Tick-borne Diseases

Laboratory Support of Diagnosis and Management

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CLINICAL BACKGROUND

Tick-borne diseases are caused by infections transmitted to humans via a tick vector such as the deer tick, dog tick, wood tick, or lone star tick. Causative agents include bacteria, viruses, parasites, and protozoa. The incidence varies by geographic location and causative agent **(Table 1)**.[1-4] Clinical manifestations also vary depending on the causative agent but frequently include fever, chills, sweating, headaches, myalgia, arthralgia, nausea, and vomiting; because of similar symptomology, tick-borne diseases have substantial clinical overlap **(Table 2)**.[5-8] Some patients develop a rash or lesion at the site of the bite. More severe disease may lead to hematologic, respiratory, cardiac, and neurologic complications, as well as kidney or liver failure and arthritis. Although tick-borne illnesses can be fatal, antimicrobial agents are usually effective for bacterial tick-borne diseases. Some ticks can harbor more than 1 infectious agent (eg, *Borrelia burgdorferi* and *Babesia microti*) that can be transmitted to humans, and coinfection may complicate the diagnosis and affect treatment selection.[9]

Preliminary differential diagnosis is primarily based on clinical presentation and a history of exposure in areas where vector ticks are endemic **(Figure)**.[10] In symptomatic patients, a rash or lesion may provide the first clue to the diagnosis; however, absence of a rash should not rule out a condition from the differential diagnosis. Identification of the tick can also be helpful, as some disease pathogens are carried by specific tick species. Because of rapid disease progression associated with some tick-borne infections (eg, tick-borne rickettsial diseases [TBRDs]), treatment should not be delayed pending the results of laboratory tests or the development of more serious symptoms.[5]

Lyme Disease

Lyme disease is by far the most common tick-borne disease in the United States **(Table 1)**,[1-4] where it is caused by *B burgdorferi* sensu stricto of the *B burgdorferi* sensu lato complex. It can also be caused by other species of the *B burgdorferi* sensu lato complex, including *B garinii* and *B afzelii*. Despite the stabilization within the last decade in the number of confirmed and probable Lyme disease cases, incidence has remained high since 2008, when about 35,000 cases were reported; more than 35,000 cases of Lyme disease were reported in 2016 and many more may go unrecognized.

Table 1. Incidence of Tick-borne Diseases, United States[1,2]

Disorder	Causative Organism	Primary Vector Tick(s)	Reported Cases, 2016	US Geographic Distribution[a]
Lyme disease	*Borrelia burgdorferi*, *B mayonii*	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	36,429	Northeast[b], Mid-Atlantic[b], upper Midwest[b]
		Western black-legged tick (*Ixodes pacificus*)		Pacific coast, northern California
Anaplasmosis/ Ehrlichiosis				
Anaplasmosis, also known as human granulocytic anaplasmosis (HGA)[c]	*Anaplasma phagocytophilum*	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	4,151	Upper Midwest, Northeast
		Western black-legged tick (*Ixodes pacificus*)		Pacific Coast of northern California
Human monocytic ehrlichiosis (HME)	*Ehrlichia chaffeensis*	Lone star tick (*Amblyomma americanum*)	1,377	Southeast, Northeast
		American dog tick (*Dermacentor variabilis*)		East of the Rocky Mountains, Pacific Coast
Human ehrlichiosis ewingii (HEE)	*Ehrlichia ewingii*	Lone star tick (*Amblyomma americanum*)	16	Southeast, East
Ehrlichia muris-like agent ehrlichiosis	*Ehrlichia muris*-like	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	12	Wisconsin
Spotted fever rickettsiosis	*Rickettsia rickettsii* (RMSF)	American dog tick (*Dermacentor variablis*)	4,269	East of the Rocky Mountains, Pacific Coast
	Rickettsia parkeri	Rocky Mountain wood tick (*Dermacentor andersoni*)		Rocky Mountain States
	Rickettsia philipii (364D)	Pacific Coast tick (*Dermacentor occidentalis*)		California
Babesiosis	*Babesia microti* and other *Babesia* species	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	1,910	Upper Midwest, Northeast
		Western black-legged tick (*Ixodes pacificus*)		Northern California along Pacific Coast
Tularemia	*Francisella tularensis*	Lone star tick (*Amblyomma americanum*)	230	Southeast, East
		Rocky Mountain wood tick (*Dermacentor andersoni*)		Rocky Mountain States
		American dog tick (*Dermacentor variabilis*)		East of the Rocky Mountains, Pacific Coast

(Continued)

Table 1. Incidence of Tick-borne Diseases, United States[1,2] *(Continued)*

Disorder	Causative Organism	Primary Vector Tick(s)	Reported Cases, 2016	US Geographic Distribution[a]
Powassan virus neuroinvasive disease/ encephalitis	*Flavivirus*	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	22	Northeast, Virginia, Wisconsin
***Borrelia miyamotoi* disease**	*Borrelia miyamotoi*	Black-legged tick, also known as deer tick (*Ixodes scapularis*)	97[d]	Upper Midwest, Northeast
		Western black-legged tick (*Ixodes pacificus*)		Northern California along Pacific Coast
Colorado tick fever	Colorado tick fever virus	Rocky Mountain wood tick (*Dermacentor andersoni*)	83[e]	Rocky Mountain States
Tick-borne relapsing fever (TBRF)	*Borrelia hermsii*	*Ornithodoros* species	20/y	West

RMSF, Rocky Mountain spotted fever.
[a] See reference 3 for detailed geographic distribution maps.
[b] In 2015, these regions accounted for roughly 95% of confirmed Lyme disease cases in the United States.[4]
[c] Formerly known as human granulocytic ehrlichiosis (HGE).
[d] First recognized cases in North America were reported in the United States in 2013-2014.
[e] Cases were reported to the CDC from 2002 through 2012.

Borrelia burgdorferi is transmitted by *Ixodes scapularis* and *I pacificus* ticks. Because *I scapularis* may also harbor *Babesia microti*, *Anaplasma phagocytophilum*, deer tick virus, *Borrelia miyamotoi*, and *Ehrlichia* species Wisconsin, a single tick bite can lead to coinfection in humans.[9,11]

Lyme disease cases are heavily centered in New England and the Mid-Atlantic. However, they are also found in Wisconsin and Minnesota and, to a lesser extent, in other states in the Great Lakes region and in Pacific Coastal regions. Lyme disease is most common among children and middle-aged adults.[11]

The clinical presentation of Lyme disease can be either localized or disseminated. Characteristic of early localized disease is the presence of erythema migrans, a round or oval erythematous skin lesion; it usually develops 3 to 30 days **(Table 3)**[9,11] after the tick bite and should be ≥5 cm in largest diameter for a firm Lyme disease diagnosis.[9,11] Untreated, lesions can grow much larger. Some but not all such lesions present with a "bulls-eye" pattern of central clearing at the site of the tick bite. Erythema migrans occurs in approximately 70% to 80% of infected persons.

Additional smaller lesions may develop if Lyme disease is untreated, and these are often the first sign of early disseminated disease. Extracutaneous involvement in early disseminated disease can include the musculoskeletal, cardiac, or nervous system. Disseminated disease can also occur in the absence of a recognized skin lesion. In late-stage disease, Lyme carditis may overlap temporally with neurologic Lyme disease.

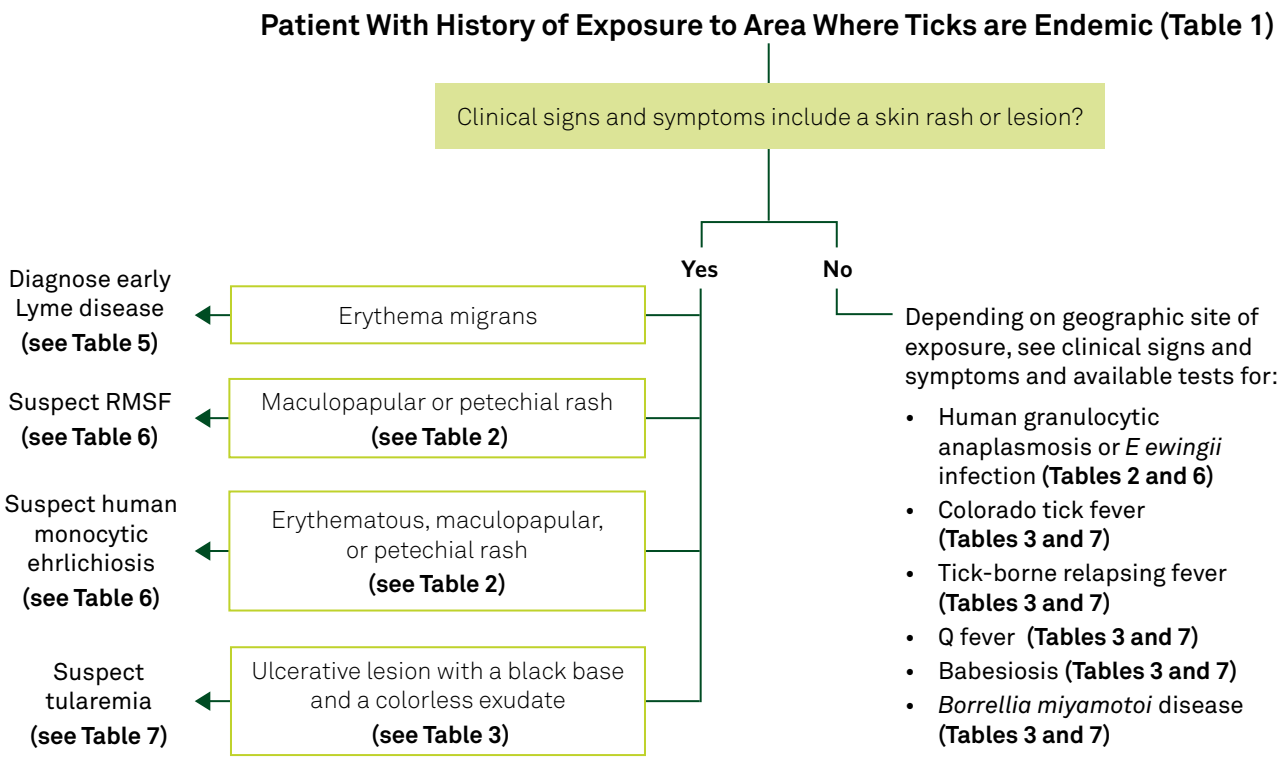
If initiated in the early stages of disease, treatment with appropriate antibiotics is usually effective.[9] Prophylaxis after a tick bite is usually not indicated in areas where less than 20% of ticks are infected—and then in only selected patients.[9]

Tick-borne Rickettsial Diseases

TBRDs include spotted fever rickettsiosis (SFR), human monocytic ehrlichiosis (HME) and other ehrlichioses, and anaplasmosis including human granulocytic anaplasmosis (HGA). TBRDs commonly manifest with an acute onset of nonspecific symptoms that mimic benign viral infections, making diagnosis difficult **(Table 2)**.[5-8] The presence or absence of a rash can be a useful diagnostic aid.

Because antibiotic treatment is most effective when given early, therapy for symptomatic patients with clinically suspected TBRDs should not be delayed pending confirmatory laboratory results.[7] Once the presumptive diagnosis of TBRD is made based on endemic exposure and clinical signs and symptoms, doxycycline is generally the drug of choice for both children and adults.[5,12]

Figure. An Approach to the Differential Diagnosis of Tick-borne Diseases



A maculopapular or petechial rash may be present in up to 15% of patients with Colorado tick fever. Rash may be present in ≈18% of patients with tick-borne relapsing fever.[10] RMSF, Rocky Mountain spotted fever.

This figure was developed by Quest Diagnostics. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

Spotted Fever Rickettsiosis

SFR includes Rocky Mountain spotted fever (RMSF) as well as *Rickettsia parkeri* and *Rickettsia philipii* (364D) rickettsiosis, which are difficult to distinguish with commonly available serologic tests. SFR has been reported from each of the 48 contiguous states and the District of Columbia, although 5 states accounted for about 63% of cases during 2008–2012 (Arkansas, Missouri, North Carolina, Oklahoma, and Tennessee).[5] The peak season for infection coincides with tick activity level for the region, but infection has been reported throughout the year.[5] The reported incidence has increased in recent decades to >11 cases per million population in 2014. This increase in incidence has been accompanied by a decrease in case fatality rate, to <0.5% in 2014.[13] Although infection is most common in the 60- to 69-year-old age group, children younger than 10 years have the highest case-fatality rate.[5]

RMSF, the most severe of the rickettsial illnesses, is caused by *Rickettsia rickettsii*. This organism infects endothelial cells and causes small-vessel vasculitis that usually results in a maculopapular or petechial rash. Symptoms tend to appear 3 to 12 days after a bite. RMSF is also the most severe of the rickettsioses in the United States. Vasculitis in organs such as the brain or lungs can lead to life-threatening complications.

Of the other SFR infections, the first case of human *R parkeri* infection was documented in 2014; as of 2015, at least 40 cases have been identified.[1] It is carried by the Gulf Coast

tick (*Amblyomma maculatum*), and its geographic distribution extends from the Southern and Mid-Atlantic regions. Human infection with *R philipii* (364D) was first documented in 2010. *Rickettsia philipii* (364D) is transmitted by the Pacific Coast tick (*Dermacentor occidentalis*), which is present in California and Oregon. Of the few (<10) cases reported as of 2013, all were from California.[1]

Anaplasmosis

Anaplasmosis, or human granulocytic anaplasmosis (HGA), is caused by infection with *Anaplasma phagocytophilum*. It is usually found in the Northeastern and upper Midwestern states. The incidence rate is 6.3 cases per million person-years in the United States, being highest in these geographical areas.[5] Anaplasmosis has substantial overlap of features with early Lyme disease but tends to be a more severe illness.[14] Infection is most common in adults 60 years and older; the case fatality rate (0.3%) is highest in adults 70 years and older, and those with compromised immune systems.[15]

The ticks that carry *A phagocytophilum* can also harbor *B burgdorferi*, *B miyamotoi* or *B microti*, and detection of coinfection is recommended as it may affect treatment choices.[5]

Ehrlichiosis

Ehrlichiosis can be caused by 3 bacteria in the United States: *Ehrlichia chaffeensis* (the cause of human monocytic ehrlichiosis), *Ehrlichia ewingii*, and the recently identified *Ehrlichia muris*-like agent that may share primary tick vectors with *B burgdorferi* in Lyme disease.[5] *Ehrlichia chaffeensis* is the most common cause of ehrlichiosis. It is mostly identified in South-central, Southeastern, and Mid-Atlantic states. Although *E ewingii* infection had been thought to primarily affect immunocompromised patients from Missouri, Oklahoma, and Tennessee, a recent study found it to be more often found in immunocompetent individuals.[16]

The incidence of ehrlichiosis increased 4-fold from between the year 2000 to the 2008–2012 period; during the 2008–2012

Table 2. Clinical Features of Tick-borne Rickettsial Diseases[5-8]

Disease	Incubation Period (Days)	Signs and Symptoms	Rash
Rocky Mountain spotted fever	2–14	Fever, headache, malaise, myalgia, nausea/vomiting	Maculopapular rash 2–4 days after fever onset in 50%–80% of adults and >90% of children; frequently on palms and soles
Rickettsia parkeri rickettsiosis	2–10	Fever, headache, malaise, myalgia	Eschar (dark scab), usually days to a week after the bite; maculopapular or papulovesicular rash, sometimes on palms and soles
Rickettsia philipii (364D) rickettsiosis	5–14	Fever, headache, malaise, myalgia	Eschar or ulcerative lesion; lymphadenitis or lymphadenopathy
Human granulocytic anaplasmosis	5–21	Fever, headache, malaise, myalgia, nausea/ abdominal pain (less frequent)	Rare
Human monocytic ehrlichiosis	5–14	Fever, severe headache, malaise, myalgia, nausea/ vomiting/diarrhea	Erythematous, maculopapular or petechial rash in <30% of adults and ~60% of children
Human ehrlichiosis ewingii	5–14	Fever, headache, myalgia, nausea/vomiting	Rare
Ehrlichia muris-like (EML) agent ehrlichiosis	5–14	Fever, malaise, headache, myalgia	Rare

period, 16 new cases per million persons were reported, or an incidence rate of 3.2 cases per million persons per year for *E chaffeensis*. The case fatality rate is highest for children younger than 5 years old (4%).[16] *Ehrlichia ewingii* had a much lower incidence rate during the same time period and was not associated with fatality.[16]

Tick-borne Non-Rickettsial Diseases

Babesiosis

Babesiosis is primarily caused by a protozoan parasite infection (*Babesia microti*). This organism infects erythrocytes, and the disease process shares clinical features with malaria. Babesiosis is now reportable in 18 states but is most common in New England, New York, New Jersey, Minnesota, and Wisconsin, which account for 97% of cases.[9,17,18] Infection is primarily transmitted by ticks, although it can also be transmitted congenitally or through transfusion; 6 transfusion-associated cases were reported in 2014. The disease may be asymptomatic, or symptoms may appear 1 to 6 weeks after the tick bite **(Table 3)**.[9,17,18] Symptoms vary widely but may include a gradual onset of irregular fever, chills, sweating, myalgia, arthralgia, nausea/vomiting, and fatigue **(Table 3)**.[9,17,18] Mild hepatosplenomegaly and mild hemolytic anemia may develop.

Treatment is not usually required for people without symptoms. For patients with clinical illness, treatment usually includes a combination of clindamycin and quinine, or atovaquone and azithromycin.[9]

Tularemia

Tularemia is caused by the bacterium, *Francisella tularensis*. Most commonly found in Missouri, Arkansas, and Oklahoma, transmission occurs through varying portals of entry including tick bites, skin contact with infected animals, and inhalation of contaminated aerosols or agricultural dusts. Infection via tick bites is characterized by an ulcerative lesion at the site of the tick bite and by lymphadenopathy. An erythematous, tender, or pruritic papule typically appears within 3 to 5 days and subsequently enlarges to form an ulcer with a black base. Additional symptoms of tularemia appear abruptly and include fever, chills, headache, and generalized myalgia and arthralgia **(Table 3)**.[19] Clinical consequences depend on the portal of entry and the extent of systemic involvement. Ulceroglandular and glandular forms account for 75% to 85% of cases; pneumonic tularemia, a pulmonary form that may be contracted by inhalation or hematogenous spread, accounts for about 18% of adult cases.[19] Less common forms include oropharyngeal and oculoglandular disease. Presumptive diagnosis is based on a history of exposure to a tick-endemic region and clinical signs and symptoms. Treatments include streptomycin, gentamicin, and doxycycline.[19] Ciprofloxacin is a non-FDA approved alternative when doxycycline is contraindicated.

Borrelia miyamotoi Disease (BMD)

First reported in the United States in 2013, BMD is caused by the transmission of *Borrelia miyamotoi* in infected *I scapularis* and *I pacificus* ticks.[22] Recent surveillance suggests that the disease may be an emerging tick-borne infection in regions of the Northeast that include Massachusetts, Rhode Island, New Jersey, and New York.[22] The clinical presentation of BMD is variable but shares a similar spectrum with other tick-borne diseases such as Lyme disease, anaplasmosis, and babesiosis.[23] A constellation of nonspecific symptoms

Table 3. Clinical Features of Tick-borne Non-Rickettsial Diseases[9-11,17-23]

Disease	Incubation Period (Days)	Common Signs and Symptoms
Lyme Disease	3–30	Fever, chills, headache, myalgia, arthralgia, lymphadenitis or lymphadenopathy, rash
Babesiosis	7–42	Fever, chills, myalgia, hemolytic anemia
Tularemia	1–14	Fever, chills, headache, myalgia, arthralgia, papule/ulceration
Powassan virus neuroinvasive disease/ encephalitis	7–30	Fever, headache, vomiting, weakness, confusion, loss of coordination, speech difficulties, seizure
Borrelia miyamotoi disease	12–16	Fever, chills, headache, myalgia, arthralgia, fatigue
Colorado tick fever	1–14	Severe headache, severe myalgia, arthralgia, saddleback fever
Tick-borne relapsing fever	4–18+	Headache, myalgia, chills, nausea/vomiting, arthralgia

commonly includes fever, severe headache, myalgia, fatigue, and arthralgia; symptoms are characteristic of Lyme disease, babesiosis, and anaplasmosis which may be included in a differential diagnosis.[23] A "toxic" appearance suggestive of sepsis is common on presentation and is often accompanied by elevated liver enzyme levels, neutropenia, and thrombocytopenia in patients hospitalized for suspected infection.[22] A 2- to 4-week course of doxycycline is used to treat BMD infection.

Colorado Tick Fever (CTF)

CTF is caused by an arbovirus (Colorado tick fever virus) that infects erythrocytes. It is found throughout the Rocky Mountain region of the United States. Although CTF is not a nationally notifiable condition, an average of approximately 8 cases per year were reported to the CDC from 2002 through 2012. After a mean incubation time of 1 to 14 days, disease onset is abrupt; presenting symptoms include intense headache, severe myalgia, arthralgia, and a characteristic biphasic pattern of fever termed "saddleback" fever. The fever lasts for 2 to 3 days, disappears, and then may recur for another 2 to 3 days. In rare instances, severe complications such as central nervous system involvement and hemorrhage may occur, especially in children. Specific antiviral treatment is not available for CTF.

Tick-borne Relapsing Fever (TBRF)

TBRF is caused by *Borrelia hermsii* and occurs west of the Mississippi River, especially in forested mountainous areas of the far Western states. Transmission typically occurs while sleeping in cabins or other rustic buildings that may house ticks in animal nests concealed in walls, attics, or crawl spaces. TBRF is characterized by recurrent acute episodes of spirochetemia and fever. Following a mean incubation period of 7 days, the onset of illness is sudden, with headache, myalgia, chills, nausea/vomiting, and arthralgias that may be severe **(Table 3)**.[10] Fever is typically ≥104° F and may be accompanied by delirium. Leukocytosis and thrombocytopenia are common, and splenomegaly may be present. Microscopy can be utilized to visualize the spirochetes in a blood smear, and thus, is useful in establishing the diagnosis.

Symptoms intensify without treatment, therefore, treatment should be administered when clinical suspicion is high. Tetracycline, or erythromycin if tetracycline is contraindicated, is recommended as the treatment of choice for TBRF.[24]

INDIVIDUALS SUITABLE FOR TESTING

- Symptomatic individuals with a history of exposure to a tick-endemic area

TEST AVAILABILITY

Laboratory tests that can help confirm the clinical diagnosis include tick identification, microscopic visualization of the causative organism in blood or other clinical specimens, various serologic techniques, culture, and polymerase chain reaction (PCR)-based assays **(Table 4)**. Panel components may be ordered individually.

TEST SELECTION AND INTERPRETATION

In most cases, presumptive diagnosis of tick-borne illnesses is based on clinical grounds. Treatment should not be delayed pending confirmatory laboratory results except in the prophylaxis of Lyme disease in persons bitten by

Table 4. Tests Available for Diagnosis and Management of Tick-borne Diseases

Test Code	Assay	Method	Clinical Use
All Tick-borne Diseases			
94322	Tick-borne Disease, Acute Molecular Panel Includes *Anaplasma phagocytophilum* DNA, Qualitative Real-Time PCR; *Babesia microti* DNA, Real-Time PCR; *Borrelia miyamotoi* DNA, Real-Time PCR, Miscellaneous; *Ehrlichia chaffeensis* DNA, Real-Time PCR; Lyme Disease (*Borrelia spp*) DNA, Qualitative, Real-Time PCR, Blood[b,c]	Real-time PCR	Diagnose tick-borne diseases when selecting tests for individual pathogen is challenging due to overlapping geographic distributions and clinical presentations of illness; especially useful to diagnose mixed infections
3946(X)	Tick (and Other Arthropods) Identification	Microscopy	Identify tick to determine risk of tick-borne disease; assist with differential diagnosis

(Continued)

Table 4. Tests Available for Diagnosis and Management of Tick-borne Diseases *(Continued)*

Test Code	Assay	Method	Clinical Use
Lyme Disease			
6646	Lyme Disease (*Borrelia* spp) Antibody with Reflex to Blot (IgG, IgM)[a]	Immunoassay	CDC recommends two-step testing to diagnose Lyme disease
34194	Lyme Disease Antibody Index for CNS Infection Includes *B burgdorferi* IgG and IgM, total IgG and IgM, and albumin (all in CSF and serum) as well as *B burgdorferi* antibody index and albumin ratio.	ELISA; Nephelometry	Diagnose neurologic Lyme disease
29477	Lyme Disease Antibody (IgG), Immunoblot	Immunoblot	Diagnose Lyme disease in patients with equivocal or positive serology
8593	Lyme Disease Antibodies (IgG, IgM), Immunoblot		
15777	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, Blood[b]	PCR	Diagnose Lyme disease
15564	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, CSF/Synovial Fluid[b]	PCR	Diagnose neurologic Lyme disease or Lyme arthritis
15510	Lyme Disease (*Borrelia* spp) DNA, Qualitative, Real-Time PCR, Tick[b]	PCR	Detect *B burgdorferi* in tick to assess risk of Lyme disease
15868	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, Urine[b]	PCR	Diagnose Lyme disease
90558	Tick ID with Reflex to Lyme Disease DNA, Real-Time PCR, Tick[a]	Microscopy; reflex to PCR	Identify tick and *B burgdorferi* to assess risk of tick-borne disease and assist with differential diagnosis
Spotted fever rickettsiosis			
70191	*Rickettsia rickettsii* DNA, Real-Time PCR[b]	PCR	Diagnose RMSF
6419	*Rickettsia* (RMSF) Antibodies (IgG, IgM) with Reflex to Titers[a]	IFA	
37507	*Rickettsia* Antibody Panel with Reflex to Titers[a] Includes IgG and IgM to causative organisms of RMSF and typhus fever.	IFA	
37478	Rickettsial Disease Panel[a] Includes IgG and IgM to causative organisms of RMSF, typhus fever, with reflex to appropriate titers	IFA	Differential diagnosis of rickettsial disease
37503	*Rickettsia* (Typhus Fever) Antibodies (IgG, IgM) with Reflex to Titers	IFA	
Anaplasmosis			
34464(X)	*Anaplasma phagocytophilum* Antibodies (IgG, IgM)[b]	IFA	Diagnose HGA
17320	*Anaplasma phagocytophilum* DNA, Qualitative, Real-Time PCR[b]	PCR	
10611(X)	*Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* Antibody Panel[b] Includes IgG and IgM for both organisms	IFA	Differential diagnosis of ehrlichiosis

(Continued)

Table 4. Tests Available for Diagnosis and Management of Tick-borne Diseases *(Continued)*

Test Code	Assay	Method	Clinical Use
Ehrlichiosis			
34271(X)	*Ehrlichia chaffeensis* Antibodies (IgG, IgM)[b]	IFA	Diagnose HME
11353	*Ehrlichia chaffeensis* DNA, Real-Time PCR[b]	PCR	
70194(X)	*Ehrlichia ewingii* DNA, Real-Time PCR[b,c]	PCR	
Babesiosis			
34300	*Babesia microti* Antibodies (IgG, IgM)[b]	IFA	Diagnose babesiosis
37314	*Babesia microti* DNA, Real-Time PCR[b]	PCR	
17231	WA1 IgG Antibody, IFA[b]	IFA	
831	Malaria/Babesia/Other Blood Parasites	Microscopy	
Tularemia			
91122	Febrile Antibodies and *Francisella* Panel[a,b] Includes IgG and IgM to causative organisms of RMSF and typhus, with reflex to appropriate titers; total antibody to *Salmonella* (*Salmonella* H types A, B, D; *Salmonella* O types D, Vi); IgG and IgM to *Brucella*, with reflex to agglutination; and antibody to *Francisella tularensis*	See individual tests	Differential diagnosis of febrile disease
35176(X)	*Francisella tularensis* Antibody, DA[b]	Direct Agglutination	Diagnose tularemia
***Borrelia miyamotoi* disease**			
93795	*Borrelia miyamotoi* DNA, Real-Time PCR, Miscellaneous[b]	PCR	Confirm diagnosis of *B miyamotoi* infection
93794	*Borrelia miyamotoi* DNA, Real-Time PCR, Tick[b]	PCR	Detect *B miyamotoi* in tick to assess risk of human infection
Colorado Tick Fever			
34986	Colorado Tick Fever Antibodies (IgG, IgM)[b]	IFA	Diagnose Colorado tick fever

EIA, enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; IFA, immunofluorescence assay; HGA, human granulocytic anaplasmosis, formerly known as human granulocytic ehrlichiosis (HGE); HME, human monocytic ehrlichiosis; TBRF, tick-borne relapsing fever.

[a] Reflex tests are performed at an additional charge and are associated with an additional CPT code(s).

[b] This test was developed and its performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the US Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.

[c] Available from Quest Diagnostics Nichols Institute.

I scapularis or *I pacificus* ticks where empiric treatment is not recommended. The clinical symptoms and type of rash or lesion, if present, guide the initial differential diagnosis among patients exposed to a tick-endemic area **(Figure)**.[10] This, in turn, guides appropriate test selection, presumably leading to confirmation of the suspected disorder. The sections below outline characteristic test results for each of the tick-borne diseases discussed.

The information in the text, tables, and the figure is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

Lyme Disease

Early Lyme Disease

Diagnosis of Lyme disease (*B burgdorferi* infection) generally relies on clinical findings and a history of exposure to the vector tick or tick habitat. Clinical findings, as well as laboratory testing, vary with the stage of the disease **(Table 5)**.[9,25,26] Diagnosis of early-stage, localized Lyme disease can sometimes be made on the basis of erythema migrans alone without laboratory testing.[9] When there is diagnostic uncertainty, positive IgG and/or IgM serology results from acute- and convalescent-phase (ie, 2 to 4 weeks after the acute phase) samples can support the diagnosis **(Table 5)**.[9,25,26]

Extracutaneous/Disseminated Lyme Disease

In the absence of erythema migrans, Lyme disease cannot be diagnosed on the basis of extracutaneous disease manifestations alone, because these symptoms are nonspecific.[4] Diagnosis requires demonstration of seropositive test results; in cases where diagnosis of Lyme disease is not clear, support or confirmatory testing by PCR may be considered.[4] Combined IgG and IgM testing by ELISA (or IFA) is recommended for patients 2 to 4 weeks after the tick bite **(Table 5)**.[9,25,26] Western blot (ie, immunoblot) testing is recommended as a confirmatory test for all specimens positive or equivocal by EIA.[4]

IgM antibodies may be present within a few weeks of disease onset, whereas large increases in IgG titers are produced months later. A positive IgM result as determined by 2-tier testing, in conjunction with a negative IgG result, is presumptive evidence of early infection unless obtained on a specimen collected more than 1 month following onset. In the latter scenario, a positive IgM finding is more likely to represent a false-positive result unless IgG is also positive. A positive IgG result by 2-tier testing is required to confirm the diagnosis of disseminated disease.[4]

Immunoassays used to detect Lyme disease antibodies utilize whole spirochete preparations or purified antigens, such as VlsE. The VlsE protein contains a C6 epitope that binds IgG and IgM antibodies produced in response to *B burgdorferi* infection. The VlsE antibody (IgG+IgM) test is specific to the C6 epitope and used to detect infection from both American and European *Borrelia* species while reducing false-positives that may be caused by antibody cross-reaction with similar organisms.[27]

The interpretation of Western blot assays is based on the number of positive bands: 2 of 3 bands (23, 39, 41 kDa) for IgM positivity and 5 of 10 bands (18, 23, 28, 30, 39, 41, 45, 58, 66, or 93 kDa) for IgG positivity. The Western blot is to be used only following initial EIA testing; positive results confirm *B burgdorferi* infection.

False-positive antibody test results may be due to vaccination, infectious mononucleosis, systemic lupus erythematosus, or other diseases caused by spirochetes such as syphilis, yaws, pinta, and relapsing fever. Positive IgG antibody test results do not differentiate between recent and past *B burgdorferi* infection.

Table 5. Lyme Disease: Clinical Features[25] and Recommended Laboratory Testing[9]

Stage of Disease	Clinical Features	Laboratory Testing
Early-stage (localized)	Erythema migrans, fever, myalgia, headache, nausea, fatigue	<2 weeks after the tick bite: if a mixed infection is clinically suspected, tick-borne PCR panel may be useful 2–4 weeks after the tick bite: acute and convalescent 2-tier[a] IgG, IgM serology (if diagnosis uncertain)
Early-stage disseminated (cardiac involvement)	Atrioventricular heart block sometimes with myopericarditis; migratory pain in joints, bone, and muscle; secondary annular lesions; malaise; fatigue	2–4 weeks after the tick bite: acute and/or convalescent 2-tier[a] IgG, IgM serology >4 weeks after the tick bite: acute and/or convalescent 2-tier[a] IgG serology
Late-stage disseminated (neurologic and/or arthritic involvement)	Encephalopathy; polyneuropathy; lymphocytic meningitis; prolonged, chronic arthritis; lymphocytoma; fatigue	Acute and/or convalescent 2-tier[a] IgG serology in serum; consider serology and/or detection of *B burgdorferi* DNA in CSF or synovial fluid

[a] Two-tier testing is a follow-up of a positive or equivocal EIA with a Western blot test as recommended by the Centers for Disease Control and Prevention, and the Association of State and Territorial Public Health Laboratory Directors.[26]

Negative serology results may indicate lack of infection or lack of seroconversion, which may occur if samples are collected too early after disease onset or when early antibiotic therapy blunts the antibody response. PCR-based assays can be useful in the workup of *B burgdorferi* infection if seroconversion has not yet occurred; these assays, however, are limited by low clinical sensitivity (18%).[28] Untreated patients who continue to be symptomatic but are seronegative for 6 to 8 weeks are unlikely to have Lyme disease, and a differential diagnosis should be considered.[9]

Expression of paired CSF and serum *B burgdorferi* antibody results as an antibody index can be used as an aid in the diagnosis of neuroborreliosis. The resulting antibody index, or AI, helps differentiate whether antibody present in CSF is due to intrathecal antibody as opposed to passive transfer across the blood-brain barrier (sensitivity, 75%; specificity, 97%).[28,29] In the absence of elevated control antibody index or albumin ratio, an elevated *B burgdorferi* antibody index indicates intrathecal production of *Borrelia* antibody production and strongly suggests neuroborreliosis. However, a negative result does not rule out CNS involvement.

DNA detection in CSF has a sensitivity of 38% (≥93% specificity) for neurologic Lyme disease.

Methods that detect *B burgdorferi* DNA may also be used to support the diagnosis of rheumatologic manifestations of Lyme disease. Positive DNA findings in synovial fluid support the diagnosis of Lyme arthritis (sensitivity, 78%; specificity,100%).[28] However, because spirochetemia is typically transient or absent, detection of *B burgdorferi* DNA in whole-blood specimens has low clinical sensitivity (14%), rendering negative results non-informative. For evaluation on rheumatologic manifestations, positive DNA results via PCR in a seropositive patient support the diagnosis of Lyme disease.[9] However, in a seronegative patient, positive results should be interpreted with caution.[9]

Tick-borne Rickettsial Diseases

Diagnosis of TBRDs is primarily clinical. However, laboratory testing can play important roles in distinguishing among these closely related diseases and in confirming infection.

Anaplasmosis and ehrlichiosis infections are characterized by infection of leukocytes, in which the causative agents multiply in cytoplasmic membrane-bound vacuoles called morulae.[30] *Anaplasma phagocytophilum* and *E ewingii* infect granulocytes, whereas *E chaffeensis* infects monocytes. Thus, visualization of morulae on a routine blood smear may provide the first clue for diagnosis and help differentiate HME from HGA and *E ewingii* infection. Positive results may be seen in up to 60% of patients with HGA and to a lesser extent in patients with HME.[5]

Routine laboratory tests are also useful in assessing patients suspected of having tick-borne illness, and can provide supportive evidence of specific illnesses; test results associated with the TBRDs are listed in **Table 6**.[5] For example, patients with HGA often present with slightly decreased platelet and WBC counts and elevated liver enzymes. Although such abnormalities are suggestive of HGA, these markers tend to stabilize over time. Therefore, normal levels do not rule out HGA, especially in patients who have had symptoms for more than 1 week.[32] Patients with early RMSF often have normal or slightly altered laboratory values. Band neutrophil counts may be increased; because such band increases are uncommon in viral infections, they can be helpful in differential diagnosis. Markers of tissue injury may arise later during the disease course.

Laboratory confirmation validates the accuracy of the presumptive clinical diagnosis and is important from an epidemiology and public health perspective. Confirmatory laboratory testing for TBRDs includes serology and nucleic acid testing **(Table 6)**.[5] IFAs are considered the gold standard for TBRD serology testing.[5] A 4-fold rise in titer of IgG or IgM in paired acute and convalescent samples collected 2 to 3 weeks apart is essential to confirm acute infection. For RMSF, IgG and IgM increase concurrently; IgM wanes after 3 to 4 months, whereas IgG persists for 7 to 8 months.

Note: although most patients have positive IgG or IgM antibody by the second week of illness, many people will be seronegative at the time of the first test (especially if done within the first week or so of illness). Therefore, negative results on serologic tests should not lead to discontinuation of therapy.

Detection of DNA in whole blood is especially useful for confirming HGA, HME, and *E ewingii* infection because these organisms infect circulating leukocytes. For RMSF, detection of *R rickettsii* in blood is more likely in advanced disease or fulminant infection. Whereas positive results confirm TBRD, negative results do not exclude the diagnosis.

Tick-borne Non-Rickettsial Diseases

Babesiosis

The current case definition for babesiosis requires the presence of clinical evidence (fever, anemia, or thrombocytopenia) and/or at least 1 subjective symptom (chills, sweats, headache, myalgia, or arthralgia). Laboratory confirmation of infection may

include microscopic identification or nucleic acid amplification detection of *Babesia* species DNA **(Table 7)**.[9,17,18,21,32] Serologic studies may provide supportive laboratory evidence **(Table 7)**.[9,17,18,21,32] Specific *B microti* antibodies are usually present by the time the patient exhibits parasitemia and invariably within 4 weeks of onset, unless the patient is immunocompromised.

Additional laboratory abnormalities may include hemolytic anemia with an elevated reticulocyte count, thrombocytopenia, proteinuria, and elevated levels of liver enzymes, blood urea nitrogen, and creatinine.

Laboratory test results, combined with clinical symptoms, are used to make treatment decisions.[9] Treatment is

Table 6. Laboratory Confirmation of Tick-borne Rickettsial Diseases[5]

Disease	Common Laboratory Abnormalities	Confirmatory Laboratory Tests	Laboratory Criteria for Confirmation of Diagnosis
Rocky Mountain spotted fever (*Rickettsia rickettsii*)	• WBC count N or slight ↑ • Immature neutrophils ↑ • Platelet count ↓ • Sodium ↓ • Transaminases slight ↑	Acute and convalescent serology *or*	4-fold increase in antibody titer
		R rickettsii DNA	Detected
Rickettsia parkeri rickettsiosis	• WBC count ↓ • Platelet count slight ↓ • Transaminases slight ↑	Acute and convalescent serology *or*	4-fold increase in antibody titer
		Rickettsia philipii (364D) DNA	Detected
Rickettsia philippi (364D) rickettsiosis	• Not documented	Acute and convalescent serology *or*	4-fold increase in antibody titer
		R parkeri DNA	Detected
Human granulocytic anaplasmosis	• WBC count ↓ in ≤53% • Platelet count ↓ • Transaminases ↑	Acute and convalescent serology *or*	4-fold increase in antibody titer
		A phagocytophilum DNA *or*	Detected
		Identification of morulae in WBCs and serology	Morulae detected and positive antibody titer
Human monocytic ehrlichiosis	• WBC count ↓ in ≤53% • Platelet count ↓ in ≤94% • Transaminases ↑ (2–8 times ULN) • Sodium ↓ • Anemia	Acute and convalescent serology *or*	4-fold increase in antibody titer
		E chaffeensis DNA *or*	Detected
		Identification of morulae in WBCs and serology	Morulae detected and positive antibody titer
Ehrlichia ewingii ehrlichiosis	• WBC count ↓ • Platelet count ↓ • Transaminases ↑	Acute and convalescent serology *or*	4-fold increase in antibody titer
		E ewingii DNA	Detected
Ehrlichia muris-like (EML) agent ehrlichiosis	• WBC count ↓ • Platelet count ↓ • Lymphocytes ↓ • Transaminases ↑ • Anemia	Acute and convalescent serology *or*	4-fold increase in antibody titer
		EML DNA	Detected

N, normal; ↑, increased; ↓, decreased; ULN, upper limit of normal.

recommended in symptomatic patients when babesial parasites have been identified in peripheral blood smears or when DNA results are positive.[9] Treatment is not recommended in symptomatic patients whose blood is negative for babesial parasites or DNA, even if serology testing is positive. Also, treatment is not recommended for asymptomatic individuals, regardless of laboratory test results. Asymptomatic individuals with positive babesial smears and/or DNA results should have these tests repeated, and treatment should be considered if repeat testing is positive >3 months later.

Tularemia

Various methods are available to assist in the diagnosis of tularemia. Positive results for *F tularensis* using direct agglutination assay provides presumptive evidence. Culture of *F tularensis* from appropriate sites provides definitive evidence of tularemia, but requires biosafety level 3 precautions. *Francisella tularensis* serology testing is the primary laboratory approach to confirm a diagnosis of tularemia **(Table 7)**.[19] A 4-fold increase in antibody titer between acute and convalescent sera (collected at least 4 weeks after onset) is considered diagnostic.[19]

Borrelia miyamotoi Disease (BMD)

PCR amplification of *B miyamotoi* DNA is part of an acute molecular panel of tests used to confirm a presumptive diagnosis of BMD based on clinical presentation. CSF, synovial fluid, whole blood or urine are acceptable specimen types for analysis. Detection of *B miyamotoi* DNA in suspected

Table 7. Laboratory Confirmation of Tick-borne Non-Rickettsial Diseases[9,17-21,32,33]

Disease	Common Laboratory Abnormalities	Confirmatory Laboratory Tests	Laboratory Criteria for Confirmation of Diagnosis
Babesiosis[a]	• Hematocrit ↓ • Reticulocyte count ↑ • Platelet count ↓ • Transaminases ↑ (±)	Light microscopy of stained blood smears (Giemsa, Wright, or Wright-Giemsa) *or*	Identification of *Babesia* organisms in RBCs
		Nucleic acid amplification of *Babesia* DNA *or*	Detected
		Isolation of *Babesia* organisms by animal inoculation from whole blood	Isolated
Tularemia	• Transaminases ↑ (±)	Acute and convalescent serology	4-fold increase in antibody titer
Borrelia miyamotoi disease	• WBC count ↓ • Transaminases ↑ • Platelet count ↓	*B miyamotoi* DNA (PCR)	Detected
Colorado tick fever	• WBC count ↓ • Platelet count ↓ (±)	Acute and convalescent serology	4-fold increase in antibody titer; positive IgM antibody titer
Tick-borne relapsing fever	• Platelet count ↓	Acute and convalescent serology	4-fold increase in antibody titer

IFA, immunofluorescence assay; PCR, polymerase chain reaction; IHC, immunohistochemistry; ULN, upper limit of normal; ↑, increased; ↓, decreased; ±, may be present.

[a] Demonstration of at least 1 of the following provides supportive but not confirmatory laboratory evidence of babesiosis: *B microti* total Ig or IgG antibody titer ≥1:256 by IFA (≥1:64 for epidemiologically-linked blood donors or recipients); *B microti* IgG by immunoblot; *B divergens* total Ig or IgG titer ≥1:256 by IFA; or *B duncani* total Ig or IgG ≥1:512 by IFA.[22]

tick specimens is supportive for the diagnosis of infection. Guidelines also suggest serologic testing to confirm the diagnosis of BMD.[34] Positive results on serology by ELISA or IgM and IgG immunoblot, however, may indicate coinfection by *Borrelia* species such as *B hermsii* and/or *B burgdorferi*. Negative results do not necessarily rule out infection and may be due to testing prior to seroconversion during the acute phase of infection.[35]

Colorado Tick Fever (CTF)

Leukopenia is characteristically seen in a CBC, and thrombocytopenia may be present. Acute and convalescent serology should be considered for patients with clinically suspected Colorado tick fever **(Table 7)**.[20] A 4-fold rise of IgG or IgM titer in paired acute and convalescent samples confirms the diagnosis; the detection of IgM indicates acute infection.

Tick-borne Relapsing Fever (TBRF)

Diagnosis of TBRF is made by the detection of spirochetes in the patient's blood during periods of high fever (sensitivity ~70%).[10] The diagnosis is confirmed by serology testing. The presence of *B hermsii* IgM titers ≥1:16 are associated with acute infection, while IgG titers ≥1:64 reflect later stages of disease. Single IgG titers ≥1:64 are considered presumptive evidence of infection and a 4-fold increase in titer between acute and convalescent sera provides evidence of recent or current infection **(Table 7)**.[33] Because other *Borrelia* and *Treponema* species cross-react in the IFA test, positive specimens should be tested for antibodies to these organisms.

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