**INTRODUCTION**

Early in the 20th century, European physicians observed patients with a red, slowly expanding rash called erythema migrans (EM) (Figure 1) (1). They associated this rash with the bite of ticks and postulated that EM was caused by a tick-borne bacterium. In the 1940s, a similar tick-borne illness was described that often began with EM and developed into a multi-system illness. In 1969, a physician in Wisconsin diagnosed a patient with EM and successfully treated the individual with penicillin. In 1975, a women in Old Lyme, Connecticut contacted the state health authorities to ask why so many children in her town had arthritis. She was alarmed by the fact that 12 cases of juvenile arthritis, a relatively rare disease, had been diagnosed in a town with a population of only 5,000. Subsequent investigations of these and similar cases led to the discovery of Lyme disease and to the identification of the causative bacterium.

**EPIDEMIOLOGY**

Lyme disease (LD), caused by infection with *Borrelia burgdorferi*, is the most common vector-borne disease in the United States, accounting for more than 95% of all reported vector-borne illnesses. From 2003-2005, 64,382 cases of Lyme disease were reported to the CDC from 46 states and the District of Columbia (2). However, a considerable level of underreporting is associated with Lyme Disease and, therefore, the true number of infected individuals is probably greater. Of the reported cases, 59,770 occurred within ten endemic states: Connecticut, Delaware, Maine, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin (2). Within these endemic states, the average annual rate of infection over this three year period was 29.2 cases per 100,000 population (2). Three counties, New York's Columbia and Dutchess counties and Massachusetts' Dukes County, were determined to have an incidence rate of 300 cases per 100,000 population (2). Evaluation of race and sex revealed a slightly higher incidence for males (54% of reported cases) and that 97% of the cases affect caucasians (2). Persons of all ages and both genders are equally susceptible, although the highest incidence of LD occurs in children ages 0-14 years and in persons 30 years of age and older (median, 28 years). LD has been reported in 49 states and the District of Columbia. Lyme borreliosis also occurs in temperate regions of the Northern Hemisphere in Europe (3), Scandinavia (4), the former Soviet Union (5), China (6) and Japan (7). In the United States, the infection is spreading and has caused focal epidemics, particularly in the northeastern United States (8-10). From 1985 to 1989, the number of counties in New York State with documented *I. scapularis* ticks increased from 4 to 22, and the number of counties endemic to LD increased from 4 to 8 (11). During the subsequent 10 years, *I. scapularis* ticks have spread to 54 of the 62 counties in the state. In the US, the majority of affected individuals have had symptoms of the illness, whereas in Europe, the majority is asymptomatic (10, 11). Of 346 people who were studied in the highly endemic area of Liso, Sweden, 41 (12%) had symptoms of the illness and 89 (26%) had evidence of subclinical infection (12). In a serosurvey of 950 Swiss orienteers, 26% had detectable IgG antibodies to *B. burgdorferi*, but only 2% to 3% had a past history of definite or probable LD (Figure 2) (1,13).
Ticks evolved 94 million years ago from mites as parasites of reptiles, birds, and mammals. More than 50 species of ticks can feed on humans (13, 14). Ticks belong to two taxonomic families, the soft ticks (Argasidae) and the hard ticks (Ixodidae). Humans are not the principal, but rather the serendipitous host for most ticks (Figure 3) (1).

Attachment:
Upon finding a suitable host, the tick raises its body at an angle to the skin surface and the chelicerae begin to cut the epidermis (17). Mouthpart lengths differ significantly, with lengths in Dermacentor (dog ticks) extending to the dermis-epidermis interface, while the mouthparts of Ixodes species extend well into the dermis (18).

Cement:
Salivary glands of Ixodid ticks generally secrete an attachment cement, which is believed to serve as an adhesive and to seal the bite lesion bridging the cleft between mouthparts and host tissue (19). Attachment cement secretion begins within minutes after skin penetration and hardens into a tube surrounding the mouthparts.

Mechanics:
Cutting action and mouthpart insertion produces an expanding hemorrhagic pool within the tissues, which is aided by anticoagulants, vasodilators, and platelet aggregation inhibitors in tick saliva (17). Mouthpart mechanical actions alone are not sufficient to account for the lesion. The lesion is characterized by a large blood-filled cavity. Ixodid tick blood feeding is not continuous (17). The first few days of hard tick feeding are referred to as the slow feeding phase, which coincides with cuticle growth to accommodate the blood meal. Next, the rapid feeding phase is characterized by the uptake of very large quantities of blood. The increase in size during the 7 to 10 day slow-feeding phase can be 10-fold, with an additional 10-fold increase during the rapid-feeding phase (20). This increase actually underestimates the amount the blood consumed, since fluid from the blood meal is reintroduced into the host during salivation (20).

**Figure 2**: Approximate geographic distributions of I. ovatus (green with horizontal lines), I. pacificus (red with horizontal lines), I. persulcatus (red), I. ricinus (green), I. scapularis (blue), R. evertsi (yellow with horizontal lines), and R. pumilio (yellow with vertical lines).

**Figure 3**: (A) Adult female lone star tick (Ameblyoma americanum). The lone star tick is broadly distributed throughout the southeastern quadrant of the United States, with ranges extending into New England and the Midwestern states. All three life cycle stages of A. americanum can bite humans. A. americanum is a recognized vector of several pathogens that cause diseases in humans, including ehrlichioses caused by E. chaffeensis and E. ewingii, and has been implicated as a vector of southern tick-associated rash illness believed to be caused by Borrelia lonestari. Other pathogens or potential pathogens have also been detected in this tick, including various spotted fever group rickettsiae, Francisella tularensis, and Coxiella burnetti, although the role of A. americanum in the transmission of these agents to humans is not well characterized. (B) Adult female American dog tick (Dermacentor variabilis). This tick is abundant in the southeastern United States and coastal New England, with limited distribution in several midwestern states, southern Canada, and western coastal regions. The tick is best known as the primary vector of Rocky Mountain spotted fever in the eastern United States. (C) Adult female black-legged or deer tick (I. scapularis). As a member of the greater I. persulcatus group that also includes I. pacificus, I. ricinus, and I. persulcatus, this tick is an important vector of several infectious agents that cause disease in humans, including Lyme borreliosis, (granulocytic) anaplasmosis, and babesiosis. I. persulcatus ticks elsewhere in the world may also transmit tick-borne flaviviruses capable of causing encephalitis, encephalomyelitis, and even hemorrhagic fevers (including tick-borne encephalitis virus, louping ill virus, Russian spring-summer encephalitis virus, Powassan virus, Kyasanur Forest Disease virus, and Langat virus). Images courtesy of G. Maupin.
Molecular and Biological Aspects of Feeding

Ticks evolved strategies to overcome host hemostasis, pain and itch responses, inflammation, and immune defenses by secreting saliva, which contains a complex array of pharmacologically active molecules (Figure 4) (1).

![Figure 4: (A-D) Hard tick morphology. (A) Engorged Female A. americanum. (B) Closeup of mouthparts of an I. scapularis nymph. C, chelicerae; P, palps; H, hypostome. The mobile distal cheliceral teeth reach beyond the hypostome. (C) During feeding, the palps are splayed laterally (female D. andersoni tick). (D) A feeding D. andersoni couple; the female tick is in front. (E) Tick feeding lesion. Adult I. scapularis female feeding on a sensitized rabbit. Predominantly mixed inflammatory infiltrate cells line the feeding cavity. Discrete dermal swelling and dilated venules are found in the proximity. The mouthparts are anchored deep into the dermis, just reaching the cavity from which the tick feeds as indicated by the imprint of the hypostome contours (trichrome stain). Photographs courtesy of S. Archibald and E. Denison.](image)

Pain and Itch: Pain and/or itching are important host responses that alert an individual to the presence of a tick, resulting in grooming behavior that removes the tick. Bradykinin is a peripheral mediator of the sensations of itching (17) and pain (22). I. scapularis saliva contains a metallopeptidyl carboxypeptidase capable of degrading active bradykinin (23). Bradykinin also has roles in inflammation and increases vascular permeability (24). Histamine is one of several mediators that transmit the sensation of itching through peripheral sensory nerve endings (17). In addition, a combination of histamine and serotonin, a further mediator of itching, inhibits tick feeding (25). Histamine binding proteins are produced by the salivary glands of many tick species like Rhipicephalus sanguineus (26) and I. scapularis (27).

Blood Coagulation and Tick Anticoagulants: Blood coagulation is a critical mechanism to prevent blood loss from the vasculature and poses a major obstacle for blood-feeding parasites, including ticks. Blood coagulation results from activation of the extrinsic tissue factor and intrinsic pathways, which converge to form activated factor X (Xa) to initiate the common pathway of coagulation (28). Ticks have developed numerous redundant and complementary ways to inhibit coagulation. Ticks block host blood coagulation predominantly by targeting factor Xa and thrombin (29). Inhibitors of factor Xa have been reported from the saliva or salivary glands of the following tick species: O. moubata, (30), R. appendiculatus, (31), and I. scapularis (32). I. scapularis saliva contains a further inhibitor of extrinsic factor X activation, Ixolaris, which is believed to utilize both factors X and Xa as scaffolds for the inhibition of activated factor VII/tissue factor complex (29). Blocking the action of thrombin prevents conversion of fibrinogen to fibrin (27). Thrombin inhibitors are produced by the salivary glands of I. holocyclus (33) and I. ricinus (34). In addition to the inhibition of blood coagulation, a successful blood feeding is highly dependent on the inhibition of platelet aggregation. Platelet aggregation can be inhibited by apyrase, which hydrolyzes both ATP and ADP to AMP and inorganic phosphate. Salivary apyrases are present in I. scapularis (35) and many other blood feeding arthropods (36). Genes encoding putative metalloproteases are present in the salivary glands of I. scapularis (27). Tick saliva metalloproteases could provide another means of preventing platelet aggregation. In addition, the disruption of the extracellular matrix likely contributes to maintaining the feeding lesion around tick mouthparts.

In addition to inhibiting platelet aggregation, prostaglandin E\textsubscript{2} is also a vasodilator. Prostaglandin E\textsubscript{2} occurs in the salivary glands of I. scapularis (35). Vasodilation increases blood flow to the bite site, which is beneficial to engorging ticks.

Proteases and Protease Inhibitors: As mentioned earlier, the mechanical activity of the chelicerae will most certainly not entirely explain the relatively large blood-filled tissue cavity that is characteristic of feeding ticks. A number of proteolytic activities and proteases have been characterized from various tick species. Multiple putative serine protease inhibitors have been identified in Ixodes ricinus (21).
TICK INTERACTION WITH THE HOST IMMUNE SYSTEM

The outcome of tick feeding and pathogen inoculation is governed by the interplay of the tick’s salivary pharmacologic repertoire and the host’s innate and adaptive immune responses. Ticks target common pro-inflammatory pathways of cell activation and recruitment, such as bioactive amines, chemokines and the complement system. For example, adult *D. reticulates* and *A. variegatum* salivary gland extracts inhibited human natural killer cells (NK), while *I. ricinus* had no effect (37, 38). IL-2, an important growth factor for NK cells and T cells, is blocked through a putative IL-2 binding protein in the saliva of *I. scapularis* (39).

In addition, chemokines like IL-8, which recruit immune cells such as neutrophils, basophils, lymphocytes and monocytes to the site of tissue, insulin are inhibited by activities in the saliva of several hard ticks (*R. appendiculatus*, *D. reticulates* and *I. ricinus*) (40).

The effects of tick saliva and tick feeding on T-cell cytokines have been studied extensively. The common denominator of these observations for several species is a reduced IFN-γ response and an upregulation of IL-4 (41, 42). Upregulation of IL-10 is not consistently observed (42, 43), but up regulation of IL-4 and IL-10, individually or combined, may account for the reduced IFN-γ induction (44).

THE ORGANISM

Based on genotyping of isolates from ticks, animals, and humans, three pathogenic groups of *Borrelia burgdorferi* (senso lato) have been identified. In the United States, the sole cause of human infection is group one, *B. burgdorferi* (senso stricto). Group one can also be found in Europe and Asia. Although all three groups are found in Europe, the predominant groups are group 2 (*B. garinii*) and group 3 (*B. afzelii*). Only groups 2 and 3 have been associated with Lyme disease in Asia. Although closely related, the disease patterns observed in humans differ greatly depending upon the particular *Borrelia* species involved.

*Borrelia* species are spirochetes, spiral-shaped organisms that have a gram-negative cell wall architecture consisting of two membranes, between which is sandwiched the peptidoglycan layer (*Figure 5*) (45). The *Borrelia* spp. are longer and more loosely coiled than other spirochetes. Of the *Borrelia* spp., *B. burgdorferi* is the longest (20 to 30 mm) and narrowest (0.2 to 0.3 mm) and has fewer flagellae (8-12, 46). The bacteria are actively motile, but the flagellae do not extend outward from the surface of the bacterium like those of most gram-negative bacteria. Rather, the flagellae are embedded into the two ends of the spirochete and are wrapped around the inside the space between the cytoplasmic membrane and the outer membrane. Periodic contractions of these internal flagellae cause the outer surface to rotate, producing a corkscrew-type motility. This type of motility may be responsible for the ability of spirochetes to move out of the skin into the bloodstream and out of the bloodstream into tissues. *Borrelia* spp. do not have LPS in their outer membranes. Instead, the outer membrane contains a number of lipoproteins, some of which are designated Osp, for outer surface proteins, A through F. Two outer surface proteins, Osp A and Osp B, are encoded by the same 50 kilobase linear plasmid and share 56% sequence homology (47) (Table 1).

![Figure 5: B. burgdorferi](image)

<table>
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<th>Protein</th>
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| OspA    | - Up-regulated in maturing tick larvae  
|         | - High homology (56%) with Osp A |
| OspB    | - Down-regulated in nymphs during blood meals  
|         | - Not expressed in mammals until late in infection |
| OspC    | - High homology (50%) with Osp A |
| OspE-OspF | - Immunogenic in some patients |
| OspD (Erps) | - Produced early during mammalian infection |
| OspD-OspF | - Known for its potential to bind mammalian protein decorin |
| VlsE    | - Surface protein that varies antigenically, similar to variable membrane proteins found in *B. hermsi* (the causative agent of relapsing fever) |

Table 1: Features of major surface proteins of *B. burgdorferi*.

THE BORRELIA GENOME

*Borrelia* species have linear plasmids as well as the conventional circular plasmids. Linear plasmids were first discovered by scientists studying *B. burgdorferi*, a feature originally thought to be unique to this genus. In recent years, however, linear plasmids and chromosomes have been found in a number of other bacterial groups. The two strands of the double stranded linear plasmids are covalently closed at the ends and have sequences similar to telomeres found on eukaryotic chromosomes. The mechanisms by which the linear plasmids of spirochetes replicate is still unknown.
The complete genome of a prototypic *B. burgdorferi* strain (B31) was sequenced (48). The total genome is small (approximately 1.5 megabases) and includes a linear chromosome of 950 kilobases along with 9 circular and 12 linear plasmids. The plasmid comprises approximately 40% of the bacterial genome. The organism has a minimal number of proteins with biosynthetic activity and therefore depends on the host for much of its nutritional requirements. Analysis of *B. burgdorferi* plasmid DNA content reveals that extensive recombination occurs between plasmids leading to genetic rearrangements. What role this variability of plasmid-borne genes plays in adaptation of the bacteria to its various hosts remains to be determined.

Analysis of the *Borrelia* genome reveals the absence of any genes encoding cytochromes or tricarboxylic acid (TCA) cycle enzymes, which is consistent with the observation that although *B. burgdorferi* can grow in the presence of oxygen, it seems not to need oxygen for growth. Because the bacteria lack a TCA cycle, they can not make any of the amino acids whose synthesis requires TCA cycle intermediates. Given this, it appears odd at first that there were few genes encoding amino acid transport proteins. This is consistent, however, with the observation that the bacteria preferentially take up peptides rather than individual amino acids.

**PATHOGENESIS**

To maintain its complex enzootic cycle, *B. burgdorferi* must adapt to two markedly different environments, the tick and the mammalian host. For example, in the mid-gut of the tick, the spirochete expresses OspA and OspB (48). When the blood meal is taken, OspC is up-regulated as the organism migrates to the tick salivary gland and the mammalian host (49). After injection of the spirochete by the tick and an incubation period of 3 to 32 days, the spirochete usually first multiplies locally in the skin at the site of the tick bite. Several days later, the spirochete begins to spread in the skin and within days to weeks it may disseminate further. Bacterial spread within the host is probably facilitated by the spirochete’s ability to bind human plasminogen and urokinase-type plasminogen activator to its surface (50, 51). Plasmin, the active proteinase form of plasminogen, can promote the tissue invasion capability of *B. burgdorferi*. To date, two binding and cellular entry mechanisms have been identified. First, the spirochete attaches to members of the integrin family of receptors and the vitronectin and fibronectin receptors (52, 53). A second pathway for cell attachment is mediated by host cell sugars, particularly glycosaminoglycans (54). In *in vitro* systems, intracellular localization of *B. burgdorferi* has been demonstrated within human endothelial cells, macrophages, and fibroblasts (55, 56, 57).

Initially, the immune response in Lyme disease seems to be suppressed (57), which may be an important mechanism in allowing the spirochete to disseminate. Within days to weeks, peripheral blood mononuclear cells begin to have heightened responsiveness to *B. burgdorferi* antigens and mitogens (58, 59). *In vitro*, *B. burgdorferi* is a potent inducer of pro-inflammatory cytokines, including tumor necrosis factor-γ and interleukin-1b from peripheral blood mononuclear cells (60).

The antibody response to *B. burgdorferi* develops slowly. The specific IgM response spikes between the third and the sixth week of infection (61) and often is associated with polyclonal activation of B cells, including elevated total serum IgM levels (62), circulating immune complexes (63), and cryoglobulins (64). Membrane lipoproteins, including OspA, are mitogenic for B cells (65). The specific IgG response develops gradually over months to an increasing array of spirochetal polypeptides (66) and non-protein antigens (67). Histologically, all affected tissues show an infiltration of lymphocytes and plasma cells (68). Some degree of vascular damage, including mild vasculitis or hypervascular occlusion, may be seen in multiple sites, suggesting that the spirochete may have been in or around blood vessels. Despite an immune response, *B. burgdorferi* may survive for years in untreated patients in certain niches within the joints, nervous system, or skin; it is not yet known how it is able to sequester itself in these sites.

**CLINICAL SIGNIFICANCE**

Lyme disease occurs in stages, with remissions, exacerbations and different clinical manifestations at each stage. Early infection consists of stage 1 (localized EM) (*Figures 1,6*) (1), followed by stage 2 (disseminated infection), and finally stage 3 (late infection or persistent infection). Stage 3 usually begins months or years after disease onset, sometimes following long periods of latent infection (69). In an individual patient, however, the infection is highly variable, ranging from brief involvement in only one system, to chronic multi-system involvement of the skin, nerves, and joints for a period of years.

![Figure 6: Erythema migrans rash in Lyme borreliosis](image-url)
Early Infection: Stage I (Localized Infection)

The first phase, or the skin phase, follows inoculation of the spirochete into the skin by a tick during its blood meal. The *Ixodes* tick that transmits Lyme disease to humans is a pool feeder in that it does not inject the bacteria directly into the bloodstream but creates a lesion into which blood flows. (Figure 4E) Thus, the bacteria are originally introduced into the lower layers of skin, EM, which occurs at the site of the bite, usually begins as a red macule or papule. In one study, only 31% of patients (N=314) recalled a tick bite at the skin site where EM developed 3 to 32 days later (70). The centers of early lesions sometimes become intensely erythematous and indurated, vascular, or necrotic. In some instances, migrating lesions remain an even intense red, have several red rings which are found within the outside ring, or the central area turns blue before it clears. It has been documented that as many as 40% of patients do not exhibit this characteristic skin manifestation. This initial skin phase may give the spirochete some protection from the neutrophils that patrol the blood stream. The ability of the spirochete to move away from the region may also play a protective role.

Early Infection: Stage II (Disseminated Infection)

The spirochete next invades the bloodstream, leading to further dissemination throughout the body. Within days after the onset of the initial EM skin lesion, patients may experience multiple annular secondary lesions (70, 71). During this period, some patients develop macular rash, conjunctivitis, or, rarely, diffuse urticaria. EM and secondary lesions usually fade within 3 to 4 weeks (range, 1 day to 14 months). EM is often accompanied by malaise, fatigue, headache, fever, chills, generalized aches, and regional lymphadenopathy (70, 71). In addition, patients may have evidence of meningeal irritation, mild encephalopathy, migratory musculoskeletal pain, sore throat and cough. Except for fatigue and lethargy, which are often constant, the early sign and symptoms are typically intermittent and changing. After several weeks to months, however, 15% of untreated patients in the United States develop frank neurologic abnormalities, including meningitis, encephalitis, and cranial neuritis, including bilateral facial palsy (72). The usual pattern consists of fluctuating symptoms of meningitis with superimposed cranial neuritis, particularly facial palsy. On examination, such patients usually have neck stiffness. Facial palsy, or Bell’s palsy, frequently occurs alone and may be the presenting manifestation of the disease (73). In Europe, the most common manifestation is Bannwarth’s syndrome, which includes neuritic pain, lymphocytic pleocytosis without headache, and sometimes cranial neuritis (74). Within several weeks after the onset of illness, about 5% of untreated patients develop cardiac involvement (75) with the most common abnormality as fluctuating degrees of atrioventricular block (75, 76). However, some patients have evidence of electrocardiographic changes compatible with acute myopericarditis (77).

During this stage, musculoskeletal pain is common. The typical pattern is one of migratory pain in joints, tendons, bursae, muscle or bones, often without joint swelling. In addition, a few patients have been described with osteomyelitis and myositis (78, 79). Conjunctivitis is the most common eye abnormality in Lyme disease, but deeper tissues in the eye may be affected as well (70).

Late Infection: Stage III (Persistent Infection)

The third stage of the infection begins when the spirochete enters tissue throughout the body. At that point, the bacteria become more difficult to find and may enter a quiescent phase with lowered metabolic activity, which makes them less likely to attract the cells of the host’s defense systems. Months after the onset of the illness, about 60% of patients begin to experience intermittent attacks of joint pain and swelling, primarily in large joints, especially the knee (80). Although the spirochete has been cultured from the joint fluid of only two patients with Lyme arthritis (69), *B. burgdorferi* DNA may be detected by polymerase chain reaction (PCR) in the synovial tissue or joint fluid of most patients (81, 82). *B. burgdorferi*–specific T cells, reactive with multiple spirochetal polypeptides, are concentrated in joint fluid (59, 83). These T cells secrete primarily the pro-inflammatory Th1 cytokine IFN-γ. This response is dominant in synovium, a pattern that leads to a delayed hypersensitivity response (84). From months to years after disease onset, sometimes following long periods of latent infection, patients may develop chronic neurologic manifestations of the disorder (84, 85). The most common form of chronic central nervous system involvement is a sub-acute encephalopathy affecting memory, mood, or sleep (87, 88). Patients with these symptoms often have CSF abnormalities, including increased CSF protein levels, evidence of *B. burgdorferi* antibodies, or both (87, 88).

*Borrelia garinii*

*Borrelia garinii* is transmitted by the ticks *Ixodes ricinus* and *Ixodes persulcatus* and appears to be the most neurotropic of the three *Borrelia* species. *Borrelia garinii* may cause an exceptionally wide range of neurologic abnormalities including borrelial encephalomyelitis, a multiple sclerosis–like illness, which is a severe neurologic disorder characterized
by spastic parapareses, ataxia, cognitive impairment, bladder dysfunction, and cranial neuropathy, accompanied by antibody production to \textit{B. burgdorferi}.

\textit{Borrelia afzelii}  
\textit{Borrelia afzelii} is transmitted by the ticks \textit{Ixodes ricinus} and \textit{Ixodes persulcatus}. In Europe and Asia, \textit{B. afzelii} may persist in the skin for decades resulting in acrodermatitis chronica atrophicans (ACA) (Figure 7) (1). ACA occurs during the third stage, or late stage, of European Lyme Borreliosis, and is a skin condition that occurs primarily on sun-exposed surfaces of distal extremities beginning with an inflammatory stage with bluish-red discoloration and cutaneous swelling and concluding several months or years later with an atrophic phase. Sclerotic skin plaques may also develop. The frequency of ACA is about 1-10\% of all European patients with Lyme Borreliosis, varying according to the region of the population sampled. ACA is probably the most common late and chronic manifestation of \textit{borreliosis} in European patients with Lyme disease. Because the clinical diagnosis of ACA is much more difficult than that of EM or \textit{Borrelia} lymphocytoma (BL), the condition is often underdiagnosed, and, in fact, the ratio of EM cases to ACA cases may be higher.

\textbf{Figure 7: Acrodermatitis chronica atrophicans, a late skin manifestation of \textit{B. afzelii} infection, shown affecting the legs of an elderly woman where the skin has become thinned and atrophic. Image courtesy of R. Muellegger.}

\textbf{DIAGNOSIS}  
\textbf{Indirect (Antibody Detection Tests)}

Antibody tests are indirect, in that they measure exposure to a pathogen. A positive test is a function of the host immunological response to a foreign antigen, \textit{B. burgdorferi} in the case of Lyme disease. Direct tests are those that detect the entire bacterium (usually by culture or staining techniques) or parts of the bacterium such as cell wall proteins, carbohydrates, intracellular proteins, or nucleic acids such as DNA or RNA. Both indirect (serology) and direct tests (PCR) are available for the accurate, sensitive, and specific laboratory detection of Lyme disease. Because of the complex nature of the disease, no single test may be sufficient for all clinical situations.

Medical Diagnostic Laboratories (MDL) offers three serology tests for the detection of antibodies to \textit{Borrelia burgdorferi}, the causative agent of Lyme disease (LD):

1. First step Enzyme-Linked Immunosorbent Assay (ELISA) screening test
2. Supplemental Western immunoblot (IgG, IgM)
3. C6 Lyme peptide assay (C6LPA)

\textbf{A. The Enzyme-Linked Immunosorbent Assay (ELISA) screening test}

Enzyme-Linked Immunosorbent Assays rely on an antigen’s ability to adsorb to a solid phase. When a serum specimen is introduced to this system, any antigen-specific antibodies which may be present in the patient serum will bind to the antigen pre-adhered to the solid support such as a microwell plate. Once excess antibody is washed from the surface of the solid phase, goat anti-human IgG/IgM conjugated with horseradish peroxidase is then introduced. It will bind to any antigen-antibody complexes which are adhered to the solid phase, forming a sandwich. Once excess conjugate is washed from the solid phase, Chromagen/Substrate Tetramethylbenzidine (TMB) is then introduced. If specific antibodies are present because they formed a sandwich with the antigen-antibody complexes, the solution will turn blue. Once the stop solution, 1N H\textsubscript{2}SO\textsubscript{4}, is added, the solution turns yellow and is measured using a spectrophotometer. Interpretation of the resulting reading is indicative of the concentration of antibody in the patient serum specimen (Figure 8) (88).
The ELISA should only be ordered for patients who have signs and symptoms consistent with Lyme disease. Equivocal or positive ELISA tests are recommended by the CDC to be followed with a supplemental Western blot. However, some clinicians may wish to use a Western blot test as a companion test to the ELISA. While positive first-step ELISA results supplemented with a positive second-step Western blot can be used to support a diagnosis of Lyme disease, negative results of either an ELISA or Western blot should not be used to exclude Lyme disease.

Early antibody responses are often due to the gp41 flagellar antigen. Due to its cross-reactive components, a false positive reaction will often occur with syphilis patients or other patients with spirochetal diseases, such as leptospirosis or periodontal disease, since these treponemes also have similar gp41 flagellar antigens. This degree of cross-reactivity results in low specificity of the ELISA assay and it is therefore recommended that positive or equivocal first-step ELISA results be supplemented by a second-step assay.

**B. Western immunoblot (IgG, IgM)** *B. burgdorferi* antibodies

Early tests for the laboratory diagnosis of Lyme disease suffered from a lack of both sensitivity and specificity. Enzyme linked immunoassays (ELISA) used whole cell lysate preparations of *B. burgdorferi* as capture antigen and were particularly susceptible to false positive results because of cross-reactive antibodies present in the patient sample. In 1989, the Centers for Disease Control and Prevention (CDC) recommended that all indeterminate and positive ELISA tests be confirmed by Western blot (88). Subsequently, tests have become available which are both more sensitive and specific but the majority of clinical laboratories still use a whole cell lysate ELISA screening test with a supplemental Western blot test performed on indeterminate and positive screening results.

The Western blot is an antibody test which is capable of differentiating the antibody response to the variety of *B. burgdorferi* antigens. The antigens are separated electrophoretically and then transferred, or “blotted”, to a nitrocellulose strip. The strips are exposed to the patient sample containing antibodies that are both specific to *B. burgdorferi* as well as other infectious agents. The presence of colored bands on the strips is indicative of an antigen/antibody reaction. The molecular weight of the antigen can be determined by its location on the strip; hence the numerical designation of the antigen, OspA (31 kDa), OspB (34 kDa), and so on. Because many of the antigens that appear on the Western blot strip are shared by other organisms in addition to *B. burgdorferi*, the interpretation of the blot becomes critically important in determining whether the Western blot test is positive or negative. In the Western blot sample illustrated in (Figure 9), the top strip is from a patient positive for IgG and the bottom strip is the band locator strip which is used for comparative purposes to help properly identify band location.

*Figure 9:* An IgG positive patient Western blot strip (top) in comparison to a band locator strip (bottom).

The CDC recommends an interpretation of Western blot based on the work of Dressler *et al.* (90). The **CDC CRITERIA** includes:

**IgM Significant Bands:**

**Reactive:** At least two of the following three bands must be present: 23, 39, 41.

**IgG Significant Bands:**

**Reactive:** At least five of the following bands must be present: 18, 23, 28, 30, 39, 41, 45, 58, 66, 93.

**Non-Reactive:** Fewer than two bands are present.

* Note that the 31 and 34 kDa *B. burgdorferi* specific bands represent outer surface proteins A and B, respectively, have been excluded from the IgG criterion. Note also, that there is no indeterminate or equivocal category—five bands are interpreted as positive while four bands constitute a negative result. Included in the IgG interpretive criterion is the 41 kDa band observed in 65% of the normal population.

Tilton *et al.* (91) and others have proposed the following **ALTERNATE CRITERIA**:

**IgM significant bands:**

**Reactive:** Two or more of the following four bands must be present: 23, 39, 41, 83/93.

**IgG significant bands:**

**Reactive:** Three or more of the following bands must be present: 20, 23, 31, 34, 35, 39, 83/93.

**Equivocal:** One of the following bands must be present: 23, 31, 34, 37, 39, 41, 83/93

**Non-reactive:** No Lyme-specific bands present.
The major differences are:

**IgM** — This interpretive criterion is similar to the CDC except that 83/93 has been included as a significant IgM band that is specific to *B. burgdorferi*. The Alternate Criteria also incorporates an “Equivocal” category that includes late appearing antibodies specific for the 31 kDa OspA and 34 kDa OspB antigens.

**IgG** — The Alternate Criterion is based on both the number of bands and the significance of the antibodies detected. For example, Osp A (31 kDa) and B (34 kDa) are important bands often seen in late stages of Lyme disease. The alternate criteria also includes an “Equivocal” category which indicates that although there are insufficient antibody bands present for the blot to be reactive, there is significant immunologic activity observed which may be related to Lyme disease.

To determine the effect of reporting results using the CDC vs. the alternate criteria (92), commercially available panels (BBI Diagnostics, CDC) of characterized serum samples were used. The results indicated that the use of the Alternate Criteria markedly increased the sensitivity of the Western blot while only decreasing the specificity slightly.

This raises the question at to whether a Western blot should be more sensitive or as specific. If the Western blot is to be used solely for confirmation of ELISA results, then specificity may be preferred over sensitivity. However, a specific Western blot with low sensitivity may invalidate a sensitive and specific ELISA. A Western blot that is both highly sensitive and specific is desirable for a two-tiered test scheme. However, despite the recommendations of the CDC for two-tiered testing, many physicians who treat patients with Lyme disease do not accept ELISA as a very sensitive screening test and, therefore, request both ELISA and Western blot be performed on their patients.

**Please note that BOTH the Alternate and the CDC criteria will appear on all Lyme Western blot result reports from Medical Diagnostic Laboratories. Occasionally, this will cause differences in results; your alternate report will also include an equivocal category.**

C. The C6 Lyme Peptide Antibody Test

Commercially available Lyme disease antibody kits, as well as most research level Lyme disease kits, use a whole cell lysate as the capture antigen. The capture antigen is used to coat a solid support such as a microwell plate and binds specific antibodies that may be present in the serum sample. These lysates contain multiple epitopes, not all of which are specific for *B. burgdorferi*. Because of the immunologic complexity of a whole cell lysate, a high background is often present and false positives are therefore a problem. This is the basis for the lack of specificity inherent to most enzyme immunoassay (EIA) kits and thereby necessitates confirmatory immunoblot tests to distinguish Lyme-specific from non-specific antibodies.

Yet another problem with whole cell ELISAs is the presence of antibodies to OspA that are vaccine induced. Separating the vaccine response from the natural host response to infection becomes very difficult with whole cell ELISAs.

Single or multiple peptide tests use only antigens that may be specific for *B. burgdorferi*. These include tests using recombinant OspA, OspB, and flagellar proteins (91), recombinant OspC, p39 and flagellar proteins, p83 antigen, the hook protein of *B. burgdorferi* (FlgE), p37, and the C6 Lyme peptide recombinant or synthetic antigen (93, 94). The single peptide Lyme C6 EIA for Lyme disease has been approved by the Food and Drug Administration (FDA). The *Borrelia burgdorferi* C6 peptide antibody test (C6 LPA) identifies antibodies to a newly discovered, conserved peptide called C6, which is a component of the variable surface antigen of *B. burgdorferi*. The C6 LPA is important because it detects both IgM and IgG antibodies in patients with LD but not in vaccinees.

The C6 LPA, like some other peptide assays (the exception being the OspA EIA), can effectively discriminate vaccinated from unvaccinated patients with Lyme disease. One hundred percent of vaccinees tested had a negative Lyme C6 EIA. The C6 LPA FDA submission reported sensitivities for acute, convalescent and late stage disease of 74%, 90%, and 100%, respectively (93, 94). More recent unpublished data (99) suggest that the sensitivity of the C6 LPA in chronic Lyme disease may not be as high as originally anticipated but no worse, and probably better, than any other available ELISA (99). These levels of sensitivity and the much improved specificity (99%) suggests that the C6 LPA might become the test of choice for screening purposes. Serum specimens from patients with early neuroborreliosis are 95% positive. The early C6 response to infection includes both IgM and IgG production and may appear soon after a tick bite (93, 94). Patients with over a dozen different diseases including systemic lupus erythematosus, non-Lyme arthritis, syphilis, other spirochetal diseases, and other autoimmune diseases were uniformly C6 negative. Antibodies produced by challenge with *B. burgdorferi*, *B. afzelii*, and *B. garinii* can all be detected with the C6 LPA. Although the latter two strains are not indigenous to the United States, both are prevalent in Europe.

All current positive serological screening tests should be confirmed by Western blot. With more clinical experience,
the high specificity of a positive C6 LPA may eliminate the need for confirmatory testing (95). The immune response to the OspA vaccine has been reported to induce antibodies that bind to multiple proteins of *B. burgdorferi* (95). Sera from vaccinated people show multiple low molecular weight bands in addition to a broad band at 31 kDa on Western blot. The utility of a Western blot in a vaccinated person is questionable. Until recently, there has been no “test of cure” for Lyme disease and the clinical determination of a “cure” is very controversial. Whether persistent symptoms (persistent Lyme disease, post-Lyme syndrome, chronic Lyme disease) after adequate therapy are due to active *B. burgdorferi* infection, existing tissue injury, a post-infectious syndrome or a condition unrelated to Lyme disease is not well defined. Routine ELISAs do not correlate with the disappearance or reappearance of symptoms. In contrast, C6 LPA antibodies diminished by a factor of >4 in successfully treated patients and <4 in patients who did not respond to appropriate therapy for Lyme disease. Control tests using a whole cell ELISA or a p39 recombinant EIA were either unchanged or showed no significant change in titer as a result of treatment (93, 94). The results suggest that quantitative C6 LPAs administered over a period of time may be useful for monitoring a response to therapy as well as to determine whether the spirochete has been eliminated. Finally, the question of active vs. inactive Lyme disease may be resolved because a positive C6 LPA suggests active infection and a negative test, even with a positive routine ELISA, suggests inactive infection or no infection at all, except in very early acute infections (93, 94).

**Direct Tests (Molecular Detection via Real-Time PCR)**

DNA detection by Real-Time PCR is available from MDL for whole blood, serum, urine, CSF, and synovial fluid samples. Four individual assays were developed to differentiate between the three *B. burgdorferi* sensu lato genospecies known to cause Lyme disease in humans, *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*, as well as the clinically related, but genomically distinct, *B. lonestari*.

The ability to distinguish among these pathogens offers a diagnostic benefit that traditional sero-based testing methods do not provide. Variations between the genetic composition of each pathogen make each species distinct from one another and, as a result, the immune response elicited by each distinct, too. Antibodies are produced against discrete regions of proteins termed epitopes that are “presented” during the immune response. Genetic variations between pathogenic epitopes allows for the generation of antibodies specific to a particular pathogen and serves as the basis of immune memory. This high degree of antibody specificity serves to limit the diagnostic value of conventional ELISAs. Real-Time PCR represents a significant advancement to traditional PCR and has improved the specificity and sensitivity of diagnostic testing of this type of testing. Like traditional PCR, real-time assays utilize sequence-specific primers that allow for the highly specific amplification of DNA sequences from minute amounts of target material. What makes this technology superior, however, is the incorporation of a sequence-specific fluorescent reporter probe that allows for an accurate quantitation of the reaction following each amplification cycle.

The limitations associated with conventional Lyme testing are especially evident when taking into consideration infections with *Borrelia lonestari*, a clinically similar, yet genomically distinct, pathogenic strain. In this instance, serum samples from individuals determined to be infected with *B. lonestari* were unable to recognize *B. burgdorferi* antigens, rendering conventional ELISAs useless (96, 97). Despite the fact that most of these organisms are separated geographically from one another and, as such, infection with these agents would be more presumptive, the high rate of travel within the Unites States and to Europe means that none of these pathogens should be discounted when a patient presents with symptoms typical of Lyme disease. The relevance of such speciation tests is further supported by the report of an individual from Westchester County, New York who was infected with *B. lonestari* during a trip to Maryland and North Carolina (98). Therefore, in instances where patient symptoms are suggestive of Lyme disease yet conventional Lyme tests return negative or ambiguous results, direct screening via PCR-based technology should be employed.

**Borrelia lonestari**

Since the mid-1980’s, physicians in non-Lyme disease endemic areas of the south eastern and south central portion of the United States have described a Lyme disease-like illness. Two to twelve days following the bite of the Lonestar tick, *Amblyomma americanum*, patients would describe an erythema migrans (EM)-like rash and mild flu-like symptoms including fever. This condition is often referred to as southern tick-associated rash illness (STARI), Master’s disease, or southern Lyme disease. Based on PCR amplification of the flagellin and 16s rRNA genes, a new spirochete, *Borrelia lonestari*, has been identified. Optimal management of this condition will depend upon further discoveries regarding its natural history and etiology.

**References:**


http://biosystemdevelopment.com/technology.htm (Accessed: 07/06/07)


