The Red Blood Cell (RBC) Special Studies Laboratory offers testing for hereditary causes of hemolytic anemia. Some of the enzyme studies are also useful in the diagnosis of red cell aplasia disorders and immunodeficiency syndromes in children.

**Hemolytic Anemia Evaluation**

Genetic abnormalities in red blood cell enzymes can cause acute or chronic hemolytic anemia. Acute or episodic hemolysis occurs with infection or certain drugs. In most cases, this is due to glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is one of the most common genetic disorders affecting millions of individuals worldwide. The gene for G6PD deficiency is located on the X chromosome and most hemolytic episodes occur in males. Chronic hemolysis due to other enzyme disorders is rare compared to G6PD deficiency. The second most common cause is pyruvate kinase (PK) deficiency, and this disorder is thought to affect thousands worldwide. Much less common enzyme defects causing hemolysis are those due to other glycolytic enzymes, alterations in glutathione metabolism and abnormalities in purine or pyrimidine metabolism. Testing for red cell enzyme abnormalities is indicated in patients with hemolytic anemia not due to an immune process (direct antiglobulin test negative) and not due to a hemoglobin or RBC membrane abnormality.

**Red Cell Enzyme Panel**

- Glucose-6-Phosphate Dehydrogenase (G6PD), Quantitative
- 6-Phosphogluconate Dehydrogenase (6PGD), Quantitative
- Pyruvate Kinase (PK), Quantitative
- Glucose Phosphate Isomerase (GPI), Quantitative
- Hexokinase (HK), Quantitative
- Pyrimidine 5’ Nucleotidase (P5’N) Screen
- Reduced Glutathione (GSH) Concentration
- Adenosine Deaminase (ADA), Quantitative

**G6PD Female Carrier Status Panel**

Glucose-6-Phosphate Dehydrogenase deficiency (G6PD) is an X-linked disorder. Females who are carriers can have low levels of G6PD activity. However, since women have another normal X chromosome, G6PD activity can be in the normal range thereby obscuring detection of the carrier state. In view of the G6PD assay procedure which includes testing of 6-Phosphogluconate Dehydrogenase (6PGD), a ratio of 6PGD to G6PD activity has become a useful screen for recognizing G6PD carrier status in females. The normal ratio of 6PGD to G6PD is less than or equal to 0.8. The ratio found in G6PD deficient carriers is greater than or equal to 1.0.

**EMA (Eosin-5-maleimide) for Spherocytosis by Flow Cytometry**

Hereditary Spherocytosis (HS) is a common cause of chronic hemolysis, particularly in people of northern European extraction. HS is due to an inherited defect in the red cell cytoskeleton. A morphological feature of this disorder is the presence of spherocytes on the peripheral blood smear. EMA is useful in detecting hereditary spherocytosis (HS) in the setting of non-immune spherocytic hemolytic anemia, and is useful in detecting mild or atypical presentations of HS. The flow cytometric test measures the fluorescence intensity of intact red cells labeled with the dye eosin-5-maleimide (EMA), which reacts covalently with band 3 protein. The flow method is a reliable, speedy diagnostic test for HS with very good sensitivity and specificity. If the pre-test probability of HS is high, a positive EMA study would confirm the diagnosis. For more complex clinical presentations, EMA together with an incubated Osmotic Fragility RBC maybe useful.

**Osmotic Fragility, RBC**

In patients with HS, there is increased fragility (i.e. hemolysis) when erythrocytes are suspended in hypotonic salt solutions. This effect is magnified after incubation for 18 hours at 37°C. This test will also be positive in patients with other causes of spherocytosis, such as autoimmune hemolytic anemia, and in a few rare congenital nonspherocytic hemolytic anemias. Disorders of increased or decreased red blood cell hydration can be recognized by abnormalities in the Osmotic Fragility. This test is usually performed in conjunction with the EMA.
**Evaluation of RBC Aplasia in Children**

*Adenosine Deaminase (ADA) Quantitative*

Two types of red cell aplasia occur in children:

- The first type of red cell aplasia is congenital hypoplastic anemia, also known as Diamond Blackfan anemia (DBA). This is a lifelong condition often requiring specific drug therapy, red cell transfusions, and in some cases bone marrow transplantation. The erythrocytes in DBA patients have several unusual features. One of the most interesting abnormalities has been an elevated level of adenosine deaminase (ADA) activity, seen in 75% of affected patients.

- The second type of red cell aplasia is due to a brief immunologic suppression of normal erythropoiesis occurring in otherwise healthy children. This transient erythroblastopenia of childhood (TEC) remits within weeks to a few months of onset. ADA activity is almost always normal.

The relationship of this enzyme alteration to the pathophysiology of RBC aplasia in DBA is not known, although it remains a useful screening marker for identifying children with this disorder.

**Immunodeficiency Enzyme Studies**

*Adenosine Deaminase (ADA) Quantitative*

*Purine Nucleoside Phosphorylase (PNP) Quantitative*

Certain forms of severe combined immunodeficiency syndrome (SCIDS) are related to adenosine deaminase deficiency in lymphocytes. This impairment in lymphocyte metabolism leads to an accumulation of purine nucleotides that inhibit normal lymphocyte function. Diagnosis of this variant form of SCIDS is facilitated because the same enzyme defect occurs in the patient's red blood cells. In addition, another immunodeficiency syndrome due to a selective defect in T-cell function is related to a deficiency of purine nucleoside phosphorylase in lymphocytes. This defect can also be detected in erythrocytes.

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Diagnostic tests performed by this laboratory, except for pure quantitative studies, include an interpretation of results based on the available clinical and laboratory data. Correlation with clinical history and other data is crucial for the interpretation of these tests. Our staff and medical directors are available to answer questions and discuss results. For patients with transfusion history, please call the lab prior to sending samples to discuss the alternate testing methods available. For consultation services please call Customer Services at 1-877-717-3733 and request the Red Blood Cell Special Studies Laboratory.

**Customer Service**

High quality customer service is key in building and maintaining client relationships. Our dedicated customer support team can provide any information that is requested or arrange for any services required by clients. The department is staffed 24 hours a day, seven days a week to provide rapid and accurate responses.

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Web portal for patient or health care facilities to view bill and/or update insurance information. Charges directed to submitting facility can be viewed prior to month end invoicing. Each month you will receive an itemized invoice indicating the date of service, patient name, CPT code, test name, and test charge. We can also bill Stanford contracted 3rd party payers for laboratory services.

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