News From Coagulation
By Marilyn Hamilton, MD, PhD

New PT and PTT reference ranges were recently announced. I want to review how the new values were determined. The entire study took many people in the lab over a year. The final values were selected in conjunction with Brian Wicklund.

The need for an evaluation became apparent when more and more coagulation workups, done for minimally elevated PT or PTT, were not finding any abnormality. The Platelet Function Analyzer was also introduced. This assay provides a good screen for von Willebrand’s disease, sometimes identified by an elevated PTT.

Finally the Hematology Laboratory installed new coagulation instruments. Although the values on the old and new instrument were sufficiently close to allow using the new instruments, they were not identical. Our old upper limit for PTT for children over 6 months old was 35.2.

The first step was to find children’s hospitals experienced with the new coagulation instrument and using the same reagents. ARUP labs had recently completed a large study of 900 normal children recruited over a one year period. Their mean PTT was 31 with a central 95% reference range of 26-38.

Children’s Memorial Hospital also uses the same instrument and reagents and has a PTT Reference Range of 25.3-38.7.

The second step was to look at our own “normal” patients who can be hard to find at CMH. Even a significant history of antibiotics, such as you might find in a tonsillectomy patient, alters the coagulation results.

The largest group of “normal” patients is at CMS. There were 61 specimens from children over 6 months old in 4 months. Eleven children with values over our current upper limit, 35.2, were evaluated. Seven cases were omitted from the analysis based on the possibility that they had a coagulation abnormality. Our mean value for the remaining was 31 with a standard deviation of 3.25.

PTT should be clearly abnormal with a Factor 8 level < 50%. This was verified. In addition, the medical records of 518 patients with PTT values between our old upper limit, 35.2, and 38 were evaluated. Only 8 coagulation abnormalities were identified and the PTT was 38.8 in the one patient with a PTT identifiable abnormality, Factor 8, 47%.

A similar study was completed for PT. The new upper limit has increased from 15.0 to 15.4. The relative INR will drop by about 0.1 point.

Values for children less the 6 months old were extrapolated from the work of Maureen Andrew et al. American Journal of Pediatric Hematology/Oncology 12:95-104, 1990.

The new higher upper limits for PTT of 37.5 and PT of 15.4 should result in fewer coagulation consultations, cancelled surgeries and less use of FFP. Within the laboratory over 400 follow-up studies will be unnecessary.

If you have any concerns about these new values, please call the laboratory.
Glycated Hemoglobins: Role of Laboratory in Management of Diabetes Mellitus.  
What are the Limitations?  
By Uttam Garg, Ph.D.

Diabetes mellitus is a group of metabolic disorders in which glucose is underutilized, producing hyperglycemia. The number of people diagnosed with the disease continues to climb in the United States (currently 16 million). The disease has been classified into several categories the major ones being Type 1 and Type 2. Type 1, formerly known as insulin-dependent or juvenile-onset diabetes mellitus is caused by autoimmune destruction of the β-cells of the pancreas rendering the pancreas unable to synthesize and secrete insulin. Type 2, formerly known as non-insulin-dependent diabetes mellitus or adult-onset diabetes, results from a combination of insulin resistance and inadequate insulin secretion. In adults Type 2 is the most common form, accounting for 90–95% of diabetes cases. Once rarely found in children, Type 2 diabetes is becoming common in children accounting for 8-45% of diabetes cases.

The Laboratory’s role in the diagnosis and follow-up of diabetes is prominent. Fasting plasma glucose levels, oral glucose tolerance tests (OGTT) and symptoms combined with random high glucose levels are used in the diagnosis of diabetes mellitus. Once the diagnosis is established, glycated hemoglobins (GHb) become a major laboratory test for follow-up of glycemic control. The measurement of GHb is important as clinical evidences suggest that tight glycemic control results in a reduced incidence of diabetic nephropathy and other long-term complications of diabetes mellitus.

GHb are heterogeneous group of hemoglobins formed by the chemical reaction between sugars and hemoglobin. The rate at which GHb is formed is proportional to the concentration of blood glucose. Since red blood cells survive an average of 120 days in the circulation, the measurement of GHb provides an index of a person's average blood glucose concentration for the last 2-3 months. The various chemical species of GHb have been named with reference to their order of elution when separated by chromatographic techniques (e.g., HbA0, HbA1a, HbA1b, HbA1c, HbA1d, HbA2, HbA3, and so forth). Some assays measure total GHb, while other assays measure a particular species. HbA1c being the major peak is generally measured or calculated in the laboratory. In an attempt to standardize the clinical measurements, most laboratory assays measure HbA1c or are calibrated to produce a result equivalent to HbA1c. To minimize intra and inter-laboratory variations, it is recommended that laboratories use only methods which are National Glycohemoglobin Standardization Program (NGSP) certified and are traceable to the Diabetes Control and Complications Trial (DCCT) reference method. Many methods are available for measurement of GHb, the most common being immunoassays, ion-exchange high performance liquid chromatography (HPLC) and boronate affinity HPLC. The former two methods measure HbA1c and the latter method measures total GHb and generally provides calculated HbA1c results.

It is important to recognize in vivo and in vitro factors affecting HbA1c results. Due to increased turn-over and shortened span of red blood cells, chronic blood loss, renal failure, and hemolytic anemia result in a misleading decrease in the HbA1c levels. Samples from patients with polycythemia or post-splenectomy exhibit increased levels of glycated HbA1c. Spurious values of HbA1c can be found with ion-exchange-HPLC from blood containing hemoglobin variants such as F, S and C. Immunoassays can also result in spurious results on blood samples with hemoglobin variants particularly those affecting the beta-chain of hemoglobin because antibodies are generally raised against the beta chain. It is recommended that the presence of hemoglobin variants be evaluated if HbA1c is >15% with these methods. Boronate affinity- HPLC is the least affected by hemoglobin variants as it measures total GHb and separation of GHb and non-GHb is relatively easy. Currently, Children’s Mercy clinical laboratory uses Boronate affinity- HPLC method.