

February 2017

Use of Laboratory Parameters for Diagnosis of Iron Deficiency Anemia

The prevalence of iron deficiency anemia is 2% in adult men, 9-12% in non-Hispanic white women, and approximately 20% in black and Mexican-American women. Iron deficiency typically produces microcytic anemia, also seen in thalassemia, sideroblastic anemia, anemia of chronic disease, and lead poisoning. Serum ferritin level, an indicator of iron stores, is the initial preferred diagnostic test. Additional tests including serum iron levels, serum transferrin receptor levels, total iron binding capacity, and transferrin saturation may be necessary especially when inflammation is present. Markers of iron metabolism are differentially affected during acute-phase reaction, for example, ferritin is a positive and transferrin a negative acute-phase reactant. For these reasons, laboratory diagnosis of iron deficiency anemia can be challenging in patients with acute or chronic inflammatory conditions.

Decrease in iron stores creates an imbalance between the erythroid marrow requirement and the actual supply, directly effecting red cell hemoglobin content. The consequence is presence of increased hypochromic microcytic mature red blood cells and reticulocytes in the circulation. Modern hematology analyzers, due to inclusion of advanced technologies such as flow cytometry, can measure parameters related to red blood cells and reticulocytes with high accuracy, efficiency and at low cost. Red blood cell parameters including percentage of hypochromic red blood cells (%Hypo), defined as percentage of red blood cells with hemoglobin <28 g/dL and reticulocyte parameters including reticulocyte hemoglobin (CHr or Ret-Hb), immature reticulocyte fraction (IRF), and absolute reticulocyte count are available (Urrechaga E. *et. al.* Int. Jnl. Lab. Hem. 2013, 35, 144-149).

The measurement of CHr or Ret-Hb reflects the incorporation of iron into erythrocyte hemoglobin and is an estimate of recent functional availability of iron into the erythron. Since the life span of a reticulocyte in circulation is short (approximately 1-2 days), this measurement provides a sensitive indicator of iron-deficient erythropoiesis. A Ret-Hb value of less than 29 pg correlates with deficient erythropoiesis. The clinical utility of Ret-Hb is well established as a reliable marker of functional iron deficiency in hemodialysis patients, exhibiting high specificity and sensitivity in the management of IV iron therapy. In fact, guidelines issued by The Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) of the National Kidney Foundation consider use of Ret-Hb as an appropriate test to assess adequacy of iron for erythropoiesis. Certain conditions, such as thalassemia and concurrent megaloblastic anemia, however require interpretation in clinical context due to disproportionate changes in Ret-Hb value.

IRF provides measurement of immature reticulocytes that are released during periods of intense erythropoiesis, such as following hemorrhage or hemolysis and in response to therapy with iron or erythropoietin-stimulating agents (ESAs). It has been proposed as an early marker of engraftment in hematopoietic stem cell transplantation and bone marrow regeneration following chemotherapy. IRF in conjunction with the reticulocyte count provides the same information as the historic reticulocyte production index (RPI). The clinical utility of IRF has been reported in anemia treatment, neonatal transfusion needs, prognosis in prematurity, in AIDS anemia, renal transplant engraftment due to erythropoietin production, the detection of occult hemorrhage or hemolysis, aplastic crisis in hemolytic anemias, and to verify aplastic anemia (Piva E. *Rev. Bras. Hematol. Hemoter.* 2015, 37(2), 73-76).

The measurement of %Hypo is a sensitive method for quantitating the hemoglobinization of mature red blood cells. Owing to long life span of red blood

cells in circulation, determination of %Hypo provides iron status in the last 2-3 months. The clinical utility of %Hypo has been recognized in patients with chronic renal failure treated with ESAs.

At Saint Luke's Laboratories, reticulocyte count is an orderable test which provides IRF, Ret-Hb, reticulocyte percentage, and absolute reticulocyte count. Specimen requirement is one lavender top tube of blood or add on testing can be done if ordered within 24 hr of specimen collection to avoid falsely low values.

Lyme Antibody Testing

Effective immediately, Lyme antibody testing will be sent to a reference laboratory, instead of being performed by Saint Luke's Regional Laboratories. Positive results will continue to have Western blot confirmation performed as reflex testing. Specimen requirement is one red-top tube of blood.

Blood Culture Contamination Data

Saint Luke's Regional Laboratories processed 31,614 blood cultures in 2016. Overall, 10% of blood cultures were positive, which is comparable to previous years. Blood culture contaminants account for 1 to nearly 3% of positive blood cultures across SLHS. All SLHS hospitals are below the national standard for blood culture contamination (<3%), although an upward trend has been noted in the last 3 years, particularly in the four metro hospitals where the majority of blood cultures are drawn.

SLHS Hospital	# Blood Cultures 2013	# Blood cultures 2016	# Contaminant 2013 (%)	# Contaminant 2016 (%)
SLH	11,403	12,462	111 (0.97%)	203 (1.63%)
SLE	5,943	8,949	106 (1.78%)	193 (2.16%)
SLN	3,091	3,447	58 (1.88%)	88 (2.55%)
SLS	2,670	2,595	55 (2.06%)	72 (2.77%)

Blood culture contaminants result from improper antiseptic preparation of the draw site prior to collection; therefore the primary organisms responsible for contamination are skin flora.

Coagulase-negative staphylococci are found to be contaminants approximately 80% of the time. Other common potential contaminants include *Propionibacterium* species, *Bacillus* species, *Corynebacteria* species, Micrococcus, and viridians streptococci. Historically, a single blood culture contaminant has been estimated to add \$5,000 to the cost of a hospitalization, due to additional diagnostic studies and unnecessary antibiotic therapy.

Proactive efforts are underway SLHS-wide to optimize blood culture collection as much as possible through a variety of means, including modification of collection techniques and heightened attention at the time of collection. Likewise, the Antimicrobial Stewardship Program collaborates with physicians at the time a positive blood culture is reported to determine the likelihood that the organism is a contaminant based on the clinical findings, and discuss the most appropriate course of action including the necessity for antibiotics.

Aminoglycoside Monitoring and Interference

Aminoglycoside antimicrobials, such as tobramycin are mostly used to treat infections with gram negative bacteria resistant to less toxic antibiotics. For serious infections with gram negative bacteria, a combination therapy with beta-lactams or quinolones may be used due to synergistic action.

Aminoglycosides require therapeutic drug monitoring due to adverse toxic effects including nephrotoxicity and ototoxicity. Accurate serum aminoglycoside level determination can be challenging in patients on combination therapy with beta-lactams. Several studies have shown that therapeutic concentrations of β -lactam antibiotics may inactivate aminoglycosides *in vivo and in vitro*, rate of which can vary with the concentration of both aminoglycoside and β -lactam, as well as the time, temperature, and pH (Hammett-Stabler C. *et al.* Clinical Chemistry, 1998, 44(5), 1129-1140). This can lead to falsely low serum aminoglycoside levels on therapeutic drug monitoring.

Specimen requirement for aminoglycoside levels is a red top or green top tube. After processing, the specimen is analyzed within 2 hours of collection or stored frozen to prevent *in vitro* inactivation of aminoglycosides.