Glycohemoglobin (GHb)

- Glycohemoglobin is superior to fasting blood glucose in monitoring diabetic compliance because it provides glycemic surveillance for an extended period of time.
- The risk for development and progression of the chronic complications of diabetes is closely related to the degree of glycemic control, as measured by glycohemoglobin.[1,2]
- Boronate affinity chromatography is the methodology of choice for glycohemoglobin testing because ion-exchange chromatography may cause falsely elevated results, especially in uremic patients.[3]
- Spectra Laboratories' methodology is consistent with the protocols established by the National Glycohemoglobin Standardization Program.[4]

Glycohemoglobin (GHb) testing is used by clinicians to evaluate diabetic patients' blood glucose control over the previous 6 to 8 weeks.[1,2] This enables physicians to determine if the patient is compliant with diabetic therapy. Glycohemoglobin testing is well-suited to an office or clinic setting, since physicians typically see and test patients infrequently—once a month at most. Blood glucose testing during an office visit may not adequately reflect true patient compliance, because it reports blood glucose levels only at the time of testing. Patients may manage blood glucose more carefully right before an office visit than during the preceding weeks.

Glycohemoglobin Biochemistry

Glycohemoglobin is a group of compounds that forms in blood when glucose attaches to the several related types of hemoglobin molecules in a process called *glycation*.[5] Since most adult hemoglobin is hemoglobin A (HbA), over 80% of all GHb is of the type HbA_{1c}.[5] Glycation of hemoglobin occurs slowly and continuously over the lifespan of red blood cells at a rate proportional to the level of blood glucose.[5,6] Glycohemoglobin levels reflect glucose control over the half-life of these cells—approximately 6 to 8 weeks.[5,6]

Glycohemoglobin (and other glycated molecules) undergo a slow chemical modification to form smaller, toxic products called *advanced glycosylation end-products* or AGEs. AGEs accumulate in blood vessel walls, nerves, and kidneys, where they cause many of the complications associated with diabetes, including nephropathy, cardiovascular breakdown, and atherosclerosis.[1,7] Conversion of GHb to AGEs occurs even after blood glucose levels are controlled.[1]

Clinical Applications

Regular glycohemoglobin testing may:

- Assist clinicians to control hyperglycemia[1,2]
- Provide a measure of long-term blood glucose control
- Identify patients with increased risk of developing vascular complications of diabetes.[1,7]

Relevance in Diabetes

The risk for development and progression of the chronic complications of diabetes is closely related to the degree of glycemic control, as measured by GHb results.[2] Regular testing of GHb indicates how effectively a diabetic patient is controlling blood glucose, enabling the clinical team to adjust therapy accordingly. Regular glycohemoglobin testing, insulin therapy, and a comprehensive diet and exercise regimen are associated with improvements in the quality of life for diabetic patients.[2]

Relevance in Kidney Disease

Kidney damage associated with diabetes is the single most common cause of chronic renal failure. The risk of death for patients with diabetic kidney failure is twice that for patients with kidney failure from other causes.[1] Glycohemoglobin and AGE levels are elevated in patients with diabetes, and further elevated in patients with both diabetes and chronic renal failure.[1] AGEs are inefficiently cleared by current dialysis therapies.[8] While transplants have proven effective in reducing AGE levels,[9] inhibitors of AGE synthesis, currently in clinical trials, have shown even greater promise.[1,7]

Spectra Laboratories Scientific Bulletin

Glycohemoglobin (GHb)

A Test for Evaluating Blood Glucose Control

Scientific Bulletin

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Clinical Intervention

Clinical management of patients with high glycohemoglobin levels may include:

- Regular testing of glycohemoglobin
- Nutritional and lifestyle counseling
- Regular testing and control of blood glucose.

Test Methodology

Two types of assays are commonly used to measure GHb: boronate affinity chromatography and ion-exchange chromatography. Boronate affinity chromatography is the preferred method because it detects only stable glycated products, whereas ion-exchange chromatography may detect unstable intermediate products and other modified forms of hemoglobin that can falsely elevate results.[3]

Boronate affinity chromatography is the preferred method because it detects only stable glycated products . . .

The lack of standardization among available GHb methodologies has led to confusion among clinicians trying to compare results from different laboratories. The National Glycohemoglobin Standardization Program (NGSP) is in the process of certifying manufacturers' equipment as being traceable to the Diabetes Control and Complications Trial, where relationships to mean blood glucose and risk for vascular complications have been established.[4] Spectra Laboratories uses equipment that has been certified by the NGSP.

Boronate affinity chromatography

Boronate affinity chromatography is based on the binding of GHb to an affinity resin. The resin contains boronic acid, which forms a specific chemical bond with the glycated hemoglobin. Non-glycated hemoglobin is not bound, and passes through the column. Sorbitol is then added to break the bond, and GHb is eluted and measured. The percentage of GHb in total hemoglobin is calculated.

Since boronate affinity chromatography detects only stable glycated products, samples do not need pre-treatment to remove unstable intermediates. Boronate affinity chromatography is not affected by factors that interfere with the accuracy of charge-based methods, such as ion-exchange chromatography.[3]

Ion-exchange chromatography

Ion-exchange chromatography is based on the different rates at which normal and glycated forms of hemoglobin migrate in a charged column. Glycohemoglobin molecules are more negatively charged than normal hemoglobin, and are eluted first. The remaining hemoglobin is then eluted. Each component is measured, and the percentage of GHb in total hemoglobin is calculated.

Ion-exchange chromatography may falsely elevate GHb results because molecules other than GHb mimic HbA_{1c} and migrate at the same rate. These include unstable intermediates and other modified hemoglobin molecules that contain non-glucose chemical groups.[3,6] The method is also sensitive to interference from hemoglobin variants and sub-optimal specimen storage.[10] While less specific than modern boronate affinity methods, many other laboratories still use ion-exchange chromatography for GHb testing.

Reference Ranges for Total Glycohemoglobin*

Non-Diabetic**	Moderate Diabetic[11]	Chronic Disease[11]
4.4 – 6.4%	11.4 – 13.4%	16.7 – 18.5%

*Boronate affinity chromatography[11]
**Spectra Laboratories' Reference Range for general population

Interpreting Results

Glycohemoglobin test results are affected by conditions that change the half-life of red blood cells. Hemolytic anemias, phlebotomy, and pregnancy generally reduce red blood cell lifespan, falsely lowering GHb results.[6] Conversely, some types of hemoglobinopathies may falsely elevate GHb results.[6]

Specimen Collection

Refer to the Spectra Reference Guide.

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For Medicare patients on dialysis, the test discussed in this bulletin is not covered under the composite rate. Medicare makes no recommendations on testing frequency. This test is separately billable with medical justification.

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