VOLUME - XXII
ISSUE - CXXXI
NOV/DEC 2025

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Lyme disease, also known as **Lyme borreliosis**, is a tick-borne disease caused by species of *Borrelia* bacteria, transmitted by blood-feeding ticks in the genus *Ixodes*. It is the most common disease spread by ticks in the Northern Hemisphere. Infections are most common in the spring and early summer.

The most common sign of infection is an expanding red rash, known as erythema migrans (EM), which appears at the site of the tick bite about a week afterwards. The rash is typically neither itchy nor painful. Approximately 70–80% of infected people develop a rash. Other early symptoms may include fever, headaches and tiredness. If untreated, symptoms may include loss of the ability to move one or both sides of the face, joint pains, severe headaches with neck stiffness or heart palpitations. Months to years later, repeated episodes of joint pain and swelling may occur. Occasionally, shooting pains or tingling in the arms and legs may develop.

Diagnosis is based on a combination of symptoms, history of tick exposure, and possibly testing for specific antibodies in the blood. If an infection develops, several antibiotics are effective, including doxycycline, amoxicillin and cefuroxime. Standard treatment usually lasts for two or three weeks. People with persistent symptoms after appropriate treatments are said to have Post-Treatment Lyme Disease Syndrome (PTLDS). The **"DISEASE DIAGNOSIS"** portion of the issue highlights all aspects of LYME DISEASE.

"UNDERSTANDING" superficially discusses PROTOZOAL DISEASES of man. While "**TROUBLESHOOTING"** talks about Preanalytical problems observed in Rapid Diagnostic tests. WE ignore nothing – "**BOUQUET"** is very much there.

DISEASE DIAGNOSIS

LYME DISEASE

Background

Lyme disease is a multisystem illness usually caused by infection with the spirochete *Borrelia burgdorferi* and the body's immune response to the infection. (See the image below.) The disease is transmitted to humans via tick bites, from infected ticks of the genus *Ixodes*.

Lyme disease. The bacterium Borrelia burgdorferi (darkfield microscopy technique, 400X)

Lyme disease is the most common vector-borne illness in the United States. An estimated 476,000 cases of Lyme disease are diagnosed and treated in the United States each year; this estimate is based on commercial insurance claims data. Lyme disease is also endemic in other parts of North America, as well as in Europe and Asia. Because most patients with early Lyme disease do not recall the tick bite, epidemiologic context is extremely important. The probability of a tick bite—and thus, the likelihood of contracting Lyme disease—is highest in persons who spend time outdoors (particularly in wooded, brushy, or grassy habitats) in a geographically endemic area, especially from May through November. Early localized Lyme disease refers to isolated erythema migrans, the characteristic skin rash of Lyme disease, and to an undifferentiated febrile illness. This stage occurs 1-30 days after the tick bite. Early disseminated Lyme disease usually develops 3-10 weeks after inoculation. Musculoskeletal and neurologic symptoms are the most common; less common are symptoms from cardiac disturbances and ocular manifestations, most often conjunctivitis. Late or chronic Lyme disease refers to manifestations that occur months to years after the initial infection, sometimes after a period of latency. Signs and symptoms of chronic Lyme disease are primarily rheumatologic and neurologic.

Etiology

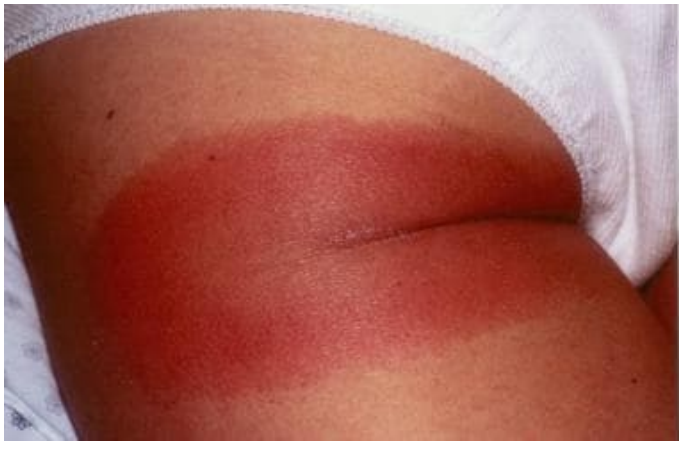
Lyme disease is usually caused by infection with the spirochete *Borrelia burgdorferi.* The complete genome of *B burgdorferi* was described in 1998.

The species *Borrelia burgdorferi* sensu lato has three well-characterized groups, as follows:

- *B burgdorferi* sensu stricto
- *B garinii*
- *B afzelii*

B burgdorferi sensu stricto is a broad category of closely related but genetically distinct genospecies that constitutes most North American isolates and is found in Europe as well. *B afzelii* is found mainly in Europe. *B garinii* is found predominantly in Eurasia, but it has also been isolated in eastern North America. These subspecies are associated with different clinical presentations, probably due to genomic variation. Infection with *B burgdorferi* sensu stricto has a particular predilection to affect joints. In European patients with erythema migrans, *B afzelii* can be isolated from about 80% of lesions and *B garinii* from 15%. *B afzelii* often infects the skin only but may persist in that site, causing various cutaneous manifestations including acrodermatitis chronica atrophicans. *B garinii* has some neurotropism and is the isolate that accounts for most cases of lymphocytic meningoradiculitis (Bannwarth syndrome) and white matter encephalitis, which is rare in North America. However, this organism can also cause all the various cutaneous manifestations of Lyme disease. Other strains, which may be sufficiently different in their genetic structure to be considered separate strains, exist; however, most of these are nonpathogenic to humans. This is an area of active research. *Borrelia miyamotoi*, which causes a febrile illness and is transmitted by ixodid ticks, was discovered in 1994 in Japan and has since been reported in North America, Europe, and Asia. *B miyamotoi* infection is widespread in *Ixodes* ticks in the northeastern, northern midwestern, and far western United States and in Canada. Common clinical manifestations of *B miyamotoi* infection are fever, fatigue, headache, chills, myalgia, arthralgia, and nausea. *B miyamotoi* infection responds to the same antibiotics used for Lyme disease. Mayo Clinic researchers reported a novel species of bacteria, provisionally named *Borrelia mayonii*, isolated in six patients in the upper Midwest of the United States with suspected Lyme disease. Clinically, disease from *B mayonii* is similar to that from *B burgdorferi*, except that *B mayonii* is associated with nausea and vomiting, diffuse rashes, and unusually high spirochetemia. Polymerase chain reaction (PCR) testing targeting the *oppA1* gene of *B burgdorferi* sensu lato proved useful in diagnosis. *B burgdorferi* is transmitted by ixodid tick species. In the northeastern and upper midwestern United States, *Ixodes scapularis* (sometimes termed *Ixodes dammini*) is the vector. In the northwestern United States, *Ixodes pacificus* is the vector. In other parts of the world, other *Ixodes* ticks serve this function. Other tick species (eg, *Amblyomma americanum*) and insects can carry *B burgdorferi*, but the vast majority of cases are believed to be caused by bites by *Ixodes* ticks.

See the images below.



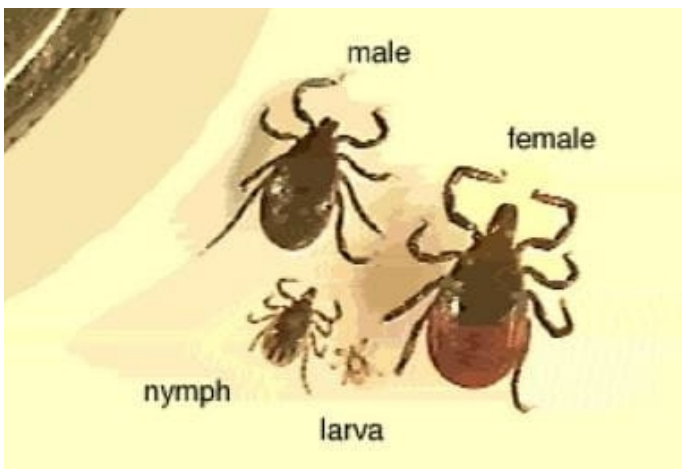
Lyme disease. This patient's erythema migrans rash demonstrates several key features of the rash, including size, location, and presence of a central punctum, which can be seen right at the lateral margin of the inferior gluteal fold. Note that the color is uniform; this pattern probably is more common than the classic pattern of central clearing. On history, this patient was found to live in an endemic area for ticks and to pull ticks off her dog daily.

Amblyomma americanum is the tick vector for monocytic ehrlichiosis and tularemia. An adult and a nymphal form are shown (common match shown for size comparison).

Pathophysiology

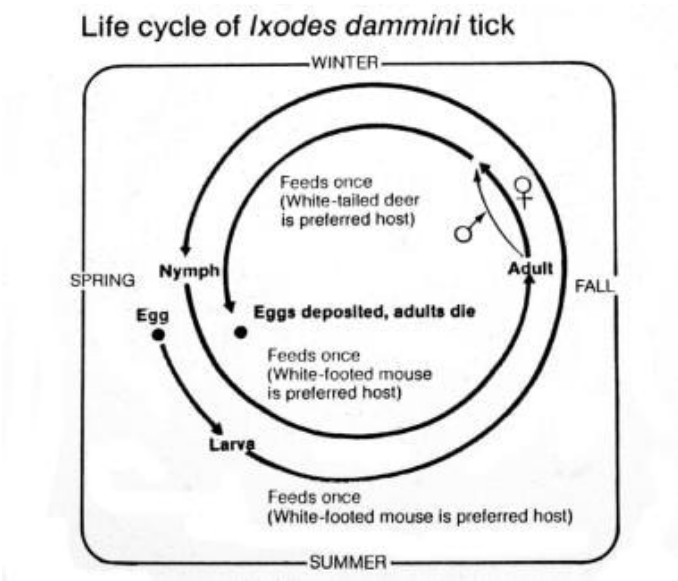
Borrelia burgdorferi infectious cycle

The infectious cycle of *B burgdorferi* involves colonization, infection of *Ixodes* ticks, and then transmission to a broad range of mammalian hosts, including humans. Variation in environmental and host conditions promotes different gene expression and changes in the composition of the membrane proteins of the spirochete. This adaptation is a critical step in the pathogenesis and transmission of Lyme disease. The *Ixodes* tick progresses through four stages of development: egg, larva, nymph, and adult (see the following image for examples of each stage). Only larvae, nymphs, and adult female ticks require blood meals, and only ticks in the nymphal and adult stages can transmit *B burgdorferi.*



Lyme disease.

The life cycle of *Ixodes* ticks spans 2 years (see the image below). The adult lays eggs in the spring, and the larvae emerge in the summer. The larvae feed once, in late summer, on any of a wide variety of small animals (eg, the white-footed mouse) . The following spring, the larvae emerge as nymphs. Nymphs feed once, in the spring and summer. The white-footed mouse is the preferred feeding source of nymphs, but other animals apparently suffice. Nymphs molt into adults the following fall and feed once on a larger animal, with the white-tailed deer being the preferred host.



Life cycle of the Ixodes dammini tick.

Ticks can acquire *B burgdorferi* from feeding on an infected animal host during any of the three life-cycle stages. Unless the tick has fed on an infected host before feeding on a person, infection cannot result from that tick bite. Even if a tick that has previously fed on an infected animal and then feeds on another animal, the animal may not acquire the infection. Mice do not appear to develop Lyme disease, but they do carry the bacteria. They may be considered infested rather than infected. Deer also are incompetent hosts for *Borrelia*. Ticks carry *B burgdorferi* organisms in their midgut. The bacteria are introduced into the skin by a bite from an infected tick, and disease is transmitted to humans as the spirochete is translocated from the gut to the salivary glands and then to the person at the site of the bite. The risk of Lyme disease is highest during the time of the year when *Ixodes* ticks in the nymphal stage are seeking a blood meal. Although the prevalence of *B burgdorferi* infection in adult ticks is twice that of nymph ticks, nymphs are responsible for 90% of human disease transmissions because of the great abundance of nymphs; the increase in human outdoor activity in the summer (the peak feeding season of nymphs); and the small size of nymphs, which makes them less likely to be detected and removed before disease transmission occurs. The risk of transmission of *B burgdorferi* from an infected tick to a human depends on the length of exposure. It takes hours for the tick to attach fully, and experimental studies have indicated that in most cases, nymphs must feed 36-48 hours and adults 48-72 hours to transmit *B burgdorferi,* since the blood meal must trigger the reproduction of the *Borrelia* to a large enough number to be infective.

Pathogenesis

Once the spirochete is in the skin, one of three events may occur:

- The spirochete may be overwhelmed and eliminated by host defense mechanisms
- The spirochete may remain viable and localized in the skin, where it produces erythema migrans, the characteristic skin lesion of Lyme

disease
- Within days to weeks, the spirochete may disseminate through the lymphatics or blood,

After entering the circulation, the organism shows a distinct tropism for the skin, heart, central nervous system (CNS), joints, and eyes. Any part of the body can be affected, however; spirochetes have also been demonstrated histologically in bone marrow, the spleen, lymph nodes, the liver, testes, and the placenta during early hematogenous dissemination. The clinical manifestations of Lyme disease generally follow three stages of disease progression: early localized, early disseminated, and chronic disseminated. All are potentially curable with antibiotic therapy. The infection progresses to disseminated disease in approximately 50% of untreated patients. Only a few genotypes of *B burgdorferi* appear to be responsible for the large majority of cases of disseminated disease.

Stage 1 disease

Stage 1 is also known as primary or early localized infection. It generally occurs within 30 days of the tick bite. Most patients present with a characteristic expanding rash (erythema migrans) at the site of the tick bite 7-14 days after the tick is removed. Nonspecific symptoms may include the following:

- Fatigue
- Myalgias
- Arthralgias
- Headache
- Fever
- Chills
- Neck stiffness

Stage 2 disease

Stage 2 is also known as early disseminated disease. It generally occurs weeks to months after the bite. Musculoskeletal and neurologic symptoms are the most common; less common symptoms are cardiac and dermatologic. *B burgdorferi* spreads throughout the body and produces symptoms by direct invasion (eg, erythema migrans), particularly in the early stages of the disease. Because growing *B burgdorferi* in culture is difficult, confirming that the organism is actually present in a specific organ that may be involved in Lyme disease is also difficult. The inflammatory response to *B burgdorferi* in the skin is probably the explanation for multiple lesions of erythema migrans, as almost all patients with multiple lesions are seropositive, regardless of duration. Antibodies against spirochetal protein membrane epitopes have been shown to cross-react with neural and connective tissues. This molecular mimicry possibly generates an autoimmune inflammatory reaction. The pathophysiology of early versus late manifestations of the disease is similar to that seen with syphilis. Early studies showed that *B burgdorferi* or its DNA can be detected in the bloodstream of roughly 10% of patients with isolated erythema migrans and no systemic symptoms. In addition, early in the course of disease and while erythema migrans still is present, spirochetal DNA has been detected in cerebrospinal fluid (CSF), indicating early CNS penetration. This can occur even in the absence of neurologic symptoms. One study found that if large-volume cultures (9 mL of plasma) were performed in early-presenting patients with erythema migrans, 93 (43.7%) of 213 had spirochetemia. Some of these patients had only isolated erythema migrans and no systemic symptoms.

Stage 3 disease

Stage 3 or chronic Lyme disease occurs months to years after infection, which sometimes involves a period of latency. Musculoskeletal (mainly joints) and neurologic systems are most commonly affected. *B burgdorferi* induces an immune response that may lead to symptoms in various organs, with little evidence of bacterial invasion. Studies of Lyme arthritis have shown that the arthritis is associated with certain immunologic factors, including the production of proinflammatory cytokines and the formation of immune complexes; and genetic factors, such as carriage of human leukocyte antigen (HLA)–DR4 and HLA-DR2. Patients with HLA-DR4 or HLA-DR2 and antibodies to OspA and OspB (outer surface protein A and B) proteins in their joint fluid may be more susceptible to long-term arthritis than persons without these characteristics. The presence of these genes is presumably related to the development of autoimmunity in the joint, which can lead to persistent inflammation even after the spirochete is apparently eradicated. Animal studies have suggested a primary role of astrocytes and microglial cells in the pathogenesis of neuroborreliosis. Interleukin 6 (IL-6) production by astrocytes and subsequent oligodendrocyte apoptosis have been proposed as mechanisms of cell injury. A study of maladaptive immune responses in 79 patients with Lyme neuroborreliosis who were followed for 1 year reported that serum levels of interferon-α (IFN-α) were elevated at study entry and remained elevated in patients whose symptoms persisted despite antibiotic therapy. The highest IFN-α levels corresponded with severe disease. In contrast, levels of 19 other cytokines and chemokines associated with innate and adaptive T-cell and B-cell immune responses were initially elevated, particularly in cerebrospinal fluid, but decreased dramatically with effective antibiotic therapy. The organism also can persist in the skin for very long periods. Experimentally, the spirochete can penetrate human fibroblasts and live intracellularly, even when the extracellular medium contains ceftriaxone at concentrations well above bactericidal levels. Although intracellular organisms have never been demonstrated in vivo, this may be a mechanism by which the organism eludes host defenses.

Epidemiology

Lyme disease is endemic in North America, Europe, and Asia, and the distribution of the vectors directly affects the incidence of the disease. *Ixodes scapularis* is the principal vector found in the Northeast and Central United States and Canada, whereas *Ixodes pacificus* is more common on the Pacific coast. *Ixodes ricinus* is the principal vector in Europe. The vector in Asia is the taiga tick, *Ixodes persulcatus.*

International statistics

Lyme disease exists throughout much of the world, including Canada, Europe, and Asia. Occasionally, cases are reported in more tropical locales, and Lyme disease may exist in Australia. In Asia, *B burgdorferi* infection has been reported in countries including China, Korea, Japan, Indonesia, Nepal, and eastern Turkey. In Europe, Lyme disease is primarily caused by *B afzelii* and *B garinii*. A systematic review of Lyme borreliosis in Europe from 2005 to 2020 found that the highest incidences (> 100 cases per 100,000 population per year [PPY]) were reported in Belgium, Finland, the Netherlands, and Switzerland. Incidences of 20-40/100,000 PPY were reported in the Czech Republic, Germany, Poland, and Scotland, and incidences of < 20/100,000 PPY were reported in Belarus, Croatia, Denmark, France, Ireland, Portugal, Russia, Slovakia, Sweden, and the United Kingdom. However, local high incidences were also noted in areas of countries with low overall incidence.

Race-, sex-, and age-related demographics

Lyme disease is reported primarily in Whites, although it occurs in individuals of all races. No genetic explanation is known for this; the disparity most likely stems from social or environmental factors (ie, a higher exposure rate to ticks in Whites than in members of other races) and possibly to the fact that erythema migrans is more difficult to diagnose in dark-skinned individuals. No strong preponderance of Lyme disease is noted in either sex. Reports from Europe indicate that, among children, the rate of Lyme disease is slightly higher in boys than in girls

aged 5-19 years, but, in adults over 30 years of age, the disease is more common in women than in men. In the United States, 53.1% of reported Lyme disease cases occurred in males. Age distribution in Lyme disease is bimodal: the first peak is in children aged 5-14 years and the second in adults aged 45-54 years. In general, this pattern is related to increased levels of outdoor activity and environmental exposure in these age groups rather than any intrinsic difference in susceptibility.

Prognosis

The prognosis for patients with Lyme disease is generally excellent when they are treated early with appropriate antibiotic regimens. However, recurrent infection is possible if the patient is again bitten by an infected tick; these infections are usually due to a different strain of the local *Borrelia*. Patients, especially adults, who receive late treatment or initial treatment with antibiotics other than doxycycline or amoxicillin may develop chronic musculoskeletal symptoms and difficulties with memory, concentration, and fatigue. These symptoms can be debilitating and difficult to eradicate. Some patients develop chronic arthritis that is driven by immunopathogenic mechanisms and not active infection. This condition is more prevalent among individuals with HLA-DR2, HLA-DR3, or HLA-DR4 allotypes. The arthritis is resistant to antibiotic treatment but typically responds to symptomatic treatment and shows eventual resolution. Cardiac involvement in Lyme disease is rarely chronic. However, patients with third-degree heart block often require a temporary pacemaker insertion and, on rare occasions, a permanent pacemaker insertion. Lyme disease appears rarely to be fatal. Many of the fatal cases reported have been in patients coinfected with other tick-borne pathogens such as *Ehrlichia* species and *B microti*, and in Europe, tick-borne encephalitis. A CDC study of death records found that only one of 114 total records listing Lyme disease as an underlying or multiple cause of death was consistent with clinical manifestations of Lyme disease. Extremely rare cases of neonatal death or stillbirth have been reported after pregnancies complicated by untreated or inadequately treated symptomatic maternal Lyme borreliosis. Subsequent findings from CDC studies suggest that congenital infection with *B burgdorferi* is unlikely and that it is not directly responsible for adverse fetal outcomes.

Post-treatment Lyme disease syndrome

Lingering symptoms, which may persist for more than 6 months, affect 10-20% of patients who receive recommended treatment for Lyme disease. Common complaints include cognitive disturbances, fatigue, joint or muscle pain, headaches, hearing loss, vertigo, mood disturbances, paresthesias, and difficulty sleeping. This condition is often termed chronic Lyme disease, but is more appropriately called post-treatment Lyme disease syndrome (PTLDS). No evidence suggests that prolonged antibiotic therapy is effective for PTLDS. Almost all patients recover with time, but recovery may take more than 6 months in some cases.

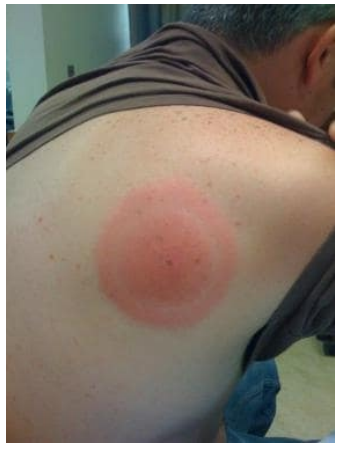
Clinical Presentation

History

Because only approximately 25-30% of US patients with early Lyme disease recall the tick bite, the clinician must direct the history toward the possibility of a tick bite. (In Europe, 64% do not remember being bitten.) Patients are generally unaware of a tick bite because these ticks are extremely small (nymphal *Ixodes* ticks are approximately the size of a poppy seed) and their bites are often painless. Epidemiologic context is extremely important. The clinician should determine where the patient lives, works, and vacations, and should ask about specific activities in which the patient participates at those locales. The probability of a tick bite—and thus, the likelihood of contracting Lyme disease—is highest in persons who spend time outdoors (particularly in wooded, brushy, or grassy habitats) in a geographically endemic area. Endemic areas can be defined as those with established populations of *Ixodes scapularis* or other vector ticks and evidence of enzootic transmission of *Borrelia burgdorferi* between the tick and the resident animal population. The season is important, especially in patients with early disease. Most cases of erythema migrans occur from late spring through early fall, because that is when ticks in the nymphal stage are seeking a blood meal, and nymphs account for 90% of Lyme disease cases. For patients presenting with later cutaneous manifestations, especially acrodermatitis chronica atrophicans, questions must be directed at assessing the risk of tick bite (or previous manifestations of Lyme disease) from many years in the past. Because people who engage in activities that put them in risk for tick bites tend to continue those activities, reinfection is not uncommon. Patients who have previously had erythema migrans can be reinfected (meaning that the first infection has been successfully treated, and they have a new infection with *B burgdorferi*). This has been clearly demonstrated in reinfected patients who were culture positive and had different serotypes isolated in the first and second infections. Reinfections manifest in much the same ways as first infections, although a tendency towards less hematogenous spread is noted. In contrast, relapse (as opposed to reinfection) is very unusual in patients who have been treated with appropriate antimicrobials. Certain manifestations of Lyme disease are related to the particular strain of *Borrelia* involved. In the United States, isolates from the East Coast are known as *B burgdorferi* sensu stricto, and infection with this organism has a particular predilection to affect joints, whereas other strains are more common in Europe, such as *B burgdorferi afzelii*, which is associated with acrodermatitis chronica atrophicans. Similar to syphilis, the manifestations of Lyme disease have been divided into three stages: localized, disseminated, and persistent. However, in individual patients, no rigid cutoffs exist between stages. The first two stages are part of the early infection, whereas persistent disease is considered late infection. Unlike syphilis, stage 3 disease may occur within 1 year of infection, not many years later.

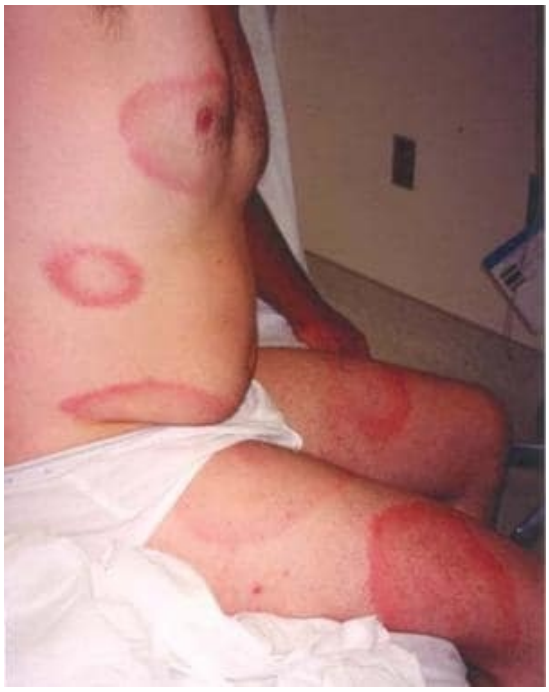
Stage 1 Lyme disease

Early localized Lyme disease refers to isolated erythema migrans and to an undifferentiated febrile illness. This stage occurs 1-30 days after the tick bite. Erythema migrans, the characteristic skin rash of Lyme disease, occurs in two thirds of patients with Lyme disease and develops at an average of 7 days after the tick bite. The rash typically occurs at or near the site of the tick bite, which may be in an area not normally visualized by individuals, such as the axilla, groin, or popliteal areas. It may be asymptomatic, or it may itch or burn. The rash typically expands over days and is not evanescent. It may not be observed until it is already full size. Clearing of portions of the rash as it expands may result in concentric rings of erythema, producing the classic bull's-eye rash (see the image below). In the United States, however, erythema migrans is more likely to have a uniform color.



Lyme disease. Bulls-eye rash.

Although many patients present with erythema migrans, others first present with extracutaneous symptoms. In those cases, erythema migrans may have never occurred or may not have been recognized by the patient or correctly diagnosed by the physician. Untreated, the rash may persist for 2-3 weeks. Eighty percent of patients with Lyme disease have only one episode of erythema migrans, whereas 20% may have recurrent episodes. Multiple lesions may occur in 20% of patients with Lyme disease; they result from hematogenous dissemination and are not the result of multiple tick bites (see the following image).



Lyme disease. Multiple lesions of erythema migrans occur in approximately 20% of patients. A carpenter from Nantucket who worked predominantly outside had been treated with clotrimazole/betamethasone for 1 week for a presumed tineal infection, but the initial lesion grew, and new ones developed. He then presented to the emergency department with the rashes seen in this photo. The patient had no fever and only mild systemic symptoms. He was treated with a 3-week course of oral antibiotics. Approximately 50% of patients describe flulike symptoms within days to 1 week of infection, characterized by fever, chills, and malaise. Fever is generally low grade. Other symptoms include fatigue and myalgia (80% of patients with Lyme disease in the United States but < 35% in Europe), as well as arthralgia, headache, and neck stiffness, which may resolve spontaneously even if specific therapy is not initiated. A paucity of respiratory and gastrointestinal tract symptoms has also been described. The most common ocular manifestations of stage 1 Lyme disease are redness and tearing. Approximately one third of all patients with erythema migrans develop no further manifestations of Lyme disease. Two thirds of patients develop further symptoms (stages 2 and 3). It is also important to consider coinfection by other organisms transmitted by the same tick bite, in areas where those are endemic. Coinfection with *Ehrlichia* species and *Babesia microti* are reported with increased frequency; in some studies, coinfection occurs in as many as 10-15% of patients with Lyme disease. When Lyme disease is strongly suggested but some of the manifestations are atypical (eg, a high fever, especially if accompanied by rigors or a toxic appearance), these other tick-borne infections or an alternative diagnosis must be considered.

Stage 2 Lyme disease

Early disseminated Lyme disease usually develops 3-10 weeks after inoculation. Approximately 25% of individuals infected with *B burgdorferi* have signs and symptoms of disseminated disease at presentation. Systemic manifestations may include fever and malaise. One or more organ systems become involved as hematologic or lymphatic spread disseminates spirochetes to distant sites. Musculoskeletal and neurologic symptoms are the most common; less common are symptoms from cardiac disturbances, such as dizziness, syncope, dyspnea, chest pain, and palpitations. Ocular manifestations include diplopia secondary to a cranial neuropathy or Bell palsy. Blurred vision and eye pain can occur from keratitis and iritis. Unilateral blindness from panophthalmitis has been reported as well.

Musculoskeletal manifestations

Intermittent inflammatory arthritis often begins as a migratory polyarticular process involving bursae, tendons, and joints, which evolves over 1-2 days into a monoarticular process involving the knee, ankle, and wrist, in decreasing frequency. When asked about symptoms after they have resolved, patients with Lyme disease are less likely to remember those symptoms that occurred before the monoarthritis. Polyarticular episodes may also occur. In two thirds of patients, the first episode occurs within 6 months of the erythema migrans lesion. Untreated, the episodes last approximately 1 week. Two thirds of patients have three recurrences approximately 2.5 months apart. The recurrences are more likely to involve more than one joint. With time, these episodes become less frequent and severe and involve fewer joints. Even without treatment, the recurrent episodes usually resolve over a 10-year period. Some patients may present with intermittent joint pain without inflammatory findings. This presentation is more common in Europe, where arthritis was not recognized as a manifestation of Lyme disease until the early reports from the United States.

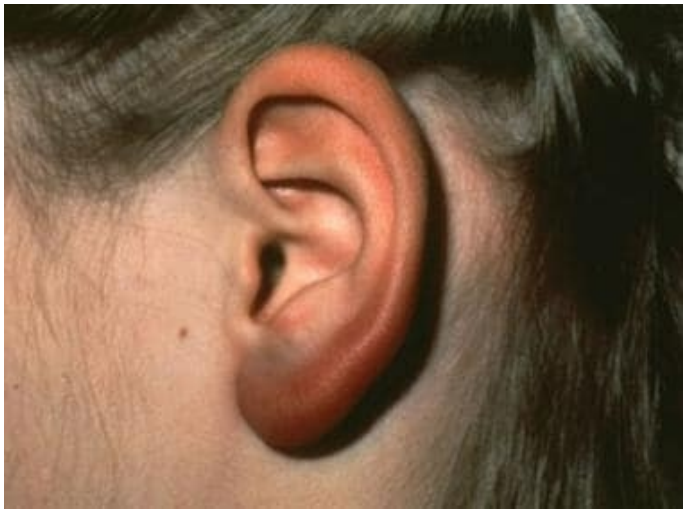
Neurologic manifestations

Neurologic involvement, also known as Lyme neuroborreliosis, is reported in 5-20% of cases. In the United States, cranial neuropathy is the most common manifestation of early neurologic Lyme disease. Other manifestations include meningitis and encephalopathy. Cranial neuropathies, especially facial nerve palsy (Bell palsy), develop in approximately 3% of patients with Lyme disease. In endemic areas, Lyme disease is the most commonly identified cause of acquired facial palsy, especially in children. Headache, absence of previous herpetic lesions, and meningeal symptoms are noted in most pediatric Lyme disease patients with facial palsy. When meningitis is involved, symptoms usually occur 2-10 weeks following infection. Headache, neck pain or stiffness, and photophobia typically indicate meningeal irritation. The headache of Lyme disease usually is described as waxing and waning, and the severity varies from mild to severe, even in patients with frank meningitis. Persistent headache alone is a rare presentation of Lyme disease but should be considered in patients in endemic areas during summertime. *Borrelia* encephalopathy most commonly manifests as a mild confusional state accompanied by disturbances in memory, concentration, mood, sleep, personality, and/or language occurring months to years after the infection. Depression and irritability are also common.

Cutaneous manifestations

In patients with cutaneous involvement, multiple erythema migrans lesions are present. These are relatively small erythematous macules (1-5 cm) and are often oval. Unlike primary single erythema migrans rashes, these lesions can be evanescent and do not show the typical

expansion over days. Borrelial lymphocytoma is an uncommon manifestation of early disseminated Lyme disease (though it may also occur very early in the disease course) that has been reported only from Europe. It is a bluish-red nodular swelling that typically occurs on the lobe of the ear in children (see the following image) or the areola of the nipple in adults. Occasionally, borrelial lymphocytoma lesions occur on the scrotum, nose, and extremities. Nipple lesions tend to be painful, possibly because of rubbing against clothing.



Lyme disease. Borrelial lymphocytoma of the earlobe, which shows a bluish red discoloration. The location is typical in children, as opposed to the nipple in adults. This manifestation of Lyme disease is uncommon and occurs only in Europe

Stage 3 Lyme disease

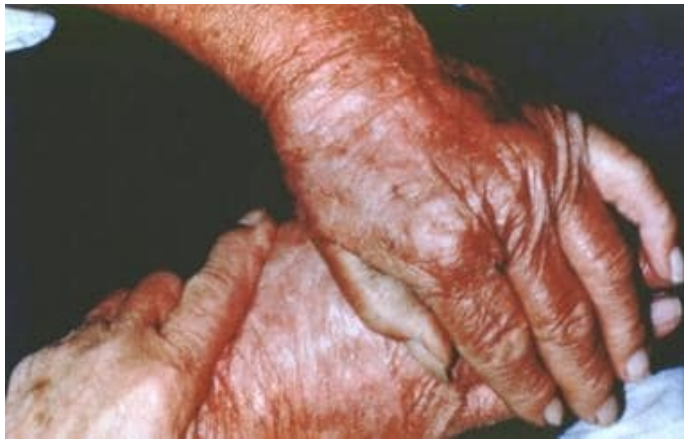
Late or chronic Lyme disease refers to manifestations that occur months to years after the initial infection, sometimes after a period of latency. Signs and symptoms of chronic Lyme disease are primarily rheumatologic and neurologic. Acrodermatitis chronica atrophicans, the cutaneous feature of late-stage Lyme disease, is found almost exclusively in European patients. Most patients presenting with late disease do not have a history of erythema migrans, because the rash typically leads to earlier treatment, which prevents the development of late disease. However, other manifestations of the disease may coexist or may have occurred in the past. Thus, a history of Bell palsy, aseptic meningitis, arthritis, acral paresthesia or dysesthesia (from peripheral neuropathy), or cognitive dysfunction (from CNS involvement) may be diagnostically useful.

Maladaptive host responses can lead to a variety of syndromes in stage 3 as follows:

- Postinfectious Lyme arthritis - Massive inflammatory synovial proliferation, usually in a knee
- Posttreatment Lyme disease syndrome - Pain, neurocognitive impairment, fatigue
- Autoimmune joint disease - Rheumatoid arthritis, psoriatic arthritis, or peripheral spondyloarthropathy
- Autoimmune neurologic disease - Chronic idiopathic demyelinating polyneuropathy

Lyme arthritis is the hallmark of stage 3 Lyme disease. It tends to involve large joints (the knee is involved in 90% of cases). Arthritis must be differentiated from arthralgia, which is common in early diseases. The neurologic abnormalities of stage 3 Lyme disease involve both the central and peripheral nervous systems. Typical presentations include subacute encephalopathy, chronic progressive encephalomyelitis, and late axonal neuropathies, as well as symptoms consistent with fibromyalgia. Radicular pain can occur and present as acute disk disease. More common in European patients, radicular pain may be associated with lymphocytic pleocytosis (Bannwarth syndrome). *Borrelia* encephalomyelitis is a rare but severe syndrome. Symptoms can progress gradually or in a relapsing-remitting pattern, with partial improvement after the attacks. The most common clinical manifestations of *Borrelia* encephalomyelitis are hemiparesis, ataxia, seizures, cognitive impairment, bladder dysfunction, and hearing loss. Myelitis is present in 50% of patients with late neuroborreliosis. Progressive spastic paraparesis or quadriparesis is common. Acrodermatitis chronica atrophicans most commonly affects older women. Unlike erythema migrans, acrodermatitis chronica atrophicans tends to occur acrally, especially on the dorsal surfaces of the hands, feet, knees, and elbows. Early on, a minimally symptomatic erythema tends to occur in these locations. Initially, there is discoloration and inflammation; later, severe atrophy is noted.

See the image below.



Acrodermatitis chronica atrophicans is found almost exclusively in European patients and comprises an early inflammatory phase and a later atrophic phase. As the term suggests, the lesion occurs acrally and ultimately results in skin described as being like cigarette paper.

Physical Examination

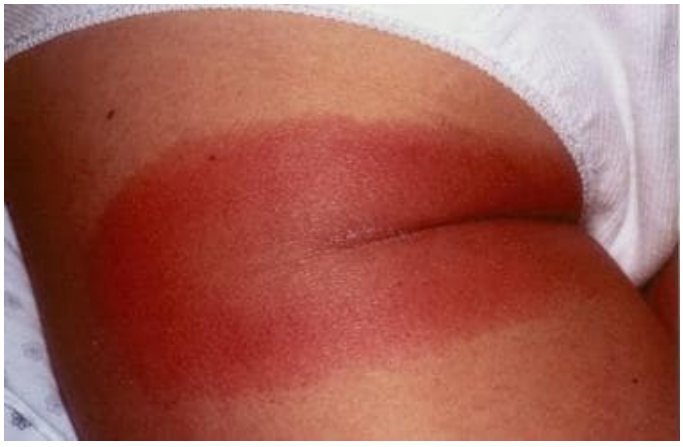
In many patients with early Lyme disease, identification of erythema migrans on physical examination alone is sufficient to establish a working diagnosis of Lyme disease. Careful attention to the details often makes the difference between the need to proceed with further confirmatory tests and an empiric course of antibiotics. In particular, the examination findings must be interpreted in the epidemiologic context; this cannot be overemphasized. The location, time of year, and patient's activities can be important diagnostic clues. Regional lymphadenopathy may be seen, and a low-grade fever is not uncommon. High fever suggests another coinfecting tick-borne organism such as *Ehrlichia* or *Babesia* species or some other diagnosis altogether, such as streptococcal cellulitis.

Erythema migrans

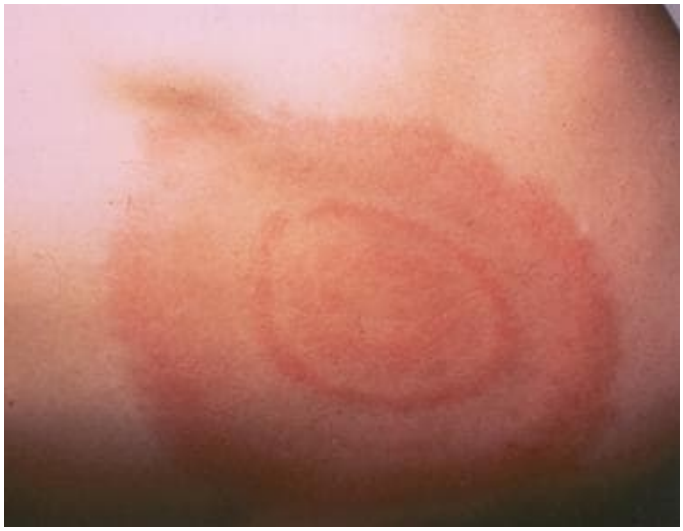
Erythema migrans is the characteristic rash of Lyme disease. Classic erythema migrans is a flat to slightly raised erythematous lesion that appears at the site of the tick bite after 1-33 days (average, 7-10 days). Without therapy, erythema migrans typically fades within 3-4 weeks. The erythema migrans lesion usually is round or oval, but can be triangular or

linear. Often, a central punctum is evident at the bite site. Erythema migrans enlarges by a few centimeters per day; single lesions typically achieve a diameter of approximately 16 cm (approximately 5-6 inches), but lesions as large as 70 cm have been reported. The case definition for Lyme disease used by the CDC specifies that erythema migrans be greater than 5 cm in size. This size cutoff is only meant to be used for epidemiologic purposes; erythema migrans smaller than 5 cm has occasionally been documented in culture-proven cases. The entire lesion may be uniform in color or have central darkening. Central clearing is more common in European patients than in North American patients. More proximal to the clearing may be additional erythema leading to a so-called bull's eye or target appearance; however, this phenomenon, emphasized in the earlier literature, occurs only in a minority of patients (37% in one North American study of culture-proven erythema migrans).

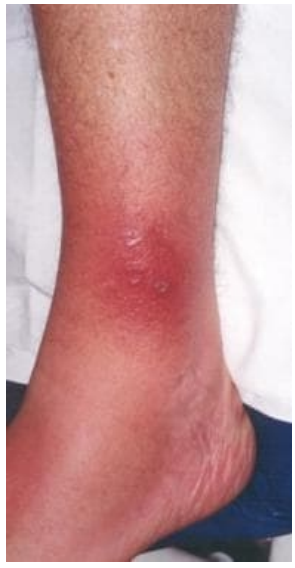
See the images below.



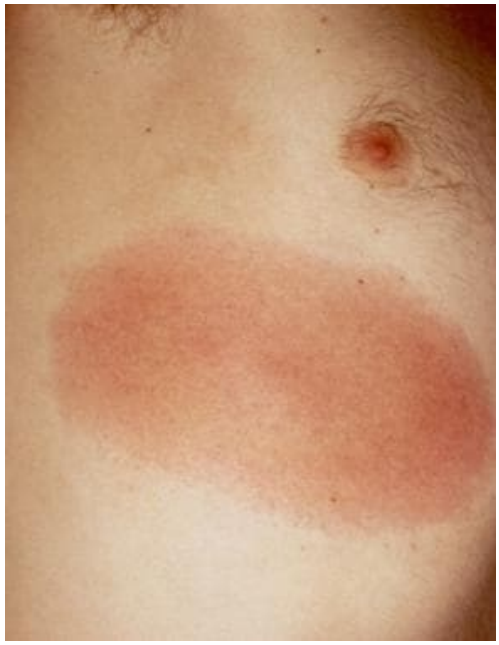
Lyme disease. This patient's erythema migrans rash demonstrates several key features of the rash, including size, location, and presence of a central punctum, which can be seen right at the lateral margin of the inferior gluteal fold. Note that the color is uniform; this pattern probably is more common than the classic pattern of central clearing. On history, this patient was found to live in an endemic area for ticks and to pull ticks off her dog daily.



Lyme disease. Classic target lesion with concentric rings of erythema, which often show central clearing. Although this morphology was emphasized in earlier North American literature, it only represents approximately 40% of erythema migrans lesions in the United States. This pattern is more common in Europe.

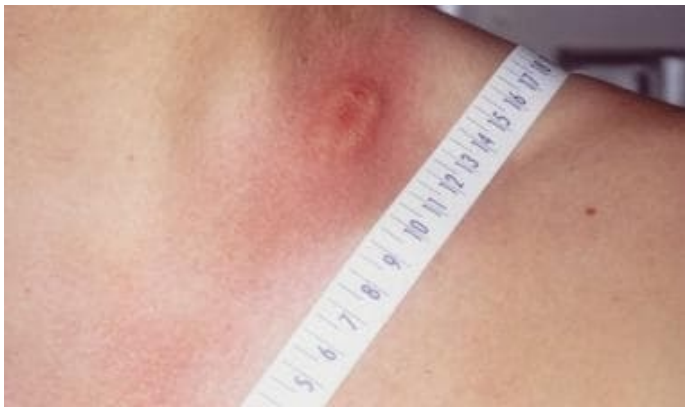


Lyme disease. The rash on the ankle seen in this photo is consistent with both cellulitis (deep red hue, acral location, mild tenderness) and erythema migrans (presentation in July, in an area highly endemic for Lyme disease). In this situation, treatment with a drug that covers both diseases (eg, cefuroxime or amoxicillin-clavulanate) is an effective strategy.



Lyme disease. The thorax and torso are typical locations for erythema migrans. The lesion is slightly darker in the center, a common variation. In addition, this patient worked outdoors in a highly endemic area. Physical examination also revealed a right axillary lymph node.

Atypical manifestations of erythema migrans include vesicular (see the image below) and centrally necrotic lesions. Scaling is unusual, but may be seen, especially in the center of the lesion.



Lyme disease. Photo of the left side of the neck of a patient who had pulled a tick from this region 7 days previously. Note the raised vesicular center, which is a variant of erythema migrans. The patient had a Jarisch-Herxheimer reaction approximately 18 hours after the first dose of doxycycline.

Rash location is another important diagnostic clue. Unlike spider and other arthropod bites, erythema migrans rarely is found on the hands or feet. Rather, ticks tend to bite where natural barriers impede their forward motion (eg, popliteal fossa, axillary or gluteal folds, hairline, areas near elastic bands in bra straps or underwear). In children, the scalp, face, and hairline are especially common locations. Approximately 20% of patients with erythema migrans have secondary lesions (see the image below). These lesions generally are smaller than the primary one, lack the central punctum, and are not necrotic or vesicular. They also tend to be more uniform in morphology than the primary lesion. Because secondary lesions result from hematogenous spread, their locations are not as restricted as those of the primary lesion. Note that rashes very similar to erythema migrans, but from which *Borrelia burgdorferi* cannot be cultured, have been reported in the southern United States. This disease is called southern tick-associated rash illness (STARI), or Master disease. As a group, distinctions can be made between classic erythema migrans and this illness, but significant overlaps exist such that the differences are not useful in diagnosing individual patients. An erythematous skin lesion present while an *Ixodes* tick is still attached most likely represents a hypersensitivity reaction rather than erythema migrans. Hypersensitivity reactions also tend to produce lesions that are smaller and more transient than erythema migrans; typically, the lesions are less than 5 cm in size and begin to resolve within 2 days.

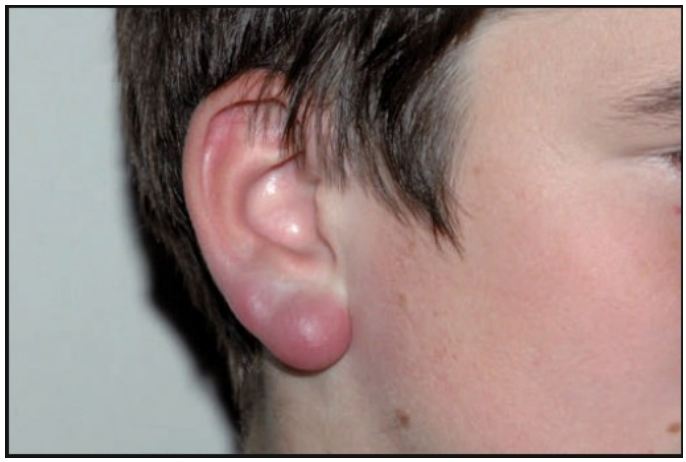
Borrelial lymphocytoma

Less than 1% of patients with stage 2 Lyme disease, almost all of them European, develop *Borrelia* lymphocytoma, described as a small, bluish-red nodule or plaque. The earlobe and scrotum are the typical location in children (see the image below), whereas the nipple is the more common location in adults.

Borrelial lymphocytoma tends to occur in areas of previous (or concurrent) erythema migrans and may be up to a few centimeters in size. Regional lymphadenopathy may be present.

Other terms used to describe borrelial lymphocytoma include the following:

- Lymphadenosis benigna cutis
- Lymphocytoma cutis
- Cutaneous lymphoid hyperplasia
- Spiegler-Fendt lymphoid hyperplasia



Other dermatologic manifestations

Other skin lesions have been associated with *B burgdorferi* infection, but whether they are part of the syndrome of Lyme disease is controversial. The lesion for which the most evidence of causality has been reported is morphea (localized scleroderma), which develops in roughly 10% of European patients with borrelial lymphocytoma and acrodermatitis chronica atrophicans.

Other European reports less commonly link the following with *B burgdorferi* infection:

- Progressive facial hemiatrophy (Parry-Romberg syndrome)
- Eosinophilic fasciitis

Musculoskeletal findings

Muscle tenderness can result from myositis. Tenderness of tendons and periarticular structures may be present. Frank arthritis can occur after weeks, months, or years and may lead to erythema, edema, synovial effusion, and tenderness of the affected joints. Usually, this is a monoarthritis or oligoarthritis involving large joints, especially the knee. Swelling often is disproportional to the tenderness.

Lyme arthritis

Approximately 60% of all untreated patients develop symptoms of intermittent migratory monoarthritis. Episodes last a mean of 3 months and very often affect the knee or temporomandibular joints, although not universally. Migratory oligoarthritis involving the small or large joints can also occur. Joint symptoms develop in approximately 80% of all untreated patients within 2 years of infection. The severity of joint involvement can range from intermittent episodes of subjective pain to frank arthritis to chronic erosive synovitis. During the attacks, the joints are swollen, hot, and painful, but they are not usually red or as severe as in a septic joint. Effusions may be large and generally recur following aspiration, as is often seen in spondyloarthropathies. Fewer than 10% of patients with arthritic sequelae develop pannus or erosion of cartilage and bone.

Neurologic involvement

Approximately 5-10% of untreated patients with Lyme disease have signs of cranial neuropathies, and up to 60% of patients with early neuroborreliosis develop cranial neuritis. Seventh nerve palsy is by far the most common. Bilateral facial palsy can be seen in 35% of patients and is a unique characteristic that is useful for distinguishing it from idiopathic Bell palsy and other disorders. Typical associated findings depend on the nerve affected and can include visual or auditory disturbances, facial paresthesia, and/or vertigo. Other neurologic manifestations include diffuse or focal mononeuropathy multiplex

(multifocal involvement of anatomically unrelated nerves), plexopathy, and/or radiculoneuropathy (more common in Europe). Less common presentations include myositis, pseudotumor cerebri, and cerebellitis. Lyme meningitis is relatively common, occurring in as many as 15% of untreated patients bitten by the *Ixodes* tick and in 30% of Lyme disease cases, and does not manifest as the usual signs of bacterial meningitis (eg, boardlike rigidity, Kernig and Brudzinski signs). Meningitis may be accompanied by cranial or peripheral radiculoneuropathy. Neck stiffness can occur early, with or without frank meningitis. Acute radiculoneuritis is reported in 50-85% of cases. Acute onset of motor deficits, severe radicular pain, and sensory loss are commonly seen after 2-4 weeks of infection. Multifocal asymmetric weakness is a common presentation. Although the presentation of inflammatory radiculoneuropathy is often indistinguishable from that of spinal-root compression, involvement of multiple dermatomes in the thorax and a lack of a precipitating injury can aid in diagnosis. Chronic radicular paresthesias are usually not associated with motor or sensory deficits. The physical examination results are normal. With peripheral neuropathy, patients usually report intermittent paresthesias. The most frequent finding on examination is decreased vibratory sensation of the distal lower extremities. A stocking-glove distribution of epicritic sensory deficits is also a common finding. Sensory findings are more pronounced than motor findings. With late axonal neuropathy, patients can report intermittent distal limb paresthesias months to years after infection. It is distinct from the neuropathy of early Lyme disease, because the symptoms are less severe. Acrodermatitis chronica atrophicans–associated neuropathy is common in Europe and manifests as neuropathic pain, paresthesias, and muscle cramps. In Europe, common manifestations of Garin-Boujadoux-Bannwarth syndrome (Bannwarth syndrome) include neuritic pain, cranial neuritis without headache, and lymphocytic pleocytosis. Bannwarth syndrome has also been called tick-borne meningopolyneuritis, lymphocytic meningoradiculitis, and chronic lymphocytic meningitis. While this manifestation is typically associated with infection with *B garinii*, a cluster was reported in Minnesota.

Neuropsychiatric findings in late-stage disease may include the following:

- Depression
- Anxiety
- Schizophrenialike psychosis
- Bipolar disorder
- Dementia

Cardiac involvement

Cardiac involvement ranges from atrial or ventricular arrhythmias to transient heart block or myopericarditis. Approximately 8% of untreated patients have acute-onset atrioventricular (AV) conduction abnormalities. Most cardiac episodes are isolated and transient, lasting less than a week. In rare instances, patients with heart block require electrical pacing. In patients with complete heart block, cannon A waves may be observed in the neck. A slow or irregular pulse may be palpated. A cardiac rub, S_3 and/or S_4, may be auscultated in patients with myocarditis or pericarditis. Signs of tamponade very rarely can occur. In patients with chronic cardiac involvement with heart failure, typical signs of chronic heart failure may be present.

Ophthalmic involvement

Ophthalmic manifestations vary by disease stage. In stage 1 Lyme disease, the ocular manifestations are conjunctivitis and photophobia. These are mild and transient, and ophthalmologists usually need not be consulted. Significant ophthalmic complications may appear during stage 2 Lyme disease. Blurred vision can be noted during stage 2, secondary to papilledema, optic atrophy, optic or retrobulbar neuritis, or pseudotumor cerebri. Optic nerve disease may be unilateral or bilateral, and solitary or associated with other neurologic or neuro-ophthalmologic manifestations. Some evidence exists that children are more predisposed to optic nerve disease than adults.

In late stage 2 or stage 3 Lyme disease, most of the severe ocular manifestations of the disease occur. These include the following:

- Episcleritis
- Keratitis
- Posterior or intermediate uveitis
- Vitritis
- Exudative retinal detachment
- Retinal pigment epithelial detachment
- Cystoid macular edema
- Retinal vasculitis
- Symblepharon
- Iritis
- Pars planitis
- Chorioretinitis
- Cranial nerve palsies
- Orbital myositis
- Branch artery occlusion

Of this group, keratitis, vitritis, and pars planitis are the most common. The keratitis usually is a bilateral, patchy, nummular stromal keratitis. Posterior segment inflammatory disease generally presents as a bilateral pars planitis associated with granulomatous iritis and vitritis. Many of these patients also have granulomatous keratic precipitates and posterior synechiae.

Diagnostic Considerations

In most patients with erythema migrans, a carefully elicited history (including definitions of epidemiologic context) and a physical examination are all that is required to establish the diagnosis of Lyme disease. However, although many patients with Lyme disease present with erythema migrans, others first present with extracutaneous symptoms. In those cases, erythema migrans may never have occurred, may not have been recognized by the patient, or may not have been correctly diagnosed by the physician. Lyme disease tends to be overdiagnosed, especially in patients with a lifestyle that puts them in a high-risk category. Performing tests when the prior probability of disease is low increases the likelihood of false-positive results. The best way to avoid problems with diagnosis is to follow CDC guidance, to use a reputable laboratory with experience in testing for Lyme disease, and to obtain the assistance of an infectious disease expert when any questions arise. Because interpretation of testing is related to the stage of disease and requires a two-stage test, laboratory results are often misinterpreted. Clinicians unfamiliar with Lyme disease or Lyme testing may falsely exclude the diagnosis by testing too early in the disease course, or falsely diagnose disease by following up negative enzyme immunoassay (EIA) results with Western blot testing (the latter is indicated only in patients with a positive or indeterminate EIA result). In addition, separating false-positive antibody tests from asymptomatic infection is impossible. Approximately 5-10% of patients in endemic areas have positive antibody results without a history of symptoms. Unfortunately, antibodies induced by the infection are not protective against further exposures to *Borrelia burgdorferi*; therefore, reinfection easily could be confused with a recurrence. Because antibodies may persist for years following an infection, repeated infection is a difficult diagnosis without specific signs of Lyme disease (eg, erythema migrans). Increasing titers after adequate treatment certainly raise suspicion of an active infection, but this is not a reason to repeat posttreatment titers, as they may remain positive for many years.

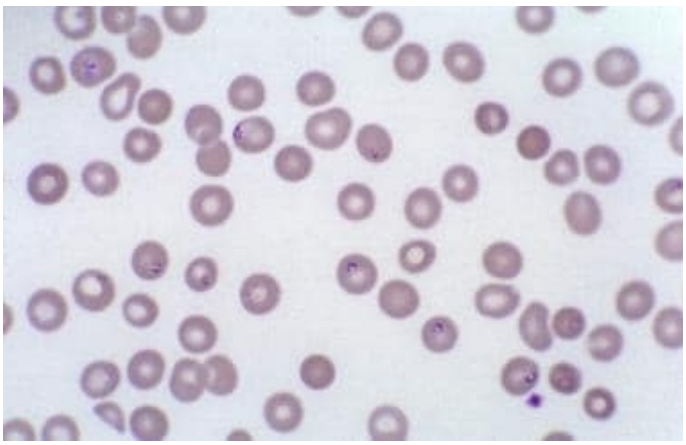
Other conditions to be considered include the following:

- Ankylosing spondylitis and rheumatoid arthritis
- Atrioventricular (AV) nodal block
- Cellulitis

- Confusional states and acute memory disorders
- Contact dermatitis
- Gout and pseudogout
- Granuloma annulare
- Prion-related diseases

Coinfection

The pathogens responsible for babesiosis and ehrlichiosis share the same tick vector as *B burgdorferi*, making coinfection possible. Severe and even fatal acute infection caused by these pathogens is more common in asplenic individuals (babesiosis) or older adults (ehrlichiosis). Unlike *B burgdorferi,* however, these pathogens do not cause chronic infection. To add to the confusion, ehrlichial infection may cause a false-positive result for Lyme disease on immunoglobulin M (IgM) Western blot analysis. In a prospective study from New York State, 52 adults with erythema migrans who had not received treatment for Lyme disease underwent testing for *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, and the deer tick virus subtype of Powassan virus. Nearly 90% of the patients showed no evidence of coinfection. Polymerase chain reaction (PCR) tests for *B microti* DNA were positive in three patients, one of whom also had a positive blood smear; these patients had clinical signs suggestive of babesiosis. An additional three patients had elevated convalescent-phase IgG titers for *B microti.* The possibility of coinfection with another tick-borne pathogen should be considered if the patient's condition does not respond to treatment as expected with ordinary early Lyme disease. Evidence suggests that coinfected patients have more symptoms of longer duration compared with other patients. In addition, these patients may be sicker than others on first observation. Results of some laboratory studies may suggest some of the other coinfecting tick-borne pathogens such as ehrlichial or babesial species. Most patients with ehrlichiosis have elevated levels of hepatic transaminases, leukopenia, and/or thrombocytopenia. In addition, some patients have morulae (intracytoplasmic inclusions) in white blood cells, as demonstrated on peripheral blood smears. Patients with babesiosis often are anemic (hemolytic type) and may have thrombocytopenia. Blood smears reveal the malarialike intraerythrocytic parasite in this disease as well (see the image below).



Blood smear showing likely babesiosis. Babesiosis can be difficult to distinguish from malaria on a blood smear.

Differential Diagnoses

- Bell Palsy
- Chronic Fatigue Syndrome (Myalgic Encephalomyelitis)
- Fibromyalgia
- Insect Bites
- Juvenile Idiopathic Arthritis
- Myocarditis
- Pediatric Contact Dermatitis
- Systemic Lupus Erythematosus (SLE)
- Third-Degree Atrioventricular Block (Complete Heart Block)
- Tick-Borne Diseases

Lyme Disease Workup

Approach Considerations

In endemic areas, patients with probable erythema migrans and a recent source of tick exposure should be started on treatment without blood tests. At this early stage (the first several weeks of illness), the clinical probability of Lyme disease is high and the sensitivity of serologic tests is low. If the lesion is indeed erythema migrans, improvement should occur within a few days after initiation of empiric antibiotics, along with resolution of any constitutional symptoms. Alternatively, observing the rash over several days is safe. In most patients with erythema migrans, some expansion of the rash is expected over 2-3 days without antibiotics. This is a reasonable alternative to immediate empiric therapy. In contrast, laboratory tests are important for establishing the diagnosis in the many patients with suspected Lyme disease who do not recall a tick bite and did not notice or do not have erythema migrans. However, much confusion can occur in the interpretation of the tests used for Lyme disease. The most widely used tests for Lyme disease are antibody detection tests, which can demonstrate that a patient has been exposed to *Borrelia burgdorferi* but cannot confirm infection. In the presence of typical clinical manifestations and laboratory results suggestive of current disease activity (eg, elevated synovial and spinal fluid cell counts), they support the clinical diagnosis. The US Centers for Disease Control and Prevention (CDC) recommends a two-step testing procedure. The first step typically consists of a sensitive enzyme immunoassay (EIA) or, less frequently, an immunofluorescence assay (IFA); if results are positive or equivocal, a Western immunoblot test is performed to confirm the results. In modified two-tier testing (MTTT), a second EIA can be used to confirm the results of the initial EIA. The US Food and Drug Administration (FDA) approved the use of concurrent or sequential EIA testing for diagnosis of Lyme disease. Clinical data show that MTTT is as accurate as testing with EIA or IFA plus Western blot. A joint guideline from the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR) suggests performing acute-phase serum antibody testing in patients with one or more skin lesions that are suggestive of erythema migrans but are atypical for it. If the initial result is negative, repeated testing can be performed on a convalescent-phase serum sample collected at least 2-3 weeks afterward. Routine use of sequential serologic testing in individual patients with early Lyme disease should be discouraged. In addition, acute and convalescent-phase serologic testing has no role in Lyme disease. Because titers may remain elevated for extended periods (as can the positivity of Western blots), convalescent testing is not helpful. Culturing *B burgdorferi* is impractical. Obtaining adequate samples requires an invasive procedure, such as biopsy or lumbar puncture, and the organism is difficult to grow. Even if the tick that bit the patient is available, testing the tick for *B burgdorferi* is not recommended. The presence or absence of *B burgdorferi* in an *Ixodes* tick does not reliably predict the likelihood of Lyme disease. Biopsy of dermatologic lesions suggestive of borrelial lymphocytoma or acrodermatitis chronica atrophicans in patients without a clear history of

other symptoms suggestive of Lyme disease may be helpful. Biopsy of other skin lesions should be restricted to research settings. Most, but not all, patients with borrelial lymphocytoma are seropositive for antiborrelial antibodies. This is true for all early disseminated manifestations of Lyme disease. In addition, essentially all patients with acrodermatitis chronica atrophicans are seropositive for antiborrelial antibodies. The diagnosis should be questioned in seronegative patients. In patients with clinical findings typical of Lyme disease, a complete blood cell (CBC) count, erythrocyte sedimentation rate (ESR), and liver function tests generally are unnecessary. However, leukopenia or thrombocytopenia suggests coinfection with *Ehrlichia* or *Babesia* species. In patients with Lyme disease, the white blood cell (WBC) count can be normal or elevated. The ESR is usually elevated. The serum aspartate transaminase (AST) level may be elevated. Elevation of at least one liver enzyme level is reported to occur in 40% of patients with Lyme disease. This finding also is common in ehrlichiosis. On complement testing in patients with Lyme disease, C3 and C4 levels are generally normal or slightly elevated. Antinuclear antibody and rheumatoid factor test results are negative. On urinalysis, microscopic hematuria and mild proteinuria have been described. Urine antigen testing has not been studied sufficiently. Because it has not been proven reliable or accurate, it should not be used as a diagnostic tool. Joint aspiration for diagnostic reasons is unnecessary if only Lyme arthritis is suspected. However, arthrocentesis may be appropriate to exclude other causes of effusions, such as septic arthritis or, in adults, gout and pseudogout. In Lyme arthritis, joint fluid may have 25,000-125,000 WBCs/µL, often with a polymorphonuclear predominance. A retrospective study of children in areas where Lyme disease is endemic who presented with knee monoarthritis found that the presence of a peripheral blood absolute neutrophil count of 10 × 10 cells/mm or higher and an ESR of 40 mm/hour or higher predicted septic arthritis; no child with values below those cutoffs had septic arthritis. These researchers suggested that those criteria could be used to identify children with knee monoarthritis who are at low risk for septic arthritis and might not require diagnostic arthrocentesis. An IDSA/AAN/ACR joint guideline recommends testing for Lyme disease in patients with plausible exposure to high-risk ticks who present with meningitis, painful radiculoneuritis, mononeuropathy multiplex, acute cranial neuropathies, or evidence of spinal cord (or rarely brain) inflammation The guideline recommends against routine Lyme disease testing for patients with other neurologic syndromes or psychiatric illnesses. In patients with Lyme disease meningitis, cerebrospinal fluid (CSF) analysis often reveals a mild pleocytosis (< 1000 cells/µL) with lymphocyte predominance. CSF antibody is considered positive when the titer is higher than in serum. In children, the "Rule of 7's" can be used to identify those patients who are unlikely to have Lyme meningitis and can be managed in an outpatient setting while awaiting Lyme serology test results. The Rule of 7's classifies children as being at low risk when they meet the following three criteria:

- < 7 days of headache
- < 70% CSF mononuclear cells
- Absence of seventh or other cranial nerve palsy

Electrocardiograms (ECGs) show fluctuating levels of atrioventricular block in patients with syncopal or near-syncopal symptoms secondary to Lyme carditis. In patients with possible exposure but without symptoms of myocardial ischemia, such changes should prompt further investigation for Lyme disease. Imaging studies are almost never indicated in patients with Lyme disease who present with early syndromes. Patients with some clinical syndromes may require imaging studies to exclude other disorders, depending on the specifics of the case. For example, a patient with fever and severe back pain, with signs of radiculopathy, might require spine imaging. Newer approaches that are being investigated to improve the diagnosis of Lyme disease include a cytokine-based immunoassay, rapid point-of-care testing using lateral flow technologies, and the use of metabolic biomarkers and biosignatures. For example, a single-tier test that pairs a multiplexed antigen assay with a machine learning algorithm has shown over 90% accuracy in identifying Lyme disease in human serum samples.

Serologic Testing

Serologic testing for Lyme disease is complex. Rational ordering and interpretation of these test results requires some understanding of the basic underlying principles and performance characteristics of the tests. The test results do not rule in or rule out Lyme disease; however, the results make a clinical diagnosis of Lyme disease more (or less) likely. The most frequently used test is an an EIA or enzyme-linked immunosorbent assay (ELISA). Much less often used for this purpose is an IFA. The principal limitation of these serologic tests has been the high frequency of both false-negative results and false-positive results. False-negative results occur during the acute phase of Lyme disease, when patients have not yet developed a sufficient antibody response to give a positive serologic test. Seroconversion can take as long as 6-8 weeks after a tick bite. The false-negative rate for ELISA is 32% in early disease. A variety of diseases, including Rocky Mountain spotted fever, syphilis, systemic lupus erythematosus, and rheumatoid arthritis, can cause false-positive ELISA results. Also, a small percentage of the healthy population has positive test results with ELISA testing. For these reasons, confirmatory Western blot testing is recommended. Patients with early Lyme disease who are treated with antibiotics may never develop positive titers. Of patients with early disseminated disease, 90% have a positive titer. Some patients with late disease are seronegative, but significant controversy exists regarding the frequency of late seronegativity. Most authorities suggest that this phenomenon is rare.

Two-tier testing

The CDC recommends a two-step testing procedure. The first step typically consists of an EIA or ELISA. The test for the first step may measure either a total Lyme titer or separate immunoglobulin G (IgG) and immunoglobulin M (IgM) titers. If the results of the initial test are positive or equivocal, the second step is to confirm the results with a Western blot. In MTTT, both assays used in the first and second steps are EIAs. If signs and symptoms have been present for 30 days or less, both IgM and IgG Western blot testing are performed; if signs and symptoms have been present for more than 30 days, only IgG Western blot testing is performed.

For IgM blots, the test is considered positive if any two of the following three bands are present, as they are the most commonly found in early disease:

- 23 kd
- 39 kd
- 41 kd

For IgG blots, any five of the following bands are considered a positive test result:

- 18 kd
- 21 kd
- 28 kd
- 30 kd
- 39 kd
- 41 kd
- 45 kd
- 58 kd

- 66 kd
- 93 kd

A positive IgM titer is reliable only when measured 30 days or less from symptom onset. In patients with a high probability of having early Lyme disease, IgM testing is 96% specific and 93% predictive. In the absence of treatment, IgM titers usually peak 6-8 weeks after infection and disappear within 4-6 months, although levels sometimes remain elevated for several months or years. IgG antibodies are typically detectable within 6-8 weeks after infection, peak within 4-6 months, and remain elevated indefinitely. In late-stage disease (>4-6 weeks after infection), IgG results are more useful than IgM results. Careful consideration of both IgG and IgM antibodies is essential because the IgG response may be negative in as many as 50% of patients (particularly those with early disease), whereas a persistence of IgM antibodies can lead to false-positive findings in patients infected for more than 1 month who subsequently receive effective treatment. Of note, serologic results can remain positive years after adequate treatment and cannot be used to distinguish active from inactive disease. Similarly, positive IgM titers with negative IgG more than 6-8 weeks after exposure in an untreated patient is thought to represent a false-positive test. Two-step testing is not indicated for patients with erythema migrans, because the rash may develop before the antibodies. Nor is it recommended for patients who have not been in endemic areas, because of the high false-positive rates in that setting. In addition, inadequate antibiotic therapy for early Lyme disease may fail to control the infection yet still suppress the antibody response, potentially yielding a false-negative result. Western blot testing should be performed only in conjunction with antibody titer testing, and only as follow-up of a recent positive or equivocal ELISA titer. Ordering a "Lyme titer with reflex testing" ensures that two-step testing is performed properly. In the United States, patients with extracutaneous involvement in the absence of treatment almost universally have positive titers. In Europe, negative serum titers have been reported in patients with neurologic Lyme disease that was confirmed by intrathecal antibody production. The results of one study noted that differing sensitivity and specificity were found between various assays used to detect anti-*Borrelia* antibodies in patients suspected of having Lyme disease. False-positive results occurred in 7% of healthy controls in two of the eight ELISA assays tested. This variability makes it very difficult to compare results from different laboratories, both among different patients and in individual patients.

C6 peptide testing

A serologic test that measures IgG to a peptide from the sixth invariant region (C6) of the variable major proteinlike sequence-expressed (VlsE) lipoprotein of *B burgdorferi* may be more sensitive in patients with erythema migrans. However, because the recommendation in patients with erythema migrans is to treat without obtaining laboratory tests, there is no clear reason to perform this assay in clinical practice.The C6 peptide test may be effective in differentiating southern tick-associated rash illness (STARI) from Lyme disease, as well as confirming infection in patients who may have been infected in Europe.

Polymerase Chain Reaction Testing

Polymerase chain reaction (PCR) testing is growing in uses and availability but is not readily available in routine practice. PCR remains a research technique in part because laboratories performing PCR tests must be meticulous in technique to minimize the likelihood of false-positive results. In addition, no large clinical series have been reported that assess the performance of Lyme disease PCR in the nonresearch setting. PCR can be used to detect *B burgdorferi* DNA in the blood, CSF, urine, or synovial fluid within weeks of infection. The result is positive in approximately 30% of patients with active Lyme disease. A notable disadvantage of PCR testing is the likelihood of false-negative results because of a sparsity of spirochetes in infected tissues. Likewise, inexperience with the PCR technique can yield false-positive findings when care is not taken to prevent contamination and when incorrect primers are used in preparing the specimen. Although most PCR results become negative within 2 weeks of antimicrobial therapy, results can remain positive for years after apparent cure. One of the most compelling uses of PCR may be in confirming persistent or recurrent disease, because a positive result is highly specific for exposure to *B burgdorferi.* With the exception of synovial fluid, PCR testing is not recommended because of unacceptable low sensitivity, especially from the CSF (though it does have high specificity if the result is positive). CSF titers to *B burgdorferi* should not be used for diagnosis of Lyme meningitis but may have value in patients who have recurrent infection or for following serial markers in patients with persistent symptoms. CSF titers should be performed and interpreted at a reference laboratory.

Synovial Fluid Analysis

In patients with Lyme arthritis, synovial fluid is usually inflammatory, with cell counts ranging from 500-98,000/µL reported. In adult patients, the fluid should also be examined for crystals to rule out gout and pseudogout. One study that included 63 patients with Lyme arthritis found that although the majority had positive PCR results for *B burgdorferi* DNA in synovial fluid, none of the samples tested were positive for *B burgdorferi* messenger RNA (a marker of spirochetal viability), even when the specimen was obtained before initiation of antibiotic treatment. These results suggest that detection of *B burgdorferi* DNA in synovial fluid is not a reliable test for active joint infection in Lyme disease.

Cerebrospinal Fluid Evaluation

A lumbar puncture should be performed if Lyme meningitis is in the differential diagnosis. Whether all patients with cranioneuropathy require lumbar puncture before treatment is controversial. Occasionally Lyme disease presents as pseudotumor cerebri, and in such cases an opening pressure is essential for diagnosis. In most patients with isolated Bell palsy and no associated signs of aseptic meningitis, most physicians do not perform a lumbar puncture. For most other patients with cranioneuropathies and suspected Lyme disease, a lumbar puncture should be performed, particularly in patients who live in an endemic area and present during peak Lyme disease season. Computed tomography (CT) or magnetic resonance imaging (MRI) should be performed before the lumbar puncture if increased intracranial pressure or mass lesion is suspected. Unlike most bacterial infections in the spinal fluid, Lyme disease produces a pleocytosis characterized by mononuclear cells. In addition, spinal fluid levels of IgM and IgG antibodies to *B burgdorferi* should be measured, and an index of cerebrospinal fluid (CSF) to serum antibody (immunoglobulin-to-albumin ratio) should be calculated. This is particularly true in patients who have no other signs of Lyme disease. Although CSF cultures are positive in fewer than 10% of Lyme disease patients with apparent meningitis, intrathecal antibodies and a lymphocytic pleocytosis (approximately 100 cells/µL) are present in more than 80%. Patients with meningitis typically have elevated protein concentrations (>50 mg/dL) but normal glucose levels (45-80 mg/dL). Oligoclonal bands specific for *B burgdorferi* may be present. Ongoing controversy surrounds the diagnosis of neurologic Lyme disease. A positive Lyme disease serology in CSF does not mean that the patient has neuroborreliosis. It could represent evidence of a previous infection or simply reflect potential leakage of serum antibodies across the blood-

brain barrier. IgG and IgM antibodies may persist in CSF long after adequate treatment and in the absence of evidence of active neurologic disease. Intrathecal anti-*Borrelia* antibody production is typically seen within 3-6 weeks of infection. Anti-*Borrelia* antibody CSF-to-serum index has been reported to show a 97% specificity and 75% sensitivity for the diagnosis of neuroborreliosis. A CSF-to-serum index greater than 1.0 suggests synthesis of antibody in the central nervous system (CNS).

It has been proposed that four of the following five criteria should be present in order to diagnose neuroborreliosis:

- No past history of neuroborreliosis
- CSF anti-*B burgdorferiantibodies*
- Positive anti-*B burgdorferi* antibody index
- Favorable clinical outcome after proper antibiotic therapy
- Absence of alternative diagnosis

Culture

Because of the fastidious growth requirements for *B burgdorferi*, culture has not been a useful test in the past. In routine practice, borrelial cultures are often unavailable. In the skin, where findings are most likely to be positive, culturing is least likely to be clinically useful, except in cases of atypical rash. In other body fluids (eg, blood, synovial fluid, CSF), the yield is lower. Although one study from an endemic area reported positive blood culture results in 43.7% of untreated adult patients with erythema migrans, this required culturing specifically for Lyme disease. In addition, all but two of the 213 patients met CDC criteria for Lyme disease and warranted treatment, regardless of culture results.

Histologic Findings

Approximately 60-80% of specimens isolated from the leading edge of a suspected erythema migrans lesion by means of saline-lavage needle aspiration or 2-mm punch biopsy reveal *B burgdorferi.* However, because the presence of a lesion along with a compatible history and clinical presentation are sufficient to initiate treatment, these skin biopsy procedures are seldom performed. Histologic findings in erythema migrans are nonspecific, usually showing a perivascular cellular infiltrate consisting of lymphocytes, plasma cells, and histiocytes. Occasionally, mast cells and neutrophils are seen. Central biopsies may show eosinophilic infiltrates consistent with a local reaction to an arthropod bite. Spirochetes occasionally may be identified using silver or antibody-labeled stains, although usually, a paucity of spirochetes is found in the tissues of patients with Lyme disease.

Borrelial lymphocytoma

Histologic examination is recommended in patients with suspected borrelial lymphocytoma, when the location of the lesion or the clinical history is not clear enough to support a diagnosis. Borrelial lymphocytoma biopsy specimens show a dense dermal lymphocytic infiltrate with lymphoid follicles and pseudogerminal centers. Lymphocytes with both B- and T-cell markers, occasional macrophages, plasma cells, and eosinophils are seen.

Acrodermatitis chronica atrophicans

In acrodermatitis chronica atrophicans, biopsy specimens from early lesions show a lymphocytic dermal infiltrate, sometimes perivascular in location, with some vascular telangiectasia and lymphedema. Plasma cells also may be seen in the cellular infiltrate. Later lesions demonstrate epidermal thinning with loss of skin appendages. At this stage, plasma cells may be the only feature to distinguish acrodermatitis chronica atrophicans from morphea. The fibrotic nodules show fibrosis of the deeper dermis and sometimes, hyalinization of collagen bundles. *B burgdorferi* occasionally can be cultivated from the lesions; in one patient, cultivation was successful more than 10 years after the lesion's first appearance.

Treatment & Management

Approach Considerations

Antibiotic selection, route of administration, and duration of therapy for Lyme disease are guided by the patient's clinical manifestations and stage of disease, as well as the presence of any concomitant medical conditions or allergies. Prompt treatment increases the likelihood of therapeutic success. With prompt and appropriate antibiotic treatment, most patients with early-stage Lyme disease recover rapidly and completely. A joint guideline from the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR) recommends administering a single dose of oral doxycycline for prophylaxis within 72 hours of removing a tick after a high-risk bite. For a bite to be considered high risk, it must be from an *Ixodes* tick, in a highly endemic area, and from a tick engorged and attached for 36 hours or more. Antibiotic prophylaxis should not be given for tick bites that are equivocal or low risk. Doxycycline has traditionally been considered contraindicated in patients younger than 8 years and in pregnant and breastfeeding women. Although more recent research suggests that doxycycline for at least up to 14 days is safe in young children, amoxicillin remains the usual first choice for pediatric patients. See the tables.

Table 1. Clinical Presentation and Therapy for the Stages of Lyme Disease

Clinical Manifestations	Adult Dose	Pediatric Dose
Erythema migrans	Doxycycline 100 mg PO twice a day for 10 days, OR Amoxicillin 500 mg PO three times a day for 14 days, OR Cefuroxime axetil 500 mg PO twice a day for 14 days Patients unable to take doxycycline or beta-lactam antibiotics: Azithromycin 500 mg PO once a day for 7 days	Doxycycline 4.4 mg/kg/day, divided into 2 doses; not to exceed 100 mg/dose for 10 days, OR Amoxicillin 50 mg/kg/day PO, divided into 3 doses; not to exceed 500 mg/dose for 14 days, OR Cefuroxime axetil 30 mg/kg/day PO, divided into 2 doses; not to exceed 500 mg/dose for 14 days
Facial palsy	Doxycycline 100 mg PO twice a day for 14-21 days	Doxycycline 4.4 mg/kg/day PO, divided into 2 doses; not to exceed 100 mg/dose for 14-21 days

Table 1. Clinical Presentation and Therapy for the Stages of Lyme Disease

Clinical Manifestations	Adult Dose	Pediatric Dose
Lyme meningitis or radiculoneuritis	Doxycycline 200 mg/day PO, divided into 1-2 doses for 14-21 days, OR Ceftriaxone 2 g IV every day for 14-21 days; may substitute oral therapy once the patient is stabilized or discharged to complete the course	Doxycycline 4.4 mg/kg/day PO, divided into 1-2 doses; not to exceed 100 mg/dose for 14-21 days, OR Ceftriaxone 50-75 mg/kg IV every day; not to exceed 2 g/day for 14-21 days; may substitute oral therapy once the patient is stabilized or discharged to complete the course
Lyme disease–associated meningitis, cranial neuropathy, radiculoneuropathy, or with other peripheral nervous system (PNS) manifestations	Without parenchymal involvement of brain or spinal cord: IV ceftriaxone, cefotaxime, penicillin G, or oral doxycycline With parenchymal involvement of brain or spinal cord: IV antibiotics are preferred	
Mild Lyme carditis (1st degree AV block with PR interval < 300 milliseconds)	Doxycycline 100 mg PO twice a day for 14-21 days, OR Amoxicillin 500 mg PO three times a day for 14-21 days, OR Cefuroxime axetil 500 mg PO twice a day for 14-21 days	Doxycycline 4.4 mg/kg/day PO, divided into 2 doses; not to exceed 100 mg/dose for 14-21 days, OR Amoxicillin 50 mg/kg/day PO, divided into 3 doses; not to exceed 500 mg/dose for 14-21 days, OR Cefuroxime axetil 30 mg/kg/day PO, divided into 2 doses; not to exceed 500 mg/dose for 14-21 days
Severe Lyme carditis (symptomatic, 1st degree AV block with PR interval ≥300 milliseconds, 2nd or 3rd degree AV block)	Ceftriaxone 2 g IV every day for 14-21 days; once symptoms and high-grade AV block resolve, consider transitioning to oral antibiotics to complete treatment course	Ceftriaxone 50-75 mg IV q day; not to exceed 2 g/day for 14-21 days; once symptoms and high-grade AV block resolve, consider transitioning to oral antibiotics to complete treatment course
Borrelial lymphocytoma	Oral antibiotic therapy for 14 days	
Arthritis	Doxycycline 100 mg PO twice a day for 28 days, OR Amoxicillin 500 mg PO three times a day for 28 days, OR Cefuroxime axetil 500 mg PO twice a day for 28 days	For ≥8 years: Doxycycline 4.4 mg/kg/day PO, divided into 2 doses; not to exceed 100 mg/dose for 28 days, OR Amoxicillin 50 mg/kg/day PO, divided into 3 doses; not to exceed 500 mg/dose for 28 days, OR Cefuroxime axetil 30 mg/kg/day PO, divided into 2 doses; not to exceed 500 mg/dose for 28 days For < 8 years: Amoxicillin 50 mg/kg/day PO, divided into 3 doses; not to exceed 500 mg/dose for 28 days, OR Cefuroxime axetil 30 mg/kg/day PO, divided into 2 doses; not to exceed 500 mg/dose for 28 days
Arthritis without any response to initial treatment	Ceftriaxone 2 g IV every day for 14-28 days	Ceftriaxone 50-75 mg IV every day; not to exceed 2 g/day for 14-28 days
Acrodermatitis chronica atrophicans	Oral antibiotic therapy 21-28 days	

AV = atrioventricular; IV = intravenous; PO = oral

Table 2. Adult and Pediatric Treatment Options, Dosages, and Routes of Administration

	Treatment	Adult Dose	Pediatric Dose
Oral Therapy	Doxycycline (patients > 8 y)	100 mg twice a day	Doxycycline 4.4 mg/kg/day (up to 100 mg twice a day)
	Amoxicillin	500 mg three times a day	50 mg/kg/day (up to 500 mg) in 3 divided doses
	Cefuroxime axetil	500 mg twice a day	30 mg/kg/day (up to 500 mg) in 2 divided doses
	Phenoxymethylpenicillin	500 mg four times a day, or 1 g three times a day	50-100 mg/kg/day in three divided doses; maximum 1 g/dose
	Azithromycin (for patients unable to take doxycycline or beta-lactams)	500 mg once a day	50-100 mg/kg/day in three divided doses; maximum 1 g/dose
Intravenous therapy	Ceftriaxone	2 g once a day	10 mg/kg/day (maximum, 500 mg/day)
	Cefotaxime	2 g every 8 h	150-200 mg/kg (up to 2 g) every 8 h
	Penicillin G	18-24 million U/d divided, every 4 h	200,000-400,000 mg/kg (up to 2 g) every 8 h

In most patients with carditis, prompt institution of appropriate antibiotics is the only treatment needed. However, occasional patients with Lyme disease–related atrioventricular (AV) block may require hospitalization for temporary cardiac pacing. The indications for cardiac pacing are the same as for any other patient with varying degrees of heart block. Permanent pacing is very rarely needed. Symptoms of arthritis may persist for a few weeks beyond adequate therapy. Repeated treatment usually is not necessary unless symptoms worsen or persist beyond 2 months. Persistent arthritis after clearance of the infection is most likely related to autoimmunity and is more prevalent among individuals with HLA-DR2, HLA-DR3, or HLA-DR4 allotypes. These patients should be treated with nonsteroidal anti-inflammatory drugs (NSAIDs), plus hydroxychloroquine if necessary. As a last resort, such patients may need a synovectomy to eradicate the inflammatory arthritis in the involved joint. Neurologic manifestations of Lyme disease in both adults and children respond well to penicillin, ceftriaxone, cefotaxime, and doxycycline. Although most studies of neuroborreliosis have used intravenous antibiotics, European studies support use of oral doxycycline in adults with meningitis, cranial neuritis, or radiculitis, with intravenous regimens reserved for patients with parenchymal central nervous system (CNS) involvement, other severe neurologic symptomatology, or failure to respond to oral treatment.

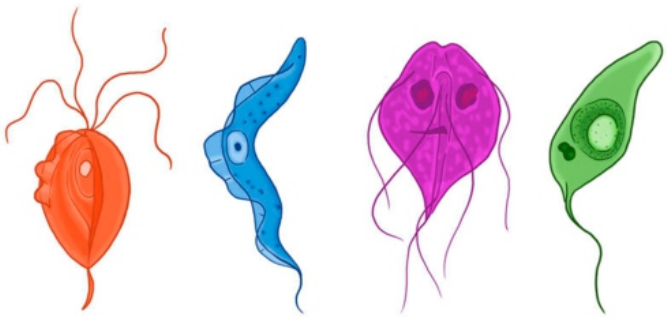
Borrelial lymphocytoma is sufficiently uncommon that no comparative trials address the ideal duration of treatment, route of administration of the antibiotic, or the choice of medication. Treatment is usually with 14-21 days of oral antibiotics. When symptoms of dissemination are noted, however, parenteral therapy sometimes is used. Physicians should observe patients closely for possible Jarisch-Herxheimer reactions after the institution of therapy. This allergic/ inflammatory response may manifest in the skin, mucous membranes, viscera, or nervous system. Several groups have published Lyme disease guidelines, including a joint guideline from the IDSA, AAN, and ACR, which is endorsed by the American Academy of Pediatrics. The International Lyme and Associated Diseases Society also issued recommendations for the management of Lyme disease. This guideline is based on "low or very low quality evidence" and uses patient preference as a major portion of the support for the recommendations. In addition, the recommendations are limited to three specific aspects of management: antibiotic prophylaxis for tick bites, erythema migrans treatment, and antibiotic retreatment for persistent manifestations of Lyme disease.

INTERPRETATION

PROTOZOAN DISEASES OF MAN

Protozoan diseases in humans are caused by single-celled organisms called protozoa. These parasites can be transmitted through contaminated food and water, insect bites, or sexual contact and range from mild intestinal infections to severe, life-threatening systemic diseases.

Common protozoan diseases



Malaria

- Cause: Plasmodium parasites, most notably *P. falciparum* and *P. vivax*.
- Transmission: Bite of an infected female Anopheles mosquito.
- Symptoms: High fever, chills, fatigue, headache, nausea, and vomiting. Severe cases can cause seizures, jaundice, organ failure, and death.
- Affected areas: Endemic in tropical and subtropical regions.

Amoebiasis

- Cause: Entamoeba histolytica, a parasite that can reside in the large intestine.
- Transmission: Ingestion of contaminated food or water containing the parasite's cysts. Poor hygiene is a significant risk factor.
- Symptoms: Mild infection can cause diarrhea and abdominal pain. In severe cases, it can lead to amoebic dysentery with bloody diarrhea, fever, and liver abscesses.

Giardiasis

- Cause: Giardia lamblia (also known as Giardia duodenalis).
- Transmission: Swallowing Giardia cysts found in contaminated water, food, or from contact with infected surfaces.
- Symptoms: Watery diarrhea, gas, stomach cramps, and nausea. The infection can be acute or chronic and may cause nutrient malabsorption.

Cryptosporidiosis

- Cause: Cryptosporidium species, parasites that live in the intestines of humans and animals.
- Transmission: Ingestion of the parasite through contaminated water or food, or contact with an infected person or animal.
- Symptoms: Watery diarrhea, stomach cramps, and nausea. While usually self-limiting in healthy people, it can cause severe, prolonged illness in immunocompromised individuals.

Toxoplasmosis

- Cause: Toxoplasma gondii, one of the most widespread parasites in the world.
- Transmission:
- Eating undercooked, contaminated meat.
- Accidentally ingesting the parasite after contact with infected cat feces.
- Mother-to-child transmission during pregnancy.
- Symptoms: **Most healthy people have no symptoms. However, it can cause serious or fatal complications in individuals with weakened immune systems and in babies born to mothers who become infected during pregnancy.

Leishmaniasis

- Cause: Leishmania parasites.
- Transmission: Bite of an infected sandfly.
- Symptoms: This complex disease has three forms, with symptoms ranging from skin ulcers (cutaneous leishmaniasis) to severe systemic illness involving fever, enlarged liver and spleen, and low red blood cell count (visceral leishmaniasis).
- Affected areas: Endemic in tropical and subtropical regions.

Trichomoniasis

- Cause: Trichomonas vaginalis, a sexually transmitted parasite.
- Transmission: Sexual intercourse.
- Symptoms: Many people are asymptomatic. When symptoms occur, they include genital itching, burning, and unusual discharge.

Chagas disease (American trypanosomiasis)

- Cause: Trypanosoma cruzi parasite.
- Transmission: Primarily through the feces of infected triatomine bugs (also called "kissing bugs").
- Symptoms: An initial acute phase may cause fever and swelling. A chronic, long-term infection can cause severe heart and gastrointestinal damage.
- Affected areas: Affects people in Latin America.

African trypanosomiasis (African sleeping sickness)

- Cause: Trypanosoma brucei parasites.
- Transmission: Bite of an infected tsetse fly.
- Symptoms: Starts with fever, headaches, joint pain, and swollen lymph nodes. Later stages involve neurological problems, including sleep-cycle disruption, which gives the disease its name.
- Affected areas: Found exclusively in Sub-Saharan Africa.

Prevention and control

Prevention is the most effective approach for many protozoan diseases, especially since specific vaccines are not available.

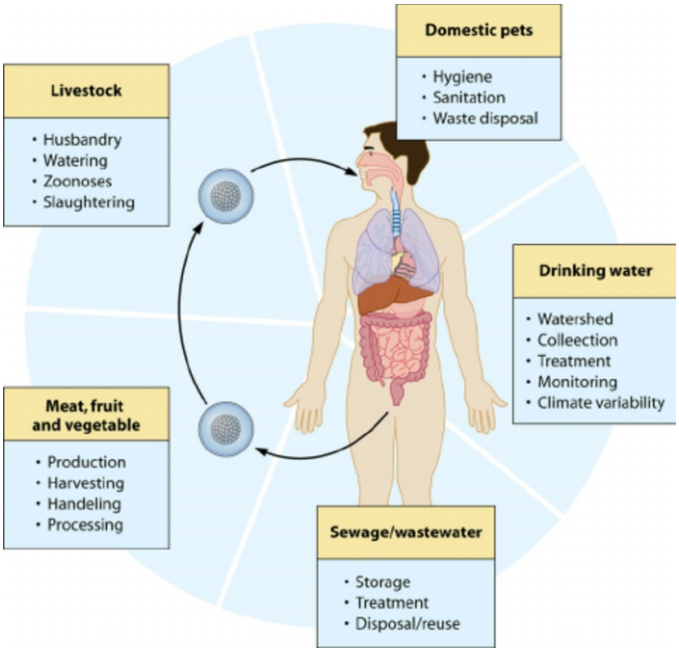
Improve hygiene and sanitation: Proper handwashing and access to clean drinking water are crucial for preventing faecal-oral transmission.

Ensure food safety: Thoroughly wash and cook food, especially in areas where protozoan infections are common.

Control vectors: Use insect repellent, wear protective clothing, and use

insecticide-treated bed nets to prevent mosquito and sandfly bites in endemic regions.

Avoid contaminated water: Do not drink untreated water from sources like lakes or rivers. Use properly filtered or boiled water.



List of Protozoan Diseases

Protozoan	Disease	Transmission
Entamoeba histolytica	Amoebiasis	Contaminated water or food
Giardia lamblia	Diarrhoea	Contaminated water or food
Plasmodium	Malaria	Mosquitoes
Leishmania	Visceral disease	Sand flies
Trichomonas vaginalis	Inflammation of urogenital tract disease	Sexually transmitted
Trypanosoma brucei gambiense	African sleeping sickness	Tsetse fly
Cryptosporidium	Watery diarrhoea	Waterborne outbreaks
Toxoplasma gondii	Toxoplasmosis	Ingestion of undercooked meat

TROUBLESHOOTING

RAPID TEST - PRE-ANALYTICAL ERRORS

INTRODUCTION

The accuracy of laboratory test results is extremely important for correct diagnosis and effective patient care. While much attention has been given to the testing (analytical) phase, many errors actually occur before testing begins — in the pre-analytical phase — which includes steps like patient preparation, sample collection, handling, and transportation. Problems such as haemolysis, lipemia, or icterus in blood samples can lead to wrong results. These errors often arise from incomplete test requests, improper sample collection, or mishandling during transport. Although modern laboratories have become more advanced and automated, they still depend on proper coordination with clinical staff to ensure samples and request forms are accurate and complete. Maintaining quality at every stage from test ordering to result interpretation is essential to avoid mistakes. Poor pre-analytical practices can lead to serious consequences such as misdiagnosis, wrong or delayed treatment, patient safety risks, and financial losses. Therefore, improving awareness, training, harmonized procedures, and collaboration among healthcare and laboratory professionals is crucial to ensure reliable results and better patient outcomes.

1. Insufficient Sample Volume

If the amount of sample collected is less than the required quantity, the concentration of the target analyte (antigen or antibody) may be too low for the test to detect. In such cases, the reagents on the test strip do not get fully activated, leading to faint or missing test lines.
Result: False-negative or weak positive results.

2. Excess Sample Volume

Using too much sample can oversaturate the test membrane, causing improper capillary flow. The reagents may not migrate as designed, leading to smearing, background staining, or invalid results. Excess sample can also dilute the buffer and alter the optimal pH needed for the antigen–antibody reaction.
Result: Invalid, smeared, or inconclusive results.

3. Incorrect Type of Sample

Each rapid test is validated for a specific sample type — for example, serum, plasma, whole blood, or nasal swab. Using an unapproved type can interfere with the test chemistry. Whole blood may contain cells that obstruct flow; saliva may lack sufficient antigen concentration; or serum might contain clotting factors that affect reaction kinetics.
Result: False-positive or false-negative outcomes.

4. Haemolyzed or Lipemic Samples

When red blood cells rupture (haemolysis), haemoglobin and other cellular materials leak into the plasma, causing discoloration that can mask or distort the test lines. Similarly, lipemic samples (high fat content) make the sample cloudy and can physically block capillary flow through the strip.
Result: Invalid, unclear, or misinterpreted results.

5. Contamination of the Sample

Sample contamination can occur from improper handling, non-sterile collection tools, or contact with alcohol, disinfectants, or other chemicals. Contaminated samples can alter antigen or antibody structures and interfere with the immunochromatographic reaction. Cross-contamination between patients' samples is especially dangerous, leading to false positives.
Result: False-positive, invalid, or inconsistent test results.

6. Delay Between Sample Collection and Testing

Antigens, antibodies, or nucleic acids in biological samples degrade over time, especially if the sample is left at room temperature or exposed to sunlight. Rapid tests are designed for fresh samples; delays can reduce analyte stability and result in low reactivity.
Result: False-negative or weak positive readings.

7. Improper Swab Technique (for Antigen Tests)

Inadequate swabbing—such as shallow nasal or throat collection—may fail to capture enough viral or bacterial material. Rapid antigen tests rely on sufficient sample load to produce visible results. Incorrect swab depth, duration, or rotation can severely compromise accuracy.
Result: False-negative test despite infection being present.

8. Improper Mixing with Buffer

The buffer provided with the kit maintains the right pH and ionic strength for optimal antigen–antibody interaction. Using the wrong volume or not mixing the sample properly with the buffer can disrupt these conditions. Inconsistent mixing also leads to uneven analyte distribution, causing partial flow or incomplete reaction on the strip.
Result: Invalid or weak positive results.

9. Wrong Storage or Temperature Conditions

Samples must be collected, stored, and transported within the manufacturer's recommended temperature range. Excess heat, freezing, or prolonged exposure to sunlight can denature proteins, degrade nucleic acids, or cause bacterial growth.
Result: False-negative results or inconsistent performance.

10. Cross-contamination Between Samples

Using the same pipette tip or sample applicator for multiple samples, or mixing up patient labels, can cause one sample to contaminate another. Even trace contamination can transfer antigen or antibody material, producing misleading results.
Result: False-positive results and unreliable interpretations.

11. Mixing Buffers of different lots

Mixing buffers from different manufacturing lots for a rapid test is generally not recommended and should be avoided. Lot-to-lot variations, even in seemingly minor components, can compromise the test's performance.
Result: Inaccurate results including false positives or false negatives.

12. Exposure to moisture

When a rapid test device is left open for too long before use, outside its sealed foil pouch it can absorb moisture from the air. Also, when a rapid test device, stored in the refrigerator (2–8°C) is opened immediately after removal, the cool surface of the device/strip is exposed to room temperature which leads to condensation, forming inside the test device and leading to degradation.
Result: Erroneous results- like invalid results.

13. Timely reading of results

Timely reading of results in rapid diagnostic test (RDT) devices is crucial because interpreting results outside the manufacturer's specified timeframe can lead to inaccurate readings, specifically false positives or false negatives.

Result: Inaccurate results such as false negative or false positive.

Summary

Improper sample collection is one of the most common causes of inaccurate or invalid results in rapid diagnostic testing. Errors such as insufficient or excessive sample volume, use of the wrong specimen type, haemolyzed or contaminated samples, and poor swab techniques can all interfere with the test's immunochemical reactions and capillary flow. Likewise, delays in testing, improper mixing with buffers, incorrect storage conditions, and cross-contamination between samples can degrade target analytes or introduce foreign material, leading to false-negative, false-positive, or invalid results. To ensure reliable and reproducible outcomes, it is essential that all samples are collected using the correct technique, appropriate sample type, and precise volume under recommended environmental and handling conditions. Proper training, adherence to manufacturer instructions, and strict quality control practices are key to maintaining the accuracy of rapid tests in clinical and field settings.

BOUQUET

In Lighter Vein

Japanese concept:

If one can do, you can do.
If none can do, you must do.

My concept:

If one can do, let him do.
If none can do, how can I do?

A lady broke the traffic signal...
Police : 'Stop....!!!!!'

Lady : 'Please...let me go. . .I am a teacher...'

Police : 'Aahaa!!.... I have waited for this moment all my life....
Now ....WRITE .....
I'll never break a signal, 100 times...'

Wisdom Whispers

There is nothing noble in being superior to some other man. The true nobility is in being superior to your previous self.

The man who has mounted an elephant will not fear the bark of a dog.

The eyes do not see what the mind does not want.

Brain Teasers

1. **What is the primary cause of Lyme disease?**
   A) Virus
   B) Bacteria
   C) Fungus
   D) Parasite

2. **Which of the following is a common early symptom of Lyme disease?**
   A) A "bull's-eye" rash (erythema migrans)
   B) A Christmas-tree shaped rash
   C) A line-like rash
   D) No rash occurs with Lyme disease

3. **What is the recommended treatment for Lyme disease?**
   A) Antivirals
   B) Antifungals
   C) Antibiotics
   D) Corticosteroids

4. **Which of the following is a way to prevent Lyme disease?**
   A) Using antifungal sprays
   B) Avoiding all outdoor activities
   C) Checking for ticks after being in high-risk areas
   D) Not relevant, as it is not preventable

ANSWER: 1-B, 2-A, 3-C, 4-C

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.