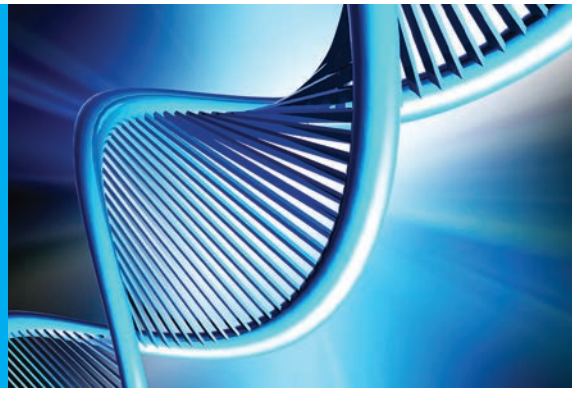
SUMMER 2016

Lab Link

THE NEWSLETTER OF MAIN LINE HEALTH LABORATORIES



Antinuclear Antibody Update

By Pradeep Bhagat, MD, Medical Director, Main Line Health Laboratories

At Main Line Health Laboratories, Antinuclear antibody (ANA) screens are performed utilizing two methodologies:

- Multiplex Bead Assay (MBA) by AtheNA MultiLyte ANA II Plus test and
- Indirect immunofluorescence (IFA).

The Multiplex Bead Assay (MBA) is the default method which, if positive, will reflex to nine subset antibody tests and an ANA IFA titer. At this time, MBA assays are not equivalent to screening ANA by IFA and the discordance rate between multiplex assay and IFA test is at least 25–30%.

Recently, we performed a study with 62 patients that have both multiplex and IFA assays ordered at the same time. We found 25 samples (40%) out of 62 samples to be positive by both methods. 30 samples (48%) to be positive for MBA and IFA negative, and 7 samples (11%) were found to be MBA negative and IFA positive.

The American College of Rheumatology considers IFA as the gold standard for ANA testing. ANA by IFA, if performed with a history and physical, identified almost all patients with SLE and with higher sensitivity for other rheumatological conditions. Since IFA is a labor intensive test, our recommendation is to order ANA IFA only in select patients with signs and symptoms suggestive of rheumatological conditions.

To order an ANA IFA in SmartChart, be sure to type “ANA IFA” to begin your

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Babesiosis

By Olarae Giger, PhD, Manager, Microbiology

In the warmer months of the year, many people spend time on outdoor activities which can put them at risk for contracting tick-borne illnesses. In the United States, babesiosis has most frequently been reported from the Northeast & the upper Midwest. However, there have recently been increasing numbers of cases reported within the Delaware Valley. In the summer of 2015, 27 patients were diagnosed with babesiosis in the Main Line Health system.

Epidemiology

Many different species of *Babesia* parasites have been found in animals, only a few of which have been found in humans. *Babesia microti*, which usually infects white-footed mice and other small mammals, is the species that causes the majority of human disease in the U.S. Occasional cases are caused by other species.

B. microti is most often transmitted to humans by the bite of infected *Ixodes scapularis* ticks, commonly referred to as deer ticks or blacklegged ticks. The young, nymph stage of the tick most actively seek blood meals during warm summer months in wooded areas, brush or tall grass. Infected people may not recall a tick bite because *I. scapularis* nymphs are very small, about the size of a poppy seed.

Babesia can also be transmitted via blood transfusion or congenitally during pregnancy. In 2012, 7 of 911 cases reported to the Centers for Disease Control & Prevention were classified as transfusion associated, and 1 case was attributed to congenital transmission. To date, no *Babesia* tests have been licensed for screening blood donors.

Disease & Risk Factors

Babesia infection can range from subclinical to severe and life-threatening. Symptoms, if any, usually develop within a few weeks or months after exposure but may appear many months later, particularly in persons who are or become immunosuppressed. Hemolytic anemia and nonspecific flu-like symptoms (fever, chills, body aches, weakness, fatigue) are the most common clinical manifestations. Some patients have splenomegaly, hepatomegaly or jaundice.

Risks factors for severe babesiosis include asplenia, advanced age and other causes of impaired immune function (malignancy, corticosteroid therapy, HIV). Severe cases can be associated with marked thrombocytopenia, disseminated intravascular coagulation, hemodynamic instability, acute respiratory distress, myocardial infarction, renal failure, hepatic failure, altered mental status and death.

For acutely ill patients, findings on routine laboratory testing frequently include hemolytic anemia and thrombocytopenia. Additional findings may include proteinuria, hemoglobinuria and elevated liver enzymes, blood urea nitrogen and creatinine.

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Preventing Hemolysis

By Donna Burkhardt, MT (ASCP), Clinical Physician Liaison

Eighty-four percent of laboratory errors can be attributed to pre-analytical variables, those processes that occur before the specimen is analyzed, hemolysis being one of the most common. By definition, hemolysis occurs when red blood cells rupture, resulting in hemoglobin being released into the serum or plasma. This can cause a false elevation in some analytes, such as potassium and LDH due to their high concentration in red blood cells. The red or pink color of a hemolyzed sample can also interfere with some test methodologies, including Vitamin D, which requires a colorless sample or it will be rejected as an unacceptable specimen, thus causing the patient the inconvenience of returning to be redrawn.

The Main reasons for hemolysis are:

- **A traumatic venipuncture**
  - Warm up the puncture site; warming increases the blood flow and prevents the need to "milk" the site, a significant cause of hemolysis.
  - Place the needle correctly in the vein; if the bevel of the needle is crowded by the inner wall of the vein, the partial occlusion exerts a dramatic force on the cells. This is typically indicated by too slow a blood flow.
  - When using a syringe, pull the plunger gently; pulling too quickly exerts excess pressure—well beyond that of a standardized evacuated tube—and will shear the cell walls.
  - Similarly, pushing hard on the syringe plunger while transferring blood to another tube exerts a destructive level of pressure, and can also cause loss of the sample if the stopper comes off.
  - Avoid drawing from catheters and lines; these are designed to deliver fluids to the patient, not drawn from the patient. Drawing blood samples from these systems involves turbulence that makes hemolysis unavoidable.
- **Vigorous shaking of tubes after collection**
  - Mix additives with the specimens gently; vigorous mixing or shaking can break the cells. Sodium citrate tubes for coagulation testing should be inverted only three or four times.
  - All other anticoagulant tubes should be gently inverted eight to 10 times.
- **Collecting samples using a small diameter needle:**
  - Use a 20-22 gauge needle for routine collection; too small a needle results in excess vacuum force, while too large a needle can cause stress on the cell walls.
- **Centrifuging samples before they have fully clotted.**
  - Clotting time cannot be rushed; centrifugation of the sample too soon will result in hemolysis.
  - For tubes with a clot activator, gently invert the specimen five times to ensure complete mixing, and allow the activator to work for 30 minutes (minimum 20) with the tube in a vertical position.
  - For serum tubes without a clot activator, don't invert—just allow the sample to clot for 60 minutes with the tube in a vertical position.

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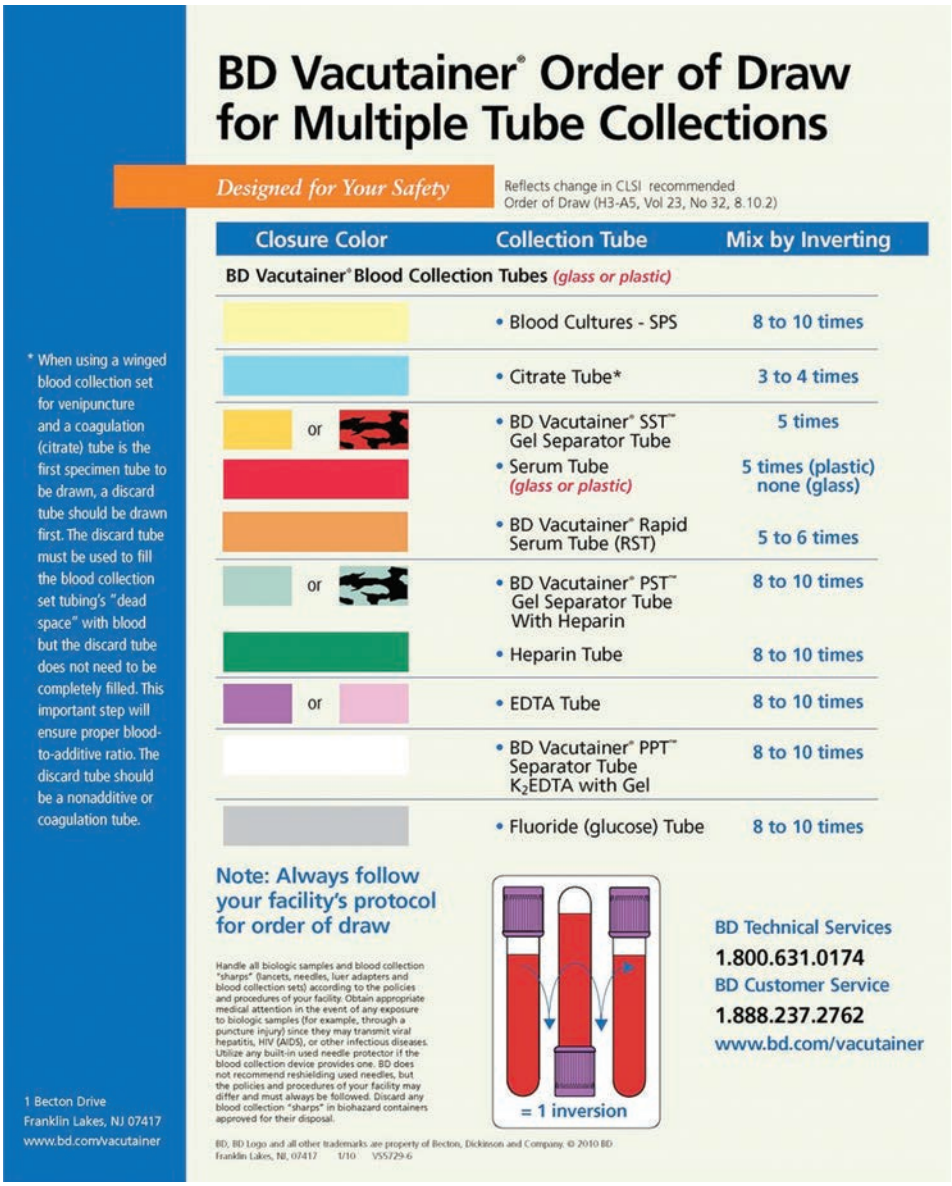

Antinuclear Antibody

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search. Check the box for "ANA, IFA Method (Titer)."

If you have any questions do not hesitate to call Dr. Bhagat at 484.476.3521, or Annemarie Brewer, Immunology supervisor, at 484.476.8408. ■

Preventing Hemolysis

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- Don't centrifuge specimens at a higher speed or for longer than necessary.

- **Not following the Order of Draw for multiple tube collection** (see chart)

- **Not allowing the site to dry completely, after cleaning the arm with alcohol**
  - Alcohol damages cell walls; allow the venipuncture site to completely air dry after cleaning it with alcohol.

- **Leaving the tourniquet on for more than one minute**
  - Prolonged tourniquet time causes the interstitial fluid to leak into the tissue, promoting hemolysis.

- **Never collect blood from an area with a hematoma (bruise)**

- **Blood collected insufficient to the amount of additive in the tube**
  - Fill tubes to correct volume; under-filling of tubes containing anticoagulant results in a higher than recommended concentration of the additive. Use a smaller tube for difficult draws.

- **Adverse conditions when samples are being transported to the laboratory**
  - Protect the specimens during shipping; exposure to inappropriate temperatures and significant jarring will cause hemolysis in transit.

Accurate results are essential for physicians and practioners to diagnose and treat patients. Lab data comprises about 70% of a patient's medical record. Therefore, the integrity of the blood sample is the vital first step for an accurate final result. It also ensures that the patient will not have to return to have their specimen recollected. ■

Vitamin D Testing Reimbursement

During the past few years the importance of Vitamin D has gained significant attention by the general public and media. More and more people are being tested for 25(OH) Vitamin D by patient request or even as part of screening during a routine physical examination. As a result, the 25(OH) Vitamin D assay has become one of the most ordered esoteric tests. It is important to note that Medicare and private health insurers will only reimburse for patients with, or being evaluated for, certain diseases or conditions that present the risk of Vitamin D deficiency.

In the past, insurers would commonly cover lab testing at 100%. Increasingly, only preventive services are being covered at 100%. Also, unless a specific, disease-related diagnosis (ICD-10 code) is present on the lab requisition, the Vitamin D level may not be covered at all and the patient may receive an unexpected lab bill.

According to Novitas Solutions*, the Medicare Administrative Carrier (MAC) for Pennsylvania, Measurement of Vitamin D levels is indicated for patients with:

- Chronic kidney disease stage III or greater;
- Cirrhosis;
- Fibromyalgia;
- Granuloma forming diseases;
- Hypocalcemia;
- Hypercalcemia;
- Hypovitaminosis D;
- Hypervitaminosis D;
- Long term use of anticonvulsants or glucocorticoids and other medications known to lower Vitamin D levels;
- Malabsorption states;
- Obstructive jaundice;
- Osteomalacia;
- Osteoporosis;
- Osteogenesis imperfect;
- Osteosclerosis;
- Psoriasis;
- Rickets;
- Vitamin D deficiency on replacement therapy; to monitor the efficacy of treatment.

Vitamin D refers to a group of fat-soluble steroid hormones responsible for increasing intestinal absorption of calcium and other nutrients. In humans, the most important compounds in this group are Vitamin D3, or cholecalciferol, and Vitamin D2, ergocalciferol.

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Vitamin D Testing

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Cholecalciferol and ergocalciferol can be consumed from diet and supplements. The major source, however, is synthesis in the skin when exposed to sunlight, specifically ultra-violet B.

Vitamin D, whether from the diet or dermal synthesis, is biologically inactive. Activation requires enzymatic conversion in the liver and kidneys. In the liver, cholecalciferol (Vitamin D3) is converted to calcidiol, or 25-hydroxyvitamin D—abbreviated 25(OH)D3. Ergocalciferol (Vitamin D2) is converted in the liver to 25-hydroxyergocalciferol or 25(OH) Vitamin D2. These two specific Vitamin D metabolites are measured in serum to determine a person's Vitamin D status.

Measurement of 1,25 Dihydroxy Vitamin D (1,25 OH Vitamin D) testing not an accurate measurement of Vitamin D stores, Vitamin D deficiency or toxicity. 1,25 OH Vitamin D is indicated in patients with hypercalcemia or decreased kidney function. ■

*The complete Local Coverage Determination (LCD), Vitamin D Assay Testing, L30273, can be found on the web at novitas-solutions.com or by emailing Jack Galamb, MLHL outreach manager at galambj@mlhs.org.

Babesiosis

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Diagnosis

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. For specimens submitted to Main Line Health Laboratories, order a *Babesia* Smear and collect blood specimens in an EDTA tube (purple top).

Babesia infection can also be diagnosed by positive Babesia polymerase chain reaction (PCR) anaylsis. *Babesia*-specific antibody detection by serologic testing can provide supportive evidence for the diagnosis but does not reliably distinguish between active and prior infection.

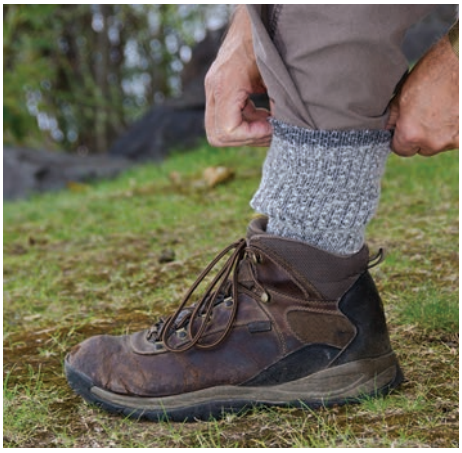
Treatment

For ill patients, babesiosis is treated for at least 7–10 days with a combination of two medications, eitheratovaquone and azithromycin or clindamycin and quinine. Treatment decisions should be individualized, especially for patients who have or are at risk for severe or relapsing infection.

Most patients without clinical manifestations of infection do not require treatment. However, consider treating persons who have had demonstrable parasitemia for more than 3 months.

Prevention

There is no vaccine to prevent Babesia infection. Preventative measures are especially important for those at increased risk for severe babesiosis. During outdoor activities in tick habitats, precautions should be taken to keep ticks off the skin:



- Walk on cleared trails & stay in the center of the trail to minimize contact with leaf litter, brush and overgrown grasses, where ticks are most likely to be found.
- Minimize the amount of exposed skin by wearing socks, long pants and a long-sleeved shirt. Tuck pant legs into socks so that ticks cannot crawl up the inside of the pants. Wear light-colored clothing to make it easier to see and remove ticks before they attach to the skin.
- Apply repellents to skin and clothing, following the instructions on the product label.

After outdoor activities, conduct tick checks and promptly remove any ticks that are found. Ticks must usually stay attached to a person for more than 36 hours to be able to transmit infection, however, *I scapularis* nymphs that typically spread Babesia infection are very small and may be easily overlooked.

If you have questions you may contact Dr. Giger at 484.476.3514 or Dr. Gary Daum, Medical Director of Microbiology, at 484.476.8013. ■

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