Chapter **2**

Glucose

1. USE

Recommendation

When glucose is used to establish the diagnosis of diabetes, it should be measured in venous plasma.
A (high)

Recommendation

When glucose is used for screening of high-risk individuals, it should be measured in venous plasma.
B (moderate)

Recommendation

Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes.
Good Practice Point (GPP)

Recommendation

Outcome studies are needed to determine the effectiveness of screening.
C (moderate)

A. Diagnosis/screening. The diagnosis of diabetes is established by identifying the presence of hyperglycemia. For many years the only method recommended for diagnosis was a direct demonstration of hyperglycemia by measuring increased glucose concentrations in the plasma *(15, 16)*. In 1979, a set of criteria based on the distribution of glucose concentrations in high-risk populations was established to standardize the diagnosis *(15)*. These recommendations were endorsed by the WHO *(16)*. In 1997, the diagnostic criteria were modified *(1)* to better identify individuals at risk of retinopathy and nephropathy *(17, 18)*. The revised criteria comprised: (*a*) an FPG value ≥7.0 mmol/L (126 mg/dL); (*b*) a 2-h postload glucose concentration ≥11.1 mmol/L (200 mg/dL) during an OGTT; or (*c*) symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose concentration ≥11.1 mmol/L (200 mg/dL) (Table 6) *(1)*. If any one of these 3 criteria is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis [note that repeat testing is not required for patients who have unequivocal hyperglycemia, i.e., >11.1 mmol/L (200 mg/dL) with symptoms consistent with hyperglycemia]. The WHO and the International Diabetes Federation (IDF) recommend either an FPG test or a 2-h postload glucose test that uses the same cutoffs as the ADA *(19)* (Table 7). In 2009, the International Expert Committee *(20)*, which comprised members appointed by the ADA, the European Association for the Study of Diabetes, and the IDF, recommended that diabetes be diagnosed by measurement of hemoglobin A_{1c} (Hb A_{1c}), which reflects long-term blood glucose concentrations (see Hb A_{1c} section below). The ADA *(21)* and the WHO have endorsed the use of Hb A_{1c} for diagnosis of diabetes.

Testing to detect type 2 diabetes in asymptomatic people, previously controversial, is now recommended for those at risk of developing the disease *(21, 22)*. The ADA proposes that all asymptomatic people ≥45 years of age be screened in a healthcare setting. An Hb A_{1c}, FPG, or 2-h OGTT evaluation is appropriate for screening *(21)*. The IDF recommends that the health service in each country decide whether to implement screening for diabetes *(23)*. FPG is the suggested test. In contrast, the International Expert Committee and the ADA have recommended that Hb A_{1c} can be used for screening for diabetes *(20, 21, 24)* (see section on Hb A_{1c} below). If an FPG result is <5.6 mmol/L (100 mg/dL) and/or a 2-h plasma glucose concentration is <7.8 mmol/L (140 mg/dL), testing should be repeated at 3-year intervals. Screening should be considered at a younger age or be carried out more frequently in individuals who are overweight (body mass index ≥25 kg/m^2) or obese and who have a least 1 additional risk factor for diabetes [see *(21)* for conditions associated ith increased risk]. Because of the increasing prevalence of type 2 diabetes in children, screening of children is now advocated *(25)*. Starting at age 10 years (or at the onset of puberty if puberty occurs at a younger age), testing should be performed every 3 years in over-weight individuals who have 2 other risk factors—namely family history, a race/ethnicity recognized to increase risk, signs of insulin resistance, and a maternal history of diabetes or GDM during the child's gestation *(25)*. Despite these recommendations and the demonstration that interventions can delay and sometimes prevent the onset of type 2 diabetes in individuals with impaired glucose tolerance *(26, 27)*, there is as yet no published evidence that treatment based on screening has an effect on long-term complications. In addition, the published literature lacks consensus as to which screening procedure (FPG, OGTT, and/or Hb A_{1c}) is the most appropriate *(20, 28–30)*. On the basis of an evaluation of NHANES III data, a strategy has been proposed to use FPG to screen whites ≥40 years and other

Table 6. Criteria for the diagnosis of diabetes.[a]

Any one of the following is diagnostic:
1. Hb A_{1c} ≥6.5% (48 mmol/mol)[b]
OR
2. FPG ≥7.0 mmol/L (126 mg/dL)[c]
OR
3. 2-h Plasma glucose ≥11.1 mmol/L (200 mg/dL) during an OGTT[d]
OR
4. Symptoms of hyperglycemia and casual plasma glucose $11.1 mmol/L (200 mg/dL)[e]

[a] In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing. From the ADA *(378)*.
[b] The test should be performed in a laboratory that is NGSP certified and standardized to the DCCT assay. Point-of-care assays should not be used for diagnosis.
[c] Fasting is defined as no caloric intake for at least 8 h.
[d] The OGTT should be performed as described by the WHO, with a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water.
[e] "Casual" is defined as any time of day without regard to time since previous meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.

populations ≥30 years of age *(31)*. The cost-effectiveness of screening for type 2 diabetes has been estimated. The incremental cost of screening all persons ≥25 years of age has been estimated to be $236 449 per life-year gained and $56 649 per quality-adjusted life-year (QALY) gained *(32)*. Interestingly, screening was more cost-effective at ages younger than the 45 years currently recommended. In contrast, screening targeted to individuals with hypertension reduces the QALY from $360 966 to $34 375, with ages between 55 and 75 years being the most cost-effective *(33)*. Modeling run on 1×10^6 individuals suggests considerable uncertainty as to whether screening for diabetes would be cost-effective *(34)*. By contrast, the results of a more recent modeling study imply that screening commencing at 30 or 45 years is highly cost-effective (<$11 000 per QALY gained) *(35)*. Longterm outcome studies are necessary to provide evidence to resolve the question of the efficacy of diabetes screening *(36)*.

In 2003, the ADA lowered the threshold for "normal" FPG from <6.1 mmol/L (110 mg/dL) to <5.6 mmol/L (100 mg/dL) *(37)*. This change has been contentious and has not been accepted by all organizations *(19, 38)*. The rationale is based on data that individuals with FPG values between 5.6 mmol/L (100 mg/dL) and 6.05 mmol/L (109 mg/dL) are at increased risk for developing type 2 diabetes *(39, 40)*. More-recent evidence indicates that FPG concentrations even lower than 5.6 mmol/L (100 mg/dL) are associated with a graded risk for type 2 diabetes *(41)*. Data were obtained from 13 163 men between 26 and 45 years of age who had FPG values <5.55 mmol/L (100 mg/dL) and were followed for a mean of 5.7 years. Men with FPG values of 4.83–5.05 mmol/L (87–91 mg/dL) have a significantly increased risk of type 2 diabetes, compared with men with FPG values <4.5 mmol/L (81 mg/dL). Although the prevalence of diabetes is low at these glucose concentrations, the data support the concept of a continuum between FPG and the risk of diabetes.

Recommendation

Routine measurement of plasma glucose concentrations in an accredited laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes.
B (low)

B. Monitoring/prognosis. There is a direct relationship between the degree of chronic plasma glucose control and the risk of late renal, retinal, and neurologic complications. This correlation has been documented in epidemiologic studies and clinical trials for both type 1 *(42)* and type 2 *(43)* diabetes. The important causal role of hyperglycemia in the development and progression of complications has been documented in clinical trials. Persons with type 1 diabetes who maintain lower mean plasma glucose concentrations exhibit a significantly lower incidence of microvascular complications—namely diabetic retinopathy, nephropathy, and neuropathy *(44)*. Although intensive insulin therapy reduced hypercholesterolemia by 34%, the risk of macrovascular disease was not significantly decreased in the original analysis *(44)*. Longer follow-up documented a significant reduction in cardiovascular disease in patients with type 1 diabetes treated with intensive glycemic control *(45)*. The effects of tight glycemic control on microvascular complications in patients with type 2 diabetes *(46)* are similar to those with

Table 7. WHO criteria for interpreting 2-h OGTT.[a]

	2-h OGTT result, mmol/L (mg/dL)	
	0 h	2 h
Impaired fasting glucose[b]	>6.1 (110 to <7.0 (126)	<7.8 (140)
Impaired glucose tolerance[c]	<7.0 (126)	>7.8 (140) to <11.1 (200)
Diabetes[d]	>7.0 (126)	>11.1 (200)

[a] Values are for venous plasma glucose using a 75 g oral glucose load. From the WHO *(19)*.
[b] If 2-h glucose is not measured, status is uncertain as diabetes or impaired glucose tolerance cannot be excluded.
[c] Both fasting and 2-h values need to meet criteria.
[d] Either fasting or 2-h measurement can be used. Any single positive result should be repeated on a separate day.

type 1 diabetes, given the differences in glycemia achieved between the active-intervention and control groups in the various trials. Intensive plasma glucose control significantly reduced microvascular complications in patients with type 2 diabetes. Although metaanalyses have suggested that intensive glycemic control reduces cardiovascular disease in individuals with type 2 diabetes *(47, 48)*, clinical trials have not consistently demonstrated a reduction in macrovascular disease (myocar-dial infarction or stroke) with intensive therapy aimed at lowering glucose concentrations in type 2 diabetes. Long-term follow-up of the United Kingdom Prospective Diabetes Study (UKPDS) population supported a benefit of intensive therapy on macrovascular disease *(49)*, but 3 other recent trials failed to demonstrate a significant difference in macrovascular disease outcomes between very intensive treatment strategies, which achieved Hb A_{1c} concentrations of approximately 6.5% (48 mmol/mol), and the control groups, which had Hb A_{1c} concentrations 0.8%–1.1% higher *(50–52)*. One study even observed higher cardiovascular mortality in the intensive-treatment arm *(50)*. In both the Diabetes Control and Complications Trial (DCCT) and the UKPDS, patients in the intensive-treatment group maintained lower median plasma glucose concentrations; however, analyses of the outcomes were linked to Hb A_{1c}, which was used to evaluate glycemic control, rather than glucose concentration. Moreover, most clinicians use the recommendations of the ADA and other organizations, which define a target Hb A_{1c} concentration as the goal for optimum glycemic control *(21, 53)*.

Neither random nor fasting glucose concentrations should be measured in an accredited laboratory as the primary means of routine outpatient monitoring of patients with diabetes. Laboratory plasma glucose testing can be used to supplement information from other testing, to test the accuracy of self-monitoring (see below), or to adjust the dosage of oral hypoglycemic agents *(22, 54)*. In addition, individuals with well-controlled type 2 diabetes who are not on insulin therapy can be monitored with periodic measurement of the FPG concentration, although analysis need not be done in an accredited laboratory *(54, 55)*.

2. RATIONALE

A. Diagnosis. The disordered carbohydrate metabolism that underlies diabetes manifests as hyperglycemia. Therefore, measurement of either plasma glucose or Hb A_{1c} is the diagnostic criterion. This strategy is indirect, because hyperglycemia reflects the consequence of the metabolic derangement, not the cause; however, until the underlying molecular pathophysiology of the disease is identified, measurement of glycemia is likely to remain an essential diagnostic modality.

B. Screening. Screening is recommended for several reasons. The onset of type 2 diabetes is estimated to occur approximately 4–7 years (or more) before clinical diagnosis *(56)*, and epidemiologic evidence indicates that complications may begin several years before clinical diagnosis. Furthermore, it is estimated that 40% of people in the US with type 2 diabetes are undiagnosed *(8)*. Notwithstanding this recommendation, there is no published evidence that population screening for hyperglycemia provides any long-term benefit. Outcome studies examining the potential long-term benefits of screening are ongoing.

3. ANALYTICAL CONSIDERATIONS

Recommendation

To minimize glycolysis, one should place the sample tube immediately in an ice–water slurry, and the plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis.
B (moderate)

Recommendation

Blood for FPG analysis should be drawn in the morning after the individual has fasted overnight (at least 8 h).
B (low)

A. Preanalytical. Blood should be drawn in the morning after an overnight fast (no caloric intake for at least 8 h), during which time the individual may consume water ad libitum *(1)*. Published evidence reveals diurnal variation in FPG, with the mean FPG being higher in the morning than in the afternoon, indicating that many diabetes cases would be missed in patients seen in the afternoon *(57)*.

Loss of glucose from sample containers is a serious and underappreciated problem *(58)*. Decreases in glucose concentrations in whole blood ex vivo are due to glycolysis. The rate of glycolysis—reported to average 5%–7%/h [approximately 0.6 mmol/L (10 mg/dL)] *(59)*—varies with the glucose concentration, temperature, leukocyte count, and other factors *(60)*. Such decreases in glucose concentration will lead to missed diabetes diagnoses in the large proportion of the population who have glucose concentrations near the cut-points for diagnosis of diabetes.

The commonly used glycolysis inhibitors are unable to prevent short-term glycolysis. Glycolysis can be attenuated by inhibiting enolase with sodium fluoride (2.5 mg/mL of blood) or, less commonly, lithium iodoacetate (0.5 mg/mL of blood). These reagents can be used alone or, more commonly, with such anticoagulants as potassium oxalate, EDTA, citrate, or lithium heparin. Unfortunately, although fluoride helps to maintain long-term glucose stability, the rates of decline in the glucose concentration in the first hour

after sample collection are virtually identical for tubes with and without fluoride, and glycolysis continues for up to 4 h in samples containing fluoride *(59)*. After 4 h, the concentration of glucose in whole blood in the presence of fluoride remains stable for 72 h at room temperature *(59)* (leukocytosis will increase glycolysis even in the presence of fluoride if the leukocyte count is very high).

Few effective and practical methods are available for prompt stabilization of glucose in whole-blood samples. Loss of glucose can be minimized in 2 classic ways: (*a*) immediate separation of plasma from blood cells after blood collection (the glucose concentration is stable for 8 h at 25 °C and 72 h at 4 °C in separated, nonhemolyzed, sterile serum without fluoride*(61)*); and (*b*) placing the blood tube in an ice–water slurry immediately after blood collection and separating the plasma from the cells within 30 min *(19, 62)*. These methods are not always practical and are not widely used.

A recent study showed that acidification of blood with citrate buffer inhibits in vitro glycolysis far more effectively than fluoride *(62)*. The mean glucose concentration in samples stored at 37 °C decreased by only 0.3% at 2 h and 1.2% at 24 h when blood was drawn into tubes containing citrate buffer, sodium fluoride, and EDTA. The use of these blood-collection tubes, where they are available, appears to offer a practical solution to the glycolysis problem.

Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended for diagnosis [note that although both the ADA and WHO recommend venous plasma, the WHO also accepts measurement of glucose in capillary blood *(19, 21)*]. The molality of glucose (i.e., the amount of glucose per unit water mass) in whole blood is identical to that in plasma. Although erythrocytes are essentially freely permeable to glucose (glucose is taken up by facilitated transport), the concentration of water (in kilograms per liter) in plasma is approximately 11% higher than in whole blood. Therefore, glucose concentrations are approximately 11% higher in plasma than in whole blood if the hematocrit is normal. Glucose concentrations in heparinized plasma were reported in 1974 to be 5% lower than in serum *(63)*. The reasons for the difference are not apparent but have been attributed to the shift in fluid from erythrocytes to plasma caused by anticoagulants. In contrast, some more recent studies found that glucose concentrations are slightly higher in plasma than in serum. The observed differences were approximately 0.2 mmol/L (3.6 mg/dL) *(64)*, or approximately 2% *(65)*, or 0.9% *(62)*. Other studies have found that glucose values measured in serum and plasma are essentially the same *(66, 67)*. Given these findings, it is unlikely that values for plasma and serum glucose will be substantially different when glucose is assayed with current instruments, and any differences will be small compared with the day-to-day biological variation of glucose. Clinical organizations do not recommend the measurement of glucose in serum (rather than plasma) for the diagnosis of diabetes *(19, 21)*. Use of plasma allows samples to be centrifuged promptly to prevent glycolysis without waiting for the blood to clot. The glucose concentrations in capillary blood obtained during an OGTT are significantly higher than those in venous blood [mean, 1.7 mmol/L (30 mg/dL), which is equivalent to 20%–25% higher *(68)*], probably owing to glucose consumption in the tissues. In contrast, the mean difference in fasting samples is only 0.1 mmol/L (2 mg/dL) *(68, 69)*.

Reference intervals. Glucose concentrations vary with age in healthy individuals. The reference interval for children is 3.3–5.6 mmol/L (60–100 mg/dL), which is similar to the adult interval of 4.1–6.1 mmol/L (74– 110 mg/dL) *(70)*. Note that the ADA and WHO criteria *(19, 21)*, not the reference intervals, are used for the diagnosis of diabetes. Moreover, the threshold for the diagnosis of hypoglycemia is variable. Reference intervals are not useful for diagnosing these conditions. In adults, the mean FPG concentration increases with increasing age from the third to the sixth decade *(71)* but does not increase significantly after 60 years of age *(72, 73)*. By contrast, glucose concentrations after a glucose challenge are substantially higher in older individuals *(72, 73)*. The evidence for an association between increasing insulin resistance and age is inconsistent *(74)*. Aging appears to influence glucose homeostasis, and visceral obesity seems to be responsible for the reported continuous decrease in glucose tolerance that begins in middle age *(75)*.

Recommendation

On the basis of biological variation, glucose measurement should have an analytical imprecision ≤2.9%, A bias ≤2.2%, and a total error ≤6.9%. To avoid misclassification of patients, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias.
B (low)

B. Analytical. Glucose is measured almost exclusively by enzymatic methods. An analysis of proficiency surveys conducted by the College of American Pathologists (CAP) reveals that hexokinase or glucose oxidase is used in virtually all analyses performed in the US *(70)*. A very few laboratories (<1%) use glucose dehydrogenase. Enzymatic methods for glucose analysis are relatively well standardized. At a plasma glucose concentration of approximately 7.5 mmol/L (135 mg/dL), the imprecision (CV) among laboratories that used the same method was ≤2.6% *(70)*. Similar findings have been reported for glucose analyses of samples from patients. The method of glucose measurement does not influence the result. A comparison of results from approximately 6000 clinical laboratories reveals that the mean glucose concentrations measured in serum samples by the hexokinase and glucose oxidase methods are essentially the same *(76)*. Compared with a reference measurement procedure, significant bias (P <0.001) was observed for 40.6% of the peer groups *(76)*. If similar biases occur with plasma, patients near the diagnostic threshold could be misclassified.

No consensus has been achieved on the goals for glucose analysis. Numerous criteria have been proposed to establish

analytical goals. These criteria include expert opinion (consensus conferences), the opinion of clinicians, regulation, the state of the art, and biological variation *(77)*. A rational and realistic recommendation that has received some support is to use biological criteria as the basis for analytical goals. It has been suggested that imprecision should not exceed one-half of the within-individual biological CV *(78, 79)*. For plasma glucose, a CV ≤2.2% has been suggested as a target for imprecision, with a 0% bias *(79)*. Although this recommendation was proposed for within-laboratory error, it would be desirable to achieve this goal for interlaboratory imprecision to minimize differences among laboratories in the diagnosis of diabetes in individuals with glucose concentrations close to the threshold value. Therefore, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias. A national or international program that uses commutable samples (e.g., fresh frozen plasma) to eliminate matrix effects and has accuracy-based grading with values derived with a reference measurement procedure should be developed to assist in achieving this objective.

4. INTERPRETATION

Despite the low analytical imprecision at the diagnostic decision limits of 7.0 mmol/L (126 mg/dL) and 11.1 mmol/L (200 mg/dL), classification errors may occur. Knowledge of intraindividual (within-person) variation in FPG concentrations is essential for meaningful interpretation of patient values (although total biological variation includes within-person and between-person variation, most discussions focus on the within-person variation). An early study, which repeated the OGTT in 31 nondiabetic adults at a 48-h interval, revealed that the FPG concentration varied between the 2 values by <10% in 22 participants (77%) and by <20% in 30 participants (97%) *(80)*. A careful evaluation of healthy individuals over several consecutive days revealed that the biological variation in FPG [mean glucose, 4.9 mmol/L (88 mg/dL)] exhibited within- and between-individual CVs of 4.8%–6.1% and 7.5%–7.8%, respectively *(81–83)*. Larger studies have revealed intraindividual CVs of 4.8% and 7.1% for FPG in 246 healthy individuals and 80 previously undiagnosed individuals with diabetes, respectively *(83)*. Similar findings were obtained from an analysis of 685 adults from NHANES III, in which the mean within-person variation in FPG measured 2–4 weeks apart was 5.7% (95% CI, 5.3%–6.1%) *(84)*. An analysis of larger numbers of individuals from the same NHANES III database yielded within- and between-person CVs of 8.3% and 12.5%, respectively, at a glucose concentration of approximately 5.1 mmol/L (92 mg/dL) *(85)*. If a within-person biological CV of 5.7% is applied to a true glucose concentration of 7.0 mmol/L (126 mg/dL), the 95% CI would encompass glucose concentrations of 6.2–7.8 mmol/L (112–140 mg/dL). If the analytical CV of the glucose assay (approximately 3%) is included, the 95% CI is approximately ±12.88%. Thus, the 95% CI for a fasting glucose concentration of 7.0 mmol/L (126 mg/dL) would be 7.0 mmol/L ± 6.4% (126 mg/dL ± 6.4%), i.e., 6.1–7.9 mmol/L (110–142 mg/dL). Use of an assay CV of 3% only (excluding biological variation) would yield a 95% CI of 6.6–7.4 mmol/L (118–134 mg/dL) among laboratories, for a true glucose concentration of 7.0 mmol/L (126 mg/dL). Performing the same calculations at the cutoff for impaired fasting glucose yields a 95% CI of 5.6 mmol/L ± 6.4% (100 mg/dL ± 6.4%), i.e., 4.9–6.3 mmol/L (87–113 mg/dL). One should bear in mind that these intervals include 95% of the results and that the remaining 5% will be outside this interval. Thus, the biological variation is substantially greater than the analytical variation. Using biological variation as the basis for deriving analytical performance characteristics *(77)*, Westgard proposed the following desirable specifications for glucose *(86)*: analytical imprecision, ≤2.9%; bias, ≤2.2%; and total error, ≤6.9%.

A. Turnaround time. A short turnaround time for glucose analysis is not usually necessary for diagnosis of diabetes. In some clinical situations, such as acute hyper- or hypoglycemic episodes in the emergency department or treatment of diabetic ketoacidosis (DKA), rapid analysis is desirable. A turnaround time of 30 min has been proposed *(87)*. This value is based on the suggestions of clinicians, however, and no outcome data that validate this time interval have been published. Inpatient management of diabetic patients on occasion may require a rapid turnaround time (minutes, not hours). Similarly, for protocols with intensive glucose control in critically ill patients *(88)*, rapid glucose results are required in order to calculate the insulin dose. Bedside monitoring with glucose meters (see below) has been adopted by many as a practical solution.

B. Frequency of measurement. The frequency of measurement of plasma glucose is dictated by the clinical situation. The ADA, WHO, and IDF recommend that an increased FPG or an abnormal OGTT result must be confirmed to establish the diagnosis of diabetes *(19, 89)*. Screening by FPG is recommended every 3 years, beginning at 45 years of age and more frequently in high-risk individuals; however, the frequency of analysis has not been specified for the latter group. Monitoring is performed by patients who measure their glucose themselves with meters and by assessment of Hb A_{1c} in an accredited laboratory (see below). The appropriate interval between glucose measurements in acute clinical situations (e.g., patients admitted to a hospital, patients with DKA, neonatal hypoglycemia, and so forth) is highly variable and may range from 30 min to 24 h or more.

5. EMERGING CONSIDERATIONS

Continuous minimally invasive and noninvasive analysis of glucose is addressed below.