MEDICAL DIAGNOSTIC LABORATORIES, L.L.C.

Vector-Borne Disease

ROCKY MOUNTAIN SPOTTED FEVER

BABESIOSIS

WEST NILE VIRUS

LA CROSSE VIRUS

MYCOPLASMAS

BARTONELLA

LYME DISEASE

EHRLICHOSIS
Rocky Mountain Spotted Fever (RMSF) is a vector-borne disease transmitted through the bite of *Dermacentor variabilis*, dog ticks, in the eastern United States and *Dermacentor andersoni* ticks in the Rocky Mountain region that are infected with the bacterium, *Rickettsia rickettsii* (4, 10). First recognized in 1896 by a United States Army physician in Boise, Idaho, RMSF has been a reportable disease since the 1920’s. The name Rocky Mountain Spotted Fever stems from the fact that all of the initial cases were clustered within mountainous states. The genus and species, *Rickettsia rickettsii*, were named after Howard Ricketts who identified it as the pathogen and determined the route of transmission in 1906 (3, 11). In the United States, RMSF is the most frequent cause of fatal vector-borne disease (11).

The genus *Rickettsia* is comprised of more than 20 different species, not all of which have been associated with human disease. Those species that are capable of infecting humans are categorized within the spotted fever and typhus groups. *R. rickettsii* is a relatively small, gram negative coccobacillus that enters the host during the blood meal of a tick and establishes itself within endothelial cells that line small and medium-sized blood vessels. As the bacteria replicate, they damage and induce the death of these infected cells, allowing for the creation of small holes that allow blood to leak from the blood vessels to the neighboring tissue. It is this process that results in the petechial rash characteristic of RMSF (Figure 1). Pathophysiological effects that occur as a result of this increased vascular permeability include edema and hypotension which adversely affect the lymphatic, circulatory, gastrointestinal, and respiratory systems (11).

**Symptoms**

The incubation period for *R. rickettsii* before the symptoms begin to emerge is typically five to ten days following a tick bite. The classical presentation of RMSF is the fulfillment of three criteria: fever, rash, and history of tick bite. Unfortunately, this classical symptomatic triad is often not manifested in the early stages of infection when medical attention is usually sought. A recent study reports that only 18% of patients present with the symptomatic triad upon initial examination and only 49% of all RMSF cases had memory of a tick bite (2). During the early stages, the presenting symptoms are non-specific and overlap with several other infectious and non-infectious diseases making a definitive diagnosis of RMSF difficult, even for physicians who have had prior experience with the disease.

Infectious symptoms vary at different stages of infection. During the early stages, symptoms are typically non-specific, consisting of nausea, headache, fever, vomiting, lack of appetite and muscle pain. The prototypical petechial rash usually emerges 2 to 5 days following onset of fever, beginning on the palms and soles of the feet. At this stage, the rash is not fulminant, appearing as small, flat, non-itchy spots on the wrists and ankles, and can easily be overlooked. In children, the rash usually emerges sooner than in adults. Later stage infections are characterized by an obvious petechial rash in 50% to 80% of patients that may be limited to the palms and soles of feet, as well as abdominal and joint pain and diarrhea. Though highly curable, if left untreated the clinical outlook and long-term symptomology of RMSF can become quite severe or even fatal (1, 7).

**Endemic Regions**

While the name suggests a localized endemic area confined to the Rocky Mountain states, in actuality RMSF is more prevalent within areas of the United States outside of the

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**Figure 1.** The petechial rash characteristic of late-stage RMSF infection (3).

**Figure 2.** Reported RMSF incidence rates by state. Adapted from the CDC (3).
mountain states and incidences have also been reported in both Central and South America. The CDC reports that the number of cases from all of the Rocky Mountain states combined account for less than 3% of all reported cases during the years spanning 1993 to 1996. During that same time, more than half of all cases were found to have originated within the south Atlantic (Delaware, Maryland, Washington DC, Virginia, West Virginia, North Carolina, South Carolina, Georgia and Florida) region of the United States (Figure 2) (3). The Pacific (Washington, Oregon and California) and west south central (Arkansas, Louisiana, Oklahoma and Texas) portions of the United States also experienced significantly higher incidences of RMSF cases than did the Rocky Mountain states. Infections have also been reported in Argentina, Brazil, Colombia, Costa Rica, Mexico and Panama and are often referred to locally by the names tick typhus and Tobia fever (Colombia), Sao Palo fiebre and fever maculosa (Brazil), and fiebre manchada (Mexico) (3).

Incidence Rate/At-Risk Populations
The annual incidence of RMSF has steadily risen from 1997 through 2002 (Figure 3) (3). Also, the CDC reported an average of 250 to 1,200 cases have occurred each year for the past fifty years, with more cases assumed to go unreported (3). This increased rate is attributed, at least in part, to increased exposure to dogs and/or living near wooded areas with tall standing grass. RMSF infections demonstrate both gender and racial predilections, with males experiencing higher rates of infection than females and Caucasians affected at higher rates than any other ethnic group. Statistics have also shown that infections occur more frequently in children under the age of 15 years, with the peak age range from 5 to 9 years (Figure 4) (3).

Laboratory Detection
Despite the ability to rapidly confirm the presence of R. rickettsii, it is a difficult and highly specialized technique that is seldom performed (3, 11). Rather, diagnoses are preferably made based upon routine laboratory findings in conjunction with epidemiological and environmental clues (3). Routine blood tests revealing abnormal white blood cell count, thrombocytopenia, hyponatremia or elevated liver enzyme levels are suggestive of RMSF infection (5). The Immunofluorescence Assay (IFA) is the most routine laboratory test in use today for the detection of either IgM or IgG-specific antibodies (6, 8). This test requires the use of both acute and convalescent sera whereby an increase in antibody titer would be indicative of infection (3). However, Polymerase Chain Reaction (PCR) remains the most reliable, sensitive and rapid means of R. rickettsii detection, capable of detecting as few as 10 R. rickettsii genomic equivalents in a biological specimen (3). Medical Diagnostic Laboratories, L.L.C. offers a highly sensitive and specific Real-Time PCR assay for the detection of RMSF DNA with a typical turnaround time between 24 and 48 hours.

Treatment
Fatality rates drop markedly, from 20% to 5%, when antibiotic treatment is initiated immediately upon suspicion of RMSF and should not be delayed until laboratory confirmation is obtained (3). The drug of choice for both children and adults is doxycycline. The duration of treatment is generally 5 to 10 days but some cases may require extended periods, with therapy continuing for at least three days following a break in fever (3). In the instances of pregnant women, chloramphenicol can serve as a suitable alternative treatment as tetracycline drugs are not recommended (9).

References:
3. CDC 2005, posting date. Rocky Mountain Spotted Fever. [Online.]
Babesiosis is caused by an intra-erythrocytic parasite, *Babesia microti*, and is one of the most common parasitic infections of free-living animals worldwide, with a prevalence rate second only to trypanosomal infections (13). The pear-shaped, plasmodium-like organisms were first detected by Victor Babes in cattle suffering from febrile hemoglobinuria in 1888 (2). Since then, nearly 100 species within the genus *Babesia* have been described, of which several are capable of infecting humans. Human babesiosis is a life-threatening, emergent tick-borne disease, caused primarily by *Babesia microti* in the United States, though human infections with *B. gibsonii*, *Babesia* WA1 and CA1 have also been reported (13). The prevalence of *B. microti* infection varies according to geographical region. Over the past thirty years, the United States has had an increasing number of confirmed babesiosis cases, presumably due to an increase in the number of deer compounded by an increase in outdoor activities. An endemic of babesiosis was recently confirmed by the Centers for Disease Control and Prevention within the state of New Jersey (1, 4, 8, 14). Significant numbers of confirmed cases have also been reported in Washington, California, Missouri and Connecticut. In contrast to the frequent reports of babesiosis in the United States, there are only sporadic reports of this disease in Europe, with seroprevalences ranging between 4% and 13% (6, 10, 11, 18).

Pathology
In order to complete their life cycle, Babesia must replicate inside erythrocytes. Several surface attached molecules have been implicated in the attachment to, and subsequent invasion of, circulating erythrocytes. After successful entrance, the parasite enters the asexual reproductive blood stage for multiplication which induces changes in the erythrocytic membrane and subsequently induces cell lysis and can result in severe anemia (3, 16, 21). Altered erythrocytic membranes also result in the retention and subsequent destruction of erythrocytes by the spleen and an expansion of the B cell population, leading to splenomegaly (12). Neutrophils, activated monocytes and CD4+ T cells are also increased following infection with *B. microti*.

Transmission
White-footed mice, meadow voles and deer all serve as the main vertebrate reservoirs for *B. microti* (9). During a blood meal on these hosts, the black-legged tick, *Ixodes scapularis*, acquires the organism. Later in the life cycle of the tick, *I. scapularis* transmits *B. microti* while taking another blood meal, thereby transmitting the pathogen to a new, possibly human, host. Although humans are dead-end hosts with little probability for subsequent transmission, human-to-human transmission via blood transfusion has been observed (8,17).

Symptoms
Babesiosis is generally benign and asymptomatic. Symptomatic babesiosis typically develops 1 to 6 weeks after a tick bite, but can take up to 3 months to manifest. Infections are dependent upon the level of parasitemia, with low level parasitemias often asymptomatic. Clinical presentation encompasses a broad spectrum of symptoms, characterized by fever, chills, headache, occasional joint pain, abdominal pain and dark urine caused by the hemolysis of red blood cells. Advanced age, immunosuppression and co-infection with other tick-transmitted pathogens can also cause more severe manifestations (15). In splenectomized patients, babesiosis is an acute, febrile, sometimes fatal hemolytic disease resembling more closely the disease observed in Europe.

Diagnosis
Laboratory methods for babesiosis detection include direct detection of the pathogen within patient erythrocytes, inoculation of small mammals with patient blood, serological detection of Babesia-specific antibodies and proteins within patient blood by Immunofluorescent Antibody Assay (IFA), Enzyme-Linked Immunosorbent Assay (ELISA) and detection of Babesia-specific DNA by Polymerase Chain Reaction (PCR) (5, 7, 19, 20).

Direct Detection
The specific diagnosis of babesiosis is based on the visualization of the parasite within Giemsa stained red blood cells (RBCs). Both thick and thin blood smears may be necessary as parasitemia usually varies only from 0.1% to 10% of total RBCs, despite the fact that parasite loads of up to 85% have been reported. The parasites resemble the malaria parasite, Plasmodium, therefore, necessitating the need for an experienced clinician to differentiate between the two. The Maltese cross formation of Babesia in RBCs is virtually pathognomonic for the disease, but is rarely seen in clinical smears. Acridine orange dye is also used to

![Figure 1: Distribution of Ixodes ticks within the United States (22).](image-url)
Inoculation of Small Mammals

Blood specimens from a suspected patient may be used to inoculate gerbils or hamsters intraperitoneally. The parasite will be detected in the animal’s blood within 1 to 3 days post inoculation. Babesia cannot be propagated in artificial media.

Immunofluorescent Antibody Assays (IFA) are widely used for the serologic detection of IgM and IgG antibodies to *B. microti* due in large part to their high degree of specificity and sensitivity. However, the procedure is both expensive and time consuming and requires specialized equipment. An IgG titer of 1:64 or greater, or a 4-fold rise in titer in specimens taken a few weeks apart, is considered positive, but some physicians and researchers have argued fewer false positives result when IgG titers of 1:128 are employed. With respect to IgM titers, Krause et al. reported an IFA with a 1:32 titer cutoff as being 91% sensitive and 99% specific for babesiosis, demonstrating IFA to be a sensitive, specific and reproducible test for the diagnosis of acute babesiosis, which are particularly useful in the absence of a positive blood smear (15).

Enzyme Linked Immunosorobent Assays (ELISA) are used to evaluate the presence of Babesia microti-specific antibodies in patient sera through their interaction with immunogenic proteins adsorbed to a solid support. When a serum specimen is introduced to this system, any antigen-specific antibodies which may be present in the patient serum will bind to the antigen. Medical Diagnostic Laboratories, L.L.C. (MDL) offers a recombinant antigen-based Enzyme-linked Immunosorobent Assay (ELISA) for the detection of Babesia microti IgG/IgM by ELISA. For serological analyses, the patient’s sera must have an index value >1.1 to be considered positive; the index value is the ratio of the patient sample to a known calibrator. Clinicians may wish to draw an acute and convalescent serum specimen 3 to 4 weeks apart to monitor disease progression as an increase in IgG antibody to >0.5 EU is indicative of active infection. IgG antibody titers may persist for up to six years after an acute infection. Although persistence of antibody does not indicate active infection, levels of antibody decline less rapidly in patients who have persistent infection.

Real-Time PCR assays for the detection of Babesia microti and Babesia WA-1-specific DNA, as well as a single serological test for the detection of Babesia have been developed by MDL. These highly sensitive and specific Real-Time PCR assays are valuable for all patients suspected of primary babesiosis or possible co-infection with one or more tick-borne pathogens (*Borrelia burgdorferi*, *Ehrlichia*, *Anaplasma*, *Mycoplasma*, or *Bartonella*). Generally, a negative Babesia PCR result is returned if the sample is obtained following the initiation of antibiotic therapy. Therefore, patient samples should be taken prior to antibiotic therapy initiation. The continual return of a positive PCR is suggestive of persistent infection.

References:

**West Nile Virus (WNV)** is a member of the arboviridae, a designation arising from the contraction of “arthropod-borne virus” having no significance regarding phylogeny or classification. The term describes the mechanism by which these viruses are transmitted and maintained in nature, through the bite of a hematophagous arthropod. Mosquitoes and ticks transmit most medically important arboviruses and, in the United States, representatives from at least five virus families can be transmitted by biting arthropods. Because the viruses are transmitted by arthropods, arboviral diseases are usually reported during the warmer months in temperate climates of the world, but can be contracted during the winter months in milder climates. Disease transmission can occur year-round in the tropics. During the milder seasons of the year, or depending on the patients’ travel histories, testing for arboviruses should be included in laboratory diagnosis of cases compatible with arbovirus infections. The primary clinical manifestation of life-threatening arboviral disease in North America has been encephalitis, which can be caused by three mosquito-borne viruses: Western equine encephalitis virus (WEE), St. Louis encephalitis virus (SLE), and West Nile virus (WNV). More specifically, WNV is taxonomically categorized as a member of the genus Flavivirus, which is currently composed of seventy-three viruses, forty of which have been associated with human disease. Of these viruses, 34 are mosquito-borne, 17 are tick-borne, and 22 are zoonotic agents transmitted with no known vector. Transmitted via the bite of infected mosquitoes, the number of cases and geographical distribution of WNV has increased every year since it was first detected within the United States (Figure 1) (3).

**Pathogenesis**

As a member of the family Flaviviridae, WNV belongs to the Japanese encephalitis (JE) virus serocomplex, which includes JE virus, SLE virus, Alfuy virus, Koutango virus, Australia Kunjin virus (KUN virus), Murray Valley encephalitis virus, Cacipacore virus, and Yaounde virus (6). Major extraneural sites of flavivirus replication include connective tissue, skeletal muscle, myocardium, smooth muscle, and lymphoreticular tissues. The mechanism by which flavivirus particles cross the blood-brain barrier during natural infection remains uncertain. Pathologic changes observed in humans and experimental animals with flaviviral encephalitis include (a) neuronal and glial damage caused directly by viral injury and characterized by central chromatolysis, cytoplasmic leakage, cell shrinkage, and neuronophagia; (b) inflammation, including perivascular infiltration on small lymphocytes, plasma cells, and macrophages; (c) cellular nodule formation composed of activated microglia and mononuclear cells; and (d) cerebral interstitial edema. At the ultra-structural level, infection of neurons is characterized by marked proliferation and hypertrophy of rough endoplasmic reticulum (RER), accumulation of vesicular structure derived from the RER and containing virus particles, and progressive degeneration of the RER and Golgi apparatus (5).

**Clinical Features**

The incubation period is 1 to 6 days post transmission. The typical case is mild and is characterized by fever, headache, backache, generalized myalgia, and anorexia. The course of fever may be biphasic. Nonirritating rash, either roseolar or maculopapular, occurs in about half of cases, with onset occurring during the febrile phase or at the end of it, and principally involves the chest, back, and upper extremities. Rash may persist for up to a week and resolves without desquamation. Generalized lymphadenopathy is a common finding and pharyngitis and gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain) may occur. The disease runs its course in 3 to 6 days, followed by a rapid recovery (4). Children generally experience milder illness than adults. Infection may also result in aseptic meningitis in a small proportion of patients, especially in the elderly (1). Severe neurological disease due to WNV infection has occurred in patients of all ages (10). Medical treatment is largely supportive as there are currently no specific therapeutic options available for WN encephalitis in humans. There is also no vaccine available for the prevention of WNV infection in humans. Attempts to eradicate the spread of this virus have relied primarily on preventative public health measures, such as a reduction of mosquito vector density by spraying pesticides and by encouraging the use of personal insect repellents. Rapid and sensitive serological diagnostic assays for the surveillance of WNV infection are critical for the effective prevention and control of this disease.

**Diagnosis**

Diagnosis of WNV infection primarily relies upon a high clinical suspicion of exposure, such as unexplained encephalitis or meningitis in late summer or early fall, and the local presence of WNV enzootic activity. West Nile virus can be isolated and identified with an Immunofluorescent Antibody Assay (IFA). However, this procedure requires
Serological Detection of Antibodies

The most efficient diagnostic method is detection of antibodies to WNV in serum or cerebrospinal fluid (CSF). The conventional Hemagglutination Inhibition (HI) assay and IFA method have low sensitivities and are labor intensive (2). At the present time, ELISAs are the method of choice for the detection of antibodies to WNV. Overall, ELISAs have been proven to be highly sensitive and specific tools for rapid immunodiagnostics for more than two decades, and have been widely applied for different pathogenic agents (11, 14). ELISAs are relatively easy to operate and large numbers of samples can be processed simultaneously. An IgM antibody capture ELISA method has been reported for detection of antibodies to WNV in CSF and serum (2, 9, 12). This method uses a commercial source of anti-human IgM as the capture antibody. The virus-specific IgM antibody complex is incubated with WNV antigen and detected using labeled monoclonal antibodies to the virus. An ELISA method using the recombinant E protein of WNV as the coating antigen was reported and evaluated with six serum samples (13). As acknowledged by the authors, a more detailed analysis of its performance with larger numbers of samples is still required. The major deficit of the currently available ELISA methods is false positive reactions caused by shared and similar flaviviral antigenic structures. Individuals vaccinated with yellow fever or JE vaccines, and others infected with a related flavivirus such as SLE virus or Dengue virus, may also exhibit positive results when tested for WNV IgM antibodies with the currently available ELISA assays. This is a diagnostic concern especially in the southern United States, such as Florida and Louisiana, that are endemic to multiple flaviviruses (9).

Plaque Reduction Neutralization Test (PRNT)

The Plaque Reduction Neutralization Test (PRNT) is the most specific test for the arthropod-borne flaviviruses. Although the PRNT is the standard test for arbovirus serology, it is frequently time consuming, labor intensive, and expensive to perform. The Plaque Reduction Neutralization Test (PRNT) is the most highly sensitive and specific tools for rapid immunodiagnostics.

The diagnostic criteria for a case of WNV infection, in combination with a compatible clinical presentation, are: (1) a two-fold higher reactivity for WNV than SLE virus by the IgM antibody capture ELISA, (2) a four-fold higher neutralizing antibody titer for WNV than SLE virus or Dengue virus by the PRNT assay, and (3) a four-fold increase of the PRNT titer to WNV from the acute to convalescence phase of the disease (10, 12).

Real-Time PCR

Alternatively, a PCR-based diagnostic assay has been developed to detect viral nucleic acid. WNV can be isolated from the blood of as many as 38% of patients (77% when the specimen is taken on the first days of the illness). Viremia was detectable up to five days after onset although titers may be low (7, 10). At MDL, a Real-Time PCR assay was validated which amplifies the gene encoding the WNV envelope protein E, that is capable of detecting as few as one hundred copies to as many as one billion copies in each reaction in a quantitative capacity. The specificity of the assay was validated by verifying that no signal is observed when the DNA from more than forty known human pathogens is utilized as a template for amplification. Cross-referencing the primers and probes against the thousands of other bacterial, viral (including other Flaviviruses), fungal, and human sequences which have been deposited into the massive genetic database at the National Library of Medicine yielded no matches aside from West Nile virus. Such results are a testament to the theoretical, as well as experimental, specificity of the assay.

References:

La Crosse virus (LAC) was first identified as a human pathogen in 1960 when it was isolated from a four year old girl who died from meningoencephalitis in Wisconsin (2). LAC is a vector-borne disease transmitted through the bite of an infected *Aedes triseriatus* tree hole mosquito. Most infections tend to occur from July to September (4). The annual number of reported cases tends to remain relatively stable at approximately 70 cases reported each year (2). Between 1964 and 2006 there have been 3,439 cases, mainly LAC encephalitis, reported in 30 states (Figure 1). Due to the typically mild nature of LAC infection, it is believed to be greatly underreported.

### Pathogenesis

Of the California serogroups of Bunyavirus that occur in North America, the La Crosse virus is the most medically important. Due to the fact that the virus can infect the mosquito’s ovarian cells and oocytes, infection can occur in offspring of both sexes. This process allows for venereal transmission as well as the ability for the virus to persist during winter months. It is thought that the stability in the number of reported cases each year is due to the abundance of both vertebrate and vector hosts to perpetuate the virus cycles in nature, as well as the virus’ ability to persist through winter.

### Clinical Significance

The Bunyaviridae family are enveloped, negative-stranded RNA viruses divided into five genera, of which La Crosse (LAC) virus is a member of the California serogroup of viruses in the genus *Orthobunyavirus*. LAC virus is the causative agent of La Crosse encephalitis. Most LAC infections are subclinical. However, when symptoms are evident the onset is abrupt. LAC virus produces an acute encephalitis that begins with a mild fever and illness lasting on average 1 to 3 days and sometimes persisting for up to one week. Patients typically present with fever, chills, abdominal pain, and headache with or without photophobia. The length of incubation period for LAC is estimated to be one week. Although LAC is the major cause of pediatric encephalitis and aseptic meningitis in children and young adults in North America, the fatality rate is less than 1% (4). Although the fatality rate is very low, 10% to 15% of survivors of severe encephalitis suffer severe neurologic sequelae such as significantly lower I.Q. scores, increased incidence of attention-deficit-hyperactivity disorder (ADHD), and seizures (1, 3).

### Diagnosis

**Direct Culturing** of viruses is a time consuming practice that requires specialized equipment and methodologies. The fact that several weeks of culturing are often required for this process precludes its use as a diagnostic tool.

**Indirect fluorescent antibody (IFA) microscopy** is the most common antibody screening test for La Crosse virus. Commercially available IFA tests can be IgG or IgM-directed and used for the analyses of patient sera and cerebrospinal fluids. Although there is some serological cross-reactivity among related arboviral species, speciation can be determined. Although commercially available IgM and IgG tests are available, the fluorescent nature of the assay limits the broad-scale availability of the test and incorporates a level of user interpretation that may lead to fluctuations in reported results.

**Polymerase Chain Reaction (PCR) methods** have been developed for the detection of La Crosse virus including viral RNA, Real-Time PCR and Quantitative-PCR. They were determined to be more sensitive than IFA diagnostic methods (5). PCR-based technologies as a whole are typically faster and more sensitive than most diagnostic methods, requiring minimal levels of infection within the host for definitive detection, and allow for speciation among highly related pathogenic organisms.

### References:

**Mycoplasmas**, of the class Mollicutes, once thought to be viruses due to their ability to pass through filters that block the passage of ordinary bacteria due to a deformable membrane, are now known to be the smallest free-living, self-replicating, fastidious bacteria. Their lack of a cell wall also renders traditional cell-wall-active antibiotics useless. Mycoplasma species are ubiquitous in nature. They are found in a wide variety of insect, plant, and animal species including humans. In recent years, certain Mycoplasma species, including *Mycoplasma fermentans*, have been identified in blood-sucking arthropods including Ixodes ticks, the same ticks that transmit Lyme disease, Babesiosis, Ehrlichiosis and Bartonellosis. In one published study, 17.4% of 230 symptomatic residents in an endemic area for Tick-Borne diseases tested positive for Mycoplasma (*M. fermentans*) (3).

**Pathogenesis**

Mycoplasmas cause infection as extracellular parasites by attaching to the surface of ciliated and nonciliated epithelial cells. Although some species of Mycoplasma have specialized attachment organelles, all are extremely efficient at attaching to and penetrating target cells. Cytotoxicity is due in part to the elaboration of Hydrogen Peroxide. Mycoplasmas stimulate lymphocytes, natural killer cells, and monocytes/macrophages to produce cytokines and chemokines which also contribute to its pathogenesis (1). They may also cause cytolysis via an inflammatory response mediated through chemotaxis of mononuclear cells, upregulation or downregulation of inflammatory cytokines, or antigen-antibody reactions (2).

**Clinical Significance**

Growing evidence has demonstrated the association of *M. fermentans* as a co-factor in rheumatoid arthritis pathogenesis, progression of HIV, and in general as a cofactor for chronic disease progression (3). In patients with congenital antibody deficiencies, Mycoplasmas are the most common etiologic agent of septic arthritis and account for up to 40% of cases (4). Mycoplasmas are associated with infection of the human respiratory and urogenital tracts. They have been implicated in severe fulminant multisystem infections in both healthy people as well as in the immunocompromised such as those with acquired immunodeficiency syndrome (AIDS) (2). *M. fermentans* has been shown to cause severe adult respiratory disease and has been recovered from the throats of children with pneumonia (4). *M. fermentans* has also been detected in lesions in the liver, brain, spleen, lymph nodes and thymus.

**Diagnosis**

Due to the fact that isolation of Mycoplasma may take 10 days or more, traditional culturing methods for the detection of Mycoplasmas are slow and difficult. Although serological methods such as Immunofluorescent Antibody (IFA) tests and Enzyme-linked Immunosorbent Assays (ELISA) are available for some species of Mycoplasma, these laboratory detection methods are not currently available for the detection of *Mycoplasma fermentans*.

As a consequence, nucleic acid amplification techniques such as the Polymerase Chain Reaction (PCR) method have been developed, and are probably the detection method of choice (2). Medical Diagnostic Laboratories, L.L.C. offers testing via Real-Time PCR allowing for species specific identification of DNA from the following Mycoplasma species in blood: *M. fermentans, M. hominis, M. penetrans* and *M. pneumoniae*.

**References:**


Bartonella species, members of the class Alphaproteobacteria, are vector-transmitted, blood-borne, intracellular, gram negative bacteria that can induce prolonged infections in the host. A wide spectrum of diseases can be attributed to Bartonella infections in both immunocompetent as well as immunocompromised individuals. There are several species capable of causing infections in human: B. bacilliformis, B. clarridgeiae, B. elizabethae, B. henselae and B. quintana. Persistent infection in humans domestic and wild animals results in a substantial reservoir of Bartonella organisms in nature that can serve as a source for inadvertent infection in humans.

Transmission
All five of the aforementioned species of Bartonella are vector transmitted. Bartonella bacilliformis is transmitted through the bite of the sand fly, genus Lutzomyia (formerly Phlebotomus). Due to the fact that its vector is only found at certain elevations of the Andes Mountains, infection with B. bacilliformis is limited in distribution. Bartonella quintana is globally distributed due to the abundance of its vector, Pediculus humanis, the human body louse. Bartonella henselae is transmitted by several arthropod vectors including the cat flea (Ctenocephalides felis) as well as other types of fleas and ixodid and Dermacentor ticks. Bartonella clarridgeiae is also spread by the cat flea. Bartonella elizabethae is spread by the Oriental rat flea.

Clinical significance
Bartonella bacilliformis is the causative agent of Carrion’s disease also known as Oroyo fever or verruga peruana. Oroyo fever is an acute disease resulting from primary bacteremia 3 to 12 weeks after inoculation from the bite of a sand fly. Infection ranges from a mild febrile illness lasting only one week to severe abrupt infection which can be fatal without proper treatment. Typically infection is accompanied by high fever, chills, diaphoresis, headache and mental status changes. The destruction of red blood cells due to infection can result in profound anemia. The eruptive lesions of B. bacilliformis infection are known as verruga peruana. They are a late stage manifestation which becomes evident weeks to months after resolution of acute infection if proper antibiotic treatment was not initiated.

Bartonella henselae has been implicated as the primary causative agent of Cat Scratch Disease (CSD) by which is transferred from cat scratches or bites to people (2). It is the most commonly recognized manifestation of human infection with Bartonella. In the United States, approximately 25,000 cases are reported annually (9). Approximately 11% of CSD cases are atypical and symptoms can include granulomatous conjunctivitis, oculoglandular syndrome, tonsillitis, visceral granulomatous disease, encephalitis, and cerebral arteritis (8). Infection with Bartonella henselae can also result in bacillary angiomatitis, or endocarditis. Children and immunocompromised individuals are especially vulnerable to this bacterium.

In immunocompromised patients, including those who have been infected with HIV-1 and have developed AIDS, infection with B. henselae can present as bacillariy angiomatisis or peliosis hepatis and may also include visceral involvement (10). The U.S. Public Health Service and Infectious Diseases Society of America has recognized the risk of contracting Bartonellosis especially in immunocompromised HIV-1 infected individuals, and have published guidelines for cat ownership as feline-to-human transmission of B. henselae is the most commonly recognized route (11).

Cats serve as a major reservoir of B. henselae. Pathogen analysis of domesticated cats in the United States have estimated that approximately 28% are chronically infected with B. henselae with no obvious clinical symptoms (6). The high carriage rates in domestic cats, combined with the close proximity in which humans and cats live, increases the likelihood of human exposure to B. henselae.

In addition to cats, Bartonella spp. have been found in 39% of deer ticks (species: Ixodes scapularis) collected in Northern New Jersey (1). This information, in conjunction with a clinical case study in which patients were co-infected with Borrelia burgdorferi, the causative agent of Lyme Disease, and B. henselae, suggests that tick bites may serve as an additional method of B. henselae transmission (5).

Bartonella clarridgeiae is also known to cause asymptomatic infection in cats and can be spread by the cat flea. Infections have been associated with endocarditis and lymphocytic hepatitis.

Bartonella elizabethae is very rare but has been associated with bacteremia, endocarditis and neuroretinitis.

Bartonella quintana was first described in association with Trench fever also known as Wolhynia fever, Meuse...
fever, His-Werner disease, shin bone fever, shank fever, and quintain or five-day fever. Trench fever is characterized by repeated attacks of fever associated with headaches, chills, skin pain, and dizziness, recurring every 4 to 6 days. B. quintana is also known to be responsible for culture negative endocarditis and bacillary angiomatosis, which occur in both human immunodeficiency virus-infected and immunocompetent patients. Infection with B. quintana is often associated with poor sanitation or personal hygiene which may predispose one to contact with its vector, the human body louse. For this reason, more recently, infection with B. quintana has presented sporadically and in small clusters among homeless populations in North America and Europe.

**Diagnosis**

**Culturing** of Bartonella from blood samples is technically challenging and is a low-yield procedure. Recommended lengthy incubation periods cause traditional bacterial isolation procedures to rarely detect Bartonella species (9). As a result, culturing of Bartonella is not considered an effective and reproducible diagnostic procedure to detect Bartonella spp. infections.

**Indirect Immunofluorescence assay (IFA)** tests employing whole bacterial cells as the antigen, are currently the most widespread diagnostic tool for *B. henselae* detection. Although they may have high sensitivity they lack overall specificity due to cross-reactivity with other human pathogens, including Coxiella burnetii, Chlamydia species, Rickettsia rickettsii, Ehrlichia chaffeensis, Treponema pallidum, Francissella tularensis, and Mycoplasma pneumoniae (3, 4, 7). In addition, IFAs rely heavily on technicians for the determination of test results, are time-consuming to score, require expensive fluorescent microscopes, and are not quantitative.

**Enzyme-linked Immunosorbent Assays (ELISA)** are used to evaluate the presence of *B. henselae*-specific antibodies in patient sera through their interaction with immunogenic proteins adsorbed to a solid support. Medical Diagnostic Laboratories, L.L.C. (MDL) offers a recombinant antigen-based test for the detection of *Bartonella henselae* IgG/IGM by ELISA. MDL uses a recombinant *B. henselae* 17-kDa antigen (r17-kDa) assay for the detection of an IgG and IgM by Capture ELISA for detecting an antibody response during different phases of infection. This is the first demonstration of an ELISA for laboratory detection of early infection with *Bartonella henselae*. The high sensitivity and specificity achievable with this assay provides a diagnostic tool capable of distinguishing between patients with active or very recent infection and those with past exposure to this organism.

**Polymerase Chain Reaction (PCR)** assays for the detection of Bartonella have been developed by MDL. This assay amplifies the bacteria’s 16S rRNA gene which permits the detection of DNA from *B. henselae*, *B. quintana*, *B. bacilliformis*, *B. elizabethae*, and *B. claridgeiae* allowing for species-specific identification (2). PCR assays utilize sequence-specific primers that allow for the highly specific amplification of DNA sequences from minute amounts of target material.

**References:**


**Lyme Disease**

Lyme disease (LD), caused by infection with *Borrelia burgdorferi*, is the most common vector-borne disease in the United States, accounting for more than 95% of all reported vector-borne illnesses. Early in the 20th century, European physicians observed patients with a red, slowly expanding rash called erythema migrans (EM) (Figure 1) (1). They associated this rash with the bite of ticks and postulated that EM was caused by a tick-borne bacterium. Subsequent investigations of these and similar cases led to the discovery of Lyme disease and to the identification of the causative bacterium, *Borrelia burgdorferi* (Figure 2). From 2003-2005, 64,382 cases of Lyme disease were reported to the CDC from 46 states and the District of Columbia (2). However, a considerable level of underreporting is associated with Lyme disease and, therefore, the true number of infected individuals is probably greater. Of the reported cases, 59,770 occurred within ten endemic states: Connecticut, Delaware, Maine, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island and Wisconsin (2). Within these endemic states, the average annual rate of infection over this three year period was 29.2 cases per 100,000 population (2). Three counties, New York’s Columbia and Dutchess counties and Massachusetts’ Dukes County, were determined to have an incidence rate of 300 cases per 100,000 population (2). Evaluation of race and sex revealed a slightly higher incidence for males (54% of reported cases) and that 97% of the cases affect caucasians (2). Persons of all ages and both genders are equally susceptible, although the highest incidence of LD occurs in children ages 0-14 years and in persons 30 years of age and older (median, 28 years). Lyme borreliosis also occurs in temperate regions of the Northern Hemisphere in Europe (3), Scandinavia (4), the former Soviet Union (5), China (6) and Japan (7).

**The Organism**

*Borrelia* species are spirochetes, spiral-shaped organisms (Figure 2) (8). The bacteria are actively motile, which may allow the spirochetes to move out of the skin into the bloodstream and out of the bloodstream into tissues. Based on genotyping of isolates from ticks, animals, and humans, three pathogenic groups of *Borrelia burgdorferi* (senso lato) have been identified. In the United States, the sole cause of human infection is group one, *B. burgdorferi* (senso stricto). Group one can also be found in Europe and Asia. Although all three groups are found in Europe, the predominant groups are group 2 (*B. garinii*) and group 3 (*B. afzelii*). Only groups 2 and 3 have been associated with Lyme disease in Asia. Although closely related, the disease patterns observed in humans differ greatly depending upon the particular *Borrelia* species involved.

**Pathogenesis**

To accomplish a successful blood meal, a tick has to (i) attach to the host, (ii) impair local itching and pain responses to stay undetected, (iii) prepare the tissue for blood extraction, (iv) prevent blood clotting, and (v) suppress the local immune and inflammatory responses (9) and, as such, evolved strategies to overcome host hemostasis, pain and itch responses, inflammation, and immune defenses by secreting saliva, which contains a complex array of pharmacologically active molecules (1). After injection of the spirochete by the tick and an incubation period of 3 to 32 days, the spirochete usually...
first multiplies locally in the skin at the site of the tick bite. Several days later, the spirochete begins to spread in the skin and within days to weeks it may disseminate further. Initially, the immune response in Lyme disease seems to be suppressed (10), which may be an important mechanism in allowing the spirochete to disseminate. The antibody response to *B. burgdorferi* develops slowly, taking between three and six weeks post-infection to mount a specific IgM response (11). Despite the mounting of an immune response, *B. burgdorferi* may survive for years in untreated patients within the joints, nervous system, or skin; it is not yet known how it is able to sequester itself in these sites.

**Clinical Significance**

Lyme disease occurs in stages, with remissions, exacerbations and different clinical manifestations at each stage. Early infection consists of stage 1 (localized EM) ([Figure 1](#)) (1), followed by stage 2 (disseminated infection), and finally stage 3 (late infection or persistent infection). Stage 3 usually begins months or years after disease onset, sometimes following long periods of latent infection (12). The infection is highly variable among individual patients, ranging from brief involvement of only one system, to chronic multi-system involvement including the skin, nerves, and joints that could last for a period of years.

**Diagnosis**

Medical Diagnostic Laboratories, L.L.C. (MDL) offers three serology tests for the detection of antibodies to *Borrelia burgdorferi*, the causative agent of Lyme disease (LD) at all stages of infection:

**Indirect (Antibody Detection Tests)**

Tests are indirect by measuring exposure to a pathogen. A positive test is a function of the host immunological response to a foreign antigen. *B. burgdorferi* is the causative agent of Lyme disease. Direct tests are those that detect the entire bacterium (usually by culture or staining techniques) or parts of the bacterium such as cell wall proteins or nucleic acids, such as DNA or RNA. Because of the complex nature of the disease, no single test may be acceptable for all clinical situations.

**Enzyme-Linked Immunosorbent Assay (ELISA) screening test.** Enzyme-Linked Immunosorbent Assays evaluate the presence of *B. burgdorferi*-specific antibodies in patient sera through their interaction with immunogenic proteins adsorbed to a solid support. When a serum specimen is introduced to this system, any antigen-specific antibodies which may be present in the patient serum will bind to the antigen. The ELISA should only be ordered for patients who have signs and symptoms consistent with Lyme disease. Early antibody responses are often due to the gp41 flagellar antigen. Due to its cross-reactive components, a false positive reaction will often occur with syphilis patients or other patients with spirochetal diseases, such as leptospirosis or periodontal disease, since these treponemes also have similar gp41 flagellar antigens. This degree of cross-reactivity results in low specificity of the ELISA assay, leading the CDC to recommend that all positive or equivocal ELISAs be supplemented by a second-step assay, the Western blot.

**Western immunoblot (IgG, IgM) for *B. burgdorferi* antibodies.** In 1989, the Centers for Disease Control and Prevention (CDC) recommended that all indeterminate and positive ELISA tests be confirmed by Western blot (13). The Western blot is an antibody test which is capable of differentiating the immune response to a variety of *B. burgdorferi* antigens. The antigens are separated electrophoretically and then transferred, or “blotted”, to a nitrocellulose strip and exposed to patient serum. The presence of colored bands on the strips is indicative of an antigen/antibody reaction. Because many of the antigens that appear on the Western blot strip are shared by other organisms in addition to *B. burgdorferi*, the interpretation of the blot becomes critically important in determining whether the Western blot test is positive or negative. MDL provides physicians with results from both CDC criteria as well as our alternate criteria to aid in the interpretation of results.

**The C6 Lyme Peptide Antibody Test.** The *Borrelia burgdorferi* C6 peptide antibody test (C6 LPA) identifies antibodies to a newly discovered, conserved peptide called C6, a component of the variable surface antigen of *B. burgdorferi*. This Food and Drug Administration (FDA) approved test is a significant advancement over both ELISA and Western blotting because it is capable of differentiating IgM and IgG antibodies in patient serum resulting from LD infection from those occurring as a result of vaccination. The high degree of specificity was demonstrated through the screening of sera from patients with over a dozen different diseases, including systemic lupus erythematous, non-Lyme arthritis, syphillis, other spirochetal diseases, and other autoimmune diseases, that were uniformly C6 negative. Quantitative C6 LPAs administered over a period of time may be useful for monitoring a response to therapy as well as to determine whether the spirochete has been eliminated and can readily address the question of active vs. inactive Lyme as a positive C6 LPA is suggestive of active infection and a negative test, even with a positive routine ELISA, is indicative of inactive infection (14,15).

**Direct Tests (Molecular Detection via Real-Time PCR).** DNA detection by Real-Time PCR is available from MDL for whole blood, serum, urine, CSF, and synovial fluid samples. Four individual assays were developed to differentiate between the three *B. burgdorferi* sensu lato genospecies known to cause Lyme disease in humans, *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*, as well as the clinically related, but genomically distinct, *B. lomestari*. Like traditional PCR, real-time assays utilize sequence-specific primers that allow for the highly specific amplification of DNA sequences from minute amounts of target material.

**References:**

Human Ehrlichiosis is an emerging tick-borne infection that became reportable to the CDC in 1999 (3, 4). The term Ehrlichiosis is a general designation encapsulating several tick-borne etiologic agents based on their similar clinical presentations. Despite this, these obligate intracellular bacteria are both epidemiologically and etiologically distinct from one another. Recognized since 1935, Ehrlichial species were primarily viewed as veterinary pathogens, infecting sheep, goats, horses, cattle and dogs (7). In 1953, Neorickettsia sennetsu (N. sennetsu) was isolated in Japan from the first human infection and identification of the next human strain did not occur until 1986. At present, five strains capable of infecting humans have been identified and three [Anaplasma (Ehrlichia) phagocytophilum, Ehrlichia chaffeensis and Ehrlichia ewingii] have been clinically characterized. Neither E. canis nor N. sennestu, the remaining human strains, have been sufficiently investigated.

Figure 1. Increased incidence of HGE and HME infections within the United States from 1997-2005. Blue bars: HME, red bars: HGE and green bars: not specified (3).

Clinical Manifestation and Transmission
Ehrlichiosis is a seasonal disease and most cases occur between the months of April and September, coinciding with the peak of tick activity (4). Clinical presentation includes fever, headache, muscle aches and chills (3, 5). Hematologic findings include thrombocytopenia and leukopenia and liver enzymes may also be elevated. In the advanced stages of Ehrlichiosis, confusion and neurologic abnormalities may occur as well as liver, spleen, kidney, lymph node, bone marrow and respiratory involvement. Unlike some of the other rarely fatal tick-borne diseases, fatalities occur at a rate of 1% to 3% in patients with Ehrlichia, particularly within immunocompromised patients. Rashes are typically not seen in patients with Ehrlichiosis, thereby distinguishing such infections from Rocky Mountain Spotted Fever (RMSF) cases. The incidence of HME and HGE has been steadily rising, with 216 and 551 cases, respectively, reported in 2002 (Figure 1) (4). Infectious incidence parallels the geographical distribution of the Ixodes ticks within the United States, making HGE a reportable disease in 16 states (Figure 2) (7). Internationally, HGE has been reported throughout Europe, with 65 confirmed cases as of March 2003 (4).

Anaplasma phagocytophilum is the causative agent of Human Granulocytic Ehrlichiosis (HGE). This organism affects the granulocytic cells (neutrophils, basophils and eosinophils) within the white blood cell repertoire. This strain was identified in 1994 as the agent responsible for twelve cases of reported Ehrlichial disease in Minnesota and Wisconsin where two patients died as a result of secondary infections. HGE is transmitted by the black-legged ticks, Ixodes scapularis, which also transmit Lyme disease and babesiosis, and possibly by Ixodes pacificus ticks native to northern California. Infectious rates coincide with peak tick activity, with the summer and fall months having the highest incidence rates (Figure 3) (7). Though infections are typically mild, complications can arise within the immunocompromised. Peripheral neuropathies, including brachial plexopathy, demyelinating polyneuropathy, and isolated facial palsy, can occur and persist for weeks or months (3).

Ehrlichia chaffeensis (Human Monocytic Ehrlichiosis, HME) was originally isolated in 1986 from a 51 year-old man in Arkansas and again in 1990 from the blood of an American Army reservist from Fort Chaffee, Arkansas, hence its designation (6). Found primarily in the southeastern, southcentral and mid-Atlantic regions of the United States, E. chaffeensis is transmitted by the Lone Star tick, Amblyomma americanum, which is indigenous to those regions. Incidences of E. chaffeensis infection have occasionally been reported in the northeastern region of the United States as well. Immunocompromised individuals are at greater risk of developing a fulminant toxic or septic-like syndrome and approximately 20% of infected individuals will demonstrate CNS involvement in the form of meningitis or meningoencephalitis; this clinical presentation also serves to distinguish HME from HGE (3).

Figure 2. States where HGE is a reportable infection (green) overlap tick-endemic regions within the Unites States (REF).
**Ehrlichia ewingii** is closely related to *E. chaffeensis* and shares the Lone Star tick vector, *A. americanum*, as a host (1). Known to cause canine granulocytotropic ehrlichiosis, this organism is also capable of infecting humans. Transmission thus far has been reported only in Missouri, Oklahoma, and Tennessee.

**Diagnosis**

A number of laboratory methods are available for the detection of Ehrlichia, including direct detection, culturing, antibody testing, Western blotting and PCR.

**Direct Detection**

Ehrlichia can be directly detected in peripheral blood smears using Diff-Quik or Giemsa staining (Figure 4). Morulae (inclusions of the organism) may be detected in monocytes (HME) and granulocytes (HGE). Infection of the monocytes and granulocytes is usually sparse so that direct detection is time consuming and requires an experienced technologist. Approximately 15% of patients that have acute ehrlichiosis will be smear positive.

**Culture**

Direct isolation of the organism is the gold standard, but this task is also difficult and time consuming. Both the agents of HME and HGE have been isolated from the blood of patients with acute disease. Utilization of this technique is hampered by the need to culture aseptically in the absence of antibiotics, and is therefore limited to a few, very specific clinical laboratories. Growth is typically observed for HGE in 7 to 12 days and for the agent of HME in 7 to 36 days.

**Antibody Tests**

The most common antibody screening test for Ehrlichia is Indirect Fluorescent Antibody (IFA) microscopy (IFA) (2). IFA tests can be IgG or IgM-directed and specific for *E. chaffeensis* or *A. phagocytophilum*. Although there is some serological cross-reactivity among species, particularly *E. ewingii* and *E. chaffeensis*, speciation can be determined based upon the cell type infected, with *E. ewingii* infecting neutrophils and eosinophils and *E. chaffeensis* infecting monocytes.

Preferably, specimens for serology should be drawn 2 to 3 weeks apart. A positive result is defined as a 4-fold rise in titer although IgG titers of >1:64 may be considered diagnostic. IgM IFA testing is available and provides a rapid, early diagnostic test for Ehrlichiosis. Diagnostic antibody titers usually appear early in the disease, typically by the second week and IgG antibodies may persist for years after the patient is cured. Several groups have reported the development of ELISA antibody tests for HGE, but none are in routine use as yet.

**Western Blots**

Some research facilities use Western blots for confirmation of positive IFA tests. The experience with Western blotting for HGE has been better than for HME as the presence an antibody specific for a 44 kDa protein is highly specific for *A. phagocytophilum*.

**Nucleic Acid Amplification (PCR)**

The Polymerase Chain Reaction (PCR) for the detection of Ehrlichia/Anaplasma DNA was determined to be 67% to 90% sensitive when compared to tissue culture isolation methods (3). PCR-based assays are most useful in patients who have received minimal doses of an antibiotic as eradication of the organism usually results in a negative PCR result. The availability of several target species allows for direct speciation in a timely manner.

To aid in the diagnoses of vector-borne diseases, MDL offers a multiplex, Real-Time PCR assay for the detection and differentiation of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* species. The use of Real-Time PCR as a diagnostic tool affords the clinician a rapid and accurate means of diagnosis that will help direct the clinical course of action, as well as monitor its efficacy.

**References:**

5. Monica Gandhi, M., MPH 3-6-2006, posting date. Ehrlichiosis. [Online.]
Vector-Borne Testing Available...

*Babesia microti* by Real-Time PCR
*Babesia microti* IgG/IgM by ELISA
*Babesia WA1* by Real-Time PCR
*Bartonella henselae* IgG/IGM by ELISA
*Bartonella* Species by Real-Time PCR (*B. henselae* & *B. quintana*)
*Borrelia afzelii* by Real-Time PCR
*Borrelia garinii* by Real-Time PCR
*Borrelia lonestari* by Real-Time PCR
*Ehrlichia chaffeensis* (HME) &
*Anaplasma phagocytophila* (HGE) by Real-Time PCR
La Crosse virus by Real-Time PCR
*Lyme disease* (*B. burgdorferi*) DNA by Real-Time PCR
*Lyme disease* C6 Peptide by ELISA
*Lyme disease* IgG/IgM by ELISA
*Lyme disease* Western blot (IgG/IgM)
*Mycoplasma general* by Qualitative PCR
*Mycoplasma fermentans* by Real-Time PCR
*Rickettsia rickettsii* (RMSF) by Real-Time PCR
*West Nile virus* by Real-Time PCR