Glucose, a monosaccharide, is the primary source of energy for the human body. It is used by the liver and other cells for energy or stored as glycogen for later use. The level of glucose in the bloodstream is regulated by 2 pancreatic hormones, insulin and glucagon. Insulin is released when glucose levels rise. Insulin acts by increasing glycogenesis, lipogenesis, and glycolysis, causing a decrease of glucose in the bloodstream. Glucagon is released when glucose levels fall, causing the liver to release stored glucose (glycogen) into the bloodstream (glycogenolysis), thereby increasing the level of glucose in the bloodstream.

Hemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}), also called glycosylated hemoglobin, is a hemoglobin compound produced when glucose reacts with the amino group on a hemoglobin molecule forming a ketoamine. The glucose molecule is attached to one or both N-terminal valines of the $\beta$-polypeptide chains of normal adult hemoglobin.\textsuperscript{1} The HbA\textsubscript{1c} formation is proportional to the blood glucose concentrations. Because the average red blood cell life is approximately 120 days, the glycosylated hemoglobin level reflects the average blood glucose level during the previous 2 to 3 months.

Clinical Significance

Glucose testing is used to determine if an individual has hyperglycemia or hypoglycemia. A high fasting glucose level ($\geq 126$ mg/dL) and/or a high HbA\textsubscript{1c} level (>6.5%) might indicate that an individual has diabetes mellitus. HbA\textsubscript{1c} is a reliable method of monitoring long-term diabetes mellitus control; it determines the average blood glucose level of an individual during a period of approximately 3 months. Normal values range from 4.0% to 6.0%. Results of a study\textsuperscript{2} have shown the strong linear relationship between average blood glucose levels and HbA\textsubscript{1c} levels. Current American Diabetes mellitus Association guidelines recommend that a HbA\textsubscript{1c} test be performed at least twice yearly on patients who are meeting treatment goals and who have stable glycemic control or quarterly in patients whose therapeutic regimen has changed or who are not meeting glycemic goals. The use of point-of-care testing for HbA\textsubscript{1c} allows for more timely decisions on therapy changes and has been shown to result in tighter glycemic control. A HbA\textsubscript{1c} level of less than 7% has been shown to reduce the microvascular, retinopathic, and neuropathic complications of diabetes mellitus.
An estimated average glucose (eAG) can be calculated from the A1C-reported value using the equation $eAG_{mg/dl} = 28.7 \times A1C – 46.3$. However, this information should be used carefully because the results of another study showed that the relationship between average plasma glucose and HbA1c can differ substantially depending on the glycemic control of the population studied and the red blood cell lifespan. If the red blood cell lifespan is decreased because of another disease state, such as hemoglobinopathy, the hemoglobin has less time to become glycosylated; as a result, the glycosylated hemoglobin level will be lower. HbA1c results from patients with hemoglobin sickle cell (HbSS) disease, hemoglobin C (HbC), and sickle cell–hemoglobin C (HbSC) disease must be interpreted with caution.

Using 2 of the following test results on subsequent days, a diagnosis of diabetes mellitus may be made: hemoglobin A1c level of greater than 6.5%, a fasting plasma glucose level of 126 mg/dL or greater, oral glucose tolerance test (OGTT) results with a 2-hour post-load (75-g glucose load) level of 200 mg/dL or greater, or symptoms of diabetes mellitus plus a random plasma glucose level of 200 mg/dL or greater.

**Specimen Requirements**

Depending on the method, glucose measurements may be performed on serum or plasma collected using heparin, ethylenediaminetetra acetic acid (EDTA), fluoride, oxalate, or citrate. The glucose concentration in whole blood is approximately 11% lower than that in plasma. For an accurate fasting blood glucose result, a patient should fast for 8 to 10 hours before blood is drawn and should be tested in the morning. Fasting plasma glucose (FPG) values display diurnal variation, with the mean FPG higher in the morning than in the afternoon; diabetes mellitus in patients tested in the afternoon may be missed because of this variation. Urine and cerebrospinal and serous fluids can also be analyzed.

Separating serum or plasma from the cells must be accomplished within 1 hour to prevent substantial loss of glucose by the cellular fraction. Sodium fluoride with oxalate (gray-top tubes) is often used as an anticoagulant and preservative for glucose testing, particularly if analysis is delayed. However, although the fluoride inhibits glycolytic enzymes and maintains long-term glucose stability, the rates of decline of glucose in the first hour after sample collection in tubes with and without fluoride are virtually identical. Therefore, the plasma should be separated from the cells as soon as possible.

The specimen requirement for HbA1c measurement is an EDTA whole blood sample. A patient’s fasting status is not required for this test.

**Glucose Testing Methods**

The most common methods of glucose analysis in the laboratory use the enzyme glucose oxidase or hexokinase. Glucose oxidase is the most specific enzyme; it reacts only with $\beta$-d-glucose. This enzyme converts $\beta$-d-glucose to gluconic acid; oxygen is consumed and hydrogen peroxide ($H_2O_2$) is produced. The reaction can be monitored polarographically by measuring the rate of disappearance of oxygen using an oxygen electrode or by consuming $H_2O_2$ in a side reaction.

Glucose + $O_2$ + $H_2O \rightarrow \text{gluconic acid} + H_2O_2$

$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen} + H_2O$

**Figure 1**

Glucose oxidase method.

The amount of glucose present can also be spectrophotometrically determined by measuring the change in the absorbance of the oxidized chromogen, which is proportional to the amount of glucose present in the specimen. Two commonly used chromogens are 3-methyl-2-benzothiazoline hydrazine and N,N-dimethylaniline. In the peroxidase reaction (Figure 1), falsely decreased glucose values can be caused by increased levels of uric acid, bilirubin, and ascorbic acid. These substances are oxidized by peroxidase and decrease the amount of the chromogen that is oxidized. The level of oxygen depletion is measured and is proportional to the amount of glucose present. The spectrophotometric measurement of oxidized chromogen is proportional to the amount of glucose present. Strong oxidizing substances, such as bleach, can cause falsely increased values. If direct measurement of oxygen by the polarography technique is used, these interferences are avoided.
The hexokinase method (Figure 2), generally accepted as the preferred reference method, is considered more accurate than the glucose oxidase methods. This reaction uses glucose-6-phosphate dehydrogenase and yields highly specific results. The hexokinase enzyme in the presence of acute thrombocytopenic purpura (ATP) converts glucose to glucose-6-phosphate. Glucose-6-phosphate and the co-factor nicotinamide adenine dinucleotide phosphate, oxidized form (NADP\(^+\)), are converted to 6-phosphogluconate and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), by glucose-6-phosphate dehydrogenase. NADPH has a strong absorbance maximum, at 340 nm. The rate of appearance of NADPH can be monitored spectrophotometrically and is proportional to the amount of glucose present in the sample. Gross hemolysis and extremely elevated bilirubin levels may cause a false decrease in results. However, this method is not affected by ascorbic acid or uric acid levels.

Point-of-care methods for glucose measure may also use glucose dehydrogenase (Figure 3). Several substrates used in this reaction include nicotinamide-adenine dinucleotide (NAD), flavin-adenine dinucleotide (FAD), and pyrroloquinolinequinone (PQQ). Maltose (found in icodextrin peritoneal dialysis solution) interferes with the PQQ test type unless specifically modified to eliminate this interference.\(^6,7\) Nonspecific methods of measuring glucose (Figure 4) are still used in the urinalysis section of the laboratory primarily to detect reducing substances other than glucose.

### Glycosylated Hemoglobin/ Hemoglobin A\(_{1c}\) Testing Methods

A common definition and method for HbA\(_{1c}\) was developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The IFCC defines HbA\(_{1c}\) as hemoglobin that is irreversibly glycated at one or both N-terminal valines of the \(\beta\) chains. Using high-performance liquid chromatography/electrospray mass spectrometry (HPLC-ESI/MS) or HPLC/capillary electrophoresis (HPLC-CE), these methods are used to standardize A\(_{1c}\) assays.\(^8\) Test results from this method are reported in Système International (SI) units (mmol HbA\(_{1c}\)/mol HbA\(_0\)). In the United States, the Diabetes mellitus Control and Complications Trial (DCCT) HPLC method is used as primary reference method and the HbA\(_{1c}\) is reported as a percentage of total hemoglobin. The SI units can be converted to percentage units using the following IFCC–National Glycohemoglobin Standardization Program (IFCC-NGSP) master equation:\(^9\)

\[
% = [0.09148 \times \text{SI value}] + 2.152
\]

Before analyzing HbA\(_{1c}\), a hemolysate must be prepared. Two potential methods used to measure HbA\(_{1c}\) include those based on the charge differences between glycated and nonglycated hemoglobin (cation-exchange chromatography, electrophoresis, and isoelectric focusing) and structural characteristics of glycoforms on hemoglobin (affinity chromatography and immunoassay). Cation-exchange chromatography uses negatively charged
hemoglobin molecules to attach to a positively charged resin bed. In this method, which is temperature dependent and affected by hemoglobinopathies, the glycosylated hemoglobin is eluted from the resin bed using a buffer of specific pH to negatively charge the hemoglobin molecules and elute them first from the column.

The affinity chromatography method, which is preferred by most technicians in the laboratory, uses a boronate resin group to attach the glycosylated hemoglobin, which is then selectively eluted from the resin bed using a buffer. This method is not temperature dependent and not affected by fetal hemoglobin (hemoglobin F), hemoglobin with sickle-cell trait (hemoglobin S), or hemoglobin C.

The HbA1c point-of-care assay is based on a latex immunooagglutination inhibition method. In this method, the concentration of HbA1c and the concentration of total hemoglobin are measured; the ratio is reported as percentage of HbA1c. Because glycated hemoglobin F is not measured using this method, very high levels of hemoglobin F (>10%) will cause the HbA1c level to be lower than expected because a greater proportion of the glycated hemoglobin will exist in the form of glycated hemoglobin F. Also, HPLC and electrophoresis methods can be used to separate the various forms of hemoglobin into A1a, A1b, and A1c.

Diagnosis and management of diabetes mellitus can be a complex matter. However, the measurement of glucose and hemoglobin A1c helps physicians to determine whether a patient has diabetes mellitus or, in already-diagnosed patients, if their diabetes mellitus is under control. The goal of management of these patients is to maintain the blood-glucose levels within or near the nondiabetic range with a minimal number of fluctuations. Serum or plasma glucose concentrations can be measured by laboratories in addition to patient self-monitoring of whole-blood-glucose concentrations. Long-term blood glucose–level regulation can be followed by measuring the level of glycosylated hemoglobin. LM

References