EDUCATIONAL COMMENTARY - GLYCOHEMOGLOBIN

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- Discuss the use of glycohemoglobin levels in the monitoring of glycemic control in diabetes.
- List and discuss assay characteristics and quality control parameters recommended for glycohemoglobin testing.
- List and discuss conditions and compounds which affect glycohemoglobin assays.

The estimated 17 million Americans who have diabetes are faced with the eventual development of microvascular (retinopathy, neuropathy, nephropathy) and macrovascular (stroke, coronary artery disease) complications. Randomized clinical trials, including the Diabetes Control and Complications Trial (DCCT), have documented that the risk of these complications decreases with improved long-term glycemic control as assessed by glycohemoglobin (GHB). The American Diabetes Association (ADA) recommends GHB testing a minimum of every 6 months for patients with stable glycemic control and quarterly in patients with unstable control or whose therapy changes.

Glycohemoglobin is a chemically modified form of the oxygen-carrying protein hemoglobin (Hgb). For the first few months after birth the approximate distribution of Hgb is: 60% Hgb F, 40% Hgb A, and a trace of HgbA2. This distribution shifts permanently by adolescence to: 97% Hgb A, 1%-3% Hgb A2 and up to 1% Hgb F. There are also numerous hemoglobin variants with Hgb S and Hgb C being the most frequently encountered in the U.S. The average lifespan of erythrocytes (red blood cells, RBC) is approximately 120 days, during which time a two-step non-enzymatic reaction occurs between hemoglobin and glucose in the blood. The first step is a reversible binding of glucose with accessible amine groups on hemoglobin to form a labile aldimine. The second step is an irreversible glycation reaction to form a stable ketoamine. The labile aldimine and stable ketoamine structures are collectively called glycohemoglobin, glycated hemoglobin, Hb A1 or Hb A1C.

In order to simplify and standardize nomenclature for glycohemoglobin testing, the term A1C was proposed in the diabetes guidelines of the American College of Endocrinology and the American Association of Clinical Endocrinologists and has been adopted by almost all of the diabetes agencies and professional organizations. Although this nomenclature facilitates public awareness and understanding, laboratorians should be aware that GHB assays may be measuring different analytes. Typically, assays identified as total glycohemoglobin tests include measurement of Hb A1 and other hemoglobin-glucose adducts. Hemoglobin A1 is made of three fractions: Hb A1A, Hb A1B, and Hb A1C. Approximately 80% of Hb A1 is Hb A1C. Methods measuring this analyte alone were used for the major trials correlating glycemic control and complications. Hb A1C is defined as Hb A with glucose attached to the amino-terminal valine of one or both beta chains. The ADA recommends that laboratories only use assay methods certified as traceable to the DCCT GHB reference because different assays can give different values. These certified assays report results as Hb A1C. Laboratorians should know the methodology, specificity, and characteristics of the assay they use.

There are currently more than 30 different GHB assay methods in use, but they may be broadly classified into two groups. The first group includes methods, such as cation-exchange chromatography and agar gel electrophoresis, that quantity GHB based on charge differences between glycated and nonglycated components. The second group includes methods, such as boronate affinity chromatography and immunoassays, that separate components based on structural differences between glycated and nonglycated components. Results from all types of assays are reported as the percent of total hemoglobin represented by the glycated fraction measured.

Conditions and interfering substances have been identified and characterized and laboratories should know these when reporting and interpreting results. Most of these effects are method dependent, but conditions, such as hemolytic anemia and recovery from acute blood loss, that shorten erythrocyte survival or decrease mean erythrocyte age cause falsely decreased GHB results regardless of assay method. Iron-deficiency anemia increases GHB results because of an increased percentage of old erythrocytes. The following chemicals or conditions have been reported to affect some assay results: vitamin E, vitamin C, hypertriglyceridemia, hyperbilirubinemia, lead poisoning, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction. In renal failure, urea reacts with hemoglobin to form carbamylated hemoglobin which affects some GHB assay methods. Those assays subject to interference from labile pre-Hb A1C (the aldimine) require a pre-treatment step prior to analysis. The presence of variant hemoglobins (F, S, C, E, D, G, H, I, J, N, etc.) interferes with some assay methods. Package inserts, product instructions, or manufacturer technical service personnel should be consulted if the...
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presence of hemoglobinopathies is suspected.

Expert groups have recommended several performance goals and quality control parameters. Intralaboratory precision of a CV <3%-5% is recommended. Two frozen or lyophilized whole blood controls with high and low mean values should be included at the beginning and end of each run. Patient results below the lower limit of the reference interval should be reassayed, and, if confirmed, red cell destruction or hemoglobin variants should be suspected. Patient results >15% GHB should be repeated, and if confirmed, possible hemoglobin variants should be considered.

Reference intervals should be established or verified by the laboratory based on an appropriate non-diabetic patient population. A typical reference interval is 4%-6% Hb A\textsubscript{1C}. Glycemic control in diabetes is evaluated using ADA target values (Table 1). A value <7% is the primary goal of therapy recommended by the ADA. Physician re-evaluation of treatment regimen should be performed for patients with Hb A\textsubscript{1C} concentrations consistently >8%. These values and the corresponding risks of complications apply only to assay methods that are certified as traceable to the DCCT reference and have a reference interval of approximately 4%-6% Hb A\textsubscript{1C} (or Hb A\textsubscript{1C} equivalent). Thus, it is important for laboratories to use only those assays that can be traceable to the standard. In the DCCT, each 10% reduction in Hb A\textsubscript{1C} was associated with approximately 45% lower risk for progression of diabetic retinopathy.

Although the lifespan of erythrocytes is 120 days, the Hb A\textsubscript{1C} results are affected more by the mean blood glucose concentration for the preceding month than for the second and third months prior. This is because of the kinetics of erythrocyte turnover. In the DCCT the mean blood glucose concentrations were determined and compared directly to Hb A\textsubscript{1C} levels. Using this data the following equation was derived to express the relationship:

\[
\text{Mean Blood Glucose (mg/dL)} = (30.9 \times \text{Hb A}1\text{C} \text{ (%)}) - 60.6
\]

Although this equation is an approximation, it may be used by the laboratory in certain circumstances as a quality assurance or troubleshooting tool. Many point of care testing (POCT) glucose instruments currently used by diabetics have the capability of storing results and calculating the mean glucose concentration. When the Hb A\textsubscript{1C} analysis is performed, the mean blood glucose value can be calculated and compared to the POCT instrument-calculated value. If these values are significantly different, it could indicate that either one or both assays (Hb A\textsubscript{1C} analysis and POCT glucose analyzer) is inaccurate.

To summarize, the glycohemoglobin level expressed as a percent of total hemoglobin is a reflection of the mean blood glucose concentration for the previous 1-3 months and is the best assay for monitoring glycemic control over that time. Current assays measure either the Hb A\textsubscript{1C} analysis, the principal glycated hemoglobin, or total glycohemoglobin. Hb A\textsubscript{1C} (or Hb A\textsubscript{1C} equivalent) results obtained using a certified assay may be interpreted using data and conclusions from reference clinical studies.

Table 1. Correlation and interpretation of Hb A\textsubscript{1C} and approximate blood glucose levels

<table>
<thead>
<tr>
<th>Hb A\textsubscript{1C} (%)</th>
<th>Approximate Mean Blood* Glucose (mg/dL)</th>
<th>Interpretation: Glycemic Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 – 6</td>
<td>63 – 125</td>
<td>Non-diabetic range or very good control</td>
</tr>
<tr>
<td>7</td>
<td>156</td>
<td>Upper limit of target for diabetes in control</td>
</tr>
<tr>
<td>8</td>
<td>187</td>
<td>Marginal control: take action above this level</td>
</tr>
<tr>
<td>&gt;8</td>
<td>&gt;187</td>
<td>Poor control: take action to lower</td>
</tr>
</tbody>
</table>

*whole blood glucose levels-- plasma glucose values are approximately 10-12% higher than whole blood levels
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