Rickettsial infections pose difficult diagnostic challenges to both clinicians and laboratorians. Rapid confirmatory assays are not commonly available to guide treatment decisions of acutely ill patients. However, confirmatory assays provide the physician with vital information that retrospectively validates the accuracy of the clinical diagnosis. Laboratory confirmation of infection is also vital to understanding the epidemiology and public health impact of TBRD.

Several laboratory methods are available to diagnose TBRD. However, they vary in the time required to obtain results and in the type of information they provide the clinician. Therefore, treatment decisions should be based on epidemiologic and clinical clues and should never be delayed while waiting for laboratory confirmation of a diagnosis. Similarly, test results should be interpreted in the context of the patient's illness and the epidemiologic setting. Misuse of specialized tests for patients with a low probability of the disease and in areas with a low prevalence of disease might result in confusion. A fundamental understanding of the signs, symptoms, and epidemiology of the disease is critical in guiding requests for tests and interpretation of test results for ehrlichiosis, anaplasmosis, and RMSF. Studies have suggested that antibiotic therapy might diminish the development of convalescent antibodies in RMSF (CDC, unpublished data, 2005). However, the degree to which doxycycline might cause this occurrence is uncertain. If molecular or culture diagnostic methods are conducted, obtaining blood for testing before antibiotics are administered is essential to obtain the best results.

**Blood-Smear Microscopy** - Microscopic examination of blood smears stained with eosin-azure type dyes (e.g., Wright-Giemsa stain) might reveal morulae in the cytoplasm of infected circulating leukocytes (1%-20%) of patients with HME and 20%-80% of patients with HGA during the first week of infection, which is highly suggestive of ehrlichial or anaplasma infection. However, blood smear examination is insensitive and should be performed by an experienced microscopist. In addition, a negative blood smear examination should not dissuade the caregiver from initiating treatment with doxycycline if the clinical presentation and routine laboratory findings support the diagnosis of ehrlichiosis or anaplasmosis. Blood smear examination is not useful for diagnosis of RMSF.

**Serologic Testing** - Serologic assays for RMSF, HME, and HGA are commonly available through multiple commercial and state public health laboratories. Serologic evaluations are commonly
conducted by using the indirect immunofluorescence antibody (IFA) assay. Antibodies in the serum bind to fixed antigens on a slide and are detected by a fluorescein-labeled conjugate. Although IFA remains the principle diagnostic tool for the diagnosis of rickettsial and ehrlichial infections, no standardized antigens, conjugates, or agreement on what constitutes a positive result among the various laboratories providing these tests exist. Individual laboratories should be consulted regarding their test threshold levels. Enzyme-linked immunosorbent assay (ELISA) is becoming a more frequently used assay. Similar to IFA, the accuracy of ELISA depends on the laboratory conducting the test, the quality and specificity of the antigen, and the threshold levels at which a positive result is considered. Available ELISA tests are qualitative and cannot be used effectively to monitor increases or decreases in antibody titer.

The sensitivity of the IFA assay is substantially dependent on the timing of collection of the sample. Early in any TBRD, a majority of serologic tests will be negative. Clinical illness nearly always precedes laboratory diagnosis by any method. As the illness progresses to 7-10 days, the sensitivity of IFA serology increases. The IFA is estimated to be 94%-100% sensitive after 14 days, and that sensitivity is increased if paired samples are tested. The IFA is considered to be the gold standard of serologic testing for rickettsial diseases, and other serologic tests have not been developed that surpass the sensitivity and specificity of these assays. Testing two sequential serum or plasma samples together to demonstrate a rising IgG or IgM antibody level is essential to confirm acute infection. Paired serum specimens taken early (i.e., acute) and later (i.e., convalescent) in the disease course represent the preferred specimens for evaluation. Typically, these specimens should be taken at least 2--3 weeks apart to examine for a four-fold or greater increase in antibody titer.

The majority of patients demonstrate increased IgM or IgG titers by the second week of the illness (patients infected with certain imported rickettsiae might not demonstrate increased titers until 4 weeks after illness onset). However, patients might lack diagnostic IgG and IgM antibody titers in the first 7 days of illness, a period when the majority of patients initially seek medical care and laboratory testing is performed. The duration of time that antibodies will persist after recovery from the infection is variable. In certain persons, high titers of antibodies against A. phagocytophilum have been observed for 3½ years after the acute illness. For RMSF, IgG and IgM titers increase concurrently by the second week of illness, and IgM antibodies wane after 3--4 months, whereas IgG titers persist for 7--8 months. The majority of commercial reference laboratories conduct testing for IgG and IgM antibodies.

Cross-reactivity of antigens results in antibody responses that are typically group-specific, but not necessarily species-specific, after infections with these pathogens. For example, serologic tests that detect antibodies reactive with R. rickettsii might have resulted from previous infections with other spotted fever group rickettsial species. Similarly, antibodies reactive with E. chaffeensis or A. phagocytophilum occasionally react with the other ehrlichial species, which might impede epidemiologic distinction between the ehrlichial infections. Most patients with E. ewingii infections develop antibodies that react with E. chaffeensis antigens. Little cross-reactivity of Rickettsia with Ehrlichia or Anaplasma species exists. Certain serologically confirmed cases of infection thought to be RMSF, HME, or HGA might represent infections with the other agent or with another antigenically related species. The predominance of non-R. rickettsii species in tick vectors collected in RMSF-endemic areas suggests that related organisms of undetermined pathogenicity might play a role in human illness. This occurrence is especially true for persons who are infected with rickettsial organisms from endemic areas outside of the United States.
Nucleic Acid Detection - Amplification of specific DNA by PCR provides a rapid method for detecting TBRD infections. These tests are available from CDC, certain state health laboratories, and a limited number of research and commercial laboratories. Conventional PCR tests have no specified standard, and diagnostic sensitivity and specificity might vary among individual assays. Doxycycline treatment, in particular, can also decrease the sensitivity of PCR. In studies of A. phagocytophilum infection, PCR was estimated as 60%-70% sensitive, and for diagnosis of infection with E. chaffeensis, PCR was estimated to be 52%-56% sensitive to 87% sensitive. For RMSF, PCR is probably more useful for detecting the etiologic agent in a skin biopsy or autopsy tissue specimen than it is in an acute blood sample because, typically, low numbers of rickettsiae circulate in the blood in the absence of advanced disease or fulminant infection. PCR testing of skin biopsies alone does not offer ideal sensitivity, and a negative result does not exclude the diagnosis because of focality of vessel involvement. Laboratory confirmation of RMSF in the acute stage is improved when PCR is used in conjunction with IHC staining. PCR of whole blood specimens is more useful for confirming HME, HGA, and E. ewingii infection because of the tropism of these pathogens for circulating WBC. However, no optimal time frame has been established that is ideal for sample collection to ensure the highest sensitivity for diagnosing ehrlichioses or anaplasmosis. New techniques (e.g., real-time PCR) might offer the advantages of speed, reproducibility, quantitative capability, and low risk for contamination, compared with conventional PCR.

IHC Staining - Another approach to diagnosing TBRD is immunohistochemical (IHC) staining of antigens in formalin-fixed, paraffin-embedded biopsy or autopsy tissues. This test can be particularly useful to diagnose fatal TBRD in those patients for whom diagnostic levels of antibodies have not developed before death. For patients with a rash, IHC or immunofluorescence staining of a skin biopsy can be a critical diagnostic technique for RMSF. Immunostaining of skin biopsy specimens has been reported to be 100% specific and 70% sensitive in diagnosing RMSF. This method has been used to diagnose fatal and nonfatal cases of RMSF. Because rickettsiae might be focally distributed in tissue, this test might not always detect the agent. Autopsy tissues also are appropriate for evaluation and include the liver, spleen, lung, heart, kidney, and brain. The IHC method is most useful in documenting the presence of organisms in patients before initiation of antibiotic therapy or within the first 48 hours after antibiotic therapy has been initiated. IHC techniques also are available for diagnosing cases of ehrlichioses and anaplasmosis from bone marrow biopsies and tissue obtained at autopsy of fatal cases, including the spleen, lymph nodes, liver, and lung. Immunostaining for spotted fever group rickettsiae, E. chaffeensis, and A. phagocytophilum is offered by CDC and certain university-based hospitals and commercial laboratories in the United States.

Culture - Because the agents that cause TBRD are obligate intracellular pathogens, they must be isolated by using cell culture techniques that are typically more labor-intensive and time-consuming than serologic, molecular, or IHC assays. Theoretically, any laboratory capable of performing routine viral isolations might have the expertise to isolate these pathogens. However, R. rickettsii is classified as a Biosafety Level-3 (BSL-3) agent, and attempts to isolate this agent should be made only in laboratories equipped to handle BSL-3 pathogens. Laboratories attempting culture of R. rickettsii bacteria need to comply completely with federal regulations (42 C.F.R. [2004]) regarding the registration and use of select agents. As a result, culture is rarely used for diagnosis, and other methods (e.g., serology, PCR, or immunostaining) are used to confirm infection.

The following is a summary of salient features of diagnostic testing:
Blood smear microscopy might reveal presence of morulae in infected leukocytes, which is highly suggestive of HGA or, less commonly, HME.

Blood smears are not useful to diagnose RMSF.

Examination of paired serum samples obtained 2–3 weeks apart that demonstrate a rise in antibody titer is the most appropriate approach to confirm TBRD.

Patients usually do not have diagnostic serum antibody titers during the first week of illness; therefore, an inability to detect antibodies (IgG or IgM) in acute-phase serum does not exclude TBRD.

Immunohistochemistry of a biopsied skin lesion or autopsy tissues is useful for RMSF diagnosis in patients for whom diagnostic titers of antibodies have not yet developed.

Whole blood specimens might be useful for a PCR confirmation of HME, HGA, and E. ewingii infection; however, a negative result does not rule out the diagnosis.

Bottom Line is Prevention

- Avoid tick bites, which is key to the prevention of TBRD.
- Limit exposure to tick habitats, including grassy and wooded areas.
- Inspect the body carefully for ticks after being in a tick habitat.
- Remove attached ticks immediately by grasping with tweezers close to skin and pulling gently with steady pressure.

If you have any questions please feel free to contact Dr. Sandy Snow at 501-661-2169 or fax to 501-661-2300 or e-mail to sandra.snow@arkansas.gov