Testing for Mosquito- or Tick-Related Infections

Policy Number: AHS – G2158 – Testing for Mosquito- or Tick-Related Infections	Prior Policy Name and Number, as applicable: Portions of this policy replaces portions of M2097- Identification of Microorganisms using Nucleic Acid Probes
Effective Date: 08/01/2023	

I. Policy Description

Arthropod vectors, including mosquitoes, ticks, fleas, and mites, that feed on vertebrate hosts can spread bacteria, protozoa, and viruses during feeding to their susceptible host, resulting in a variety of infections and diseases. Arboviruses (arthropod-borne viruses) include Zika virus, West Nile virus (WNV), chikungunya virus, dengue virus (DENV), yellow fever virus (YFV), and Colorado tick fever virus (CTF) to name a few. Malaria and babesiosis are both conditions caused by arthropod-borne protozoan parasites, Plasmodium and Babesia, respectively. Conditions caused by arthropod-borne bacteria include rickettsial diseases, ehrlichiosis, anaplasmosis, and Lyme disease, as well as other Borrelia-associated disorders (Calisher, 1994; CDC, 2021a). Isolation, identification, and characterization of these various infections depend on the causative agent. Identification methods may include culture testing, microscopy, and staining techniques; moreover, molecular testing, such as nucleic acid amplification testing (NAAT), and serologic testing, including immunofluorescence antibody assays and enzyme-linked immunosorbent assays (ELISA), can be used for laboratory diagnosis (Miller et al., 2018).

For Lyme disease and testing for *Borrelia burgdorferi*, please see AHS-G2143 Lyme Disease.

For Zika virus testing, please see AHS-G2133 Zika Virus Risk Assessment.

II. Related Policies

Policy Number	Policy Title
AHS-G2133	Zika Virus Risk Assessment
AHS-G2143	Lyme Disease
AHS-M2097	Identification of Microorganisms Using Nucleic Acid Probes

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request.

1) For individuals suspected of having a rickettsial disease (see Note 1), the use of an indirect immunofluorescence antibody (IFA) assay for IgG antibodies (limited to two units) **MEETS COVERAGE CRITERIA.**

2) For individuals suspected of having a rickettsial disease (see Note 1), the use of standard blood culture, nucleic acid amplification testing (NAAT) (including PCR), **or** IFA assay for IgM antibodies **DOES NOT MEET COVERAGE CRITERIA.**

3) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 2), the use of NAAT (including PCR) of whole blood, IFA assay for IgG antibodies, **or** microscopy for morulae detection **MEETS COVERAGE CRITERIA**.

4) For individuals suspected of having ehrlichiosis and/or anaplasmosis (see Note 2), the use of an IFA assay for IgM antibodies **or** standard blood culture **DOES NOT MEET COVERAGE CRITERIA.**

5) For individuals suspected of having a tick-borne relapsing fever (TBRF) (see Note 3), the use of dark-field microscopy of a peripheral blood smear, microscopy of a Wright- or Giemsa-stained blood smear, PCR testing, **or** indirect immunofluorescence antibody (IFA) for IgG for *Borrelia* **MEETS COVERAGE CRITERIA**.

6) For individuals suspected of having a tick-borne relapsing fever (TBRF) (see Note 3), the use of an IFA assay for IgM for *Borrelia* **or** culture testing for *Borrelia* **DOES NOT MEET COVERAGE CRITERIA.**

7) For individuals suspected of having babesiosis (see Note 4), the use of a Giemsa- or Wright-stain of a blood smear **or** NAAT (including PCR) **MEETS COVERAGE CRITERIA.**

8) For individuals suspected of having babesiosis (see Note 4), the use of either an IgG or IgM IFA assay for Babesia **DOES NOT MEET COVERAGE CRITERIA.**

9) For individuals suspected of having malaria (see Note 5), the use of a rapid immunochromatographic diagnostic test **or** smear microscopy to diagnose malaria, determine the species of Plasmodium, identify the parasitic life-cycle stage, and/or quantify the parasitemia (can be repeated up to three times within three days if initial microscopy is negative in suspected cases of malaria) **MEETS COVERAGE CRITERIA.**

10) For individuals suspected of having malaria (see Note 5), the use of NAAT (including PCR) **or** IFA for *Plasmodium* antibodies **DOES NOT MEET COVERAGE CRITERIA.**

11) For individuals suspected of having chikungunya virus (see Note 6), the use of viral culture for diagnosis, NAAT (including PCR) for the presence of chikungunya in a serum sample, **or** IFA assay for IgM antibodies during both the acute and convalescent phases **MEETS COVERAGE CRITERIA.**

12) For individuals suspected of having West Nile Virus (WNV) (see Note 7), the use of IFA for WNV-specific IgM antibodies in either serum or CSF and a confirmatory plaque reduction neutralization test for WNV **MEETS COVERAGE CRITERIA.**

13) For individuals suspected of having WNV (see Note 7), the use of NAAT (including PCR) for WNV **or** IFA for WNV-specific IgG antibodies in either serum or CSF **DOES NOT MEET COVERAGE CRITERIA**

14) For individuals suspected of having yellow fever virus (YFV) (see Note 8), the use of NAAT (including PCR) for YFV **or** serologic assays to detect virus-specific IgM and IgG antibodies, as well as a confirmatory plaque reduction neutralization test for YFV, **MEETS COVERAGE CRITERIA**

15) For individuals suspected of having Dengue virus (DENV) (see Note 9), the use of NAAT (including PCR) for DENV, IgM antibody capture ELISA (MAC-ELISA), **or** NS1 ELISA, as well as a confirmatory plaque reduction neutralization test for DENV, **MEETS COVERAGE CRITERIA.**

16) For individuals suspected of having DENV (see Note 9), the use of IgG ELISA **or** hemagglutination testing **DOES NOT MEET COVERAGE CRITERIA.**

17) For individuals suspected of having Colorado tick fever (CTF) (see Note 10), the use of virus-specific IFA-stained blood smears **or** IFA for CTF-specific antibodies **MEETS COVERAGE CRITERIA.**

NOTES:

Note 1: Typical signs and symptoms of rickettsial diseases (including Rocky Mountain spotted fever, *Rickettsia parkeri* rickettsiosis, *Rickettsia* species 364D rickettsiosis, *Rickettsia* spp (mild spotted fever), and *R. akari* (rickettsialpox)) usually begin 3 – 12 days after initial bite and can include (Biggs et al., 2016):

- Fever
- Headache
- Chills
- Malaise
- Myalgia
- Nausea
- Vomiting
- Abdominal pain
- Photophobia
- Anorexia
- Skin rash
- Ulcerative lesion with regional lymphadenopathy (for *Rickettsia* species 364D rickettsiosis)

Note 2: Typical signs and symptoms of ehrlichiosis and/or anaplasmosis usually begin 5-14 days after an infected tick bite, and they include (Biggs et al., 2016):

- Fever
- Headache
- Malaise
- Myalgia
- Shaking chills
- Gastrointestinal issues, including nausea, vomiting, and diarrhea, in ehrlichiosis

Note 3: Typical signs and symptoms of tick-borne relapsing fever (caused by *Borrelia hermsii, B. parkerii, B. mazzottii,* or *B. turicatae*) include (CDC, 2018c):

- Recurring febrile episodes that last approximately 3 days separated by approximately 7 days
- Nonspecific symptoms that occur in at least 50% of cases include headache, myalgia, chills, nausea, arthralgia, and vomiting

Note 4: Typical signs and symptoms of babesiosis can include (CDC, 2019):

- Hemolytic anemia
- Splenomegaly
- Hepatomegaly
- Jaundice
- Nonspecific flu-like symptoms such as fever, chills, body aches, weakness, and fatigue

Note 5: Typical signs and symptoms of malaria can include (Arguin & Tan, 2019):

- Fever
- Influenza-like symptoms such as chills, headache, body aches, and so on
- Anemia
- Jaundice
- Seizures
- Mental confusion
- Kidney failure
- Acute respiratory distress syndrome

Note 6: Typical signs and symptoms of chikungunya include (Staples et al., 2020):

- High fever (>102˚F or 39˚C)
- Joint pains (usually multiple joints, bilateral, and symmetric)
- Headache
- Myalgia
- Arthritis
- Conjunctivitis
- Nausea
- Vomiting
- Maculopapular rash

Note 7: Typical signs and symptoms of WNV include (Nasci et al., 2013):

- Headache

- Myalgia
- Arthralgia
- Gastrointestinal symptoms
- Maculopapular rash
- Less than 1% develop neuroinvasive WNV with symptoms of meningitis, encephalitis, or acute flaccid paralysis

Note 8: Typical signs and symptoms of yellow fever include (Gershman & Staples, 2019):

- Nonspecific influenza like syndrome including fever, chills, headache, backache, myalgia, prostration, nausea, and vomiting in initial illness
- Toxic form of disease symptoms includes jaundice, hemorrhagic symptoms, and multisystem organ failure

Note 9: Typical signs and symptoms of dengue can include (CDC, 2021e):

- Fever
- Headache
- Retro-orbital eye pain
- Myalgia
- Arthralgia
- Erythematous maculopapular rash
- Petechiae
- Leukopenia
- Nausea and/or vomiting

Note 10: Typical signs and symptoms of CTF can include (CDC, 2021b):

- Fever
- Chills
- Headache
- Myalgia
- Malaise
- Sore throat
- Vomiting
- Abdominal Pain
- Maculopapular or petechial rash

IV. Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
ASM	American Society for Microbiology
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare and Medicaid Services
CSF	Cerebrospinal fluid
CTF/CTFV	Colorado tick fever /virus
CV	Coefficient of variation
DENV	Dengue virus

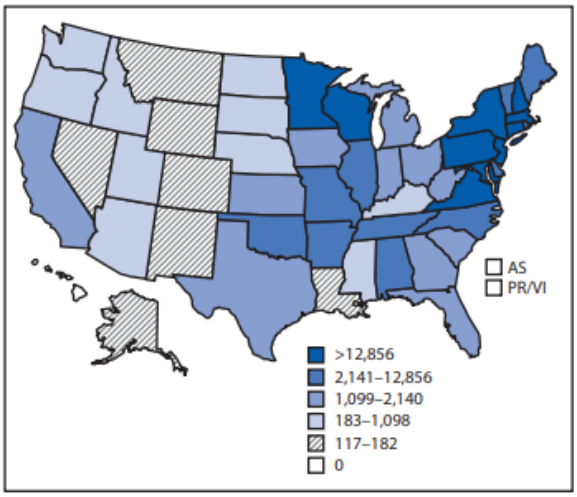
Term	Definition
DENV NS1	Dengue virus nonstructural protein 1
DHF	Dengue hemorrhagic fever
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assays
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescent in situ hybridization
GlpQ	Glycerophosphodiester phosphodiesterase gene
HAI	Hemagglutination inhibition test
IDSA	Infectious Diseases Society of America
IEC	International Encephalitis Consortium
IFA	Indirect immunofluorescence antibody
IFAs	Immunofluorescence assays
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IMCA	Immunochemiluminometric assay
LBRF	Louse-borne relapsing fever
LDTs	Laboratory developed tests
MAC-ELISA	IgM antibody capture enzyme-linked immunosorbent assay
MIF	Microimmunofluorescent
NAAT	Nucleic acid amplification testing
NDPH	New daily persistent headache
NNDSS	National Notifiable Disease Surveillance System
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
PRNTs	Plaque reduction neutralization tests
PT	Prothrombin time
PTT	Partial thromboplastin time
qPCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic testing
RMSF	Rocky Mountain spotted fever
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction
SFG	Spotted fever group
TBRF	Tick-borne relapsing fever
WHO	World Health Organization
WNV	West Nile virus
YFV	Yellow fever virus

V. Scientific Background

Hematophagous arthropods, such as mosquitoes, ticks, fleas, and mites, can spread opportunistic bacteria, protozoa, and viruses to host organisms when feeding. Numerous outbreaks of arthropod-borne disease have been documented, including plague, an acute febrile disease caused by *Yersinia pestis* through the bite of infected fleas, which resulted in more than 50 million deaths in Europe alone during the "Black Death" outbreak. More than 3000 cases of plague were reported to the World Health Organization (WHO) between 2010 and 2015 with 584 deaths. Today, most cases of plague occur in the Democratic Republic of Congo, Madagascar, and Peru (WHO, 2017).

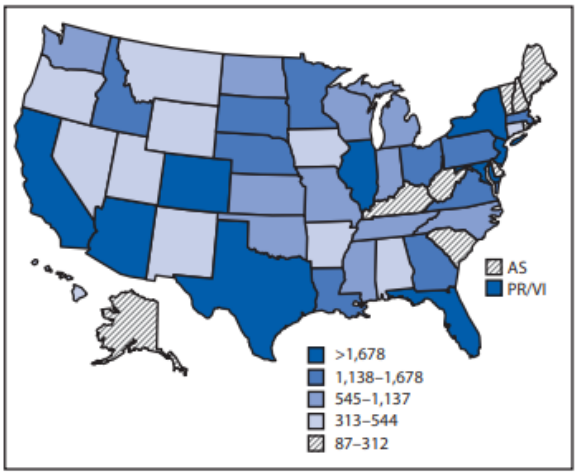
The Centers for Disease Control and Prevention (CDC) reported a large increase in the number of vector-borne diseases within the United States and its territories between 2004-2016. More than 640,000 cases were reported during that time; in fact, infections of tick-borne bacteria and protozoa more than doubled from 2004 to 2016. "In the United States, 16 vectorborne diseases are reportable to state and territorial health departments, which are encouraged to report them to the National Notifiable Disease Surveillance System (NNDSS). Among the diseases on the list that are caused by indigenous pathogens are Lyme disease (*Borrelia burgdorferi*); West Nile, dengue, and Zika virus diseases; plague (*Yersinia pestis*); and spotted fever rickettsioses (e.g., *Rickettsia rickettsii*). Malaria and yellow fever are no longer transmitted in the United States but have the potential to be reintroduced" (Rosenberg et al., 2018). New vector-borne infections are emerging; for example, two unknown, life-threatening RNA viruses spread by ticks have been identified in the U.S. since 2004. Although both tick- and mosquito-borne diseases are increasing across the U.S., the CDC reports that these two vectors are showing different trends. The mosquito-borne diseases are characterized by epidemics; for example, West Nile Virus is essentially limited to the continental U.S. but has spread rapidly since its introduction to New York in 1999, whereas chikungunya and dengue primarily occur within the U.S. territories. On the other hand, the tick-borne disease increase occurs in the continental U.S. and has experienced a gradual, steady rate increase with Lyme disease comprising 82% of all tick-borne diseases (Rosenberg et al., 2018). Figure 1 and 2 below, taken from Rosenberg et al. (2018), show the reported cases of tickborne and mosquitoborne disease in the United States from 2004-2016.

FIGURE 1. Reported cases* of tickborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

FIGURE 2. Reported cases* of mosquitoborne disease — U.S. states and territories, 2004–2016



Sources: CDC, National Notifiable Diseases Surveillance System, 2016 Annual Tables of Infectious Disease Data. https://wwwn.cdc.gov/nndss/infectious-tables.html. CDC, Division of Health Informatics and Surveillance. CDC, ArboNET.
Abbreviations: AS = American Samoa; PR/VI = Puerto Rico/U.S. Virgin Islands.
* Data classified by quintile.

Rickettsial infections

Rocky Mountain spotted fever (RMSF) is the most common rickettsial infection in the U.S. with 6,248 cases reported to the CDC alone in 2017 (CDC, 2020b). RMSF is caused by *Rickettsia rickettsia*, spread in the U.S. predominantly by *Dermacentor variabilis* (the American dog tick*)* and *D. andersoni* (the Rocky Mountain wood tick), and can be found throughout North America as well as parts of South America. The Council for State and Territorial Epidemiologists combined RMSF with other rickettsial diseases into the more broad "spotted fever rickettsiosis" designation in 2010 (CDC, 2020b). Besides the obligatory tick bite, typical symptoms of RMSF include fever, headache, and rash with the characteristic rash occurring in approximately 90% of patients within five days of illness. If left untreated, RMSF can be fatal but can easily be treated with antimicrobial therapy upon timely diagnosis. Definitive diagnosis of RMSF cannot usually be made via culture because *Rickettsia* cannot be grown in cell-free culture media; they are obligate intracellular bacteria requiring living host cells. RMSF diagnosis can be made via either skin biopsy prior to treatment with antibiotics or through serologic testing using indirect immunofluorescence assays (IFAs). Immunoglobulin G (IgG) antibodies are more specific than immunoglobulin M (IgM) antibodies since the latter can give false-positive results due to cross-reactivity with other bacterial pathogens. A drawback of IFA is that usually it is unreliable for the first five days of infection until antibody levels are high enough for detection. The CDC and major clinical labs do offer a polymerase chain reaction (PCR)-based assay for RMSF (Sexton & McClain, 2020).

Since 2001, thirteen other human rickettsiae belonging to the spotted fever group (SFG) have been identified. All SFGs can cause fever, headache, and myalgia and are arthropod-borne (primarily ticks and mites). Most patients with an SFG display a rash and/or a localized eschar. Rickettsialpox, caused by *R. akari*, is transmitted from the bite of a house mouse mite, usually after mouse extermination programs result in a decrease of the mite's food supply. Rickettsialpox is typically a relatively mild disease that can resolve itself without treatment within three weeks,

but treatment hastens improvement. Rickettsiosis can also be due to infection with *R. parkeri*, *R. amblyommii*, and *Rickettsia* species 364D (also called *R. philipii*). Isolation of SFG rickettsiae is rare in clinical practice due to the difficulty of obtaining culture; consequently, serology, immunologic detection from tissue, and PCR are more often used for diagnosis. Microimmunofluorescent (MIF) antibody tests, enzyme-linked immunosorbent assays (ELISAs), and Western blot immunoassays can be used to detect convalescent IgG and IgM antibodies, but these methods can only be used at least 10-14 days after the onset of illness when antibody concentrations are high enough for detection. McQuiston et al. (2014) concluded that the "use of IgM antibodies should be reconsidered as a basis for diagnosis and public health reporting of RMSF and other spotted fever group rickettsia in the United States" in one small study; the study demonstrated that IgM findings often resulted in false positives for Rock Mountain Spotted Fever and questioned the value of IgM testing (McQuiston et al., 2014).PCR is a very specific technique. PCR using tissue samples has higher specificity than whole blood PCR. Immunologic detection from a tissue biopsy requires the use of special laboratory equipment so it is not as frequently used as either the serologic or PCR detection methods (Sexton & McClain, 2021).

Ehrlichiosis and Anaplasmosis

Human ehrlichiosis was first reported in 1986, and the causative agent for human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, was identified in 1994. Both ehrlichiosis and anaplasmosis are transmitted from the bite of infected ticks and have similar clinical and laboratory manifestations. Ehrlichiosis can be caused by *Ehrlichia chaffeensis, E. ewingii, and E. muris.* Typically, patients have a fever within an incubation period of one to two weeks. Other symptoms can include malaise, myalgia, headache, chills, gastrointestinal distress, and cough. Both leukopenia and thrombocytopenia can occur. Diagnosis via culture is extremely difficult. "Until 1995, only two isolates of *E. chaffeensis* had been recovered from humans; in both cases, this process required over 30 days of cultivation. The isolation of *A. phagocytophilum* from three additional patients has been accomplished using a cell culture system derived from human promyelocytic leukemia cells (Sexton & McClain, 2022b). IFA testing for bacteria-specific antibodies is the most common method for diagnosing ehrlichiosis and anaplasmosis, but similar to rickettsiae, ELISA, PCR, and immunochemical tissue staining can be used as well. Unlike rickettsiosis, ehrlichiosis and anaplasmosis can also be detected by the presence of characteristic intraleukocytic morulae in a peripheral blood smear or buffy coat smear (Sexton & McClain, 2022a).

Borrelia Infections

Besides Lyme disease, caused by *Borrelia burgdorferi*, *Borrelia* can cause relapsing fever. Tick-borne relapsing fever (TBRF) in North America is primarily caused by *B. hermsii*, *B. turicatae*, *B. parkeri*, *B. miyamotoi*, and *B.* mazzottii, and louse-borne relapsing fever (LBRF) is an infection caused by *B. recurrentis* (Barbour, 2020b; Miller et al., 2018). The characteristic feature of these infections is the relapsing fever due to cyclical spirochetemia caused by antigenic variation of the spirochetes. Each bout of fever lasts 3 to 12 days with temperatures ranged from 39˚C to 43˚C (102.2˚F to 109.4˚F). Visual analysis by Giemsa or Wright staining blood smears taken during a febrile episode is common practice. PCR can also be used on a variety of samples, including cerebrospinal fluid (CSF), blood, tissue, or even culture medium. According to the

CDC, "a change in serology results from negative to positive, or the development of an IgG response in the convalescent sample, is supportive of a TBRF diagnosis" (CDC, 2018b). One exception is using antibodies to the GlpQ protein characteristic of these *Borrelia* species but not to *B. burgdorferi* (Lyme disease) (Barbour, 2020a).

Protozoa infections

Babesiosis is due to primarily *Babesia microti* in the U.S, but *B. divergens* and *B. venatorum* are the primary causative agents of babesiosis in Europe and China, respectively. The incubation period of *Babesia* depends on the mode of transfection: 1-4 weeks following a tick bite or up to 9 weeks following a blood transfusion, even though incubation periods up to six months have been reported. The most common symptoms of infection include a fever, fatigue, malaise, chills, sweats, headache, and myalgia. Immunocompromised individuals can develop relapsing babesiosis due to an absent or impaired production of antibodies with approximately 20% mortality rate for patients who develop relapsing babesiosis. A majority of patients with babesiosis are also co-infected with other tick-borne bacterial pathogens. "Preferred tools for diagnosis of babesiosis include blood smear for identification of Babesia organisms and polymerase chain reaction (PCR) for detection of Babesia DNA. Serology can be a useful adjunct to blood smear and PCR" (Krause & Vannier, 2021). Serology is not ideal in diagnosing an acute infection since antibody concentrations remain elevated post-recovery.

Plasmodium falciparum, P. vivax, and *P. ovale* are responsible for malaria. They are spread by the bite of an *Anopheles* mosquito where their sporozoites infect the liver within one to two hours. Within the hepatocyte, they form merozoites. Upon rupturing into the bloodstream, the merozoites infect red blood cells for trophozoite formation, causing the erythrocytic stage of the life cycle where additional merozoites are released. During this stage of the cycle, the symptoms of malaria, including fever, occur. This process usually takes 12 to 35 days, but clinical manifestations can be delayed in individuals with partial immunity or those who are taking ineffective prophylaxis. Other initial symptoms can include irregular heartbeat, cough, anorexia, gastrointestinal distress, sweating, chills, malaise, arthralgia, and myalgia. Malaria, if left untreated, can also include acidosis, hypoglycemia, severe anemia, renal and hepatic impairment, edema, and death (Cohee & Seydel, 2022). Parasite-based diagnosis may include microscopic examination of blood smears, which can often identify the species of *Plasmodium* as well as the parasite density, and antigen-based tests. Rapid diagnostic testing (RDT) of the antigens using immunochromatographic methods is available, but the accuracy of the RDT can vary considerably. NAATs can also be used to identify a malarial infection, and NAATs "are typically used as a gold standard in efficacy studies for antimalarial drugs, vaccines, and evaluation of other diagnostic agents" with a "theoretical limit of detection for PCR…estimated at 0.02 to 1 parasite/microL" (Hopkins, 2022). The Mayo Clinic Laboratories indicates that "PCR is an alternative method of malaria diagnosis that allows for sensitive and specific detection of Plasmodium species DNA from peripheral blood. PCR may be more sensitive than conventional microscopy in very low parasitemias, and is more specific for species identification…Malaria PCR can be used in conjunction with traditional blood film or Babesia PCR when the clinical or morphologic differential includes both babesiosis and malaria" "Test ID: LCMAL Malaria, Molecular Detection, PCR, Varies" (2021).

Viral infections

Examples of arthropod-borne viruses (arboviruses) include West Nile virus (WNV), dengue, yellow fever virus (YFV), chikungunya, and Colorado tick fever virus. In the United States, WNV is the most common arbovirus reported to the CDC. In 2016, 96% of the reported 2,240 cases of domestic arboviruses were WNV with 61% of the WNV cases reported being neuroinvasive. Neuroinvasive WNV includes meningitis, encephalitis, and acute flaccid paralysis (Burakoff et al., 2018). In general, most infected individuals are asymptomatic with only 20-40% of infected patients showing any characteristic symptoms of WNV, including fever, headache, malaise, myalgia, anorexia, and rash. Diagnosis of WNV of a symptomatic individual usually occurs with a WNV IgM antibody capture ELISA (MAC-ELISA) assay. A patient with symptoms of a neurologic infection does require a lumbar puncture. Confirmatory testing can include a plaque reduction neutralization test (PRNT). PCR testing is primarily used with immunocompromised patients who have delayed or absent antibody production, patients with a history of prior flavivirus infections, and blood donors who may be asymptomatic (Lyle R Petersen, 2022).

Dengue virus (DENV) infection is a result of being bitten by an infected *Aedes aegypti* or *A. albopictus* mosquito. Four distinct DENV types of *Flavivirus* are known: DENV-1, DENV-2, DENV-3, and DENV-4. DENV is endemic throughout much of the tropical regions of the world, but the only region of the U.S. endemic for DENV is Puerto Rico. The last major outbreak occurred in Puerto Rico in 2010 where 26,766 cases of suspected DENV were reported and 47% of all laboratory tested specimen were positive (CDC, 2021d, 2021e). "Dengue fever…is an acute febrile illness defined by the presence of fever and two or more of the following but not meeting the case definition of dengue hemorrhagic fever: headache, retro-orbital or ocular pain, myalgia and/or bone pain, arthralgia, rash, hemorrhagic manifestations…[and] leukopenia. The cardinal feature of dengue hemorrhagic fever is plasma leakage due to increased vascular permeability as evidenced by hemoconcentration (≥20 percent rise in hematocrit above baseline), pleural effusion, or ascites. DHF [dengue hemorrhagic fever] is also characterized by fever, thrombocytopenia, and hemorrhagic manifestations…. (Thomas et al., 2021)." Laboratory diagnostic testing includes direct detection of viral components in serum or indirect serologic assays. "Detection of viral nucleic acid or viral antigen has high specificity but is more labor intensive and costly; serology has lower specificity but is more accessible and less costly" (Thomas et al., 2021). Culture testing as a diagnostic tool usually is time-prohibitive.

Colorado tick fever virus (CTFV) is a *Reoviridae* transmitted primarily by the Rocky Mountain wood tick (*Dermacentor andersoni*) in the western U.S. and Canada. Transmission of CTFV has also been reported in blood transfusions. The incubation period can last up to 14 days, and symptoms include fever, headache, chills, myalgia, leukopenia, and prostration. Only 15% of symptomatic patients demonstrate a rash. Serologic tests are usually not helpful until at least 10-14 days for antibody production whereas real-time PCR (RT-PCR) can be used on the first day of symptoms (L. R. Petersen, 2022).

Yellow fever, occurring primarily in sub-Saharan Africa and South America, is a flavivirus spread by mosquitoes that causes hemorrhagic fever with a high fatality rate. An outbreak in Brazil in January-March 2018 resulted in 4 of 10 patients infected with YFV dying. None of those showing symptoms had been vaccinated against YFV. Yellow fever causes hemorrhagic diathesis due to decreased synthesis of vitamin K-dependent coagulation factors as well as hepatic dysfunction, renal failure, and coagulopathy. Yellow fever diagnosis is typically made

by a serologic test using an ELISA-IgM assay; however, this assay does cross-react with other flaviviruses and with the YFV vaccination. Rapid diagnostic testing using either PCR or immunoassay is available. Viral isolation and culture can be performed, but it requires inoculation of mosquitoes or mammalian cell culture. Tissue biopsy, such as liver, cannot be performed on the living patient due to possible fatal hemorrhaging; biopsy would be performed during the post-mortem workup (Wilder-Smith, 2022).

Chikungunya virus, endemic in many tropical and subtropical regions of the world, is transmitted by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Within the U.S., chikungunya is prevalent in Puerto Rico where approximately 25% of blood donors were seropositive; it has also been reported in Florida. Both dengue and Zika are transmitted by the same vectors, so these viruses often co-circulate geographically Chikungunya can cause acute febrile polyarthralgia and arthritis. The predominant testing method for diagnosis of chikungunya is the detection of viral RNA via either RT-PCR or virus serology using either ELISA or IFA. Viral culture is typically not used as a diagnostic tool but is used for epidemiologic research (Wilson & Lenschow, 2020).

Types of Testing

Test	Description	Rationale
Culture	Culture growth depends on the pathogen being studied. If the pathogen is an obligate intracellular organism, then it must be isolated using more sophisticated cell culture techniques. In many circumstances, culture is used for research and/or epidemiology rather than as a diagnostic tool (Biggs et al., 2016; Miller et al., 2018).	At times, culture testing is not as sensitive as either NAAT or serologic testing and can be time-intensive when treatment should not be delayed. Depending on the organism, this may require high biosafety level laboratory for culture growth (Biggs et al., 2016).
Indirect immunofluorescene antibody (IFA) assays	IFA is a serologic assay that can be used to test for the presence of antibodies, such as IgG and IgM, reactive against the pathogen (Biggs et al., 2016).	Depending on the pathogen, IFA can be a useful tool. At times, though, it can cross-react with either a prior vaccination or infection (Wilder-Smith, 2022). An acute infection can often be determined by performing IFA in both the acute phase and convalescent phase where at least a fourfold increase in antibodies is indicative of an acute infection (Biggs et al., 2016).

Darkfield microscopy	Darkfield microscopy can be used to detect the presence of microorganisms, such as motile spirochetes (Miller et al., 2018).	This technique is not widely available, and transport of sample must be done immediately if testing of motile specimen is desired (Miller et al., 2018).
Blood-smear microscopy	Blood-smear microscopy can be either thick or thin and is typically performed on a sample stained with an eosin-azure-type dye, such as Giemsa, to look at intracellular structures or morphological features (Biggs et al., 2016).	This technique should be performed by an experienced microscopist since it can be inconsistent. As compared to other techniques, this technique is relatively inexpensive (Biggs et al., 2016).
Nucleic acid amplification testing (NAAT)	NAATs can include polymerase chain reaction (PCR), real-time PCR (RT-PCR), or other enzyme-dependent amplification testing for the presence of nucleic acids (DNA or RNA).	NAATs can be specific and sensitive; however, they may not be available at all laboratories and/or can be costly. Some NAATs are available as rapid diagnostic tools. NAATs have been used on serum, whole blood, tissue, CSF, and even formalin-fixed, paraffin-embedded biopsies from autopsy tissues. The sensitivity of the technique can vary depending on the sample; for example, whole blood PCR for *R. rickettsii* is less sensitive than a similar sample test for *E. chaffeensis* (Biggs et al., 2016).

Analytical and Clinical Validity

The use of antibodies to detect and diagnose arthropod-associated infections and diseases is a common practice. Johnson et al. (2000) first reported the use of monoclonal antibody-based capture ELISA testing for a variety of alphaviruses, including chikungunya, flaviviruses, including dengue and yellow fever, and bunyaviruses. The researchers concluded, "IgG ELISA results correlated with those of the standard plaque-reduction neutralization assays. As expected, some test cross-reactivity was encountered within the individual genera, and tests were interpreted within the context of these reactions. The tests were standardized for laboratory diagnosis of arboviral infections, with the intent that they be used in tandem with the corresponding IgM antibody-capture ELISAs" (Johnson et al., 2000). Kalish and associates also

demonstrated that IgG and/or IgM antibody responses can still occur up to 20 years post-infection; consequently, a rise in antibody titer does not necessarily indicate a current, acute infection (Kalish et al., 2001).

In 2013, Kato and colleagues tested the sensitivity of two different RT-PCR-based assays for Rickettsia—PanR8, an assay that tests for Rickettsia in general, and RRi6, an assay specific for *R. rickettsii*. Both of these methods were more sensitive in testing for Rickettsia than the nested PCR method of the CDC; moreover, both of these methods are faster than the nested PCR method (1 hr versus 1-2 days, respectively) (Kato et al., 2013). These results were corroborated in 2014 by Denison and colleagues. They used a multiplex PCR assay to correctly identify all cell controls for *R. rickettsii*, *R. parkeri*, and *R. akari*; moreover, no false-positive results were reported using this methodology. "This multiplex real-time PCR demonstrates greater sensitivity than nested PCR assays in FFPE [formalin-fixed, paraffin-embedded] tissues and provides an effective method to specifically identify cases of Rocky Mountain spotted fever, rickettsialpox, and *R. parkeri* rickettsiosis by using skin biopsy specimens" (Denison et al., 2014).

The FDA has approved the use of the BinaxNOW malaria test for screening and diagnosing malaria. Even though this testing method is considerably faster than other methods (as low as 1.1-1.7 hours complete turnaround time (Ota-Sullivan & Blecker-Shelly, 2013), the use of BinaxNOW in non-endemic areas is a point of controversy due to relatively low sensitivity (84.2%) and for misclassifying Plasmodium falciparum malaria as non-falciparum (Dimaio et al., 2012). Moreover, it has been reported that Salmonella typhi can give a false-positive for malaria using the BinaxNOW test (Meatherall et al., 2014).

Leski et al. (2020) performed a 2020 study published in Malaria Journal that compared traditional diagnostic methods such as rapid diagnostic tests (RDTs) and DNA-based methods to polymerase chain reaction (PCR). The results indicated consistency with "previous observations that PCR-based tests have a significantly higher sensitivity when compared with both microscopy and RDTs" Leski et al. (2020).

Mathison and Pritt (2017) reviewed current standards for malaria testing and the most used methods for laboratory diagnosis. The most common tests "are microscopic examination of stained blood flims and detection of parasite antigen or nucleic acid… Rapid antigen detection methods and molecular amplification tests are also increasingly employed for malaria diagnosis and are useful adjunctive tests." According to the algorithm developed in "Update on Malaria Diagnostics and Test Utilization," NAAT tests are one of three tests recommended for use if malaria is suspected based on clinical findings and exposure history (Mathison & Pritt, 2017).

van Bergen et al. (2021) evaluated a novel real-time PCR assay for clinical validity. The authors used reference samples, patient samples, and synthetic controls. The analytical performance details of the MC004 assay were considered: "analytical specificity, limit of detection, the ability to detect mixed infections, and the potential to determine the level of parasitaemia of P. falciparum, including assessment of within-run and between-run precisions." The authors reported "zero false positive or false negative results." As far as precision, "the within-run and between-run precisions were less than 20% CV at the tested parasitaemia levels of 0.09%, 0.16%, 2.15% and 27.27%." Based on these results, the authors reported that "the entry of PCR-based techniques into malaria diagnostics has improved the sensitivity and specificity of the detection

of Plasmodium infections… Based upon the analytical performance characteristics that were determined, the MC004 assay showed performance suitable for use in clinical settings, as well as epidemiological studies" (van Bergen et al., 2021).

Akoolo et al. (2017) compared qPCR results in the detection of Babesia infection against currently available non-NAAT tests (FISH and microscopy). Blood samples were analyzed from 192 patients. The researchers report that "Of 28 samples that were positive by FISH, 27 (96%) were also positive by qPCR indicating high congruency between nucleic acid-based tests. Interestingly, of 78 asymptomatic samples not tested by FISH, 22 were positive by our qPCR" (Akoolo et al., 2017). Overall, the qPCR method was found to have a sensitivity of 96.2% and a specificity of 70.5%. The authors conclude, "Robust qPCR using specific probes can be highly useful for efficient and appropriate diagnosis of babesiosis in patients in conjunction with conventional diagnostics, or as a stand-alone test, especially for donated blood screening" (Akoolo et al., 2017).

VI. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

Diagnosis and Management of Tickborne Rickettsial Diseases (Biggs et al., 2016): In 2016, the CDC released their guidelines and recommendations concerning Rickettsial diseases, including Rocky Mountain spotted fever, in the MMWR. The table below summarizes their recommended diagnostic tests for tickborne rickettsial diseases:

TABLE 4. Recommended diagnostic tests for tickborne rickettsial diseases

Disease	PCR			Microscopy for morulae detection	IFA assay for IgG antibodies (acute and convalescent)*
	Whole blood	Eschar biopsy or swab	Rash biopsy		
Rocky Mountain spotted fever	Yes†	—	Yes	—	Yes
Rickettsia parkeri rickettsiosis	—	Yes	Yes	—	Yes
Rickettsia species 364D rickettsiosis	—	Yes	—	—	Yes
Ehrlichia chaffeensis ehrlichiosis (human monocytic ehrlichiosis)	Yes	—	—	Yes	Yes
Ehrlichia ewingii ehrlichiosis	Yes	—	—	Yes	Yes
Ehrlichia muris-like agent ehrlichiosis	Yes	—	—	—	Yes
Human anaplasmosis (human granulocytic anaplasmosis)	Yes	—	—	Yes	Yes

Abbreviations: IFA = indirect immunofluorescence antibody; IgG = immunoglobulin G; PCR = polymerase chain reaction.
* IFA assay is insensitive during the first week of illness for most tickborne rickettsial diseases; a sample should be collected during this interval (acute specimen), and a second sample should be collected 2–4 weeks later (convalescent specimen) for comparison. Elevated titers alone are not sufficient to diagnose infection with tickborne rickettsial diseases; serial titers are needed for confirmation. Demonstration of at least a fourfold rise in antibody titer is considered confirmatory evidence of acute infection.
† PCR of whole blood samples for *Rickettsia rickettsii* has low sensitivity; sensitivity increases in patients with severe disease.

To summarize their recommendations, even though indirect immunofluorescence antibody assays (IFAs) are insensitive typically during the first week of an acute infection, they are the standard reference for tickborne rickettsial infections; in addition, a minimum of two tests are to be performed for a diagnosis. Usually, one sample is taken early after the initial symptoms are present, and a second sample is taken 2-4 weeks later. A minimum of a fourfold rise in antibody titer is required to confirm diagnosis. In cases of ehrlichiosis and anaplasmosis, during the first week, PCR amplification can be used on whole blood for diagnosis, but PCR has low sensitivity in Rocky Mountain spotted fever except in patients with severe disease. Morulae detection via

either blood-smear or buffy-coat preparation microscopy can also be indicative of ehrlichiosis or anaplasmosis. "Rickettsiae cannot be isolated with standard blood culture techniques because they are obligate intracellular pathogens; specialized cell culture methods are required. Because of limitations in availability and facilities, culture is not often used as a routine confirmatory diagnostic method for tickborne rickettsial diseases" (Biggs et al., 2016).

Tick-borne relapsing fever (TBRF) (CDC, 2018c): In the U.S., TBRF can be caused by *Borrelia hermsii*, *B. parkerii,* and *B. turicatae* with *B. hermsii* being the most common causative agent. TBRF often presents with a relapsing nature (usually ~3 days per febrile episode followed by an afebrile period of approximately one week). "Spirochetemia (spirochetes in blood) in TBRF patients often reaches high concentrations (>10^6 spirochetes/ml). Thus, microscopy is a useful diagnostic tool for TBRF. The diagnosis of TBRF may be based on direct microscopic observation of relapsing fever spirochetes using dark field microscopy or stained peripheral blood smears. Spirochetes are more readily detected by microscopy in symptomatic, untreated patients early in the course of infection. Other bacteria, such as Helicobacter, may appear morphologically similar, so it is important to consider clinical and geographical characteristics of the case when making a diagnosis of TBRF based on microscopy. Additional testing, such as serology or culture, is recommended."

"Serologic testing for TBRF is not standardized and results may vary by laboratory. Serum taken early in infection may be negative, so it is important to also obtain a serum sample during the convalescent period (at least 21 days after symptom onset). A change in serology results from negative to positive, or the development of an IgG response in the convalescent sample, is supportive of a TBRF diagnosis. However, early antibiotic treatment may limit the antibody response. Patients with TBRF may have false-positive tests for Lyme disease because of the similarity of proteins between the causative organisms. A diagnosis of TBRF should be considered for patients with positive Lyme disease serology who have not been in areas endemic for Lyme disease. Incidental laboratory findings include normal to increased white blood cell count with a left shift towards immature cells, a mildly increased serum bilirubin level, mild to moderate thrombocytopenia, elevated erythrocyte sedimentation rate (ESR), and slightly prolonged prothrombin time (PT) and partial thromboplastin time (PTT) (CDC, 2018c)."

Colorado Tick Fever (CTF) (CDC, 2021c): As of 2015, CTF was reportable in Arizona, Colorado, Montana, Oregon, Utah, and Wyoming. "Laboratory diagnosis of CTF is generally accomplished by testing of serum to detect viral RNA or virus-specific immunoglobulin (Ig) M and neutralizing antibodies. Antibody production can be delayed with CTF, so tests that measure antibodies may not be positive for 14–21 days after the onset of symptoms. RT-PCR (reverse-transcriptase polymerase chain reaction) is a more sensitive test early in the course of disease. CTF testing is available at some commercial and state health department laboratories and at CDC. Contact your state or local health department for assistance with diagnostic testing. They can help you determine if samples should be sent to the CDC Arbovirus Diagnostic Laboratory for further testing (CDC, 2021c)."

Babesiosis (CDC, 2019): According to the CDC website, the most recent update about babesiosis for health professionals is from 2012 (with revision in 2018). Diagnosis can be challenging due to the nonspecific clinical manifestations of the disease. "For acutely ill patients, the findings on routine laboratory testing frequently include hemolytic anemia and thrombocytopenia.

Additional findings may include proteinuria, hemoglobinuria, and elevated levels of liver enzymes, blood urea nitrogen, and creatinine. If the diagnosis of babesiosis is being considered, manual (non-automated) review of blood smears should be requested explicitly. In symptomatic patients with acute infection, *Babesia* parasites typically can be detected by light-microscopic examination of blood smears, although multiple smears may need to be examined. Sometimes it can be difficult to distinguish between *Babesia* and *Plasmodium* (especially *P. falciparum*) parasites and even between parasites and artifacts (such as stain or platelet debris). Consider having a reference laboratory confirm the diagnosis—by blood-smear examination and, if indicated, by other means, such as molecular and/or serologic methods tailored to the setting/species (CDC, 2019)."

Malaria (Arguin & Tan, 2017, 2019): The CDC considers smear microscopy as the gold standard in diagnosing malaria since it can determine the species, identify the stage of parasitic life-cycle, and quantify the parasitemia. The CDC states, "Blood smear microscopy remains the most important method for malaria diagnosis. Microscopy can provide immediate information about the presence of parasites, allow quantification of the density of the infection, and allow determination of the species of the malaria parasite—all of which are necessary for providing the most appropriate treatment. Microscopy results should ideally be available within a few hours. These tests should be performed immediately when ordered by a health care provider. They should not be saved for the most qualified staff to perform or batched for convenience. In addition, these tests should not be sent out to reference laboratories with results available only days to weeks later. Assistance with speciation of malaria on smears is available from CDC" (Arguin & Tan, 2019). The CDC also notes that rapid diagnostic tests (RDTs) for malaria can detect malaria parasitic antigens. However, "RDTs offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not immediately available. Although RDTs can detect malaria antigens within minutes, they have several limitations. RDTs cannot distinguish between all of the *Plasmodium* species that affect humans, they may be less sensitive than expert microscopy or PCR for diagnosis, they cannot quantify parasitemia, and an RDT-positive test result may persist for days or weeks after an infection has been treated and cleared. Thus, RDTs are not useful for assessing response to therapy. Both positive and negative RDT results must always be confirmed by microscopy. Microscopy confirmation of the RDT result should occur as soon as possible because the information on the presence, density, and parasite species is critical for optimal management of malaria"(Arguin & Tan, 2019). Regarding PCR, the CDC states that "Although these tests are more sensitive than routine microscopy, results are not usually available as quickly as microscopy results, thus limiting the utility of this test for acute diagnosis and initial clinical management. Use of PCR testing is encouraged to confirm the species of malaria parasite and detect mixed infections" (Arguin & Tan, 2019).

The CDC also provided an update to malaria diagnosis in 2018. Although microscopy remained the "gold standard" for confirmation of malaria, other tests such as RDTs and PCR-based tests remained useful in certain situations (namely if microscopy is unavailable). PCR is considered most useful for "confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT." Finally, the CDC recommends that all cases of malaria be evaluated for drug resistance, typically through molecular characterization (PCR, gene sequencing) or in vitro tests (CDC, 2018a).

Chikungunya (Staples et al., 2017, 2020): In the CDC Yellow Book, concerning the chikungunya virus, they recommend that "the differential diagnosis of chikungunya virus infection depends on the clinical signs and symptoms as well as where the person was suspected of being infected." The other diseases to consider include: Zika, malaria, leptospirosis, parvovirus, group A *Streptococcus*, rubella, measles, dengue, enterovirus, adenovirus, alphavirus infections, post-infectious arthritis, and rheumatic conditions. Laboratory diagnosis is done by serum testing for detection of virus, viral nucleic acids, or virus-specific IgM and neutralizing antibodies. "During the first week after onset of symptoms, chikungunya can often be diagnosed by performing viral culture or nucleic acid amplification on serum. Virus-specific IgM and neutralizing antibodies normally develop toward the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative. Testing for chikungunya virus is performed at CDC, several state health department laboratories, and several commercial laboratories (Staples et al., 2017, 2020)."

West Nile Virus (WNV) (Nasci et al., 2013): "WNV infections are most frequently confirmed by detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or cerebrospinal fluid (CSF). The presence of anti-WNV IgM is usually good evidence of recent WNV infection but may indicate infection with another closely related flavivirus (e.g., St. Louis encephalitis). Because anti-WNV IgM can persist in some patients for >1 year, a positive test result occasionally may reflect past infection unrelated to current disease manifestations. Serum collected within 8 days of illness onset may lack detectable IgM, and the test should be repeated on a convalescent-phase sample. IgG antibody generally is detectable shortly after the appearance of IgM and persists for years. Plaque-reduction neutralization tests (PRNT) can be performed to measure specific virus-neutralizing antibodies. A fourfold or greater rise in neutralizing antibody titer between acute- and convalescent-phase serum specimens collected 2 to 3 weeks apart may be used to confirm recent WNV infection and to discriminate between cross-reacting antibodies from closely related flaviviruses." NAAT may not be suitable in most cases since the concentrations of WNV RNA are so low by the time a patient begins to show symptoms of infection; however, NAAT may be suitable in immunocompromised individuals who have either delayed or absent antibody development.

Yellow Fever Virus (YFV) (Gershman & Staples, 2019): Isolation of the virus or NAAT should be performed as early as possible in suspected cases of YFV. "By the time more overt symptoms are recognized, the virus or viral RNA may no longer be undetectable. Therefore, virus isolation and nucleic acid amplification should not be used to rule out a diagnosis of YF… Serologic assays to detect virus-specific IgM and IgG antibodies (*sic*). Because of cross-reactivity between antibodies raised against other flaviviruses, more specific antibody testing, such as a plaque reduction neutralization test, should be done to confirm the infection (Gershman & Staples, 2019)." Since YFV is a nationally notifiable disease, clinicians should contact their state and/or local health departments according to their respective local, state, and/or federal guidelines.

Dengue (CDC, 2020a, 2021a, 2021d, 2021e): Diagnosis of dengue can be via isolation of virus, serological tests such as immunoassays, and molecular methods, including RT-PCR. The CDC recommends testing symptomatic individuals that have recently traveled to or lives in areas where dengue is transmitted, as well as symptomatic pregnant women with possible dengue or Zika exposure. The CDC's testing algorithm for dengue is as follows:

"Patients with symptoms consistent with dengue can be tested with both molecular and serologic diagnostic tests during the first 7 days of illness. After the first 7 days of illness, test only with serologic diagnostic tests" (CDC, 2020a).

Finally, the CDC does not recommend testing asymptomatic patients or preconception screening for dengue (CDC, 2020a, 2021d, 2021e).

Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)

Laboratory Diagnosis of Tick-borne Infections: The information given below outlines the diagnostic procedures for tick-borne infections and is taken from Table 47 of the 2018 IDSA/ASM guidelines.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
Bacteria		
Relapsing fever borreliae ***Borrelia hermsii* (western US)** ***Borrelia parkeri* (western US)** ***Borrelia turicatae* (southwestern US)** ***Borrelia mazzottii* (southern US)**	Primary test: Darkfield microscopy or Wright, Giemsa, or Diff-Quik stains of peripheral thin or/ and thick blood smears. Can be seen in direct wet preparation of blood in some cases.	Blood or bone marrow
	Other testing: NAAT, Culture, Serologic testing	Blood or body fluids for NAAT. Serum for culture or serologic testing.
***Borrelia miyamotoi (B. miyamotoi* infection, hard tick-borne relapsing fever)**	Primary test: NAAT	Blood
	Serology: EIA for detection of antibodies to recombinant GlpQ antigen	Serum
***Anaplasma phagocytophilum* (human granulocytotropic anaplasmosis)**	Primary test: NAAT Alternate Primary (if NAAT is unavailable): Wright or Giemsa stain of peripheral blood or buffy coat leukocytes during week first week of infection.	Blood
	Serology: Acute and convalescent IFA titers for IgG-class antibodies to A. phagocytophilum antibodies	Serum
	Immunohistochemical staining of Anaplasma antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver, and lung)

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
Ehrlichia chaffeensis* (human monocytotropic ehrlichiosis)** ***Ehrlichia muris ***Ehrlichia ewingii***	Primary test: NAAT (Only definitive diagnostic assay for *E. ewingii*) Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection	Whole blood for NAAT Blood for Wright or Giemsa stain
	Serology: acute and convalescent IFA titers for Ehrlichia IgG-class antibodies	Serum
	Immunohistochemical staining of Ehrlichia antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)
***Rickettsia rickettsii* (RMSF)** **Other spotted fever group *Rickettsia* spp (mild spotted fever)** ***R. typhi* (murine typhus)** ***R. akari* (rickettsialpox)** ***R. prowazekii* (epidemic typhus)**	Serology: acute and convalescent IFA for *Rickettsia* sp IgM and IgG antibodies	Serum
	NAAT	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
	Immunohistochemical staining of spotted fever group rickettsiae antigens (up to first 24 h after antibiotic therapy initiated) in formalin-fixed, paraffin-embedded specimens	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)
Protozoa		
Babesia microti ***Babesia* spp**	Primary test: Giemsa, Wright, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood (EDTA vacutainer tube is a second choice)
	Primary test for acute infection: NAAT	Blood
	Serology: acute and convalescent IFA titers for Babesia IgG-class antibodies **NOTE: Not recommended for acute infection.**	Serum
Virus		

Etiologic Agents	Diagnostic Procedures	Optimum Specimens
Colorado tick fever virus	Virus-specific IFA-stained blood smears	Blood
	Serology: IFA titers or complement fixation	Serum
Powassan/deer tick virus	Primary test: IgM capture EIA (available only through state departments of public health)	Serum
	NAAT	Blood, CSF, brain (biopsy or autopsy)

The IDSA/ASM does note that most PCR-based assays for babesiosis only detect *B. microti* even though there are at least three other species of *Babesia* that can cause the infection. "Real time PCR available from CDC and reference labs… Serology does not distinguish between acute and past infection" (Miller et al., 2018).

Their recommendation for the main diagnostic testing for malaria due to *Plasmodium falciparum, P. ovale, P. vivax, P. malariae, and P. knowlesi* is "Stat microscopic examination of Giemsa-stained thick and thin blood films (repeat testing every 12–24 h for a total of 3 exams before ruling out malaria); rapid antigen detection tests followed by confirmatory blood films within 12–24 h." They make the following special remark: "Antigen tests lack sensitivity with low parasitemia and non-falciparum malaria and do not differentiate all species. PCR from some reference laboratories will detect and differentiate all species. Calculation of percentage parasitemia (using thick or thin blood films) is required for determining patient management and following response to therapy (Miller et al., 2018)." Concerning dengue virus DENV, "Plaque reduction neutralization tests (PRNTs) are considered the reference standard for detection of antibodies to arthropod-borne viruses (arboviruses) and provide improved specificity over commercial serologic assays; however, due to the complexity of testing, PRNT is currently only available at select public health laboratories and the CDC." They note that false positives for antibodies to DENV may not necessarily indicate DENV infection since it can also be indicative of a prior flavivirus infection, such as West Nile virus or Zika virus. They do state the following concerning the use of NAAT, "Detection of DENV RNA by NAAT is preferred for acutely ill patients. Recently, detection of the DENV NS1 antigen, which is secreted from infected host cells as early as 1 day after symptom onset and up to 10 days thereafter, has become an acceptable alternative to NAAT for diagnosis of acute DENV infection (Miller et al., 2018)."

For West Nile Virus (WNV), they state: "Laboratory diagnosis of WNV, and most other arboviruses, is typically accomplished by detecting virus-specific IgM- and/or IgG-class antibodies in serum and/or CSF." Possible false positives can occur if a patient has been vaccinated against yellow fever or if they have had a previous infection of another flavivirus. They do note that WNV RNA detection via NAAT can be performed on either the serum or CSF for immunosuppressed patients.

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Babesiosis (AAP, 2021a): "Acute, symptomatic cases of babesiosis typically are diagnosed by microscopic identification of *Babesia* parasites on Giemsa- or Wright-stained blood smears… If

the diagnosis of babesiosis is being considered, manual (nonautomated) review of blood smears for parasites should be requested explicitly. If seen, the tetrad (Maltese-cross) form is pathognomonic. *B microti* and other *Babesia* species can be difficult to distinguish…examination of blood smears by a reference laboratory should be considered for confirmation of the diagnosis." They do state that antibody testing can be useful in distinguishing between *Babesia* and *Plasmodium* infections whenever blood smear examinations and travel histories are inconclusive or for detecting individuals with very low levels of parasitemia.

Non-Lyme Borrelia Infections (AAP, 2021b): Dark-field microscopy and Wright-, Giemsa-, or acridine orange-stained preparations of blood smears can be used to observe the presence of spirochetes in the initial febrile episode, but their presence is more difficult to determine in future recurrences. Both enzyme immunoassay and Western immunoblot analysis can detect serum antibodies; however, "Antibody tests are not standardized and are affected by antigenic variations among and within *Borrelia* species and strains." As of publication, PCR and antibody-based testing were still under development and were not widely available.

Ehrlichia, Anaplasma, and Related Infections (AAP, 2021e): PCR testing should be performed within the first week of illness to diagnose anaplasmosis, ehrlichiosis, and other *Anaplasmataceae* infections because doxycycline treatment rapidly decreases the sensitivity of PCR. Consequently, negative PCR results do not necessarily indicate a lack of infection. Occasionally, Giemsa- or Wright staining of blood smears can be performed to identify the presence of the morulae of *Anaplasma* in the first week of illness. Culture testing for isolation is not performed. "Serologic testing may be used to demonstrate a fourfold change in immunoglobulin (Ig) G-specific antibody titer by indirect immunofluorescence antibody (IFA) assay between paired acute and convalescent specimens taken 2 to 4 weeks apart. A single mildly elevated IgG titer may not be diagnostic, particularly in regions with high prevalence. IgM serologic assays are prone to false-positive reactions, and IgM can remain elevated for lengthy periods of time, reducing its diagnostic utility. "

Rocky Mountain Spotted Fever (RMSF) (AAP, 2021h): "The gold standard confirmatory test is indirect immunofluorescence antibody (IFA) to *R rickettsii* antigen. Both immunoglobulin (Ig) G and IgM antibodies begin to increase around 7 to 10 days after onset of symptoms; IgM is less specific, and IgG is the preferred test. Confirmation requires a fourfold or greater increase in antigen-specific IgG between acute (first 1–2 weeks of illness while symptomatic) and convalescent (2–4 weeks later) sera."

Rickettsialpox (AAP, 2021g): Rickettsialpox can be mistaken for other rickettsial infections. Ideally, the use of *R. akari*-specific antigen is recommended for serologic diagnosis, but it has limited availability. Otherwise, indirect IFA for *R. rickettsia*, the causative agent of RMSF, since *R. akari* has extensive cross-reactivity. Again, a demonstration of at least a fourfold increase in antibody titers taken 2-6 weeks apart is indicative of infection.

Chikungunya (AAP, 2021c): "Laboratory diagnosis generally is accompanied by testing serum to detect virus, viral nucleic acid, or virus-specific immunoglobulin (Ig) M and neutralizing antibodies." RT-PCR can be used to diagnose chikungunya during the first week after onset of symptoms since chikungunya-specific antibodies have not formed at that time. After the first week, serum testing of IgM or a plaque-reduction neutralization test can be performed.

Dengue (AAP, 2021d): "Dengue virus is detectable by RT-PCR or NS1 antigen EIAs from the beginning of the febrile phase until day 7 to 10 after illness onset." Cross-reactivity occurs

between anti-dengue virus IgM and other flaviviruses, including Zika. IgG EIA and hemagglutination testing is not specific for diagnosis of dengue, and IgG antibodies remain elevated for life; consequently, a fourfold increase in IgG between the acute and convalescent phase can confirm recent infection. "Reference testing is available from the Dengue Branch of the Centers for Disease Control and Prevention."

Malaria (AAP, 2021f): Microscopic identification of *Plasmodium* on both thick and thin blood films should be performed. "If initial blood smears test negative for *Plasmodium* species but malaria remains a possibility, the smear should be repeated every 12 to 24 hours during a 72-hour period… Serologic testing generally is not helpful, except in epidemiologic surveys… Species confirmation and antimalarial drug resistance testing are available free of charge at the Centers for Disease Control and Prevention (CDC) for all cases of malaria diagnosed in the United States." One FDA-approved RADT is available in the U.S. to hospitals and commercial labs; however, both positive and negative test results must be corroborated by microscopic examination.

West Nile Virus (WNV) (AAP, 2021i): PCR is not recommended for diagnosis of WNV in immunocompetent patients since WNV RNA is usually no longer detectable by the initial onset of symptoms. "Detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or CSF is the most common way to diagnose WNV infection." Anti-WNV IgM levels can remain elevated for longer than 1 year so a positive test result may be indicative of a prior infection. "Plaque-reduction neutralization tests can be performed to measure virus-specific neutralizing antibodies and to discriminate between cross-reacting antibodies from closely related flaviviruses. A fourfold or greater increase in virus-specific neutralizing antibodies between acute-and convalescent-phase serum specimens collected 2 or 3 weeks apart may be used to confirm recent WNV infection."

International Encephalitis Consortium (IEC)

In 2013, the IEC released their *Case Definitions, Diagnostic Algorithms, and Priorities in Encephalitis*. Concerning arboviruses, they state the following: "For most arboviruses, serologic testing of serum and CSF is preferred to molecular testing, since the peak of viremia typically occurs prior to symptom onset. For example, in patients with West Nile virus (WNV) associated with neuroinvasive disease, CSF PCR is relatively insensitive (57%) compared with detection of WNV IgM in CSF. The cumulative percentage of seropositive patients increases by approximately 10% per day during the first week of illness suggesting the need for repeat testing if the suspicion for disease is strong in those with initially negative results. Notably, arbovirus IgM antibodies may be persistently detectable in the serum and, less commonly, in the CSF, for many months after acute infection, and therefore may not be indicative of a current infection. Therefore, if possible, documentation of acute infection by seroconversion and/or 4-fold or greater rises in titre using paired sera is recommended" (Venkatesan et al., 2013).

VII. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

On 6/29/2017, the FDA approved the Rickettsia Real-Time PCR Assay (K170940) by the Centers for Disease Control and Prevention (CDC) with the following definition: "An in vitro diagnostic test for the detection of Rickettsia spp. nucleic acids in specimens from individuals

with signs or symptoms of rickettsial infection and epidemiological risk factors consistent with potential exposure. Test results are used in conjunction with other diagnostic assays and clinical observations to aid in the diagnosis infection, in accordance with criteria defined by the appropriate public health authorities in the Federal government (FDA, 2018)."

On 9/1/2009, the FDA approved the BinaxNOW Malaria Positive Control Kit (K083744) rapid diagnostic test (RDT), an in vitro qualitative immunochromatographic assay, for use by hospital and commercial laboratories, but it is not approved for individual or physician offices (Arguin & Tan, 2017; FDA, 2018).

As of 8/7/2018, the FDA has approved the following assays for the detection of West Nile Virus (FDA, 2018): West Nile Virus ELISA IgG model EL0300G and West Nile Virus IgM Capture ELISA model EL0300M by Focus Technologies, Inc., West Nile Virus IgM Capture ELISA model E-WNV02M and West Nile Virus IgG Indirect ELISA by Panbio Limited, West Nile Detect IgM ELISA by Inbios Intl, Inc., Spectral West Nile Virus IgM Status Test by Spectral Diagnostics, Inc., and the EUROIMMUN Anti-West Nile Virus ELISA (IgG) and EUROIMMUN Anti-West Nile Virus ELISA (IgM) by Euroimmun US, Inc.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
86757	Antibody; Rickettsia; Immunoassay Indirect Fluorescent Antibody (IFA)
87040	Culture, bacterial; blood, aerobic, with isolation and presumptive identification of isolates
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
86280	Hemagglutination inhibition test (HAI)
86382	Neutralization test, viral
86753	IFA assay Babesia microti Antibodies (IgG, IgM)
86666	Antibody; Ehrlichia
86619	Antibody; Borrelia (relapsing fever)
87207	Smear, primary source with interpretation; special stain for inclusion bodies or parasites (eg, malaria, coccidia, microsporidia, trypanosomes, herpes viruses
86750	Antibody; Plasmodium (malaria)

CPT	Code Description
86753	Antibody; protozoa, not elsewhere specified
86788	Antibody; West Nile virus, IgM
86789	Antibody; West Nile virus
86790	Antibody; virus, not elsewhere specified antibody; **(*Dengue Fever Antibodies; CTF Antibodies; Yellow Fever Antibodies)***
87449	Infectious agent antigen detection by immunoassay technique, Dengue Virus NS1 Antigen (eg, enzyme immunoassay [EIA], enzyme-linked immunosorbent assay [ELISA], immunochemiluminometric assay [IMCA]), qualitative or semiquantitative; multiple-step method, not otherwise specified, each organism
87899	Infectious agent antigen detection by immunoassay with direct optical (ie, visual) observation; not otherwise specified
0043U	Tick-borne relapsing fever Borrelia group, antibody detection to 4 recombinant protein groups, by immunoblot, IgM Proprietary test: Tick-Borne Relapsing Fever Borrelia (TBRF) ImmunoBlots IgM Test Lab/Manufacturer: IGeneX Inc
0044U	Tick-borne relapsing fever Borrelia group, antibody detection to 4 recombinant protein groups, by immunoblot, IgG Proprietary test: Tick-Borne Relapsing Fever Borrelia (TBRF) ImmunoBlots IgG Test Lab/Manufacturer: IGeneX Inc

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X. Revision History

Revision Date	Summary of Changes
01/01/2022	Initial Effective Date
05/20/2022	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate any modifications to the coverage criteria.
04/04/2023	Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. The following edits were made for clarity: Removed former note, as instructions to see AHS-G2143 and G2133 for Lyme and Zika, respectively, are already in the Policy Description. All CC were edited for better clarity of which tests MCC and which DNMCC. When a CC had both MCC and DNMCC as subcriteria, one criteria became two. Sign and symptoms were moved to being notes, as falling within a CC does not meet the overall formatting of our CC. Signs and symptoms of rickettsial diseases (CC1/2) moved to Note 1 Signs and symptoms of ehrlichiosis and/or anaplasmosis (CC3/4) moved to Note 2 Signs and symptoms of tick-borne relapsing fever (CC5/6) moved to Note 3 Signs and symptoms of babesiosis (CC7/8) moved to Note 4

Revision Date	Summary of Changes
	Signs and symptoms of malaria (CC9/10) moved to Note 5 Signs and symptoms of chikungunya virus (CC11) moved to Note 6 Signs and symptoms of West Nile Virus (WNV) (CC12/13) moved to Note 7 Signs and symptoms of Yellow Fever Virus (CC14) moved to Note 8 Signs and symptoms of Dengue virus (CC15/16) moved to Note 9 Signs and symptoms of Colorado Tick Fever (CC17) moved to Note 10 Coding Enhancement: Removed CPT codes 85060, 87254 Committee approved 4/4/2023