**Hemoglobin Electrophoresis: The Hematology Laboratory approach to screening and identification of hemoglobinopathies and Thalassemias.**

Short of amino acid sequencing or genotyping, no single or combination electrophoretic or other routine clinical laboratory methods are capable of definitively identifying all of the various hemoglobin variants 100% of the time. From a practical point of view, when a request for hemoglobin electrophoresis is received, our clinical laboratory’s approach is targeted at identifying the clinically significant and major hemoglobin variants that are common (S, C, E,) and not so common (D, G, others). Thalassemia results in the production of only normal hemoglobin variants in abnormal proportions beyond the newborn period. Therefore, when the patient is microcytic, we reflexively quantify the proportion of normal hemoglobin variants (hemoglobin A, A2 and F) in the sample for assessment of possible Beta Thalassemia. Alpha Thalassemia is recognized by the presence of high levels of gamma chain or beta chain tetramers (i.e. Hemoglobin Barts and Hemoglobin H respectively) in the newborn period or provisionally recognized by the absence of other causes of microcytosis outside the newborn period. Interestingly, the proportions of our samples that contain hemoglobin variants with no hematologic consequences (e.g. hemoglobin J, N, etc.) have increased in recent years, primarily as a result of follow up testing for abnormal newborn hemoglobin screening tests. Correct interpretation of the results of testing are reported based on an integration of hemoglobin test results with the CBC, peripheral blood smear review, and demographic information when available including race and ethnicity and clinical data. Particular attention is directed towards those patients with thalassemic indices (hi RBC, low MCV, normal RDW) and abnormal RBC forms (targets, hypochromia, sickle cells, and crystals) for recommending further testing (e.g. ferritin, parental testing, etc.) and correct interpretation. Hemoglobin isoelectric focusing currently serves as our primary screening method and abnormal hemoglobin variants are confirmed by High Performance Liquid Chromatography (HPLC). We are investigating switching to HPLC as the primary screen method to reduce costs and expedite turn around time. Reflex quantitation of hemoglobin F, hemoglobin A2 of abnormal hemoglobin variants are performed by HPLC.

When only normal hemoglobin variants are present and the patient is not microcytic, hemoglobin types are reported qualitatively in decreasing order of their relative concentration within a sample (e.g. A, F, A2; or F, A, A2 meaning that A>F>A2 or F>A2>A respectively). Abnormal hemoglobin variants as well as normal hemoglobin variants in a microcytic patient are reported quantitatively based on HPLC testing. When not known, the sample is presumed to represent the patient’s own blood (i.e. absence of previous transfusions). Please make note on the requisition if the sample is acquired after transfusion.
News From Biochemical Genetics
by Uttam Garg, PhD

Laboratory Diagnosis of Medium Chain ACYL-CoA Dehydrogenase (MCAD) Deficiency

Once glycogen stores are depleted during prolonged fasting, fatty acids are the major source of energy. Fatty acids are oxidized, in mitochondria, to ketone bodies using a number of enzymes including various acyl-CoA dehydrogenases. MCAD, one of the acyl-CoA dehydrogenases, is an important enzyme involved in the oxidation of medium chain fatty acids. Deficiency of MCAD results in accumulation of medium chain acylcarnitines, which can be measured in blood. Analysis of acylcarnitine is generally performed by tandem mass spectrometry (MS-MS). Recently a number of states have begun expanded newborn screening by MS-MS. Over 20 metabolic disorders, including MCAD can be screened from a newborn drop of blood on newborn filter paper with this technique. Plasma acylcarnitine may miss the diagnosis on patients with secondary deficiency of carnitine. Therefore, it is recommended if plasma acylcarnitine analysis is normal, urine organic acids and acylglycines be performed on patients with suspected MCAD deficiency.

Urine organic acids and acylglycines analysis: In the patients with MCAD deficiency, dicarboxylic acids are increased in urine particularly during acute episodes. Increase in adipic, suberic and sebacic dicarboxylic acids are also increased in urine particularly during acute episodes. Patients with MCAD deficiency, dicarboxylic acids are increased in urine particularly during acute episodes. Urine organic acids and acylglycines analysis is performed on patients with suspected MCAD deficiency. In the patients with MCAD deficiency, dicarboxylic acids are increased in urine particularly during acute episodes. Increase in adipic, suberic and sebacic dicarboxylic acids are also increased in urine particularly during acute episodes. Urine organic acids and acylglycines testing is available at the Children’s Mercy Hospitals Laboratories and is performed by using gas chromatography mass spectrometry (GC-MS). Please call 816-234-3295 with any questions.

The other uncommon methods for MCAD diagnosis and confirmation include analysis of fatty acids oxidation in cultured cells and mutational analysis of MCAD gene.

Laboratory CME Series
Tuesday, March 18, 2003
12:00 p.m.-1:00 p.m.
Location: Laboratory Conference Room 
#2206.10
Speaker: Robert Garola, MD
Topic: “Histological Techniques in Dermatopathology”