

Recommended Laboratory Assays for Select Tickborne Diseases

	Laboratory Test	Advantages	Comments
Lyme Disease	Two-tier test: Enzyme Immunoassay (EIA) OR Immunofluorescence Assay (IFA) reflexed to Western Blot (WB) IgM or IgG	Higher specificity than single-tier EIA/IFA	If signs or symptoms ≤ 30 days, second test with IgM and IgG Western Blot If signs or symptoms ≥ 30 days, second test with IgG Western Blot ONLY
Ehrlichiosis or Anaplasmosis	Multiplex PCR of whole blood for pathogen DNA during acute illness phase	Identifies specific illness cause during acute phase; negates need for serological testing	Collect blood prior to antibiotic administration
Ehrlichiosis or Anaplasmosis	Quantitative IFA for IgG (Acute & Convalescent recommended)	Allows comparison of acute and convalescent titers	Collect both acute & convalescent samples Serologic tests are cross-reactive among <i>Ehrlichia</i> and <i>Anaplasma</i> species
Rocky Mountain spotted fever (RMSF) or other Spotted Fever Rickettsiosis (SFR)*	Quantitative IFA for IgG (Acute & Convalescent recommended)	Allows comparison of acute and convalescent titers to distinguish pathogenic from non-pathogenic SFR	Collect both acute & convalescent samples Serologic tests are cross-reactive among all agents of SFR

*If tick exposure is noted or RMSF is suspected, treatment should be started based on suspicion of infection and not delayed pending the outcome of diagnostic tests.

Lyme Disease Laboratory Testing

- Laboratory assays for Lyme disease such as the Lyme enzyme-linked immunosorbent assay (ELISA) or the WB IgM, yield thousands of positive test results in Virginia, but when used alone, the specificities of each of these tests are low enough that the probability of false positive results are significant from each assay.
- When the enzyme immunoassay (EIA) test and the Western Blot IgM are used in concert as a “two-tier test” within 30 days of a patient’s illness onset, their combined positivity provide a more specific and reliable test result.
- The WB IgG has high specificity and yields strong laboratory evidence of infection. Keep in mind that the WB IgG will not detect an immune response until about 30 days after illness onset.

Rickettsial Disease Laboratory Testing

- The agents of ehrlichiosis and anaplasmosis circulate in human blood during the acute phase of illness, and can be detected and specifically identified by DNA testing of whole blood collected during the acute illness phase.
- DNA testing for ehrlichial agents uses a multiplex PCR assay that tests for multiple agents at once. Multiplex PCR assays are available though several commercial laboratories, provide accurate and specific results, and negate the need for acute and convalescent serum testing for ehrlichiosis or anaplasmosis.
- Additionally, as ehrlichiosis and anaplasmosis are serologically cross-reactive, PCR testing will provide a true indication of which agent caused the illness.

Recommended Laboratory Assays for Select Tickborne Diseases (continued)

- Despite patients feeling terrible within the first four days of illness onset, they rarely have a detectable immune response to any rickettsial disease until 7 to 10 days after symptom onset. Any positive immune responses detected in the first four days of illness, and most positive results from samples collected in the fifth and sixth days of illness are likely due to prior (old) exposures to rickettsial agents, and are unrelated to the current illness.
 - For example, when patients are tested for RMSF in the first six days of illness, most positive results are due to prior exposures to the ubiquitous, non-pathogenic spotted fever group *Rickettsia* (SFGR) carried by Virginia's most common tick, the lone star tick. Greater than 55% of the lone star tick population in Virginia is infected with this SFGR, and based on statewide tick surveys conducted, lone star ticks likely cause the majority of human tick bites in the Commonwealth. All non-pathogenic species of SFGR are serologically cross-reactive on the test for RMSF and may cause thousands of low positive RMSF test results in Virginia each year.
- Patients with a severe illness such as RMSF will typically develop titers $\geq 1:1024$ within 15 days of illness onset, but patients who have been exposed to non-pathogenic SFGR rarely develop titers $> 1:256$ in the months after their exposure.
- Serum testing done in the acute phase of illness should always be followed by collection and testing of a convalescent serum sample to clearly demonstrate an increase in titer.
- When testing for ehrlichiosis, anaplasmosis or spotted fever rickettsiosis, the use of a quantitative IFA for IgG will yield useful results, particularly if both acute and convalescent testing is done.
- The use of IgM assays for rickettsial diseases yields unreliable results.
- Non-quantitative IgG assays and quantitative EIA assays for IgG yield results that are not interpretable with respect to rising titers.

References

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