgdorferi* remains the only definitive proof of active ongoing infection.

The diagnosis of Lyme borreliosis in a pregnant woman should also be made according to the currently accepted CDC or EUCALB case definitions (see Tables 11–10 and 11–11 and section on Clinical Manifestations), with the additional recommendation that laboratory confirmation of the diagnosis is advisable, even for the clinical presentation as classic erythema migrans, to avoid later uncertainty, which might arise if only a clinical diagnosis were made. Because specific IgM seropositivity may be transient and development of specific IgG seropositivity may be prevented by early antibiotic therapy, immediate acute serum and several convalescent sera should be collected at approximately 2-week intervals over a period of approximately 8 weeks and at delivery. The initial acute sera should be sent for polyvalent or IgM and IgG Lyme ELISA, and also IgM and IgG Western blot; the remaining convalescent sera may be sent if no confirmation is obtained with the first sera. It is advisable to save aliquots of sera for possible future testing with more sensitive assays if they become available. The Western blot is advisable in evaluation of pregnant women with early Lyme disease, in whom early serologic confirmation is preferable, because of its increased sensitivity during the first 2 weeks of illness,[232, 233, 236] and because it may provide useful information regarding the time course of the infection.[232, 233] Although it is not recommended that biopsies of involved tissues be performed routinely in pregnancy, this could be done for diagnostic confirmation if clinically indicated. It is important to determine whether dissemination has occurred as this influences selection of antibiotic therapy and may affect pregnancy outcome; blood evaluation for evidence of spirochetemia, by culture, or possibly PCR, and CSF examination for evidence of early neuroborreliosis may be indicated for this purpose in some pregnant women.

There is no basis for routine *B. burgdorferi* antibody screening of asymptomatic healthy persons, because the incidence of false positivity exceeds the incidence of active Lyme disease in this group.[748] For the same reason, in the absence of studies indicating otherwise, there is no indication for routine prenatal *B. burgdorferi* antibody screening of asymptomatic healthy women. *B. burgdorferi* antibody serosurveys have demonstrated that seroprevalence in pregnant women[27, 37, 40, 45–47] reflects community seroprevalence; the rate of asymptomatic seroconversion was only 0.8% in one study during pregnancy.[45]

Any infant with possible congenital Lyme borreliosis should undergo evaluation by *B. burgdorferi* IgM and IgG ELISA and IgM and IgG Western blot on paired maternal and cord blood at delivery, and on the infant's blood and preferably CSF after birth, and if possible, *B. burgdorferi* culture and PCR as well on these samples. If the index of suspicion is high for congenital Lyme borreliosis and these assays are negative, the LPA should be performed (at a research center), as it appears to be more sensitive than serologic testing for confirmation of Lyme borreliosis in congenitally infected patients. Histopathology, Bosma-Steiner or Warthin-Starry silver stains, *B. burgdorferi*–specific antibody stains, culture, and PCR of the placenta are recommended. If biopsy specimens of involved tissues, such as skin, are obtained, they should be sent for the same studies, as these may be useful in diagnosis. Cardiac and neurologic evaluation should be obtained if there is a clinical suspicion of congenital heart disease or neurologic involvement. It is also advisable to store samples of sera, CSF, or tissues for possible additional future testing.

A full histopathologic evaluation is recommended of any placenta, miscarriage, stillbirth, or perinatal death from a pregnancy complicated by Lyme borreliosis. In addition, Bosma-Steiner or Warthin-Starry silver stains, *B. burgdorferi*–specific antibody stains, culture and PCR of the brain, heart, lungs, kidneys, liver, spleen, lymph nodes, bone marrow, synovium, and any other histologically abnormal tissues, and antibody assays, PCR, and culture of any blood or CSF available are recommended. Serum, CSF, and other samples should also be stored for future diagnostic tests.

Because the incidence of congenital Lyme disease is quite low, and needs more complete characterization, it is important to evaluate any suspected cases as fully as possible. The physician may wish to contact a center engaged in Lyme research for help in processing of these samples. The author has agreed to be available, by prearrangement, for discussion of infants suspected of having congenital Lyme borreliosis: Tessa Gardner, M.D., 314–727–9101.

Differential Diagnosis of Lyme Borreliosis

The differential diagnosis of Lyme borreliosis (Table 11–18), including gestational Lyme borreliosis, is extensive and depends on the particular stage and manifesta-

TABLE 11–18
Differential Diagnosis of Lyme Borreliosis[a] (LB)

DISEASE	RASH	FLULIKE ILLNESS	MUSCULOSKELETAL SYMPTOMS	CARDIAC SYMPTOMS	NEUROLOGIC SYMPTOMS	REFERENCES
Granuloma annulare	+					596, 639
Ringworm	+					599, 639, 763, 768, 870
Cellulitis	+					595, 599, 639, 767, 768, 870
Impetigo	+					595
Pityriasis rosea	+					763
Contact/atopic dermatitis	+					595, 639, 763, 768, 870
Erythema annulare centrifugum	+					639
Tick/insect bite reaction	+					595, 599, 639, 768, 870
Cutaneous malignancy	+					599, 644
Circulatory insufficiency[b]	+					599
Brown recluse spider bite	+	+				871
Serum sickness	+	+	+			243, 768, 870
Erythema nodosum	+					596, 598, 599
Erythema multiforme/urticaria	+	+	+			243, 274, 277, 279, 639, 763, 767, 768
Henoch-Schönlein purpura	+					596
JRA/RA[c]	+	+	+	+		274, 279, 651, 763, 767, 768
Lupus	+	+	+		+	243, 599
Dermatomyositis	+	+	+			599, 617
Scleroderma	+				+	596
Reiter's syndrome			+			651
Fibromyalgia		+	+		+	279, 715, 763, 767, 769, 770
Chronic fatigue syndrome		+	+			279, 763, 767, 768
Inflammatory bowel disease		+	+			279
Rheumatic fever	+	+	+	+	+	243, 687, 872
Bacterial endocarditis		+	+	+		693, 745
Acute myocarditis		+		+		279, 687, 785
Chronic cardiomyopathy				+		368, 607, 692
Syphilis	+		+	+	+	286
Relapsing fever		+				873
Sarcoidosis		+			+	211, 599
Mycoplasma pneumoniae infection	+	+	+	+	+	
Urinary retention, bladder neuropathy					+	669, 678
Diaphragmatic paralysis					+	679
Epstein-Barr virus mono	+	+	+	+	+	243, 763
Cytomegalovirus mono	+	+	+	+	+	763
Echo/coxsackievirus infection	+	+	+	+	+	687

Table continued on following page