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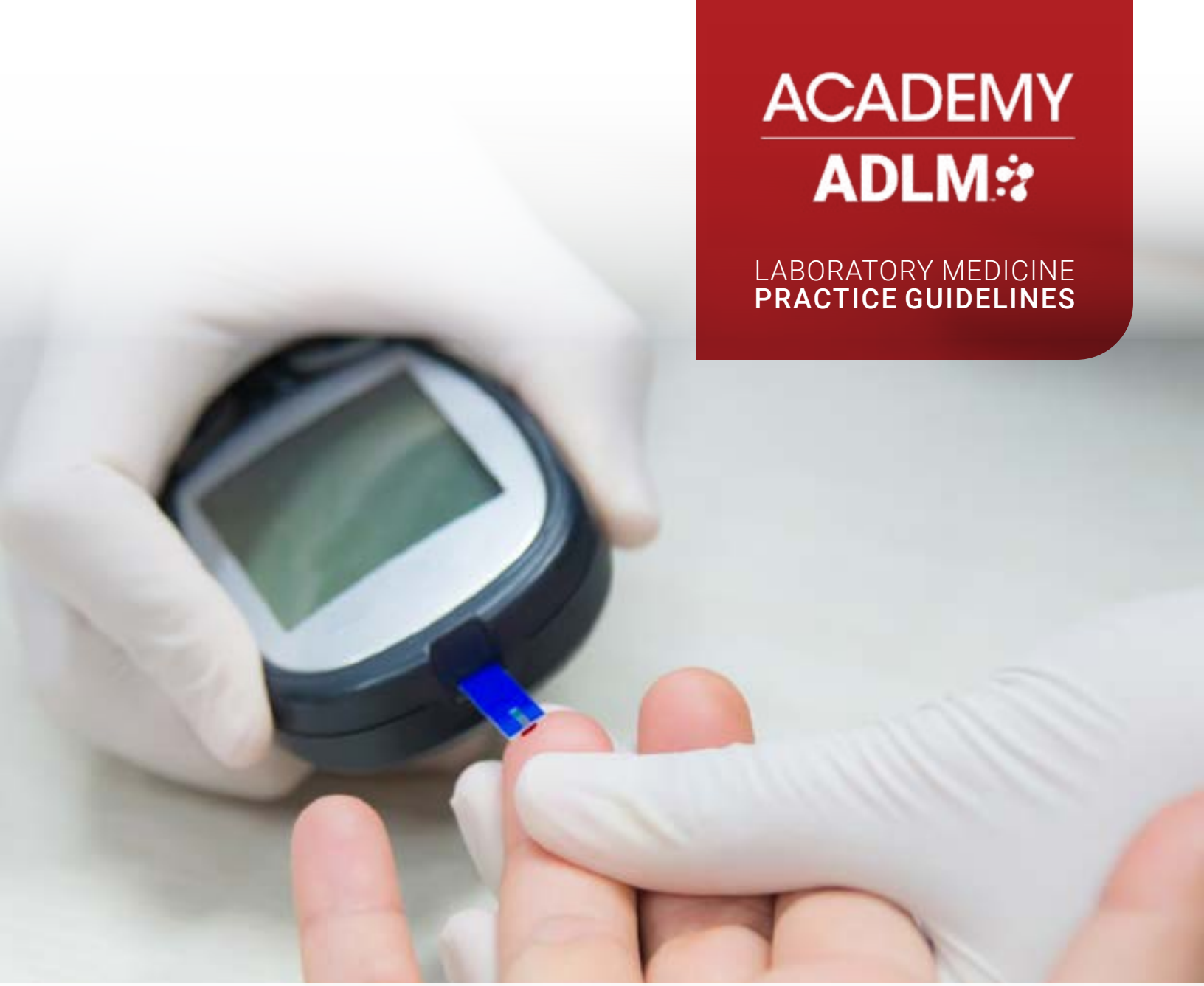

Guidelines and Recommendations for

Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

ADLM Association for Diagnostics & Laboratory Medicine™
Formerly AACC

The Academy of Diagnostics & Laboratory Medicine
Presents
Laboratory Medicine Practice Guidelines:

Guidelines and Recommendations for **Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus**

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Laboratory Medicine Practice Guidelines:

Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is both underutilized and overproduced, resulting in hyperglycemia. The disease is classified conventionally into several clinical categories. Type 1 diabetes mellitus is usually caused by autoimmune destruction of the pancreatic islet β-cells, rendering the pancreas unable to synthesize and secrete insulin (1). Type 2 diabetes mellitus results from a combination of insulin resistance and inadequate insulin secretion (2, 3). Gestational diabetes mellitus (GDM) develops during approximately 17% of pregnancies, usually remits after delivery, and is a major risk factor for the development of type 2 diabetes later in life. Type 2 is the most common form of diabetes, accounting for 85% to 95% of diabetes in developed countries.

Diabetes is a common disease. Worldwide prevalence in 2021 was estimated to be approximately 537 million (10.5% of the global population) and is forecast to reach 783 million by 2045 (4). Based on 2017-2020 National Health and Nutrition Examination Survey data and 2018-2019 National Health Interview Survey data, the US Centers for Disease Control and Prevention estimated that there were 37.3 million people (11.3% of the US population) with diabetes (5). The number of adults with diabetes has also increased in other parts of the world. For example, China and India were thought to have 140.9 and 74.2 million adults with diabetes in 2021 and are expected to have 174.4 and 124.9 million, respectively, by 2045 (4). Approximately 45% of people with diabetes worldwide are thought to be undiagnosed (4).

The cost of diabetes in the US in 2012 was approximately $245 billion and increased to $327 billion by 2017 (6). The mean annual per capita healthcare costs for an individual with diabetes are approximately 2.3-fold higher than those for individuals who do not have diabetes (7). Worldwide spending in 2021 was thought to be $966 billion. The high costs of diabetes are attributable primarily to the chronic debilitating microvascular and macrovascular complications (6), which make diabetes the fourth most common cause of death in the developed world (8). About 6.7 million adults worldwide were thought to have died from diabetes-related causes in 2021 (4).

Table 1. Rating scale for the quality of the evidence.
High: Further research is very unlikely to change our confidence in the estimate of effect. The body of evidence comes from high-level individual studies that are sufficiently powered and provide precise, consistent, and directly applicable results in a relevant population.
Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate and the recommendation. The body of evidence comes from high/moderate-level individual studies that are sufficient to determine effects but the strength of the evidence is limited by the number, quality, or consistency of the included studies; generalizability of results to routine practice; or indirect nature of the evidence.
Low: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate and the recommendation. The body of evidence is of low level and comes from studies with serious design flaws, or evidence is indirect.
Very low: Any estimate of effect is very uncertain. Recommendation may change when higher quality evidence becomes available. Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

AACC and the American Diabetes Association issued "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus" in 2002 (9, 10) and 2011 (11, 12). Here we review and update these recommendations using an evidence-based approach, especially in those areas where new evidence has emerged since the 2011 publications (13). The guideline committee, whose membership was predominantly from the US, included clinical, laboratory, and evidence-based guideline methodology experts. Members of the guideline committee have disclosed any financial, personal, or professional relationships that may constitute conflicts of interest with this guideline and received no direct funding related to the development of the recommendations. The perspectives and views of various international and national organizations, as well as other potential stakeholders (e.g., healthcare providers, people with diabetes, policymakers, regulatory bodies, health insurance companies, researchers, and industry), were taken into account during the public consultation process. The system developed in 2011 to grade both the overall quality of the evidence (Table 1) and the strength of recommendations (Table 2) was used, and the key steps and evidence summaries are detailed in the guideline and in the Data Supplement that accompanies the online version of this report (13). The literature was reviewed to the end of 2021.

This guideline focuses on the practical aspects of care in order to assist with decisions regarding the use or interpretation of laboratory tests while screening, diagnosing, or monitoring patients with diabetes. It covers the rationale, preanalytical,

Table 2. Grading the strength of recommendations.	
A. Strongly Recommend	a. adoption when: • There is high-quality evidence and strong or very strong agreement of experts that the intervention improves important health outcomes and that benefits substantially outweigh harms; or • There is moderate-quality evidence and strong or very strong agreement of experts that the intervention improves important health outcomes and that benefits substantially outweigh harms.
	b. *against* adoption when: • There is high-quality evidence and strong or very strong agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms clearly outweigh benefits; or • There is moderate-quality evidence and strong or very strong agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.
B. Recommend	a. adoption when: • There is moderate-quality evidence and level of agreement of experts that the intervention improves important health outcomes and that benefits outweigh harms; or • There is low-quality evidence but strong or very strong agreement and high level of confidence of experts that the intervention improves important health outcomes and that benefits outweigh harms; or • There is very low quality evidence but very strong agreement and very high level of confidence of experts that the intervention improves important health outcomes and that benefits outweigh harms.
	b. *against* adoption when: • There is moderate-quality evidence and level of agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits; or • There is low-quality evidence but strong or very strong agreement and high level of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits; or • There is very low quality evidence but very strong agreement and very high levels of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.
	Continued

Table 2. Grading the strength of recommendations. (cont'd)	
C. There is Insufficient Information to Make a Recommendation	Grade C is applied in the following circumstances: • Evidence is lacking or scarce or of very low quality, and the balance of benefits and harms cannot be determined, and there is no or very low level of agreement of experts for or against adoption of the recommendation. • At any level of evidence—particularly if the evidence is heterogeneous or inconsistent, indirect, or inconclusive—if there is no agreement of experts for or against adoption of the recommendation.
Good Practice Point	Good Practice Points are recommendations mostly driven by expert consensus and professional agreement and are based on the below listed information and/or professional experience, or widely accepted standards of best practice. This category mostly applies to technical (e.g., pre-analytical, analytical, post-analytical), organizational, economic, or quality management aspects of laboratory practice. In these cases, evidence often comes from observational studies, audit reports, case series, or case studies, nonsystematic reviews, guidance or technical documents, nonevidence-based guidelines, personal opinions, expert consensus, or position statements. Recommendations are often based on empirical data, usual practice, quality requirements, and standards set by professional or legislative authorities or accreditation bodies, etc.

analytical, post-analytical, and, where applicable, emerging considerations, which alert the reader to ongoing studies and potential future aspects relevant to each analyte. The recommendations intend to supplement the American Diabetes Association guidelines and thus do not address any issues related to the clinical management of patients. The full version of this guideline and its accompanying supplements are available as a Special Report (13). Key recommendations are summarized.

These recommendations primarily target laboratory professionals, general practitioners, physicians, nurses, and other healthcare practitioners involved in the care of people with diabetes. The guidelines can be used by individuals with diabetes (where relevant, e.g., self–monitoring of blood glucose), policymakers, and payers for healthcare, as well as by researchers and manufacturers. Although recommendations were developed for national and international use and are intended to be generic, certain recommendations may not reflect views that are universally held or may have limited applicability in healthcare settings with differing organizational, cultural, and economic backgrounds. The guideline committee therefore advises users to adapt recommendations to local settings.

BACKGROUND

Numerous laboratory tests are used in the diagnosis and management of patients with diabetes mellitus. The quality of the scientific evidence supporting the use of these assays varies substantially. An expert committee compiled evidence-based recommendations for laboratory analysis in patients with diabetes. The overall quality of the evidence and the strength of the recommendations were evaluated. The draft consensus recommendations were evaluated by invited reviewers and presented for public comment. Suggestions were incorporated as deemed appropriate by the authors (see Acknowledgments in the full version of the guideline). The guidelines were reviewed by the Evidence Based Laboratory Medicine Committee and the Board of Directors of the American Association of Clinical Chemistry and by the Professional Practice Committee of the American Diabetes Association.

RECOMMENDATIONS

Capital letters denote the grade of recommendations and categories in parentheses refer to the quality of the underlying body of evidence supporting each recommendation. The grading system is described in Tables 1 and 2.

1. Glucose

a. Fasting glucose should be measured in venous plasma when used to establish the diagnosis of diabetes, with a value >7.0 mmol/L (>126 mg/dL) diagnostic of diabetes. **A (high)**
b. Screening by hemoglobin A_{1c} (Hb A_{1c}), fasting plasma glucose (FPG), or 2-h oral glucose tolerance test is recommended for individuals who are at high risk of diabetes. If Hb A_{1c} is <5.7% (<39 mmol/mol), fasting plasma glucose is <5.6 mmol/L (<100 mg/dL), and/ or 2-h plasma glucose is <7.8 mmol/L (<140 mg/ dL), testing should be repeated at 3-year intervals. B (moderate)
c. Glucose should be measured in venous plasma when used for screening of high-risk individuals. **B (moderate)**
d. Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes. **Good Practice Point (GPP**)
e. Routine measurement of plasma glucose concentrations in a laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes. **B (moderate)**
f. Blood for fasting plasma glucose analysis should be drawn in the morning after the subject has fasted overnight (at least 8 h). **B (low)**
g. To minimize glycolysis, a tube containing a rapidly effective glycolytic inhibitor such as granulated citrate buffer should be used for collecting the sample. If this cannot be achieved, the sample tube should immediately be placed

in an ice-water slurry and subjected to centrifugation to remove the cells within 15 to 30 min. Tubes with only enolase inhibitors such as sodium fluoride should not be relied on to prevent glycolysis. **B (moderate)**

h. Based on biological variation, glucose measurement should have analytical imprecision <2.4%, bias <2.1%, and total error <6.1%. To avoid misclassification of individuals, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias. **B (moderate)**

2. Glucose Meters

a. Portable glucose meters should not be used in the diagnosis of diabetes, including gestational diabetes. **B (moderate)**

b. Frequent blood glucose monitoring (BGM) is recommended for all people with diabetes who use intensive insulin regimens (with multiple daily injections or insulin pump therapy) and who are not using continuous glucose monitoring (CGM). **A (high)**

c. Routine use of BGM is not recommended for people with type 2 diabetes treated with diet and/or oral agents alone. **A (high)**

d. Individuals with diabetes should be instructed in the correct use of glucose meters, including technique of sample collection and use of quality control. **GPP**

e. Glucose meters should report the glucose concentrations in plasma rather than in whole blood to facilitate comparison with plasma results of assays performed in accredited laboratories. **GPP**

f. Glucose meters should meet relevant accuracy standards of the FDA in the US or comparable analytical performance specifications in other locations. **GPP**

g. In hospitals and acute-care facilities, point-of-care testing personnel, including nurses, should use glucose meters that are intended for professional use. **GPP**

h. When testing newborns, personnel should use only meters that are intended for use in newborns. **GPP**

i. Unless CGM is used, people using multiple daily injections of insulin should be encouraged to perform BGM at a frequency appropriate for their insulin dosage regimen, typically at least 4 times per day. **B (moderate)**

j. Manufacturers should continue to improve the analytical performance of meters. **GPP**

3. Continuous Glucose Monitoring

a. Real-time CGM should be used in conjunction with insulin as a tool to lower Hb A_{1c} concentrations and/or reduce hypoglycemia in teens and adults with type 1 diabetes who are not meeting glycemic targets or have hypoglycemia unawareness and/or episodes of hypoglycemia. **A (high)**

b. Consider using intermittently scanned CGM in conjunction with insulin as a tool to lower Hb A_{1c} concentrations and/or reduce hypoglycemia in adults with type 1 diabetes who are not meeting glycemic targets or have hypoglycemia unawareness and/or episodes of hypoglycemia. **B (moderate)**

c. Consider using real-time CGM to improve Hb A_{1c} levels, time in range, and neonatal outcomes in pregnant women with type 1 diabetes. **B (moderate)**

d. Consider using real-time CGM and intermittently scanned CGM to lower Hb A_{1c} and/or reduce hypoglycemia in adults with type 2 diabetes who are using insulin and not meeting glycemic targets. **B (moderate)**

e. Consider real-time-CGM or intermittently scanned CGM in children with type 1 diabetes, based on regulatory approval, as an additional tool to help improve glucose control and reduce the risk of hypoglycemia. **B (low)**

f. Consider using professional CGM data coupled with diabetes self-management education and medication dose adjustment to identify and address patterns of hyper- and hypoglycemia in people with type 1 or type 2 diabetes. **GPP**

g. For individuals using CGM devices that require calibration by users, a blood glucose meter should be used to calibrate the CGM. Calibration should be done at a time when glucose is not rising or falling rapidly. For all individuals using CGM, BGM should be done during periods when CGM results are not available or are incomplete, or when the CGM results are inconsistent with the clinical state or suspected to be inaccurate. **GPP**

h. CGM data reports should be available in consistent formats that include standard metrics such as time in range, time in hyperglycemia, time in hypoglycemia, mean glucose, and coefficient of variation. **GPP**

4. Noninvasive Glucose Sensing

a. Overall, noninvasive glucose measurement systems cannot be recommended as replacements for either BGM or CGM technologies at this time. **C (very low)**

5. Gestational Diabetes Mellitus

a. All pregnant women with risk factors for diabetes should be tested for undiagnosed prediabetes and diabetes at the first prenatal visit using standard diagnostic criteria. **A (moderate)**

b. All pregnant women not previously known to have diabetes should be evaluated for gestational diabetes mellitus (GDM) at 24 to 28 weeks of gestation. **A (high)**

c. Either the one-step or two-step protocol may be used, depending on regional preferences. **A (moderate)**

d. Women with GDM should perform fasting and postprandial BGM for optimal glucose control. **B (low)**

e. Target glucose values are fasting plasma glucose <5.3 mmol/L (<95 mg/dL) and either 1-h postprandial <7.8 mmol/L (<140 mg/dL) or 2-h postprandial <6.7 mmol/L (<120 mg/dL). **B (low**)

f. Women with GDM should be tested for prediabetes or diabetes 4 to 12 weeks postpartum using nonpregnant oral glucose tolerance test criteria. **A (moderate)**

g. Lifelong screening for diabetes should be performed in women with a history of GDM using standard nonpregnant criteria at least every 3 years. **A (high)**

h. There is ongoing research, but insufficient evidence at this time, to recommend testing for GDM before 20 weeks of gestation. **C (low)**

6. Urine Glucose

a. Urine glucose testing is not recommended for routine care of patients with diabetes mellitus. **B (low)**

7. Ketone Testing

a. Individuals who are prone to ketosis [those with type 1 diabetes, history of diabetic ketoacidosis (DKA), or treated with sodium-glucose transport protein 2 inhibitors] should measure ketones in urine or blood if they have unexplained hyperglycemia or symptoms of ketosis (abdominal pain, nausea) and implement sick-day rules and/or seek medical advice if urine or blood ketones are increased. **B (moderate)**

b. Specific measurement of β-hydroxybutyrate in blood should be used for diagnosis of DKA and may be used for monitoring during treatment of DKA. **B (moderate)**

c. Blood ketone determinations that rely on the nitroprusside reaction should not be used to monitor treatment of DKA. **B (low)**

8. Hemoglobin A_{1c}

a. Laboratory-based Hb A_{1c} testing can be used to diagnose (a) diabetes, with a value >6.5% (>48 mmol/mol) diagnostic of diabetes, and (b) prediabetes (or high risk for diabetes) with a Hb A_{1c} level of 5.7% to 6.4% (39-46 mmol/mol). An NGSP-certified method should be performed in an accredited laboratory. **A (moderate)**

b. Point-of-care Hb A_{1c} testing for diabetes screening and diagnosis should be restricted to FDA-approved devices at CLIA-certified laboratories that perform testing of moderate complexity or higher. **B (low)**

c. Hb A_{1c} should be measured routinely (usually every 3 months until acceptable, individualized targets are achieved and then no less than every 6 months) in most individuals with diabetes mellitus to document their degree of glycemic control. **A (moderate)**

d. Treatment goals should be based on American Diabetes Association recommendations which include maintaining Hb A_{1c} concentrations <7% (<53 mmol/mol) for many nonpregnant people with diabetes and more stringent goals in selected individuals if this can be achieved without significant hypoglycemia or other adverse effects of treatment. (Note that these values are applicable only if the assay method is certified by the NGSP as traceable to the Diabetes Control and Complications Trial reference.) **A (high)**

e. Higher target ranges are recommended for children and adolescents and are appropriate for individuals with limited life expectancy, extensive comorbid illnesses, a history of severe hypoglycemia, and advanced complications. **A (high)**

f. During pregnancy and in preparation for pregnancy, women with diabetes should try to achieve Hb A_{1c} goals that are more stringent than in the nonpregnant state, aiming ideally for <6.0% (<42 mmol/mol) during pregnancy to protect the fetus from congenital malformations and the baby and mother from perinatal trauma and morbidity owing to large-for-date babies. **A (moderate)**

g. Laboratories should be aware of potential interferences, including hemoglobin variants that may affect Hb A_{1c} test results depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population. **GPP**

h. Hb A_{1c} measurements in individuals with disorders that affect red blood cell turnover may provide spurious (generally falsely low) results regardless of the method used, and glucose testing will be necessary for screening, diagnosis, and management. **GPP**

i. Assays of other glycated proteins, such as fructosamine or glycated albumin, may be used in clinical settings where abnormalities in red blood cell turnover, hemoglobin variants, or other interfering factors compromise interpretation of Hb A_{1c} test results, although they reflect a shorter period of average glycemia than Hb A_{1c}. **GPP**

j. Hb A_{1c} cannot be measured and should not be reported in individuals who do not have Hb A, e.g., those with homozygous hemoglobin variants, such as Hb SS or Hb EE; glycated proteins, such as fructosamine or glycated albumin, may be used. **GPP**

k. Laboratories should use only Hb A_{1c} assay methods that are certified by the NGSP as traceable to the Diabetes Control and Complications Trial reference. The manufacturers of Hb A_{1c} assays should also show traceability to the International Federation of Clinical Chemistry and Laboratory Medicine reference method. **GPP**

l. Laboratories that measure Hb A_{1c} should participate in an accuracy-based proficiency-testing program that uses fresh whole blood samples with targets set by the NGSP Laboratory Network. **GPP**

m. The goals for imprecision for Hb A_{1c} measurement are intra-laboratory CV <1.5% and inter–laboratory CV <2.5%

(using at least 2 control samples with different Hb A_{1c} concentrations) and ideally no measurable bias. B (low)

n. Hb A_{1c} should be reported as a percentage of total hemoglobin or as mmol/mol of total hemoglobin. **GPP**
o. Hb A_{1c} may also be reported as estimated average glucose to facilitate comparison with the self–monitoring results obtained by patients and make the interpretation of the Hb A_{1c} more accessible to people with diabetes. **GPP**
p. Laboratories should verify by repeat testing specimens with Hb A_{1c} results below the lower limit of the reference interval or greater than 15% (140 mmol/mol) Hb A_{1c}. **GPP**

9. Genetic Markers

a. Routine determination of genetic markers such as HLA genes or single nucleotide polymorphisms is of no value at this time for the diagnosis or management of patients with type 1 diabetes. Typing for genetic markers and the use of genetic risk scores are recommended for individuals who cannot be clearly classified as having type 1 or type 2 diabetes. **A (moderate)**
b. For selected diabetes syndromes, including neonatal diabetes and maturity onset diabetes of the young, valuable information including treatment options can be obtained with definition of diabetes-associated mutations. **A (moderate)**
c. There is no role for routine genetic testing in people with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes. **A (moderate)**

10. Autoimmune Markers

a. Standardized islet autoantibody tests are recommended for classification of diabetes in adults in whom there is phenotypic overlap between type 1 and type 2 diabetes and uncertainty as to the type of diabetes. **GPP**
b. Islet autoantibodies are not recommended for routine diagnosis of diabetes. **B (low)**
c. Longitudinal follow-up of subjects with two or more islet autoantibodies is recommended to stage diabetes into stage 1: two or more islet autoantibodies, normoglycemia, no symptoms; stage 2: two or more islet autoantibodies, dysglycemia, no symptoms; and stage 3: two or more islet autoantibodies, diabetes and symptoms. **GPP**
d. Standardized islet autoantibody tests are recommended in prospective studies of children at increased genetic risk of type 1 diabetes following HLA typing at birth or in first-degree relatives of individuals with type 1 diabetes. **B (low)**
e. Screening for islet autoantibodies in relatives of individuals with type 1 diabetes or in persons in the general population is recommended in the setting of a research study or can be offered as an option for first-degree relatives of a proband with type 1 diabetes. **B (low)**
f. Routine screening for islet autoantibodies in people with type 2 diabetes is not recommended at present. **B (low)**
g. There is currently no role for measurement of islet autoantibodies in the monitoring of individuals with established type 1 diabetes. **B (low)**
h. It is important that islet autoantibodies be measured only in an accredited laboratory with an established quality control program and participation in a proficiency testing program. **GPP**

11. Urine Albumin

a. Annual testing for albuminuria should begin in pubertal or post-pubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment. **A (high)**
b. Urine albumin should be measured annually in adults with diabetes using morning spot urine albumin to creatinine ratio (uACR). **A (high)**
c. If estimated glomerular filtration rate is <60 mL/min/1.73 m^2 and/or albuminuria is >30 mg/g creatinine in a spot urine sample, the uACR should be repeated every 6 months to assess change among people with diabetes and hypertension. **A (moderate)**
d. First morning void urine sample should be used for measurement of albumin:creatinine ratio. **A (moderate)**
e. If first morning void sample is difficult to obtain, to minimize variability in test results, all urine collections should be at the same time of day. The individual should be well hydrated and should not have ingested food within the preceding 2 hours or have exercised. **GPP**
f. Timed collection for urine albumin should be done only in research settings and should not be used to guide clinical practice. **GPP**
g. The analytical performance goals for urine albumin measurement should be between-day precision <6%, bias <7% to 13% and total allowable error of <24% to 30%. **GPP**
h. Semiquantitative uACR dipsticks can be used to detect early kidney disease and assess cardiovascular risk when quantitative tests are not available. **B (moderate)**
i. Semiquantitative or qualitative screening tests should be positive in >85% of individuals with moderately increased albuminuria to be useful for patient screening. **B (moderate)**
j. j. Practitioners should strictly adhere to manufacturer's instructions when using a semi-quantitative uACR dipstick test and repeat it for confirmation to achieve adequate sensitivity for detecting moderately increased albuminuria. **B (moderate)**
k. k. Positive urine albumin screening results by semi–quantitative tests should be confirmed by quantitative analysis in an accredited laboratory. **GPP**
l. l. Currently available proteinuria dipstick tests should not be used to assess albuminuria. **B (moderate)**

12. Miscellaneous Potentially Important Analytes

a. In most people with diabetes or at risk for diabetes or cardiovascular disease, routine testing for insulin or proinsulin is not recommended. These assays are useful primarily for research purposes. **B (moderate)**
b. Although differentiation between type 1 and type 2 diabetes can usually be made based on the clinical presentation and subsequent course, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as individuals who have a type 2 phenotype but present in ketoacidosis. **B (moderate)**
c. If required by the payer for coverage of insulin pump therapy, measure fasting C-peptide level when simultaneous fasting plasma glucose is <12.5 mmol/L (< 220 mg/dL). **GPP**
d. Recommendation: Insulin and C-peptide assays should be standardized to facilitate measures of insulin secretion and sensitivity that will be comparable across research studies. **GPP**
e. There is no published evidence to support the use of insulin antibody testing for routine care of people with diabetes. **C (very low)**

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Introduction

BACKGROUND

Numerous laboratory tests are used in the diagnosis and management of diabetes mellitus. The quality of the scientific evidence supporting the use of these assays varies substantially.

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is both underutilized and over-produced, resulting in hyperglycemia. The disease is classified conventionally into several clinical categories, although these are being reconsidered based on genetic, metabolomic, and other characteristics and underlying pathophysiology. The revised classification published in 2014 (1) is indicated in Table 1. Type 1 diabetes mellitus is usually caused by autoimmune destruction of the pancreatic islet β-cells, rendering the pancreas unable to synthesize and secrete insulin (3). Type 2 diabetes mellitus results from a combination of insulin resistance and inadequate insulin secretion (4, 5). Gestational diabetes mellitus (GDM), which resembles type 2 diabetes more than type 1, develops during approximately 17% (ranging from 5% to 30%, depending on the screening method, diagnostic criteria used, and maternal age) of pregnancies, usually remits after delivery, and is a major risk factor for the development of type 2 diabetes later in life. Type 2 diabetes is the most common form, accounting for 85%-95% of diabetes in developed countries. Monogenic subtypes of type 2 diabetes have been identified but are rare. Some individuals cannot be clearly classified as type 1 or type 2 diabetes (6) and an increasing fraction of people with type 1 diabetes may have superimposed metabolic characteristics of type 2 diabetes owing to the increasing prevalence of obesity.

Diabetes is a common disease. Worldwide prevalence in 2021 was estimated to be approximately 537 million and is forecast to reach 783 million by 2045 (7). Based on 2017-2020 National Health and Nutrition Examination Survey (NHANES) data and 2018-2019 National Health Interview Survey (NHIS) data, the US Centers for Disease Control and Prevention (CDC) estimated that there were 37.3 million people (11.3% of the US population) with diabetes (8). The number of adults with diabetes has also increased in other parts of the world. For example, China and India were thought to have 140.9 million and 74.2 million adults with diabetes in 2021 and are expected to have 174.4 million and 124.9 million, respectively, by 2045 (7). Approximately 45% of people with diabetes worldwide are thought to be undiagnosed (7).

The cost of diabetes in the US in 2012 was approximately $245 billion and increased to $327 billion by 2017 (9). The mean annual per capita health care costs for an individual with diabetes are approximately 2.3-fold higher than those for individuals who do not have diabetes (10). Similarly, in the UK diabetes accounts for roughly 10% of the National Health Service budget (equivalent in 2014 to $14 billion per year), while worldwide spending in 2021 was thought to be $966 billion. The high costs of diabetes are attributable primarily to treating the chronic debilitating complications (9), which can be divided into microvascular complications—predominantly retinopathy, nephropathy, and neuropathy—and macrovascular complications, particularly stroke and coronary artery disease. Together these result in diabetes being the fourth most common cause of death in the developed world (11). About 6.7 million adults worldwide were thought to have died from diabetes-related causes in 2021 (7).

The American Association for Clinical Chemistry (AACC) and American Diabetes Association (ADA) issued "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus" in 2002 (12, 13) and 2011 (14, 15). Here we review and update these recommendations, especially in those areas where new evidence has emerged since the 2011 publications using an evidence-based approach. The guideline committee, whose membership was predominantly from the US, included clinical, laboratory, and evidence-based guideline methodology experts. Members of the guideline committee have disclosed any financial, personal, or professional relationships that may constitute conflicts of interest with this guideline and received no direct funding related to the development of the recommendations. The perspectives and views of various international and national organizations, as well as other potential stakeholders (e.g., healthcare providers, people with diabetes, policy makers, regulatory bodies, health insurance companies, researchers, and industry), were taken into account during the public consultation process. The system developed in 2011 (14, 15) to grade both the overall quality of the evidence (Table 2) and the strength of recommendations (Table 3) was used and the key steps and evidence summaries are detailed in the guideline and in the Data Supplement that accompanies the online version of this report. The literature was reviewed to the end of 2021.

This guideline focuses primarily on the laboratory aspects of testing in diabetes. It does not deal with any issues related to the clinical management of diabetes which are already covered in the ADA guidelines. This guideline intends to supplement the ADA guidelines in order to avoid duplication or repetition of information. Therefore, it focuses on practical aspects of care to assist decisions related to the use or interpretation of laboratory tests while screening, diagnosing, or monitoring diabetes.

These recommendations primarily target laboratory professionals, physicians, nurses, and other healthcare practitioners involved in the care of people with diabetes. The guidelines can be used by individuals with diabetes (where relevant), policy makers, and payers for health care, as well as by researchers and manufacturers. Although recommendations were developed for national and international use and are intended to be generic, certain recommendations may not reflect views that are universally held or may have limited applicability in healthcare settings with differing organizational, cultural, and economic backgrounds. The guideline committee therefore advises users to adapt recommendations to local settings.

To facilitate comprehension and assist the reader, each analyte is divided into several headings and, where pertinent, subheadings (listed in parentheses). These are description/introduction/terminology, use and rationale (diagnosis, screening, monitoring, and prognosis), preanalytical (including sample types, frequency of measurement), analytical considerations (including methods), interpretation (including reference intervals, decision limits, therapeutic targets, and turnaround time) and, where applicable, emerging considerations, which alert the reader to ongoing studies and potential future aspects relevant to that analyte.

Table 1. Classification of diabetes mellitus.[a]

I. Type 1 diabetes A. Immune-mediated B. Idiopathic
II. Type 2 diabetes
III. Other specific types A. Genetic defects of β-cell function B. Genetic defects in insulin action C. Diseases of the exocrine pancreas D. Endocrinopathies E. Drug- or chemical-induced F. Infections G. Uncommon forms of immune-mediated diabetes H. Other genetic syndromes sometimes associated with diabetes
IV. GDM

[a]From the ADA (2).

Table 2. Rating scale for the quality of the evidence.

High: Further research is very unlikely to change our confidence in the estimate of effect. The body of evidence comes from high-level individual studies which are sufficiently powered; provide precise, consistent, and directly applicable results in a relevant population.
Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate and the recommendation. The body of evidence comes from high/moderate-level individual studies which are sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the included studies; generalizability of results to routine practice; or indirect nature of the evidence.
Low: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate and the recommendation. The body of evidence is of low level and comes from studies with serious design flaws, or evidence is indirect.
Very low: Any estimate of effect is very uncertain. Recommendation may change when higher quality evidence becomes available. Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

Glucose

DESCRIPTION/INTRODUCTION/TERMINOLOGY

The disordered carbohydrate metabolism that underlies diabetes manifests as hyperglycemia. Therefore, measurement of blood glucose was for many years the sole diagnostic criterion. This strategy is indirect as hyperglycemia reflects the consequence of the metabolic derangement, not the cause. Nevertheless, until the underlying molecular pathophysiology of the disease is identified, measurement of glycemia is likely to remain an essential diagnostic modality.

USE/RATIONALE

Diagnosis.

RECOMMENDATION: *Fasting glucose should be measured in venous plasma when used to establish the diagnosis of diabetes, with a value ≥7.0 mmol/L (≥126mg/dL) diagnostic of diabetes.* ***A (high)***

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 (16, 17). In 1979, a set of criteria based on the distribution of glucose concentrations in high-risk populations was established to standardize the diagnosis (16). These recommendations were endorsed by the World Health Organization (WHO) (17). In 1997, the diagnostic criteria were modified (18) to better identify sub-

jects at risk of retinopathy and nephropathy (19, 20). The revised criteria comprised: (*a*) fasting plasma glucose (FPG) ≥7.0 mmol/L (126 mg/dL), (*b*) 2-h post load glucose >11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT), or (*c*) symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose ≥11.1 mmol/L (200 mg/dL) (Table 4) (18). The WHO and International Diabetes Federation (IDF) recommend either FPG or 2-h post load glucose using the same cutoffs as the ADA (21) (Table 5). In 2009 an International Expert Committee (22), with members appointed by the ADA, European Association for the Study of Diabetes (EASD) and IDF, recommended that diabetes could also 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 (23), EASD, IDF, and the WHO (24) have endorsed the use of Hb A_{1c} for diagnosis of diabetes.

If any one of the criteria in Table 4 is met, confirmation is necessary to establish the diagnosis. This can be accomplished by repeating the same assay (either glucose or Hb A_{1c}) on a different blood sample drawn on a subsequent day. Alternatively, the confirmatory test can be different to the initial assay, e.g., if glucose is the initial measurement, Hb A_{1c} can be the confirmatory test in the subsequent sample or Hb A_{1c} initially, followed by glucose. A third option is to measure 2 different analytes, namely glucose and Hb A_{1c}, in samples obtained on the same day. Note that repeat testing is not required in symptomatic individuals who have unequivocal hyperglycemia i.e., >11.1 mmol/L (200 mg/dL).

Screening

RECOMMENDATION: *Screening by hemoglobin A_{1c} (Hb A_{1c}), FPG or 2-h OGTT is recommended for individuals who are at high risk of diabetes. If Hb A_{1c} is <5.7% (<39 mmol/mol), FPG is <5.6mmol/L (<100 mg/dL), and/or 2-h plasma glucose is <7.8 mmol/L (<140 mg/dL), testing should be repeated at 3-year intervals.* ***B (moderate)***

RECOMMENDATION: *Glucose should be measured in venous plasma when used for screening of high-risk individuals.* ***B (moderate)***

RECOMMENDATION: *Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes.*
GPP (good practice point)

Testing to detect type 2 diabetes in asymptomatic people, previously controversial, is now recommended for those at risk of developing the disease (25). Screening is recommended for several reasons. In the past, the onset of type 2 diabetes has been estimated to occur approximately 4 to 7 (or more) years before clinical diagnosis (26) and epidemiological evidence indicates that complications may begin several years before clinical diagnosis. More consistent screening in high-risk populations in subsequent years may reduce both the period of undiagnosed diabetes and the prevalence of complications at the time of diagnosis. Nevertheless, it is estimated that approximately 25% of people in the US (and nearly half of Asian and Hispanic Americans) with type 2 diabetes are undiagnosed (2). Global estimates are that approximately 50% of people with diabetes are undiagnosed (7). Notwithstanding this recommendation, the evidence that population screening for hyperglycemia and subsequent prevention efforts will provide long-term benefit is inconsistent (27).

The ADA proposes that all asymptomatic people 35 years of age or more should be screened in a healthcare setting. Hb A_{1c}, FPG or 2-h OGTT are appropriate for screening (2). If FPG is <5.6 mmol/L (<100 mg/dL), 2-h plasma glucose is <7.8 mmol/L (<140 mg/dL), and/or Hb A_{1c} is <5.7% (<39 mmol/mol), testing should be repeated at 3-year intervals. The ADA suggests that screening be considered at a younger age or be carried out more frequently in individuals who are overweight or obese (BMI ≥25 kg/m^2) and who have 1 or more other risk factors for diabetes (2). Individuals with prediabetes (i.e., glucose concentrations that do not meet the criteria for diabetes, but are above normal) should be tested annually (2).

Because of the increasing prevalence of type 2 diabetes in children, screening of children is now advocated (2, 28). Starting at 10 years of age (or at the onset of puberty if puberty occurs at a younger age), testing should be performed every 3 years in overweight youths (BMI >85th percentile) who have 1 or more risk factors, namely family history, race/ethnicity recognized to increase risk, signs of insulin resistance or conditions associated with insulin resistance, and maternal history of diabetes or GDM during the child's gestation (2).

Table 3. Grading the strength of recommendations.

A. Strongly Recommend	a. adoption when: • There is high-quality evidence and strong or very strong agreement of experts that the intervention improves important health outcomes and that benefits substantially outweigh harms; or • There is moderate-quality evidence and strong or very strong agreement of experts that the intervention improves important health outcomes and that benefits substantially outweigh harms.
	b. *against* adoption when: • There is high-quality evidence and strong or very strong agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms clearly outweigh benefits; or • There is moderate-quality evidence and strong or very strong agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.

Continued

Table 3. Grading the strength of recommendations. (cont'd)

B. Recommend	a. adoption when: • There is moderate-quality evidence and level of agreement of experts that the intervention improves important health outcomes and that benefits outweigh harms; or • There is low-quality evidence but strong or very strong agreement and high level of confidence of experts that the intervention improves important health outcomes and that benefits outweigh harms; or • There is very low quality evidence but very strong agreement and very high level of confidence of experts that the intervention improves important health outcomes and that benefits outweigh harms.
	b. *against* adoption when: • There is moderate-quality evidence and level of agreement of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits; or • There is low-quality evidence but strong or very strong agreement and high level of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits; or • There is very low quality evidence but very strong agreement and very high levels of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.
C. There is Insufficient Information to Make a Recommendation	Grade C is applied in the following circumstances: • Evidence is lacking or scarce or of very low quality, and the balance of benefits and harms cannot be determined, and there is no or very low level of agreement of experts for or against adoption of the recommendation. • At any level of evidence—particularly if the evidence is heterogeneous or inconsistent, indirect, or inconclusive—if there is no agreement of experts for or against adoption of the recommendation.
Good Practice Point	Good Practice Points are recommendations mostly driven by expert consensus and professional agreement and are based on the below listed information and/or professional experience, or widely accepted standards of best practice. This category mostly applies to technical (e.g., pre-analytical, analytical, post-analytical), organizational, economic, or quality management aspects of laboratory practice. In these cases, evidence often comes from observational studies, audit reports, case series, or case studies, nonsystematic reviews, guidance or technical documents, nonevidence-based guidelines, personal opinions, expert consensus, or position statements. Recommendations are often based on empirical data, usual practice, quality requirements, and standards set by professional or legislative authorities or accreditation bodies, etc.

Table 4[f]. Criteria for the diagnosis of diabetes.[a]

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. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥11.1 mmol/L (200 mg/dL)[e]

[a]In the absence of unequivocal hyperglycemia, diagnosis requires abnormal results on 2 different tests (glucose and Hb A_{1c}) on the same day or 2 abnormal results from samples obtained on different days.
[b]The test should be performed in a laboratory using a method 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, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
[e]Random" is any time of the day without regard to time since previous meal.
The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.
[f] Adapted from the ADA (2).

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 (IGT) or impaired fasting glucose (IFG) (29-31), there is yet no published evidence that treatment based on screening influences long-term complications. In addition, there is a lack of consensus in the published literature as to which screening procedure, FPG, OGTT and/or Hb A_{1c} is the most appropriate (22, 32-34).

The cost-effectiveness of screening for type 2 diabetes has been estimated. The incremental cost of screening all persons 25 years of age or older was estimated to be $236 449 per life-year gained and $56 649 per quality-adjusted life-year (QALY) gained (35). Interestingly, screening was more cost-effective at ages younger than 45 years. In contrast, screening targeted to individuals with hypertension reduces the cost per QALY from $360 966 to $34 375, with ages 55 to 75 years being most cost-effective (36). Modeling run on 1 million individuals suggests there is considerable uncertainty as to whether screening for diabetes would be cost-effective (37). By contrast, a subsequent modeling study implies that screening commencing at age 30 or age 45 is highly cost-effective (<$11 000 per QALY gained) (38). Cohort studies support cost-effectiveness of screening (39). Long-term outcome studies are necessary to provide evidence to resolve the question of the clinical effectiveness of screening for diabetes (40). Screening and prevention of diabetes based on the Diabetes Prevention Program has been shown to be cost–effective and even cost-sav-

Table 5. 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 (21).
[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.

ing with metformin (41) and has been endorsed by the Center for Medicaid/Medicare Services based on independent cost-effective analyses.

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) (42). This change remains contentious and has not been accepted by all organizations (21, 43). 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 the development of type 2 diabetes (44, 45). Subsequent 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 (46). Data were obtained from evaluation of 13 163 men of 26 to 45 years of age with FPG <5.55 mmol/L (100 mg/dL) who were followed for a mean of 5.7 years. Men with FPG 4.83 to 5.05 mmol/L (87 to 91 mg/dL) have a significantly increased risk of type 2 diabetes compared to those with FPG <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. In a population of 117 193 Danish individuals without diagnosed diabetes, random (nonfasting) increments of glucose concentrations in the normoglycemic range and higher were associated with progressively increased risks of retinopathy, neuropathy, diabetic nephropathy, and myocardial infarction (47). The risk ratio for a 1 mmol/L (18 mg/ dL) higher glucose concentration was 2.01 for retinopathy, 2.15 for neuropathy, 1.58 for diabetic nephropathy, and 1.49 for myocardial infarction.

Monitoring/prognosis.

RECOMMENDATION: *Routine measurement of plasma glucose concentrations in a laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes.*
B (moderate)

There is a direct relationship between the degree of glycemia and the risk of renal, retinal, and neurological complications. This correlation has been documented in epidemiologic studies and in clinical trials for both type 1 (48) and type 2 (49) diabetes. In the Diabetes Control and Complications Trial (DCCT), adults and adolescents with type 1 diabetes randomized to maintain lower average blood glucose concentrations exhibited a significantly lower incidence of microvascular complications, namely diabetic retinopathy, nephropathy, and neuropathy (50). Although intensive insulin therapy reduced hypercholesterolemia by 34%, the risk of macrovascular disease was not significantly decreased in the original analysis, probably related to the limited number of events and low power (50). Longer follow-up documented a significant reduction in cardiovascular disease in participants originally randomized to intensive glycemic control (51). The effects of tight glycemic control on microvascular complications in people with type 2 diabetes (52) are similar to those with type 1 diabetes, considering the differences in glycemia achieved between the active intervention and control groups in the various trials. The United Kingdom Prospective Diabetes Study (UKPDS) in people with short-duration type 2 diabetes showed that intensive blood glucose control significantly reduced microvascular complications (52). While meta-analyses suggest that intensive glycemic control in individuals with type 2 diabetes reduces cardiovascular disease (53, 54), clinical trials have not consistently demonstrated a reduction in macrovascular disease (myocardial infarction or stroke) with intensive therapy aimed at lowering glucose concentrations in type 2 diabetes. Long-term (10 year) follow-up of the UKPDS population supported a benefit of intensive therapy on macrovascular disease (55), but 3 other trials failed to demonstrate a significant difference in macrovascular disease outcomes between very intensive treatment strategies achieving Hb A_{1c} concentrations of approximately 6.5% (48 mmol/mol) compared with the control groups who had Hb A_{1c} concentrations 0.8% to 1.1% higher (56—58). One study even observed higher cardiovascular mortality in the intensive treatment arm (56). In both the DCCT (50) and UKPDS (52), participants in the intensive group maintained lower median capillary blood 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 (25, 59).

Laboratory measurements of random or fasting glucose concentrations should not be performed as the primary means of routine outpatient monitoring of people with diabetes. Laboratory plasma glucose testing can be used to supplement information from other testing or to assess the accuracy of self-monitoring (see below) (60).

ANALYTICAL CONSIDERATIONS

Preanalytical.

RECOMMENDATION: *Blood for fasting plasma glucose analysis should be drawn in the morning after the subject has fasted overnight (at least 8 h).* ***B (low)***

RECOMMENDATION: *To minimize glycolysis, a tube containing a rapidly effective glycolytic inhibitor such as granulated citrate buffer should be used for collecting the sample. If this cannot be achieved, the sample tube should immediately be placed in an ice-water slurry and subjected to centrifugation to remove the cells within 15 to 30 min. Tubes with only enolase inhibitors such as sodium fluoride should not be relied on to prevent glycolysis.*
B (moderate)

Blood should be drawn in the morning after an overnight fast (no caloric intake for at least 8 h) during which time the subject may consume water as desired (18). Published evidence reveals a diurnal variation in FPG, with mean FPG higher in the morning than in the afternoon, indicating that many cases of diabetes would be missed in individuals screened with FPG in the afternoon (61).

Decrease in glucose concentration in the sample due to glycolysis is a serious and underappreciated problem (62, 63). Glucose concentrations decrease ex vivo in whole blood due to glucose consumption predominantly by red and white blood cells. The rate of glycolysis—reported to average 5% to 7% (approximately 0.6 mmol/L; 10 mg/dL) per hour (64) —varies with the glucose concentration, temperature, white blood cell count, and other factors (62, 65). Such a decrease of glucose will lead to missed diagnoses of diabetes in the large proportion of the population who have glucose concentrations near the cutpoints for diagnosis of diabetes.

The commonly used inhibitors of glycolysis are unable to prevent short-term glycolysis. Glycolysis can be attenuated by inhibiting enolase with sodium fluoride (2.5 mg fluoride/mL of blood) or, less commonly, lithium iodoacetate (0.5 mg/mL of blood). These inhibitors can be used alone or, more commonly, with anticoagulants such as potassium oxalate, EDTA, citrate, or lithium heparin. Unfortunately, although fluoride helps to maintain 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 and glycolysis continues for up to 4 h in samples containing only fluoride (64). After 4 h, the glucose concentration is stable in whole blood for 72 h at room temperature in the presence of fluoride (64). Leukocytosis will increase glycolysis even in the presence of fluoride if the white cell count is very high (65).

Few effective and practical methods have been available for prompt stabilization of glucose in whole blood specimens. Reduction in glucose concentration from hemolysis can be minimized in 2 ways. The first is to immediately separate blood cells after blood collection (66) [in separated, nonhemolyzed, sterile serum without fluoride the glucose concentration is stable for 8 h at 25 °C and 72 h at 4 °C (66-68)]. Alternatively, the blood tube should be placed in an ice-water slurry immediately after blood collection followed by separation of plasma from the cells within 30 min (69, 70). These methods are not always practical and are not widely used.

The use of blood collection tubes containing citrate, sodium fluoride, and EDTA offers a practical solution to the problem of glycolysis. A 2009 study showed that acidification of blood using citrate buffer inhibits in vitro glycolysis far more effectively than fluoride (70). The mean glucose concentration in samples 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 (citric acid and sodium citrate), sodium fluoride, and sodium EDTA. Acidification (pH 5.3 to 5.9) immediately blocks the activity of glycolytic enzymes, thereby preventing glycolysis (71). Subsequently, several other studies also demonstrated the effectiveness of tubes containing citrate/ fluoride/EDTA (CFE) to inhibit glycolysis (72, 73).

A few studies noted that glucose concentrations were higher in samples collected in tubes containing citrate than in control samples (74, 75). While some suggest the increase is spurious (74, 75), others state that the difference is likely due to glycolysis in the samples without citrate (70, 76). In contrast, other studies observe no difference in glucose concentrations between samples collected in tubes containing citrate compared to those with stringent sample handling to prevent glycolysis (70, 76). Importantly, use of the citrate–containing tubes has implications for diagnosis of diabetes. Widespread adoption of these tubes is likely to increase the detection of diabetes, while cases of artifactual hypoglycemia will probably decrease (77). Importantly, elimination of glycolysis is likely to substantially reduce the variability in glucose measurements that is attributable to the wide variation in sample handling prior to analysis in both routine patient care and multicenter research studies. Although commercially available in several countries, particularly in Europe, at the time of writing tubes containing CFE were not available in the US. We strongly encourage manufacturers of blood collection tubes to make these available worldwide.

Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended for diagnosis. [Note that while both the ADA and WHO recommend venous plasma, the WHO also accepts measurement of glucose in capillary (skin-puncture or "fingerstick") blood (2, 21).] The molality of glucose (i.e., amount of glucose per unit water mass) in whole blood is identical to that in plasma. Although red blood cells (RBCs) are essentially freely

permeable to glucose (glucose is taken up by facilitated transport), the concentration of water (kg/L) in plasma is approximately 11% higher than that of whole blood. Therefore, glucose concentrations in plasma are approximately 11% higher 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 (78). The reasons for the difference are not apparent, but have been attributed to the shift in fluid from RBCs to plasma caused by anticoagulants. In contrast, some subsequent studies found that glucose concentrations in plasma are slightly higher than serum. The differences observed were approximately 0.2 mmol/L (3.6 mg/dL) (79), approximately 2% (80), or 0.9% (70). Other studies indicate that glucose values measured in serum and plasma are essentially the same (81, 82) Based on these findings, it is unlikely that there is a substantial difference between glucose values in plasma and serum when assayed on current instruments, and any differences are small compared with the day-to-day biological variation of glucose. Measurement of glucose in serum (rather than plasma) is not recommended by clinical organizations for the diagnosis of diabetes (2, 21). Use of plasma allows samples to be centrifuged promptly to prevent glycolysis without waiting for the blood to clot. The glucose concentrations during an OGTT in capillary (fingerstick) blood are significantly higher than those in venous blood (mean of 1.7 mmol/L or 30 mg/dL), equivalent to 20% to 25% (83, 84), probably due to glucose consumption in the tissues. In contrast, the mean difference in fasting samples is only 0.1 mmol/L (2 mg/dL) (83, 84).

Frequency of measurement.

The frequency of measurement of blood glucose is dictated by the clinical situation. The ADA, WHO, and IDF recommend that an increased FPG or abnormal OGTT must be confirmed to establish the diagnosis of diabetes (2, 21). Screening by FPG is recommended by the ADA every 3 years beginning at age 35, more frequently in high-risk individuals; however, frequency of analysis in the latter group is not specified. Monitoring is performed by patients themselves who measure glucose with meters or continuous glucose monitoring (CGM) and by assessment of Hb A_{1c} in an accredited laboratory (see below). Appropriate intervals between measurements of glucose in acute clinical situations (e.g., hospitalization, DKA, neonatal hypoglycemia) are highly variable and may range from 30 min to 24 h or more.

Analytical.

RECOMMENDATION: *Based on biological variation, glucose measurement should have analytical imprecision ≤2.4%, bias ≤2.1% and total error ≤6.1%. To avoid misclassification of individuals, the goal for glucose analysis should be to minimize total analytical error and methods should be without measurable bias.*
B (moderate)

Glucose is measured almost exclusively by enzymatic methods. Analysis of proficiency surveys conducted in 2019 by the College of American Pathologists (CAP) reveals that hexokinase or glucose oxidase is used in virtually all the analyses performed in the US (85). A very few laboratories (<1%) use glucose dehydrogenase. Enzymatic methods for glucose analysis are relatively well standardized. The CAP data revealed that at a plasma glucose concentration of approximately 7.1 mmol/L (128 mg/dL), imprecision among laboratories using the same method had a CV ≤2.7% (85). Similar findings have been reported for glucose analysis in samples from patients. The method of glucose measurement did not influence the result. 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 (86). However, compared to a reference measurement procedure, significant ($P < 0.001$) bias (up to 13%) was observed for 40.6% of the peer groups (86). If, as is likely, similar biases occur with plasma, individuals near the diagnostic threshold could be misclassified.

No consensus has been achieved on the analytic goals for glucose analysis although numerous criteria have been proposed. These include expert opinion (consensus conferences), opinion of clinicians, regulation, state of the art, and biological variation (87). A rational and realistic recommendation that has received some support is to use biological criteria as the basis for analytic goals. It has been suggested that imprecision should not exceed one half of the within-subject biological CV (88, 89). For plasma glucose, a CV ≤2.2% has been suggested as a target for imprecision, with 0% bias (89). 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 whose glucose concentrations are 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 using commutable samples (e.g., fresh frozen plasma) that eliminate matrix effects, with accuracy–based grading using values derived with a reference measurement procedure, should be developed to assist in the achievement of this objective.

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) variability of FPG concentrations is essential for meaningful interpretation of patient values. Careful evaluation over several consecutive days in normoglycemic individuals revealed that biological variation of FPG [mean glucose of 4.9 mmol/L (88 mg/dL)] exhibited within- and between-subject CVs of 4.8% to 6.1% and 7.5% to 7.8%, respectively (90-92). Measurement of FPG in 246 normal and 80 previously undiagnosed individuals with diabetes revealed mean intraindividual CVs of 4.8% and 7.1%, respectively (91). Similar findings were obtained with analysis of 685 adults from NHANES III where mean within-person variability of FPG measured 2to 4 weeks apart was 5.7% (95% CI, 5.3%-6.1%) (93). 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) (94). A study published in 2018, which measured fasting serum glucose in 89 healthy individuals for 10 consecutive weeks (mean of 9 samples per subject), observed within- and between- person CVs of 4.7% and 8.1%, respectively, at a glucose concentration of approximately 4.6 mmol/L (83 mg/dL) (95). A meta-analysis published in 2019 (96) identified 23 publications that delivered 46 different estimates of glucose biological variation. Estimates of biological variation from 11 studies deemed suitable for inclusion in the meta-analysis (main reasons for exclusion were unhealthy or elderly individuals) yielded within- and between-person CVs of 4.8% and 7.9%, respectively. If a within-person biological CV of 5.7% (from the NHANES study) is applied to a true glucose concentration of 7.0 mmol/L (126 mg/dL), the 95% CI would encompass glucose concentrations of 6.27.8 mmol/L (112-140 mg/dL). If the CV (analytical) 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%), namely 6.1-7.9 mmol/L (110-142 mg/dL). Using assay imprecision of 3% (CV) only (excluding biological variability), would yield 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 IFG yields 95% CI of 5.6 ± 6.4% (100 ± 6.4%), namely 4.9-6.3 mmol/L (87-113 mg/dL). One should bear in mind that these ranges include 95% of results and the remaining 5% will be outside this range. Thus, the biological variability within an individual is substantially greater than analytic variability; analytic imprecision makes a negligible contribution to variation in patient results. Using biological variation as the basis for deriving analytical performance characteristics (87), the following desirable specifications for glucose have been proposed (95, 96): analytical imprecision ≤2.4%, bias ≤2.1%, and total error ≤6.1%.

Reference intervals.

Glucose concentrations in healthy individuals vary with age. Reference intervals in children are 3.3 to 5.6 mmol/L (60 to 100 mg/dL), similar to the adult range of 4.1 to 5.5 mmol/L (74 to 99 mg/dL) (66). Note that the ADA and WHO criteria (2, 21), not the reference values, are used for the diagnosis of diabetes.

The ADA classifies hypoglycemia in diabetes into 3 levels: Level 1, glucose <70 mg/dL (3.9 mmol/L) and ≥54 mg/dL (3.0 mmol/L); Level 2, glucose <54 mg/dL (3.0 mmol/L) and Level 3, severe event with altered mental/physical status that requires assistance for treatment of hypoglycemia (59). However, there is no general consensus for the threshold for diagnosis of hypoglycemia. Glucose homeostasis is impaired with aging. FPG increases with increasing age beginning in the third to fourth decade (97, 98). FPG does not increase significantly after age 60, but 2-h glucose concentrations during a 75-g OGTT are considerably higher in older persons (98, 99). Many factors participate in the metabolic dysregulation that develops with increasing age, and changes in body composition make an important contribution (100).

Turnaround time.

A short turnaround time for glucose analysis is not usually necessary for the diagnosis of diabetes. In some clinical situations, such as acute hyper- or hypoglycemic episodes in the Emergency Department (Casualty) or treatment of diabetic ketoacidosis (DKA), rapid analysis is desirable. A turnaround time of 30 min has been proposed (101). However, this value is based on suggestions of clinicians and no outcome data have been published that validate this figure. Inpatient management of individuals with hyperglycemia may on occasion require a rapid turnaround time (minutes, not hours). Similarly, for protocols with intensive glucose control in critically ill patients (102), glucose results are required rapidly to calculate the dose of insulin. Bedside monitoring with glucose meters (see below) or blood gas analyzers has been adopted by many as a practical solution.

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

CGM and noninvasive analysis of glucose are addressed below.

Glucose Meters

DESCRIPTION/INTRODUCTION/TERMINOLOGY

Portable meters for measurement of blood glucose concentrations are used in 3 major settings: (a) by people with diabetes in everyday activities; (b) in outpatient clinics; and (c) in acute and chronic care facilities. The capillary blood samples used with glucose meters typically are obtained by skin puncture, usually of a fingertip. Use of glucose meters by people with diabetes was for years referred to as self-monitoring of blood glucose (SMBG), but the ADA has replaced this term with blood glucose monitoring (BGM). Glucose meter measurements are used to guide therapy, especially adjustments of insulin dosing.

The ADA summarized uses of BGM as early as 1987 [see reference (103) and references therein], and by 1993 BGM was being performed at least once a day by 40% and 26% of individuals with type 1 and 2 diabetes, respectively, in the US (104). The ADA currently recommends that most people with type 1 diabetes use intensive insulin regimens, aiming for glycemia as close to the nondiabetes range as safely possible (usually a Hb A_{1c} <7% for many nonpregnant individuals), with multiple daily injections or an insulin pump, and with selection of doses guided by BGM, CGM, or by both (105).

The benefit of BGM is less clear for people who are not using intensive insulin therapy, although the financial costs are large.

Glucose meters and their associated supplies are thought to represent a multi-billion-dollar expense for diabetes care worldwide.

USE/RATIONALE

Diagnosis/screening.

RECOMMENDATION: *Portable glucose meters should not be used in the diagnosis of diabetes, including gestational diabetes.* ***B (moderate)***

The glucose-based criteria for the diagnosis of dia-betes (Table 4) (2) are informed by studies that defined the relationship between risk of long-term complications (retinopathy) and premorbid venous plasma glucose concentrations or Hb A_{1c}. Application of the diagnostic criteria in clinical practice relies on measurements of glucose in the same sample type (venous plasma) in an accredited laboratory (2). Similarly, the recommendations of the ADA (2) and of the U.S. Preventive Service Task Force on screening for diabetes (106) rely on measure-ments of glucose in plasma (or measurement of Hb A_{1c}). By contrast, portable meters typically use skin puncture (capillary) samples (not venous samples) of whole blood (not plasma). Most portable meters have been programmed to report an estimated plasma glucose concentration, but the estimate depends on factors in addition to the glucose concentration in the plasma portion of the finger-stick samples of whole blood. Moreover, the variability among meters (see Analytical Considerations below) precludes recommending their use in the diagnosis of diabetes.

Glucose meters have limited if any documented role in screening for diabetes in healthcare settings. The ADA Standards of Medical Care in Diabetes—2022 (2) recommends that screening, typically by risk assessment with or without use of a questionnaire, be performed in a healthcare setting. This approach allows for follow-up and treatment, and it typically assures that measurements of glucose can be made by methods that are appropriate for diagnosis of diabetes.

Community screening outside a health care setting is generally not recommended because of the risk that people with positive tests will be lost to follow-up (2). The ADA Standards (2) indicate that, in specific situations where an adequate referral system is established beforehand for positive tests, community screening may be considered. Although the benefits of such programs are difficult to document, glucose meters may have a role in such screening, particularly in resource-poor areas and regions where access to laboratory testing is impractical. Diagnosis of diabetes in people who screen positive requires testing in an accredited laboratory. Citrate-containing blood collection tubes that stabilize glucose concentrations (71) may provide another option for screening in remote areas when venipuncture is available.

Monitoring/prognosis.

RECOMMENDATION: *Frequent blood glucose monitoring (BGM) is recommended for all people with diabetes who use intensive insulin regimens (with multiple daily injections or insulin pump therapy) and who are not using continuous glucose monitoring (CGM).* ***A (high)***

RECOMMENDATION: *Routine use of BGM is not recommended for people with type 2 diabetes treated with diet and/or oral agents alone.* ***A (high)***

Intensive glycemic control can decrease microvascular complications as shown by the DCCT for individuals with type 1 (50) diabetes and by the UKPDS for type 2 (52) diabetes. In the DCCT, participants with type 1 achieved glycemic control by performing BGM at least 4 times per day to guide insulin therapy (50). Therapy in participants with type 2 diabetes in the UKPDS (52) was adjusted according to FPG concentrations— BGM was not utilized.

People using insulin, particularly those with type 1 diabetes, use knowledge of ambient capillary (with BGM) or interstitial (with CGM) glucose concentrations as an aid in determining basal insulin requirements and in selecting appropriate insulin doses for meals and at different times of the day (107). Frequent use of BGM (or CGM) is particularly important for tight glycemic control and avoidance of frequent hypoglycemia in type 1 diabetes.

Hypoglycemia is a major risk in treatment of dia-betes, and BGM or CGM may help to detect and avoid this potentially life-threatening complication. The risk of hypoglycemia is seen primarily in individuals treated with insulin or insulin secretagogues, and risk increases significantly when pharmacologic therapy is directed to-wards maintaining glucose concentrations close to those found in individuals without diabetes (52). The incidence of major hypoglycemic episodes—requiring third-party help or medical intervention—was 2- to 3-fold higher in the intensive group than in the conventional group in clin-ical trials of participants with type 1 and type 2 diabetes, with the absolute rate far higher in type 1 diabetes than in type 2 (52). Furthermore, many individuals with diabetes, particularly those with type 1, lose the autonomic warning symptoms that normally precede neuroglycopenia ("hypoglycemia unawareness") (108), increasing the risk of hypoglycemia. BGM and CGM can be useful for detecting asymptomatic hypoglycemia and allowing people to avoid severe hypoglycemic episodes, especially when insulin is used in treatment.

For those using CGM devices that require calibra-tion by users, BGM should be used to calibrate the CGM device. For all individuals using CGM, BGM should be done during periods when CGM results are not available or are incomplete (e.g., no trend arrows) and when the CGM results are inconsistent with the clinical state or suspected to be inaccurate. For discussion of these topics, see the section on CGM.

Table 6. Comparison of selected accuracy standards for glucose meters.

	Required meter results	At glucose concentrations[a]
Home-use meters		
ISO 15197 Standard (2013, reviewed 2018)	95% within 15 mg/dL of laboratory result	<100 mg/dL
	95% within 15% of laboratory result	≥100 mg/dL
	99% within zones A/B of consensus error grid	Reported results
FDA 2020 Standard	95% within 15% of laboratory result	In reportable range of meter
	99% within 20% of laboratory result	In reportable range
Hospital-use meters		
FDA 2020 Standard	95% within 12 mg/dL of laboratory result	<75 mg/dL
	95% within 12% of laboratory result	≥75 mg/dL
	98% within 15 mg/dL of laboratory result	<75 mg/dL
	98% within 15% of laboratory result	≥75 mg/dL
CLSI POCT12-A3 (2013)	95% within 12 mg/dL of laboratory result	<100 mg/dL
	95% within 12.5% of laboratory result	≥100 mg/dL
	98% within 15 mg/dL of laboratory result	<75 mg/dL
	98% within 20% of laboratory result	≥75 mg/dL

[a]To convert mg/dL to mmol/L, multiply by 0.0555 or divide by 18. Concentrations in this table: 12 mg/dL = 0.67 mmol/L; 15 mg/dL = 0.83 mmol/L; 75 mg/dL = 4.16 mmol/L; 100 mg/dL = 5.56 mmol/L.

The role of BGM in individuals with type 2 diabetes who are treated with only basal insulin or no insulin has generated considerable controversy (109). Intensive glycemic control is well established as beneficial in reducing the risk for microvascular complications. However, except for the potential use of BGM in people with type 2 diabetes using insulin, BGM likely adds cost without benefit (110). Four meta-analyses have reported the effects of BGM on Hb A_{1c} in people with type 2 dia-betes who were not using insulin (111-114). The de-creases of Hb A_{1c} in those using BGM were similar to the decreases in comparably treated people who did not use BGM. For example, the meta-analysis by Farmer et al. (112) found that the mean pooled reduction in Hb A_{1c} was 0.88% in BGM-assigned groups and 0.69% in the usual care groups. Meta-analyses also reported that, by 1 year of use of BGM, the improvements in Hb A_{1c} seen at earlier time points were lost (111, 113). There is insufficient evidence to conclude whether the observed small and transient differences in Hb A_{1c}-lowering associated with BGM improved clinically important outcomes.

A pragmatic, open-label randomized trial, conducted in 15 primary care practices, evaluated use of once-daily BGM in individuals with non-insulin-treated type 2 dia-betes (115). The study found no clinically or statistically significant differences at 1 year in glycemic control (as as-sessed by Hb A_{1c}) or health-related quality of life between patients who performed BGM, with or without enhanced feedback, and those who did not.

In summary, the evidence is insufficient to recom-mend routine use of BGM for people with type 2 dia-betes whose diabetes is treated without use of insulin.

The ADA Standards of Care suggests that nonroutine use of BGM is beneficial in specific situations for some individuals with diabetes who are not using mul-tiple injections of insulin (105). These situations include sick days and stressful periods, and when altering diet, physical activity, and/or medications (particularly med-ications that can cause hypoglycemia) in conjunction with a treatment adjustment program.

ANALYTICAL CONSIDERATIONS

Preanalytical.

RECOMMENDATION: *Individuals with diabetes should be instructed in the correct use of glucose meters, including technique of sample collection and use of quality control.* ***GPP***

Recurrent education at clinic visits and comparison of BGM with concurrent laboratory glucose analysis have been shown to improve the accuracy of BGM (116). It is important to evaluate BGM technique at regular intervals (105).

The anatomical site from which skin puncture samples are obtained influences results: Use of blood from so–called alternate sites (such as forearm or thigh rather than fingertip) for testing may exhibit a temporal lag between the circulating and measured concentrations of glucose when blood glucose is changing in vivo (117).

Analytical.

RECOMMENDATION: *Glucose meters should report the glucose concentrations in plasma rather than in whole blood to facilitate comparison with plasma results of assays performed in accredited laboratories.* ***GPP***

RECOMMENDATION: *Glucose meters should meet relevant accuracy standards of the FDA in the US or comparable analytical performance specifications in other locations.* ***GPP***

Meters can be calibrated to report glucose concentrations in plasma or whole blood. A working group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommended that glucose meters report concentrations of glucose in plasma, irrespective of the sample type or technology (118, 119); this approach can improve harmonization and allows comparison with laboratory-generated results (120).

Numerous analytical goals have been proposed for the performance of glucose meters, but the ones that most broadly affect the manufacture, sale, and availability of meters are the standards of the U.S. Food and Drug Administration (FDA) in the US (121, 122) and the similar standards of the International Organization for Standardization (ISO) (123) and the Clinical Laboratory Standards Institute (CLSI) (124). The accuracy standards of these organizations are summarized in Table 6. The FDA has separate standards for meters used for home BGM (121) and meters used in healthcare facilities (122). By contrast, the ISO standard applies only to glucose meters used for home BGM and the CLSI document applies only to meters used in healthcare facilities.

These criteria serve as de facto minimal quality re-quirements for manufacturers. In a 2017 study, however, only 2 of 17 commercial meters intended for home BGM use met the ISO standard (125).

The FDA and ISO standards agree on an allowable error of approximately 15% for home BGM meters. Both standards rely largely on expert opinion, as clinical studies of the effect of meter error are lacking. The standards are supported by in silico studies that have estimated the clin-ical impact of meter errors during BGM. A simulation modeling study quantified the effect of meter errors on the rate of insulin doses differing from the dose intended for the actual glucose concentration (126). That study re-vealed that meters that achieve both an imprecision (as coefficient of variation, CV) <5% and a bias <5% rarely lead to major errors in insulin dosing. With such a meter (CV <5% and bias <5%) approximately 95% of results fall within 15% of laboratory results, which corresponds to the 15% allowable error in the FDA and ISO standards for BGM meters (Table 6).

In subsequent studies of meters for BGM, Breton and colleagues used the UVA-PADOVA Type 1 Diabetes Simulator in 2 studies (127, 128) to assess the effects of meter inaccuracy on outcomes and costs. The first study (127) addressed use of blood glucose meters for twice-daily calibration of continuous glucose monitors. The modeling demonstrated that increasing inaccuracy of the glucose measurements progressively increased (a) the number of severe hypoglycemic episodes over 30 days, (b) the total daily insulin use, and (c) the number of finger-sticks per day. Analytical errors of meters that meet the 2013 ISO standard have only limited impact on the 3 outcome measures, or on Hb A_{1c}. The second modeling study (128) demonstrated that meter inaccuracy increased the total cost of healthcare (including costs associated with hypoglycemic episodes), with the least accurate meters producing the greatest costs. Use of meters that meet the current ISO standard reduced the financial consequences of inaccuracy of glucose meters by more than £178 ($238) per patient-year. It is important to recognize that, for both studies, the reported relationships of outcomes to the ISO standard depend on the meter meeting the ISO standard in the hands of people with diabetes during routine use, not to a meter's performance in the hands of trained workers or the performance reported by manufacturers.

RECOMMENDATION: *In hospitals and acute-care facilities, point-of-care testing personnel, including nurses, should use glucose meters that are intended for professional use.* ***GPP***

RECOMMENDATIONS: *When testing newborns, personnel should use only meters that are intended for use in newborns.* ***GPP***

Meters that are designed for home BGM often do not meet the needs of testing in hospitals, especially be-cause of the danger of transmission of pathogens from one patient to another via the meters. Professional-use meters that are cleared by the FDA for use in healthcare settings address this problem and offer additional fea-tures such as the ability to communicate the results to an electronic medical record. Moreover, these meters are held to a higher standard for accuracy. Accuracy standards (analytical performance specifications) of the FDA and of CLSI for professional-use meters are shown in Table 6. Meters that are designed for professional use have been shown in published studies to be accurate on samples of whole blood (129-131). Changing from one meter to a meter with less meter error (bias) was associated with decreased glycemic variability and increased percentage of values in target glucose range in patients following cardiovascular surgery (131).

For use in newborns, glucose meters must be accur-ate in the presence of the high hematocrits that are com-mon in this population. High hematocrit will increase or decrease the measured glucose, or will have minimal ef-fect, depending on the design of the measuring system (132, 133). Analytical bias and/or imprecision at low con-centrations can lead to frequent false alarms of neonatal hypoglycemia or missed cases of true hypoglycemia (134). Professional-use meters that are selected on the basis of their performance in a population outside the newborn nursery and newborn intensive care unit (ICU) are not necessarily the optimal choice for use in newborns (132).

INTERPRETATION

Interferences. Numerous interfering factors have been reported to influence the results of blood glucose meters (135, 136). Many meters incorporate changes that elim-inate or greatly ameliorate most interferences, but inter-ferences persist (137, 138).

Several sugars—notably maltose, galactose, and xylose—falsely increase results of some glucose meters. Maltose interferes with measurements by some glucose meters that use glucose dehydrogenase (139). Maltose is present in some medications; and it, along with maltotriose and maltotetraose, is produced in vivo by me-tabolism of icodextrin that is used in some peritoneal dia-lysis solutions (139). Interference from these sugars has been essentially eliminated as a threat in meters that use a modified glucose dehydrogenase (133). Galactose (133, 140) and xylose (141, 142) have been reported to falsely increase results of some glucose meters.

Hematocrit affects the glucose results of some meters, with falsely high glucose results at low hematocrits and falsely low results at high hematocrits (143, 144). Various methods have been developed to minimize the hematocrit effect (145) and numerous glucose meters have minimal hematocrit interference (137, 143, 146). Nonetheless, hematocrit interference persists in other meters (137).

Numerous additional factors have been reported as interferences for some meters and not others. These interfering factors include vitamin C (137), acetaminophen [paracetamol (140, 143, 147)], N-acetylcysteine (148), environmental factors—such as altitude, environmental temperature, and humidity—and pathophysiological factors, such as hypotension, hypoxia, high blood oxygen tension, and high concentrations of triglycerides or creatinine in the sample (136). The product labeling should be reviewed for interferences that are specific to the currently used meter and current lot number of strips. New interferences are reported periodically, particularly interferences from new drugs, and the effects of an interfering factor may be eliminated by manufacturers shortly after the interference is described in the literature (149).

Frequency of measurement.

RECOMMENDATION: *Unless CGM is used, people using multiple daily injections of insulin should be encouraged to perform BGM at a frequency appropriate for their insulin dosage regimen, typically at least 4 times per day.* ***B (moderate)***

Frequent monitoring of blood glucose to guide insulin therapy is part of the standard of care for people with type 1 diabetes (105). Monitoring of blood glucose less frequently than 3 to 4 times per day in adults and adolescents has been associated with less effective control of glycemia as measured by Hb A_{1c} (150-152). A study of individuals age 1 to over 65 years and treated with insulin, Hb A_{1c} showed greater improvement with BGM performed 4 or more times per day than with BGM performed less frequently (152). (This association was not found in the those who were treated with diet or with oral drugs alone.) A later study found a strong, continuous association of BGM frequency with improved glycemic control as measured by Hb A_{1c} (150). This association was seen in all age groups including in infants and children younger than 6 years and children 6 to 12 years old. Testing more frequently than 10 times per day was not associated with greater control of glycemia as Hb A_{1c} levels were similar in participants testing 10 to 12 times per day and in those testing 13 or more times per day (7.8% and 7.7%, respectively). In a study of individuals under 18 years of age with type 1 dia-betes, the frequency of BGM was found to correlate in-versely with Hb A_{1c} and with the incidence of diabetic ketoacidosis (151).

The ADA recommends that most people using intensive insulin regimens (multiple daily injections or insulin pump therapy) be encouraged to assess glucose concentrations using BGM (and/or CGM) (a) prior to meals and snacks, (b) at bedtime, (c) prior to exercise, (d) when they suspect low blood glucose, (e) after treating low blood glucose until they are normoglycemic, and (f) prior to and while performing critical tasks such as driving (105).

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

RECOMMENDATION: *Manufacturers should continue to improve the analytical performance of meters.* ***GPP***

Manufacturers have improved the analytical performance of glucose meters while also decreasing sample volume requirements and increasing speed and ease of testing. Despite these advances, and despite techniques to prevent user errors, the analytical performance reported in clinical studies of meters sometimes does not meet relevant accuracy standards (125, 153). Moreover, modeling studies predict that use of meters that have performance that exceeds the quality specifications of the FDA will improve clinical outcomes and be cost-effective (154, 155). Further research to identify and address barriers to achieving optimal per-formance of BGM meters has potential to improve the glycemic control achieved by people using insulin to treat diabetes.

Continuous Glucose Monitoring

DESCRIPTION/INTRODUCTION/TERMINOLOGY

In type 1 diabetes, as well as insulin-treated type 2 diabetes, frequent assessments of blood glucose concentrations are needed to adjust insulin and detect impending or current hyper- or hypoglycemia. CGM devices measure interstitial glucose (which correlates highly with blood glucose) every 5 to 15 min. CGM devices for the most part also inform users of trends in blood

glucose over several hours, as well as alert them to current or impending high or low glucose. Current CGM systems consist of: a glucose sensor placed under the skin (either through a catheter that remains in place for 1 to 2 weeks or as a free-standing device implanted into the subcutaneous space for a period of months), a transmitter worn on the skin, and a receiver for the data (either a dedicated receiver or a smart phone or smart watch).

Several types of CGM can be used by people with diabetes. These include real-time CGM (rt-CGM), which provides the user with glucose measurements and trends in real time. Such devices also provide alerts and alarms to notify the user that glucose concentration is approaching or in the hyper- or hypoglycemic range, as well as trend arrows that show whether glucose is stable, increasing rapidly or very rapidly, or decreasing rapidly or very rapidly. Intermittently scanned CGM systems (is-CGM, sometimes called "flash" glucose monitors) measure glucose continuously, but only display glucose readings when the user swipes a reader or smart phone over the sensor/transmitter. The first generation of the is-CGM did not have alerts for hyper- or hypoglycemia, but the second generation has the option of turning on such alerts. The final type of CGM available is the so-called professional CGM, in which blinded or unblinded CGM devices are placed at the healthcare provider's office. These devices are worn for the duration of the sensor and then returned to the healthcare provider's office, where data can be downloaded and analyzed after the fact (105). Some continuous glucose monitors require calibration with a blood glucose meter at least every 12 h, while others are "factory calibrated" and do not require calibration by the user or healthcare provider. Confirmation of the CGM readings by blood glucose meter is advised when CGM results are not available, when data are incomplete (such as an absence of trend arrows), or when results reported do not correlate with the clinical scenario. Most CGM devices for home use include the ability to "share" data with a caregiver and/or the healthcare professional office via the cloud.

USE/RATIONALE

RECOMMENDATION: *Real-time CGM should be used in conjunction with insulin as a tool to lower Hb A_{1c} levels and/or reduce hypoglycemia in teens and adults with type 1 diabetes who are not meeting glycemic targets, have hypoglycemia unawareness, and/or episodes of hypoglycemia.* ***A (high)***

RECOMMENDATION: *Consider using intermittently scanned CGM in conjunction with insulin as a tool to lower Hb A_{1c} levels and/or reduce hypoglycemia in adults with type 1 diabetes who are not meeting glycemic targets, have hypoglycemia unawareness and/or episodes of hypoglycemia.* ***B (moderate)***

RECOMMENDATION: *Consider using real-time continuous glucose monitoring to improve Hb A_{1c} levels, time in range, and neonatal outcomes in pregnant women with type 1 diabetes.* ***B (moderate)***

RECOMMENDATION: *Consider using real-time CGM or intermittently scanned CGM to lower Hb A_{1c} and/or reduce hypoglycemia in adults with type 2 diabetes who are using insulin and not meeting glycemic targets.* ***B (moderate)***

RECOMMENDATION: *Consider real-time CGM or intermittently scanned CGM in children with type 1 diabetes, based on regulatory approval, as an additional tool to help improve glucose control and reduce the risk of hypoglycemia.* ***B (low)***

RECOMMENDATION: *Consider using professional CGM data coupled with diabetes self-management education and medication dose adjustment to identify and address patterns of hyper- and hypoglycemia in people with type 1 or type 2 diabetes.* ***GPP***

Most randomized controlled trials (RCTs) in adults with type 1 diabetes show that rt-CGM leads to lower Hb A_{1c} (156-159) and reduced time in the hypoglycemic range (160, 161). Although most RCTs have not been powered to detect reductions in the rate of severe hypoglycemia, a study in people over the age of 60 with type 1 diabetes (a population at high risk of hypoglycemia) showed significant reductions in both time in the hypoglycemic range and severe hypoglycemic events (162).

There are less rigorous data on the use of is-CGM in adults with type 1 diabetes. One RCT showed less time in the hypoglycemic range, without significant change in Hb A_{1c} (163). Several observational studies have shown Hb A_{1c} reduction (164) or reductions in hypoglycemia without change in Hb A_{1c} (165). A systematic review of RCTs in adults with type 1 or type 2 diabetes suggested that is-CGM may reduce Hb A_{1c} in those with type 1 diabetes or insulin-treated type 2 diabetes (166), while another systematic review of studies (primarily in type 1 diabetes) with randomized or cohort designs suggested a small (0.26%), but statistically significant, reduction in Hb A_{1c} (167). A meta-analysis of nonrandomized studies in adults suggested that Hb A_{1c} was lowered by approximately 0.5% at 12 months with the technology (168).

Randomized controlled trials of the use of rt-CGM, compared to standard blood glucose monitoring, in adults with type 2 diabetes have generally shown reductions in Hb A_{1c} with no significant change in time in hypoglycemia (169-172). These studies have typically been done in people taking insulin, and the interventions often included substantial patient education. Studies of is-CGM use in people with type 2 diabetes have shown mixed results for both outcomes (167, 173, 174).

In a large trial of rt-CGM in people with type 1 diabetes showing significant reductions in Hb A_{1c} in adults (159), improved glucose control was not seen in children (ages 8 to 14 years) or adolescents and young adults (ages 15 to 24 years). These younger participants wore the CGM device significantly less than adults of 25 years and up, and consistency of CGM use was highly correlated with lower Hb A_{1c} in all participants. A subsequent RCT specifically targeting adolescents and young adults, which included considerable education and support, showed that those randomized to rt-CGM had significantly reduced Hb A_{1c} after 6 months compared to those randomized to BGM (175).

The evidence for rt-CGM use in young children (less than age 8 years) with type 1 diabetes is limited. Although registry studies show an association of use with lower Hb A_{1c} (176, 177), a single RCT in young children showed no impact on Hb A_{1c} (178). An uncon-trolled study in toddlers with type 1 diabetes showed no evidence of glycemic improvement over 6 months, but high levels of parental satisfaction (179). There are no RCTs of is-CGM use in children, although observational studies suggest better quality of life and/or treatment satisfaction in children or their caregivers (180-183).

One RCT of rt-CGM use during pregnancy in wo-men with type 1 diabetes showed a modest but statistic-ally significant reduction of Hb A_{1c} in women randomized to rt-CGM compared to those randomized to continuing to use blood glucose meters, with no dif-ferences in severe hypoglycemia. Rates of several adverse neonatal outcomes (large-for-gestational-age infants, newborn intensive care unit admissions, neonatal hypoglycemia) were lower in the group randomized to rt-CGM (184). One RCT of rt-CGM vs blood glucose monitoring in women with gestational diabetes showed no significant differences in Hb A_{1c} or neonatal out-comes, but less weight gain with CGM use (185).

Professional CGM, along with professional inter-pretation, patient education, and therapy adjustments, may help reduce hyper- and/or hypoglycemia, but rigorous data are lacking (105).

ANALYTICAL CONSIDERATIONS

RECOMMENDATION: *For individuals using CGM devices that require calibration by users, a blood glucose meter should be used to calibrate the CGM. Calibration should be done at a time when glucose is not rising or falling rapidly. For all individuals using CGM, BGM should be done during periods when CGM results are not available or are incomplete, or when the CGM results are inconsistent with the clinical state or sus-pected to be inaccurate.* ***GPP***

Most CGM devices measure interstitial glucose using a glucose oxidase-impregnated sensor, with electrochemical conversion into glucose concentrations transmitted to a reader. One CGM system with a sensor surgically implanted for months utilizes a nonenzymatic glucose-indicating polymer to measure interstitial glucose. The range of glucose detected by current rt-CGM systems is from 40 mg/dL to 400 mg/dL (2.2 to 22 mmol/L), while the range for the current is-CGM system is 40 to 500 mg/dL (2.2 to 27.8 mmol/L). Acetaminophen in therapeutic doses caused positive bias in several older, and one current, CGM systems. Other current systems have positive bias only with supra-therapeutic blood concentrations of acetaminophen (one system) or have no significant bias with acetaminophen (186-189). For updated information about interferences, consult device manufacturer's package inserts.

The accuracy of CGM devices has improved significantly over time, with manufacturers of current devices reporting mean absolute relative deviation (MARD) proportions of 8.1% to 12.3%, compared to 5% to 10% for current BGM devices (and 22% for the first intermittently read interstitial glucose monitor brought to market in 2001) (190). Concerns about accuracy resulted in early generations of CGM being approved only for adjunctive use (e.g., capillary glucose was to be measured by a blood glucose meter to make treatment decisions, such as deciding how much insulin to take). However, the increasing accuracy of the devices and at least one RCT comparing nonadjunctive to adjunctive use (191) has led the FDA to approve most current CGM devices for nonadjunctive use in the US. Additionally, several rt-CGM devices are approved for use in hybrid closed-loop systems, wherein CGM data are fed into an algorithm that controls insulin doses via a linked insulin pump.

Early CGM devices required calibration with capillary glucose readings several times daily. However, several currently approved devices are factory-calibrated and do not require home calibration. Regardless of whether user calibration is required, all individuals using CGM should be advised to verify CGM readings that appear to be spurious or not consistent with the clinical scenario (105).

INTERPRETATION

RECOMMENDATION: *CGM data reports should be available in consistent formats that include standard metrics such as time in range, time in hyperglycemia, time in hypoglycemia, mean glucose, and coefficient of variation.* ***GPP***

Users of rt-CGM or is-CGM can see their current glucose at a glance, accompanied by arrows that suggest glucose is changing by less than 1 mg/dL/min (horizon-tal arrow), or is changing at progressively greater rates (1, 2, or in some systems 3 arrows up or down). In addition, users of rt-CGM can view glucose trends over the past several hours on their receiver or smart phone. Several current CGM systems allow users to share glucose data for remote view by others (such as a parent of a child). People using CGM need initial and ongoing education about how to respond to and make treatment decisions based on the plethora of data they can access.

CGM devices can be downloaded at the time of clinic visits (or by users at home) to obtain useful data about antecedent glucose control. In the past, each CGM manufacturer structured these downloads differently. A consensus arose that CGM data should

be reported in a standard format, called the Ambulatory Glucose Profile (AGP). The standardized metrics on the AGP include (among others): days of CGM wear, mean glucose, estimated Hb A_{1c} based on the CGM data, glucose variability (% CV or SD), two measures of "time above range" (>250 mg/dL [13.9 mmol/L] and >180 mg/dL [10.0 mmol/L]), "time in range" (70 to 180 mg/dL or 3.9 to 10.0 mmol/L), and 2 measures of "time below range" or hypoglycemia (<70 mg/dL or 3.9 mmol/L, and <54 mg/dL or 3.0 mmol/L) (59, 192). A subsequent international consensus defined targets for most of the measures on the AGP that would correspond to individualized Hb A_{1c} targets (193).

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

Although the accuracy of CGM devices has improved over time, their use to make treatment decisions and in closed-loop systems demands that accuracy and precision continue to improve.

Further studies are needed to determine whether CGM (compared to BGM) improves outcomes in people with type 2 diabetes, young children with type 1 diabetes, or pregnant women with pre-existing diabetes or gestational diabetes.

CGM devices have not been approved for use in hospitalized patients, in part due to concerns about accuracy, concomitant medication use, or theoretical alterations in the usually high correlation between interstitial and blood glucose concentrations caused by serious illness. However, during the COVID-19 pandemic, the FDA allowed use of CGM devices with remote monitoring in hospitals in the US to potentially reduce transmission of the virus (194). Although this guidance was only in effect during the declared public health emergency of the pandemic, use of CGM in hospitalized patients (and of closed-loop insulin delivery systems based on CGM) has theoretical benefits and warrants future study.

Noninvasive Glucose Sensing

RECOMMENDATION: *Overall, noninvasive glucose measurement systems cannot be recommended as replacements for either BGM or CGM technologies at this time.* ***C (very low)***

DESCRIPTION

Broadly defined, noninvasive glucose sensing is a measurement technique whereby the blood glucose concentration is obtained without invasively collecting a sample or invasively inserting an analytical device into the body. The objective is to provide a measurement that tracks blood glucose concentrations in a painless manner that avoids puncturing the skin. Approaches include spectroscopy (195), bio-impedance (196), optical coherence tomography (197, 198), photoplethysmography (199), plasmonic devices (200-203), multi-sensing devices (204-207), and direct glucose measurements in noninvasively accessible fluids, such as tears or sweat (208, 209).

RATIONALE

Spectroscopy is the predominant approach and includes techniques associated with absorption spectroscopy over near-infrared (210-216) and mid-infrared (217, 218) wa-velengths, Raman scattering spectroscopy (219-223), and microwave spectroscopy (224-228). Exploration of the photoacoustic spectroscopic technique has received considerable attention since 2015 (229-234). For these spectroscopic approaches, noninvasive measurements involve passing nonionizing electromagnetic radiation through the skin and then extracting the concentration of glucose from the resulting spectrum by using multivariate chemometric methods (235). Glucose information for near-infrared, mid-infrared, and Raman measurements originates from unique vibrational modes within the chemical structure of the glucose molecule.

ANALYTICAL CONSIDERATIONS

To date, *no noninvasive glucose device is approved by the FDA for clinical measurements in the US.*

The peer-reviewed literature contains numerous re-ports of noninvasive glucose measurements from research-grade instruments or engineering prototypes. In general, these systems lack the ability to provide accurate glucose concentration measurements after system calibration. Typically, a system is calibrated based on analytical information combined with blood glucose concentrations observed during an OGTT. The resulting calibration models cannot measure glucose concen-trations accurately during subsequent OGTTs, thereby severely limiting clinical utility. Issues of concern remain (a) over-modelling of the calibration data, (b) uncontrolled variations associated with skin, and (c) poor specificity for indirect methods. Indirect methods correspond to systems where the measured signal does not originate directly from glucose molecules, but rather reflects a secondary impact of glucose concentrations on the measured parameter, heart rate variability for example (236).

A technology described in both the peer-reviewed (237, 238) and patent (239) literature over the last 5 years purports successful noninvasive glucose measurements from color bands measured over visible wavelengths from human fingers, described by the authors as "real-time color photography related to glucose levels in capillary tissues." However, Heise and co-workers provide a complete analysis of these measurements and conclude that direct measurement of glucose is not possible at the measured wavelength bands and that the system, as described, lacks the ability to produce stable calibration functions required for practical clinical operation (240).

Considerable attention has been given over the last few years to noninvasive glucose measurements in tear fluid (241, 242). Conceptually, a screen-printed glucose biosensor or a colloidal crystalline material can be placed on the inner surface of a contact lens to measure the concentration of glucose in a film of tear fluid. A key unanswered question is: Does the concentration of glucose in a film of tear fluid track that in blood sufficiently well for clinical purposes? Studies designed to establish correlations between blood and tear glucose concentrations are inconclusive from both human (243-245) and animal studies Variability is reported in the ratio between glucose concentrations in blood and tear fluid for individual rabbits (247). The same source of variability, if present in human tears, may be at least partly responsible for the inability to establish a clinically sound blood-to-tear correlation in human subjects (247).

Table 7. Screening for and diagnosis of GDM[a,b]

1-step strategy
Perform a 75-g OGTT, with plasma glucose measurement when patient is fasting and at 1 and 2 h, at 24 to 28 weeks of gestation in women not previously diagnosed with diabetes.
The OGTT should be performed in the morning after an overnight fast of at least 8 h.
The diagnosis of GDM is made when any of the following plasma glucose values are met or exceeded:
• fasting: 92 mg/dL (5.1 mmol/L)
• 1 h: 180 mg/dL (10.0 mmol/L)
• 2 h: 153 mg/dL (8.5 mmol/L)
2-step strategy
Step 1: Perform a 50-g GLT (nonfasting), with plasma glucose measurement at 1 h, at 24 to 28 weeks of gestation in women not previously diagnosed with diabetes
If the plasma glucose level measured 1 h after the load is ≥130, 135, or 140 mg/dL (7.2, 7.5, or 7.8 mmol/L, respectively)c, proceed to a 100-g OGTT.
Step 2: The 100-g OGTT should be performed when the patient is fasting.
The diagnosis of GDM is made when at least 2[d] of the following 4 plasma glucose levels (measured fasting and at 1, 2, and 3 h during OGTT) are met or exceeded [Carpenter–Coustan criteria (243)]:
• Fasting: 95 mg/dL (5.3 mmol/L)
• 1 h: 180 mg/dL (10.0 mmol/L)
• 2 h: 155 mg/dL (8.6 mmol/L)
• 3 h: 140 mg/dL (7.8 mmol/L)

[a]Abbreviations: GDM, gestational diabetes mellitus; GLT, glucose load test; OGTT, oral glucose tolerance test.
[b]From the ADA (2).
[c]The screening threshold is set by local consensus.
[d]American College of Obstetricians and Gynecologists notes that 1 elevated value can be used for diagnosis (250).

Gestational Diabetes Mellitus

DESCRIPTION/INTRODUCTION/TERMINOLOGY

For many years, gestational diabetes mellitus (GDM) was defined as any degree of glucose intolerance with onset or first recognition during pregnancy. This included undiagnosed diabetes. However, with increasing prevalence of undiagnosed type 2 diabetes in women of childbearing age, the definition changed to exclude diabetes found (by standard nonpregnancy criteria) at an early prenatal visit. While estimates of the prevalence of GDM vary widely due to the use of different diagnostic criteria (see below), the number is increasing. In 2021 hyperglycemia in preg-nancy was thought to affect approximately 21 million live births worldwide (7). The interest in GDM is motivated by the adverse effects on both the mother and baby (248).

USE/RATIONALE

Screening/diagnosis.

RECOMMENDATION: *All pregnant women with risk factors for diabetes should be tested for undiagnosed prediabetes and diabetes at the first prenatal visit using standard diagnostic criteria.* ***A (moderate)***

RECOMMENDATION: *All pregnant women not previously known to have diabetes should be evaluated for GDM at 24 to 28 weeks of gestation.* ***A (high)***

RECOMMENDATION: *Either the 1-step or 2-step protocol may be used, depending on regional preferences.* ***A (moderate)***

As the prevalence of obesity and type 2 diabetes has increased, the number of women of reproductive age with undiagnosed diabetes has risen. In the US, approxi-mately 4.5% of women in this age group have diabetes, and 30% of those are unaware (249). Prevalence of undiagnosed diabetes is markedly increased in women aged 35 to 44 years, in those with race/ethnicity other than non-Hispanic White, and those with obesity (249). Therefore, the ADA and some other organizations rec-ommend that women with risk factors for type 2 dia-betes should be screened for diabetes using standard diagnostic criteria (Table 4) at the first prenatal visit (2, 250). This should be in the first trimester, i.e., up to 12 weeks of pregnancy. Women identified with diabetes using this approach should receive a diagnosis of diabetes complicating pregnancy and should be managed accordingly (251). Other women should be rescreened for GDM at 24 to 28 weeks of gestation.

Numerous criteria have been proposed for screening and diagnosis of GDM, since the first proposed criteria in 1964. The original O'Sullivan and Mahan diagnostic criteria were based on blood glucose values in a 3-h 100-g OGTT predictive of later risk of diabetes mellitus in the women (252). A few years later a two-step approach was advocated, in which a screening 50-g glucose challenge test was introduced to rule out women who would not need a full OGTT; only women who failed the screening test went on to an OGTT (250). Different screening and diagnostic approaches have been proposed over the years by other organizations (253-255).

Because of the risks to the mother and the neonate, for many years the ADA has endorsed screening for GDM at 24 to 28 weeks gestation in all women not pre-viously known to have diabetes

(251). The American College of Obstetricians and Gynecologists (ACOG) recommends GDM screening in women with risk factors for diabetes (250). Since the vast majority of pregnant women in the US have one or more risk factors for diabetes, universal screening is now considered the norm.

In 2008, results of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study were published (248). HAPO was a large (approximately 25 000 preg-nant women) prospective multinational epidemiologic study to assess adverse outcomes as a function of mater-nal glycemia. The study revealed strong, graded, pre-dominantly linear associations between maternal glycemia and primary study outcomes, namely frequency of birthweight >90th percentile, delivery by Cesarean section, clinically identified neonatal hypoglycemia and cord serum insulin (assessed by measuring C-peptide) concentrations >90th percentile of values in the HAPO study population. Associations remained strong after adjustments for multiple, potentially confounding factors. Strong associations were also found with infant adiposity (248). Neonatal hypoglycemia (detected clinically or biochemically) was also significantly associated with maternal glycemia (256). Some secondary outcomes, including risks of shoulder dystocia and/ or birth injury and preeclampsia, were also associated with maternal glycemia (257).

On the strength of the HAPO study results, an expert Consensus Panel appointed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended "outcome based" criteria for the classification of glucose concentrations in pregnancy (258). These were adopted by the ADA in 2011 (109), WHO, IDF (259), and other groups, and are widely used in many countries around the world. Diagnostic cutpoints for plasma glucose concentrations are indicated in Table 7, 1-step strategy (2). Using the IADPSG criteria substantially increases the incidence of GDM, mainly because only 1 increased glucose value is required to diagnose GDM rather than 2. Treatment may require additional resources and many clinicians indicate that treatment outcome studies are necessary to ascertain whether intervention is beneficial in GDM diagnosed with the IADPSG criteria.

In 2013 an NIH Consensus Development Conference Statement recommended that the 2-step ap-proach for detection and diagnosis of GDM, predomin-ately used in the US, should continue to be used rather than the 1-step approach and criteria proposed by IADPSG (253, 254). This continues to be the recom-mendation of ACOG (250); however, they indicate that only 1 increased glucose value may be used to diagnose GDM. In 2014 the ADA acknowledged that consensus had not been reached concerning detection and diagnosis of GDM and endorsed the use of either the 1-step or the 2-step approach (260).

Concerns about criteria, frequency of diagnosis, and economic impact of GDM continue to be aired. A large (23 792 women) cohort study in which participants were assigned to detection and diagnosis of GDM via either the 1-step or the 2-step process using IADPSG/WHO or Carpenter-Coustan criteria, respectively, was published in 2021 (261). Treatment and self monitoring of blood glucose were the same in both groups. The objective was to compare the frequency of GDM detected in the 1-step and 2-step groups and frequencies of some specific outcomes such as macrosomia and large-for-gestational-age births as well as a composite outcome in the entire groups, not specifically among those with GDM. The frequency of GDM detected with the 1-step process was approximately twice that found with the 2-step process, but no significant differences in pre-specified single or composite outcomes were found between the two groups. Unfortunately, approximately 25% of those assigned to the 1-step group went through the 2-step process and the caregivers were not blinded to assignment of the participants. Moreover, different glucose cutoffs for the 2-step screening were applied at the 2 sites. Significant limitations of this study have been identified (262, 263).

RCT evidence that treatment of "mild" GDM im-proves perinatal outcome was not provided until the 21st century (264, 265). Although two RCTs found that treatment of GDM can reduce perinatal morbidity (264, 265), it is not known whether treatment reduces long-term risks in children. Follow-up of the children in both these studies at 4 to 5 (264-266) and 7 years of age (267), respectively, failed to observe differences in limited indicators of child adiposity between children of treated and untreated GDM. Thus, more information on the metabolic health of children of mothers with GDM is needed. A HAPO Follow Up Study (HAPO FUS) was carried out in a subset of the HAPO cohort (2013 to 2016) when the children were on average 11.4 years of age. The results clearly demonstrate that maternal glycemia is associated with immediate and long-term adverse outcomes for both mother and offspring. The HAPO FUS documented in both groups that risk of disorders of glucose metabolism at follow-up were associated with GDM and continuously with maternal glucose concentrations (268, 269).

Monitoring/prognosis
Blood glucose.

RECOMMENDATION: *Women with GDMshouldperform fasting and postprandial BGM for optimal glucose control.* ***B (low)***

RECOMMENDATION: *Target glucose values are FPG <5.3 mmol/L (<95 mg/dL) and either 1-h postprandial <7.8 mmol/L (<140 mg/dL) or 2-h postprandial <6.7mmol/L (<120 mg/dL).* ***B (low)***

Glucose homeostasis in pregnancy differs from the nonpregnant state. Insulin-independent glucose uptake by the fetus and placenta leads to lower fasting glucose values, while diabetogenic placental hormones produce postprandial hyperglycemia and carbohydrate intolerance. Therefore, the ADA recommends that in GDM glucose be measured both fasting and postprandially by BGM (251). Women with GDM should try to achieve the following glucose targets: FPG <5.3 mmol/L (<95 mg/dL) and either 1-h postprandial <7.8 mmol/L (<140 mg/ dL) or 2-h postprandial <6.7 mmol/L (<120 mg/dL). These target values are stricter than in nonpregnant individuals. ACOG advises that, on commencing nutrition therapy, women with GDM should measure blood glucose concentrations to confirm that glycemic control has been established (250). The vast majority of women with GDM can be treated with lifestyle modification, comprising nutrition, exercise, and weight management. Insulin should be added if lifestyle alone fails to achieve the objectives. None of the recommendations regarding frequency of testing or glycemic targets is backed by formal RCT evidence. However, one report did find a lower frequency of large-for-gestational-age babies in GDM mothers who did BGM 4 times daily compared to a group with measurement of plasma glucose in the laboratory at the time of an office visit every 1 to 2 weeks (270). Another study observed that the decision whether to add pharmacological therapy in GDM could be made with BGM every other or every 3rd day instead of daily (271).

Hb A_{1c}

Hb A_{1c} concentrations decrease during normal pregnancy due to increased red cell turnover (272). Moreover, macrosomia results primarily from postpran-dial hyperglycemia, which may not be adequately detected by Hb A_{1c}. Therefore, while Hb A_{1c} may provide valuable information, it should not replace BGM. An Hb A_{1c} value <6% (<42 mmol/mol) is optimal in pregnancy, if it can be achieved without significant hypoglycemia (251). Due to the altered red cell turnover in pregnancy, Hb A_{1c} should be measured monthly.

Postpartum testing.

RECOMMENDATION: *Women with GDM should be tested for prediabetes or diabetes 4 to 12 weeks postpartum using nonpregnant OGTT criteria.* ***A (moderate)***

RECOMMENDATION: *Lifelong screening for diabetes should be performed in women with a history of GDM using standard nonpregnant criteria at least every 3 years.* ***A (high)***

Although most cases of GDM resolve after delivery, some do not. Moreover, some cases of GDM may represent pre-existing, but undiagnosed, type 2 diabetes. In addition, women with GDM have a considerably increased risk of developing type 2 diabetes after pregnancy (273) and the Diabetes Prevention Program (DPP) found that progression to diabetes can be delayed or prevented by intervention (274); thus, long-term follow-up is important. A 75-g OGTT, interpreted by nonpregnant criteria, is recommended to find persistent hyperglycemia at 4 to 12 weeks postpartum. Hb A_{1c} is not recommended at this visit because the concentration may still be influenced by changes during pregnancy and/or peripartum blood loss. Since the cumulative risk of progression to diabetes after GDM is linear over time [reaching 50% to 60% (273, 275)], women should be evaluated every 1 to 3 years with any recommended test of glycemia, e.g., annual Hb A_{1c}, annual FPG, or triennial 75-g OGTT (with nonpregnant cutoffs) (251).

Many women with GDM will have subsequent pregnancies. If possible, preconception evaluation should be done and include measurement of glucose or Hb A_{1c} because of the risks of prediabetes or diabetes in women with prior GDM (250, 251).

ANALYTICAL CONSIDERATIONS

These issues are covered comprehensively in the glucose section above. A summary of aspects that particularly pertain to GDM is provided here.

Preanalytical.

The diagnosis of GDM is totally dependent on accurate measurement of glucose. The diagnostic thresholds for GDM, especially for FPG, are substantially lower than those for diabetes i.e., 92 mg/dL (5.1 mmol/L) or 95 mg/dL (5.3 mmol/L) by IADPSG or Carpenter-Coustan criteria, respectively (Table 7). Furthermore, in view of the relatively short interval between diagnosis of GDM and delivery, confirmatory diagnostic testing is not routinely recommended as it is in nonpregnant individuals. Therefore, preparation and timing of testing and analytical accuracy of glucose measurements are important for correct classification of GDM. Screening and diagnostic testing should not be done in febrile or recently ill persons. Individuals should have normal meals without carbohydrate restriction for at least 3 consecutive days before testing. An 8 to 10 h period of fasting must precede an OGTT which must be conducted during the morning because of circadian influences on circulating glucose (276).

Stringent sample handling procedures to minimize glycolysis after phlebotomy are essential. As discussed in the glucose section above, the best method is to collect blood in a tube containing granulated citrate buffer. Sodium fluoride alone is not adequate to prevent glycolysis. Separating plasma from cells by centrifugation within a few minutes of phlebotomy will attenuate glycolysis. Alternatively, blood drawn into sodium fluoride-containing tubes can be placed in an ice-water slurry until centrifugation (provided cells are separated within 15 to 30 min), as was done in the HAPO study (269). Unfortunately, several studies have reported inaccurate GDM detection by failure to handle specimens properly to prevent glycolysis. For example, comparison of glucose measured in samples collected in sodium fluoride-containing tubes kept in an ice-water slurry, as recommended (109), with those kept at room temperature increased the rate of diagnosis of GDM by 2.7-fold (277), entirely due to control of glycolysis. Similarly, in 121 women screened for GDM with OGTTs, collecting samples in tubes containing citrate buffer doubled the diagnostic sensitivity for GDM compared to samples collected in sodium fluoride-containing tubes (73).

Analytical.

Analytical goals and methods of glucose ana-lysis are addressed in the glucose section. Based on the strict cutoffs used in the di-

agnosis of GDM, it is very important that, in addition to careful preanalytical processing to minimize glycolysis, close attention is paid to accuracy.

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

Early detection of GDM.

RECOMMENDATION: *There is ongoing research, but insufficient evidence at this time, to recommend testing for GDM before 20 weeks of gestation.* ***C (low)***

The high prevalence of diabetes and prediabetes in nonpregnant women, coupled with the increasing prevalence of type 2 diabetes detected before or during pregnancy (278) and limited population surveys in early pregnancy (279), indicate that many women in early pregnancy have high glucose values and will be found to have GDM when tested in the second or third trimester. Evaluating early pregnancy metabolism and determining if GDM can be consistently identified before 20 weeks of gestation has become the focus of considerable attention (280). For example, the NIH has funded a study, termed "Go Moms", to address this issue. Several other studies are also underway to explore screening, diagnosis, and treatment of GDM before 20 weeks gestation.

There is evidence that women diagnosed with GDM early in pregnancy are more likely to have adverse outcomes. For example, outcomes for women with GDM diagnosed before 12 weeks of gestation are similar to those in women with pre-existing diabetes (281). However, there is no consensus on the glucose cutoff that should be used for diagnosis. The glycemic thresh-olds for the diagnosis of GDM in the second and third trimester may not be appropriate for early pregnancy be-cause FPG normally declines in early pregnancy (282, 283). For example, in a large Chinese cohort many wo-men with FPG in the first trimester above the IADPSG threshold for GDM did not have GDM when tested later in gestation (279).

Efforts to detect GDM earlier than 24 weeks gestation by methods other than glucose have been reported (284). For example, the Hb A_{1c} concentration at the first prenatal visit identifies risk of adverse pregnancy outcomes and diabetes during pregnancy, but is less effective for ascertainment of GDM (285, 286). Other studies suggest that biomarkers such as CD59 (287) or serum secreted frizzle-related protein-5 (288) may be useful in early identification of women in whom GDM will be identified later in pregnancy. There is an ongoing search to identify the optimum method to detect GDM in early pregnancy.

Towards a consensus on detection and diagnosis.

Based on analysis of OGTT results from the Danish Odense Cohort Study (289, 290), McIntyre et al. (289) have questioned the universal use of the value ≥92 mg/dL (5.1 mmol/L) as the FPG threshold for a diagnosis of GDM by the IADPSG (258) and WHO (259) criteria for GDM. In an attempt to reduce the need to perform a full OGTT in all cases, some efforts have focused on an initial measurement of FPG under circumstances where an accurate measurement can be obtained quickly and high and low thresholds employed to eliminate the need for an OGTT (291, 292).

The International Federation of Gynecology and Obstetrics (FIGO) is strongly supporting an effort to reach a global consensus on an optimal strategy for the detection and diagnosis of GDM (293). This approach also includes recommendations for low-resource settings that are pragmatic, but not proven by prospective studies. In some circumstances, a glucose load is administered without formal fasting and only a single plasma glucose is measured 2 h later. In circumstances of very limited resources or in remote locations far from laboratories, the only way of estimating glycemia is by point-of-care finger stick.

The controversy surrounding the optimal way to diagnose GDM continues, despite calls for global agreement on a common approach. In 2021 a group of obstetricians reviewed the strengths and weaknesses of the 1-step and 2-step approaches to diagnose GDM (294). The authors favored the 1-step procedure, but concluded that diagnostic thresholds should be confirmed by a large multi-institutional RCT. However, there is no assurance that such a RCT would end the GDM controversy. Definitive prospective clinical trials are needed to unequivocally establish a universal and pragmatic strategy to diagnose and follow-up GDM.

Urine Glucose

RECOMMENDATION: *Urine glucose testing is not recommended for routine care of patients with diabetes mellitus.* ***B (low)***

DESCRIPTION/INTRODUCTION/TERMINOLOGY

Testing urine for glucose is inexpensive, noninvasive, and rapid. Analysis can be performed with paper test strips at home, in healthcare providers' offices or in clinics.

USE/RATIONALE

Measurement of glucose in the urine, once the hallmark of diabetes care in the home setting, has now been re-placed by blood or interstitial glucose monitoring (see above). Semiquantitative urine glucose monitoring does not accurately reflect plasma glucose concentration (295). Notwithstanding these limitations, urine glucose monitoring is supported by the IDF in situations where blood glucose monitoring is not accessible or affordable, particularly in resource-poor settings (296). In addition, due to its high specificity, urine glucose is advocated by the IDF as a screening test for undiagnosed diabetes in low-resource settings where other procedures are not available (297).

Although urine glucose is detectable in individuals with grossly increased blood glucose concentrations, it provides no information about blood glucose concentrations below the variable renal glucose threshold [approximately 10 mmol/L (180 mg/dL)]. This alone limits its usefulness for monitoring diabetes under modern care recommendations. Semiquantitative urine glucose tests also cannot distinguish between euglycemia and hypoglycemia. Furthermore, the extent of renal concentration of the urine will affect urine glucose concentrations and only average glucose values between voidings are reflected, further minimizing the value of urine glucose determinations.

ANALYTICAL CONSIDERATIONS

Qualitative, semiquantitative, and quantitative methods are available to measure glucose in urine (85). Semiquantitative test-strip methods that utilize specific reactions for glucose are recommended. Commercially available strips use the glucose oxidase reaction (85). The strip is moistened with freshly voided urine and after 10 s the color is compared to a color chart. Test methods that detect reducing substances are not recommended as they are subject to numerous interferences, including numerous drugs, and nonglucose sugars. When used, single-voided urine samples are recommended (295).

INTERPRETATION

Because of the limited use of urine glucose determina-tions, semiquantitative specific reaction-based test strip methods are adequate.

Ketone Testing

DESCRIPTION/INTRODUCTION/TERMINOLOGY

The ketone bodies, acetoacetate (AcAc), acetone, and β-hydroxybutyrate (βOHB), are catabolic products of free fatty acids. Determinations of ketones in urine and blood are widely used in the management of people with diabetes mellitus as adjuncts for both diagnosis and ongoing monitoring of DKA. Measurements of ketone bodies are performed both in an office/hospital setting and by individuals at home. Additionally, some people following very-low-carbohydrate (ketogenic) diets for weight loss or diabetes control may check blood or urine ketones at home.

USE/RATIONALE

RECOMMENDATION: *Individuals who are prone to ketosis (those with type 1 diabetes, history of diabetic ketoacidosis [DKA], or treated with sodium-glucose co-transporter-2 (SGLT-2) inhibitors should measure ketones in urine or blood if they have unexplained hyperglycemia or symptoms of ketosis (abdominal pain, nausea), and implement sick day rules and/or seek medical advice if urine or blood ketones are in-creased.* ***B (moderate)***

Ketone bodies are normally present in urine and blood, but in very low concentrations (e.g., total serum ketones <0.5 mmol/L). Increased ketone concentrations in those with known diabetes mellitus or in previously undiagnosed individuals presenting with hyperglycemia suggest impending or established DKA, a medical emer-gency. The two major mechanisms responsible for the high ketone concentrations in people with diabetes are increased production from triglycerides and decreased utilization in the liver, both a result of absolute or relative insulin deficiency and increased counter-regulatory hormones including cortisol, epinephrine, glucagon, and growth hormone (298).

The principal ketone bodies, βOHB and AcAc, are typically present in approximately equimolar amounts. Acetone, usually present in only small quantities, is de-rived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βOHB is shifted towards formation of βOHB in any condition that alters the redox state of hepatic mitochondria to increase concentrations of NADH such as hypoxia, fasting, metabolic disorders (including DKA), and alcoholic ketoacidosis. Thus, assay methods for ketones that do not include measurement of βOHB may provide misleading clinical information by underestimating total ketone body concentration (295, 299).

The presence of urine ketones is highly sensitive for DKA or significant ketosis, with high negative predictive value suggesting utility in ruling out DKA (300, 301). Some blood glucose meters also have the capacity to measure blood ketones. Compared to testing urine ketones, children with type 1 diabetes (and caregivers) were more likely to measure blood ketones during periods of illness, and those randomized to blood ketone testing had almost half the number of emergency de-partment visits or hospitalizations (302). The ADA re-commends that ketosis-prone people with diabetes mellitus check urine or blood ketones in situations characterized by symptoms of illness and/or deterioration in glycemic control, in order to detect and preempt DKA ketoacidosis (303). Ketosis-prone individuals and/or their caregivers should receive periodic education about what to do when they have symptoms of ketosis or increased ketones. Often called "sick day rules," these interventions include oral hydration, taking additional short- or rapid-acting insulin and oral carbohydrates, frequent monitoring of blood glucose and urine or blood ketones, seeking medical advice if symptoms worsen or ketone concentrations increase, and presenting to an emergency room if sufficient oral hydration cannot be maintained due to vomiting or mental status changes (303).

ANALYTICAL CONSIDERATIONS

Urine ketones

Preanalytical.

Normally, the concentrations of ketones in the urine are below the detection limits of commercially available testing methods. False-positive results have been reported with highly colored urine and in the presence of several sulfhydryl-containing drugs, including angiotensin-converting enzyme inhibitors (301). Urine test reagents deteriorate with exposure to air, giving false-negative readings; testing material should be stored in tightly sealed containers and discarded after the expiration date on the manufacturer's label. False-negative readings have also been reported with highly acidic urine specimens, such as after large intakes of ascorbic acid. Loss of ketones from urine attributable to microbial action can also cause false-negative readings. Since acetone is

a highly volatile substance, specimens should be kept in a closed container. For point-of-care analyses in medical facilities and for ketone monitoring in home setting, control materials (giving both negative and positive readings) are commercially available.

Analytical.
Several assay principles have been described. Frequently used is the colorimetric reaction that occurs between AcAc and nitroprusside (sodium nitroferricyanide), resulting in a purple color (301). This method is widely available in the form of dipsticks and tablets and is used to measure ketones in both urine and blood (either serum or plasma). Several manufacturers offer dipsticks that measure glucose and ketones; a combination dipstick is necessary only if the individual monitors urine glucose instead of or in addition to blood glucose. The nitroprusside method measures only AcAc unless the reagent contains glycine, in which case acetone is also measured. The nitroprusside-containing reagent is much more sensitive to AcAc than acetone with respect to color generation. Importantly, this reagent does not measure βOHB (295, 304).

Blood ketones.

RECOMMENDATION: *Specific measurement of β-hydroxybutyrate (βOHB) in blood should be used for diagnosis of DKA and may be used for monitoring during treatment of DKA.* ***B (moderate)***

RECOMMENDATION: *Blood ketone determinations that rely on the nitroprusside reaction should not be used to monitor treatment of DKA.* ***B (low)***

Preanalytical.
Serum/plasma ketones can be measured using tablets or dipsticks routinely used for urine ketone determinations. Although specimens can be diluted with saline to "titer" the ketone concentration (results are typ-ically reported as "positive at a 1/x dilution"), as with ur-ine ketone testing, βOHB, the predominant ketone body in DKA, is not detected.

For specific determinations of βOHB, as described below, specimen requirements differ among methods. In general, blood samples can be collected into heparin, EDTA, fluoride, citrate, or oxalate. Ascorbic acid inter-feres with some assay methods. AcAc interferes with some assay methods unless specimens are highly dilute. Specimen stability differs among methods, but in gen-eral, whole blood specimens are stable at 4 °C for up to 24 h. Serum/plasma specimens are stable for up to 1 week at room temperature, 2 weeks at 4 °C and for at least several weeks at -20 °C (long-term stability data are not available for most assay methods) (305).

Analytical.
Although several different assay methods (e.g., colorimetric, gas chromatography, capillary electrophoresis and enzymatic) have been described for blood ketones, including specific measurement of βOHB, enzymatic methods for quantification of βOHB appear to be the most widely used for routine clinical management (301). The principle of the enzymatic methods is that βOHB in the presence of NAD is converted to AcAc and NADH by β-hydroxybutyrate dehydrogenase (304). Under alkaline conditions (pH 8.5 to 9.5), the reaction favors formation of AcAc from βOHB. The NADH produced can be quantified spectrophotometrically (usually kinetically) using a peroxidase reagent. Most methods permit use of whole blood, plasma, or serum specimens (required volumes are generally 200 µL or less). Some methods permit analysis of multiple analytes and are designed for point-of-care testing. Several methods are available as handheld meters, which are FDA-approved in the US for both laboratory use or for home use by people with diabetes. These methods utilize dry chemistry test strips to which a drop of whole blood, serum, or plasma is added. Results are displayed on the instruments within approximately 2 min (301, 306).

INTERPRETATION

Urine ketone determinations.
In a person with known diabetes mellitus, or in an individual not previously di-agnosed with diabetes who presents with typical symp-toms of diabetes and hyperglycemia, the presence of positive urine ketone readings suggests the possibility of impending or established DKA. Diagnosis of DKA in clinical settings should not rely on urine ketone deter-minations, but requires the presence of hyperglycemia, increased blood ketone bodies or βOHB, and acidosis with increased anion gap.

Although DKA is most commonly associated with type 1 diabetes, it may rarely occur in people with type 2 diabetes (307). SGLT-2 inhibitors increase the risk of DKA in individuals with type 2 diabetes and impart even higher risk in individuals with type 1 diabetes treated off-label. Since the SGLT-2 inhibitors decrease the hyperglycemia that typically attends DKA, people using these drugs are often instructed to check urine ketone concentrations (or blood ketones or βOHB) at any sign of illness (307). Individuals with alcoholic ketoacidosis will have positive urine ketone readings, but hyperglycemia is not usually present. Positive urine ketone readings are found in up to 30% of first morning urine specimens from pregnant women (with or without diabetes), during starvation, and after hypoglycemia (295).

Blood ketone determinations.
Blood ketone determinations that rely on the nitroprusside reaction should generally not be used for diagnosis of DKA as results do not quantify βOHB, the predominant ketone in DKA. If βOHB measurements are not readily available, increased blood ketones by the nitroprusside reaction, when combined with hyperglycemia and tests confirming metabolic acidosis, would confirm the presence of DKA. Blood ketone determinations that use the nitroprusside reaction should not be used to monitor the course of therapy in any setting, since AcAc and acetone may increase as βOHB falls during successful therapy (295, 298). Blood ketone determinations that measure βOHB specifically are useful for both diagnosis (299, 301) and ongoing monitoring of DKA (298, 299). Resolution of acidosis or reduction in blood βOHB is traditionally the marker for successful treatment of DKA, rather than serial measurement of ketones by the nitroprusside reaction. One small study in children with DKA found that use of a POC assay for βOHB decreased time to conversion from intravenous to subcutaneous insulin. However, the comparator was conversion when urine ketones were negative, which is not a typical marker for resolution (308). Although some guidelines specifically recommend use of point-of-care (POC) blood βOHB to follow the course of treatment for DKA, others do not. A systematic review of the components of DKA management protocols in adults did not find strong evidence for any specific measurements in assessing the treatment course of DKA (309).

Reference intervals.
βOHB reference intervals differ among assay methods, but concentrations in healthy individuals fasted overnight are generally <0.5 mmol/L. Individuals with well-documented diabetic ketoacidosis (serum bicarbonate <15 mmol/L, arterial pH <7.3, plasma glucose >14.9 mmol/L [250 mg/dL]) generally have βOHB concentrations >2 mmol/L (299).

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

Since hospitalization rates for DKA are increasing (310), further studies are needed to determine more optimal home testing strategies to detect impending ketonemia. Studies are needed to establish cutoffs for βOHB for diagnosing DKA and to evaluate whether following βOHB concentrations during treatment of DKA offers any clinical advantage over more traditional management approaches (e.g., measurements of serum bicarbonate, anion gap, or pH) (299).

Hemoglobin A_{1c}

DESCRIPTION/INTRODUCTION/TERMINOLOGY

Glycation refers to the nonenzymatic attachment of glucose to available amino groups on proteins. The extent of glycation reflects the exposure of the protein to mean glycemia integrated over time as a function of the lifespan and turnover of the protein. Hemoglobin in the RBC has an average circulating lifespan of approximately 120 days and glycated hemoglobin therefore usually indicates the average glucose concentration over the preceding 60 to 90 days. The terms glycated hemoglobin, glycohemoglobin, glycosylated, and glucosylated hemoglobin, Hb A_1, Hb A_{1c}, and A_{1c} have all been used; however, these terms are not interchangeable. The current acceptable term for glycation of hemoglobin in general is glycated hemoglobin (GHb). Hb A_{1c} is the specific glycated species that is modified by glucose on the N-terminal valine of the hemoglobin beta chain. Assay methods that measure total glycated hemoglobins (e.g., boronate affinity methods) should be calibrated to report results equivalent to Hb A_{1c} to harmonize results. Hb A1 is composed of Hb A_{1a}, Hb A_{1b}, and Hb A_{1c} and should not be measured or reported. The term "A1C test" is commonly used and recommended by the ADA in place of Hb A_{1c} to facilitate communication with people with diabetes. As described herein, most of the clinical outcome data that are available for the effects of metabolic control on complications [at least for the DCCT (50) and UKPDS (49, 52)] used assay methods that quantified Hb A_{1c}. In order to harmonize results, most clinical studies of glucose control recommend the use of Hb A_{1c} assays that are traceable to the DCCT assay, as was done in the UKPDS. In this paper, we use the abbreviation GHb to include all forms of glycated hemoglobin and Hb A_{1c} to describe the consensus accepted measurement to which all assays are translated and reported for use in clinical practice.

In addition to GHb assays, approved and commercially available assays that measure total glycated protein (termed fructosamine) or glycated albumin in the serum or plasma are available. Concentrations of these glycated proteins also reflect mean glycemia, but over a much shorter time (15 to 30 days, reflecting the turnover of albumin) than GHb (60 to 90 days) (295, 311-316). However, the clinical utility of glycated proteins other than hemoglobin has not been clearly established. Few published studies have convincingly demonstrated a relationship between glycated protein levels and the chronic complications of diabetes (317).

USE/RATIONALE

Screening/diagnosis.

RECOMMENDATION: *Laboratory-based Hb A_{1c} testing can be used to diagnose*
a. diabetes, with a value ≥6.5% (≥48 mmol/mol) diagnostic of diabetes, and
b. prediabetes (or high risk for diabetes) with an Hb A_{1c} level of 5.7% to 6.4% (39 to 46 mmol/mol). An NGSP-certified method should be performed in an accredited laboratory. ***A (moderate)***

The role of Hb A_{1c} in the diagnosis of diabetes was first proposed and implemented in 2009 (22), made possible by improved assay standardization through the National Glycohemoglobin Standardization Program (NGSP) and IFCC, and new data demonstrating the association between Hb A_{1c} concentrations and risk for retinopathy (22). Guidelines have been updated over time (2). Several technical advantages of Hb A_{1c} testing compared with glucose testing, such as its pre-analytic stability and decreased biological variability (318), also played a role. Finally, the clinical convenience of the Hb A_{1c} assay, which requires no fasting or glucose challenge, has led to increasing use of Hb A_{1c} testing for

diagnosis. A Hb A_{1c} value of 6.5% (48 mmol/mol) or greater is considered diagnostic. Confirmation with a repeated Hb A_{1c} test on a different sample or a glucose-based test is recommended (2, 319). The frequency of Hb A_{1c} testing for diagnosis has not been established, but guidelines similar to those for glucose-based testing seem appropriate (2). Hb A_{1c} assays are not recommended for screening for or diagnosis of gestational diabetes (see GDM section). Screening for diabetes will also identify populations with Hb A_{1c} that are increased but not high enough to qualify as diabetes (≥6.5%). Although the risk for developing diabetes follows Hb A_{1c} levels as a continuum, i.e., higher values are associated with higher risk for future development of diabetes (320—322), an International Expert Committee (22) recommended Hb A_{1c} levels from 6.0% to 6.4% and the ADA has recommended Hb A_{1c} levels from 5.7% to 6.4% (2) as those that define high risk to develop future diabetes (prediabetes). The concentration chosen to define high risk may depend on resources available to address prevention.

RECOMMENDATION: *Point-of-care Hb A_{1c} testing for diabetes screening and diagnosis should be restricted to FDA-approved devices at CLIA-certified laboratories that perform testing of moderate complexity or higher.*
B (low)

Only Hb A_{1c} methods that are NGSP-certified should be used to diagnose (or screen for) diabetes. The ADA has cautioned that POCT devices for Hb A_{1c} should not be used for diagnosis (59). Although several point-of-care Hb A_{1c} assays are NGSP-certified, the test is CLIA-waived in the US and proficiency testing is not necessary. Therefore, minimal objective information is available concerning their performance in the hands of nonlaboratory personnel who often measure Hb A_{1c} with POCT devices. Several published evaluations revealed that few POCT devices for Hb A_{1c} met acceptable analytical performance criteria (323). A meta-analysis published in 2017 revealed continuing problems with the accuracy of POCT devices (324). Analysis of 60 studies with 13 devices showed that most devices had negative bias (all the others had positive bias) and large standard deviations. A later study suggests improved accuracy with 1 device, including when it was used by nonlaboratory clinical staff (325). In contrast to POCT, laboratories that measure Hb A_{1c} need to have a CLIA certificate, be inspected, and meet the CLIA quality standards (326). These standards include specified personnel requirements (including documented annual competency assessments) and participation 3 times per year in an approved proficiency testing program. Absent objective—and ongoing— documentation of acceptable performance by those performing the assay using accuracy-based proficiency testing that employs whole blood (or other suitable material that is free from matrix effects), point-of-care Hb A_{1c} devices should not be used for diagnosis of or screening for diabetes.

Monitoring.

RECOMMENDATION: *Hb A_{1c} should be measured routinely (usually every 3 months until acceptable, individualized targets are achieved and then no less than every 6 months) in most individuals with diabetes mellitus to document their degree of glycemic control.*
A (moderate)

Measurement of Hb A_{1c} is widely used for routine monitoring of long-term glycemic status in people with diabetes mellitus. Hb A_{1c} is used as an index of mean glycemia, as a measure of risk for the development of diabetes complications and, most importantly, to set goals of therapy for people with diabetes (295, 318, 327). The ADA, virtually all other endocrinology specialty organizations, and nonspecialty organizations have recommended measurement of Hb A_{1c} in all individuals with diabetes to document the degree of glycemic control and assess response to therapy (59, 328). The recommended specific treatment goals for Hb A_{1c} are based on the results of prospective randomized clinical trials, most notably the DCCT in type 1 diabetes (50) and the UKPDS in type 2 diabetes (52). These trials have documented an association between glycemic control, as quantified by longitudinal determinations of Hb A_{1c}, and risks for the development and progression of chronic complications of diabetes (48, 49). More importantly, they have established a salutary role of "intensive" glycemic control aimed at achieving near-normal glycemia, as measured by Hb A_{1c} levels, on long-term complications of diabetes (50, 52).

Frequency of measurement.
There is no consensus on the optimal frequency of Hb A_{1c} testing. The ADA recom-mends (59): "The frequency of HbA_{1c} testing should depend on the clinical situation, the treatment regimen used and the clinician's judgment." In the absence of well-controlled studies that suggest a definite testing protocol, expert opinion recommends Hb A_{1c} testing "at least two times a year in patients who are meeting treatment goal (and who have stable glycemic control) and at least quarterly and as needed in patients whose therapy has changed and/or who are not meeting glycemic goals" (59). These testing recommendations are for nonpregnant individuals with either type 1 or type 2 diabetes. In addition, people with diabetes who are admitted to hospital should have Hb A_{1c} measured if the result of testing in the previous 3 months is not available (59). Studies have established that serial (quarterly for 1 year) measurements of Hb A_{1c} are associated with significant reductions in Hb A_{1c} values in people with type 1 diabetes (329).

Target levels/treatment goals.

RECOMMENDATION: *Treatment goals should be based on ADA recommendations which include maintaining Hb A_{1c} concentrations <7% (<53 mmol/mol) for many nonpregnant people with diabetes and more stringent goals in selected individuals if this can be achieved without significant hypoglycemia or other adverse effects of treatment. (Note that these values are applicable only if the assay method is certified by the NGSP as traceable to the DCCT reference.)* ***A (high)***

RECOMMENDATION: *Higher target ranges are recommended for children and adolescents, and are appropriate for individuals with limited life expectancy, extensive co-morbid illnesses, a history of severe hypoglycemia, and advanced complications.* ***A (high)***

The ADA recommends that in general a Hb A_{1c} tar-get less than 7% (53 mmol/mol) is desirable for many nonpregnant adults, with higher values recommended for children and adolescents (2), balancing the acute risks of hypoglycemia against the long-term benefits on complications. Hb A_{1c} measurements are a routine component of the clinical management of patients with diabetes mellitus. Based principally on the results of the DCCT in type 1 diabetes and the UKPDS in type 2 diabetes, the ADA has recommended that a primary goal of therapy is a Hb A_{1c} value < 7% (53 mmol/mol) for many people with diabetes (59). Other endocrine specialty clinical organizations recommend Hb A_{1c} targets similar to the ADA, ranging from 6.5% to 7% (48 to 53 mmol/mol), although higher levels have been sug-gested by nonspecialty organizations (330, 331). These Hb A_{1c} values apply only to assay methods that are certi-fied as traceable to the DCCT reference, with nondiabetic reference interval approximately 4% to 6% Hb A_{1c} (20 to 42 mmol/mol). In the DCCT, each 10% reduction in Hb A_{1c} (e.g., 12% vs 10.8% or 8% vs 7.2%) was associated with a 44% lower risk for the progression of diabetic retinopathy (49). Comparable risk reductions were found in the UKPDS (52). It should also be noted that in the DCCT and UKPDS decreased Hb A_{1c} was associated with increased risk for severe hypoglycemia.

Hb A_{1c} goals should be individualized based on the potential for benefit regarding long-term complications balanced against the increased risk for hypoglycemia and burden and cost that may attend intensive therapy. For selected individuals, more stringent targets than 7% (53 mmol/mol) can be pursued, provided this goal can be achieved without substantial hypoglycemia or other adverse effects of treatment. Such individuals might in-clude those with short duration of diabetes, diet-treated type 2 diabetes, and long life expectancy (59). Moreover, the introduction of CGM devices that alarm with low blood glucose concentrations and semi-automated pumps that suspend insulin infusion as glucose concentrations decrease have facilitated achieving target Hb A_{1c} levels with less risk for hypoglycemia (332). Conversely, in individuals with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications or extensive comorbid conditions, higher Hb A_{1c} goals should be chosen (59).

RECOMMENDATION: *During pregnancy and in preparation for pregnancy, women with diabetes should try to achieve Hb A_{1c} goals that are more stringent than in the nonpregnant state, aiming ideally for <6.0% (<42 mmol/mol) during pregnancy to protect the fetus from congenital malformations and the baby and mother from perinatal trauma and morbidity owing to large-for-date babies.*
A (moderate)

During pregnancy and in preparation for pregnancy, Hb A_{1c} testing and maintenance of specified con-centrations in individuals with pre-existing type 1 or type 2 diabetes are important for maximizing the health of the newborn and decreasing perinatal risks for the mother. Specifically, stringent control of Hb A_{1c} values during pregnancy decreases congenital malformations, large-for-date infants, and the complications of pregnancy and delivery that can otherwise occur when glycemic control is not carefully managed (333). ADA recommendations include a Hb A_{1c} < 6% (42 mmol/ mol) during pregnancy in women with preexisting dia-betes (recognizing that changes in RBC turnover during pregnancy in women without diabetes lower usual Hb A_{1c} concentrations), if this can be achieved without sig-nificant hypoglycemia (251).

ANALYTICAL CONSIDERATIONS

Preanalytical

Patient variables—age and race.
Hb A_{1c} results are not significantly affected by acute fluctuations in blood glu-cose concentrations, such as those that occur with illness or after meals. However, age and race are reported to influence Hb A_{1c}. Population data show age-related in-creases in mean Hb A_{1c} in people without diabetes of approximately 0.1% per decade after age 30 years (334, 335). Careful phenotyping of subjects with OGTT sup-ports an increase in Hb A_{1c} with age, even after removing those with otherwise undiagnosed diabetes and persons with impaired glucose tolerance from the study popula-tion (336). The increase in Hb A_{1c} levels with age gener-ally parallel other measures of glycemia. The clinical implications of the small, but statistically significant, progressive increase of "normal" Hb A_{1c} levels with aging remains to be determined (337).

The effects of race on Hb A_{1c} values remain contro-versial. Several studies have suggested a relatively higher Hb A_{1c} in Black and Hispanic populations than in White populations at the same level of glycemia, although glucose levels have not always been measured comprehensively to be confident that they capture true average glycemia (335, 338, 339). An analysis of 11 092 adults showed that Black individuals had mean Hb A_{1c} values 0.4% higher than White individuals (336). However, race did not modify the association between the Hb A_{1c} concentration and adverse cardiovascular outcomes or death (336). In addition, a study among races showed that all measures of glycemia,

including Hb A_{1c}, fructosamine, and glycated albumin, were on average higher among Black participants compared with White participants, and that the measures were similarly associated with risk of nephropathy, retinopathy, and cardiovascular disease (CVD) in the different races (340). The consistency of glycemic measurements within races and the similar relationship of each glycemic measurement with complications in Black compared to White populations suggests that higher Hb A_{1c} measurements in Black populations reflects, at least in part, higher glycemic exposure and not just a difference in the relationship between mean glycemia and Hb A_{1c} levels. The Hb A_{1c}-derived average glucose (ADAG) study, which included frequent measures of glucose, did not show a significantly different relationship between calculated mean glucose over 3 months and Hb A_{1c} at the end of the 3 months between Black and White participants; however, the size of the Black population was relatively small, limiting the interpretation of this finding (341). A study in type 1 diabetes demonstrated a difference in the relationship between mean average glucose measured with CGM and Hb A_{1c} in Black compared with White participants (342). At the same average glucose values, Hb A_{1c} was approximately 0.4% higher in the former compared to the latter.

Other patient-related factors and interfering factors.

RECOMMENDATION: *Laboratories should be aware of potential interferences, including hemoglobin variants that may affect Hb A_{1c} test results depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population.* ***GPP***

RECOMMENDATION: *Hb A_{1c} measurements in individuals with disorders that affect red blood cell turnover may provide spurious (generally falsely low) results regardless of the method used and glucose testing will be necessary for screening, diagnosis, and management.* ***GPP***

RECOMMENDATION: *Assays of other glycated proteins, such as fructosamine or glycated albumin, may be used in clinical settings where abnormalities in red blood cell turnover, hemoglobin variants, or other interfering factors compromise interpretation of Hb A_{1c} test results, although they reflect a shorter period of average glycemia than Hb A_{1c}.* ***GPP***

RECOMMENDATION: *Hb A_{1c} cannot be measured and should not be reported in individuals who do not have Hb A, e.g., those with homozygous hemoglobin variants, such as Hb SS or Hb EE; glycated proteins, such as fructosamine or glycated albumin, may be used.* ***GPP***

Any condition that shortens RBC survival or de-creases mean RBC age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers Hb A_{1c} test results, compared with mean glycemia, regardless of the assay method (295). One study has suggested that differences in mean red cell half-life, which may range from approximately 48 to 68 days (mean 58 days and 1 SD of 4.5 to 6.5 days), may explain some of the inter-individual variability in the relationship between measured average glucose and Hb A_{1c} levels (343).

Vitamins C and E are reported to lower Hb A_{1c} results falsely, possibly by inhibiting glycation of hemoglobin (344, 345). Iron-deficiency anemia is reported to increase test results (346). Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction are reported to interfere with some assay methods, falsely increasing results (312, 347). These studies are old and the findings may not pertain to modern methods. For example, interference by uremia has been eliminated.

Several hemoglobin variants (e.g., hemoglobins S, C, D, and E) and chemically modified derivatives of hemoglobin interfere with some assay methods (inde-pendent of any effects due to shortened RBC survival) (348-350): for a review, see (347). Depending on the particular hemoglobinopathy and assay method, results can be either falsely increased or decreased. Boronate af-finity chromatographic assay methods are generally con-sidered to be less affected by hemoglobin variants than other methods. In capillary electrophoresis and in some cation-exchange high-performance liquid chromatographic methods, manual inspection of chromatograms, or an automated report by the device, can alert the laboratory to the presence of either a variant or a possible interference. If an appropriate method is used, Hb A_{1c} can be measured accurately in most individuals het-erozygous for hemoglobin variants (see http://www. ngsp.org/factors.asp for a summary of published stud-ies). It is important to emphasize that Hb A_{1c} cannot be measured in individuals with homozygous hemoglobin variants (e.g., Hb SS, Hb CC, Hb EE) or 2 variant hemoglobins, like Hb SC; they have no Hb A and there-fore do not have Hb A_{1c}. In this situation, or if altered RBC turnover interferes with the relationship between mean blood glucose values and Hb A_{1c}, or if a suitable assay method is not available for interfering hemoglobin variants, alternative non-hemoglobin-based methods for assessing long-term glycemic control (such as fructosamine or glycated albumin) may be useful.

Since analytical interferences are generally method specific, product instructions from the manufacturer should be reviewed before use of the Hb A_{1c} assay meth-od. A list of interfering factors for specific assays is main-tained on the NGSP website (www. ngsp.org). In selecting an assay method, the laboratory should take into consideration characteristics of the patient population served, e.g., high prevalence of hemoglobin variants.

Sample collection, handling, and storage.

Blood can be obtained by venipuncture or by finger-stick capillary sampling. Blood tubes should contain anticoagulant as specified by the manufacturer of the Hb A_{1c} assay method (EDTA can be used unless otherwise specified by the manufacturer). Sample stability is assay method-specific (351, 352). In general, whole blood samples are stable for up to 1 week at 4 °C (352). For most methods, whole blood samples stored at -70 °C or colder are stable long-term (at least 1 year), but specimens are not as stable at -20 °C. Improper handling of specimens, such as storage at high temperatures, can introduce large artifacts that may not be detectable, depending on the assay method.

Several convenient capillary blood collection systems have been introduced, including filter paper, capillary tubes, and small vials containing stabilizing/lysing reagent (353355). These systems are designed for field collection of specimens with routine mailing to the laboratory and are generally matched to specific assay methods. They are generally used in field research settings and should be used only if studies have been performed to establish comparability of test results using these collection systems with standard sample collection and handling methods for the specific assay method em-ployed. The accuracy of such collection methods has been validated in several large research cohorts (353, 354).

Analytical

Traceability of Hb A_{1c} methods.

RECOMMENDATION: *Laboratories should use only Hb A_{1c} assay methods that are certified by the NGSP as traceable to the DCCT reference. The manufacturers of Hb A_{1c} assays should also show traceability to the IFCC reference method.* ***GPP***

There are >300 Hb A_{1c} assay methods in current clinical use. Many of these use high-throughput auto-mated systems dedicated to Hb A_{1c} determinations. Most methods can be classified into groups based on assay principle (66, 295, 312). The first group includes methods that quantify GHb based on charge differences between the glycated and nonglycated components. Examples include cation-exchange chromatography and capillary electrophoresis. The second group includes methods that separate components based on structural differences between the glycated and nonglycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify Hb A_{1c}, defined as hemoglobin A with glucose attached to the NH2-terminus valine of one or both beta chains. Other methods quantify "total glycated hemoglobin," which includes both Hb A_{1c} and other hemoglobin-glucose adducts (i.e., internal glucose-lysine adducts, and terminal glucose-alpha chain NH2-terminus valine adducts). Enzymatic methods to specifically measure Hb A_{1c} are also commercially available. Generally, results of methods using different assay principles show excellent inter-assay correlation, and there are no convincing data to show that any one method type or analyte is clinically superior to any other. The ADA recommends that laboratories use only assay methods that are certified as traceable to the DCCT GHb reference (59); these results are reported as Hb A_{1c} (295, 312, 330, 356).

RECOMMENDATION: *Laboratories that measure Hb A_{1c} should participate in an accuracy-based proficiency-testing program that uses fresh whole blood samples with targets set by the NGSP Laboratory Network.* ***GPP***

Since 1996, the NGSP, initiated under the auspices of the AACC and endorsed by the ADA, has standar-dized GHb test results among laboratories to DCCT-equivalent Hb A_{1c} values (357-359) and focused on improving worldwide assay performance. The NGSP laboratory network includes laboratories using a variety of certified assay methods that are calibrated specifically to the NGSP. The NGSP reference method, which was the DCCT primary reference, is a cation-exchange HPLC method that quantifies Hb A_{1c} and is a CLSI-designated comparison method (360). Secondary reference laboratories in the network interact with manufacturers of GHb methods to assist them, first in calibrating their methods, and then in providing comparison data for certification of traceability to the DCCT. Since initiation of the NGSP in 1996, the College of American Pathologists proficiency testing survey has documented a steady improvement in comparability of GHb values among laboratories, both within-method and between-method (357, 358, 361). The NGSP website provides detailed information on the certification process and maintains a listing of certified assay methods (updated monthly) and factors that are known to interfere with specific methods (NGSP website: http://www.ngsp.org).

The IFCC has developed a higher order reference method and reference materials for Hb A_{1c} analysis that was approved in 2001 (362, 363). Analysis is performed by cleaving hemoglobin with endoproteinase Glu-C and separating the resulting glycated and nonglycated N-terminal β chain hexapeptides by HPLC (363). Quantification of the hexapeptides is performed with electrospray ionization mass spectrometry or capillary electrophoresis. The 2 methods use the same primary reference materials and the results are essentially identical. Hb A_{1c} is measured as the ratio of glycated to non-glycated N-terminal peptide and is reported as mmol beta N1-deoxyfructosyl-hemoglobin per mol hemoglobin. Of note, the preparation and measurement of samples using this method is laborious, expensive, and time-consuming and was never envisioned as a practical means of assaying clinical samples. It is only used for manufacturers to standardize the assays. Like the NGSP, the IFCC has established a network of reference laboratories (364). The IFCC offers manufacturers calibrators and controls and a monitoring program (364).

Analytical performance goals and quality control

RECOMMENDATION: *The goals for imprecision for Hb A_{1c} measurement are intra-laboratory CV <1.5% and inter-laboratory CV<2.5% (using at least 2 control samples with*

different Hb A_{1c} levels), and ideally no measurable bias.
B (low)

Several expert groups have presented recommendations for assay performance. For example, intra-laboratory CVs <3% (365) or <2% (14) and inter-laboratory CV <5% (365) have been proposed. The prior version of these guidelines recommended intra-laboratory CV <2% and inter-laboratory CV <3.5% (14, 15). Intraindividual CVs in healthy persons are very small (<2%) and many current assay methods can achieve intra-laboratory CVs <1.5% and inter-laboratory CVs <2.0% among different laboratories using the same method (366). Using the reference change value (also termed critical difference), an analytical CV ≤2% will result in a 95% probability that a difference of ≥0.5% Hb A_{1c} between successive patient samples is due to a significant change in glycemic control [when Hb A_{1c} is 7% (53 mmol/mol)] (361). In addition, if a method has no bias, a CV of 3.5% is necessary to have 95% confidence that the Hb A_{1c} result for an individual with a "true" Hb A_{1c} of 7% (53 mmol/mol) will be between 6.5% and 7.5% (48 and 58 mmol/mol) (361). Based on the currently available technologies and the clinical need for low CVs, we recommend intra-laboratory CV <1.5% and inter-laboratory CV <2.5%.

Bias is the deviation of a result from the true value. Criteria based on biological variation have been suggested to establish analytic performance targets. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation database, which uses a systematic review that is regularly updated, recommends a desirable bias no more than 1.2% for Hb A_{1c} (367). To minimize differences among laboratories in the diagnosis of diabetes in individuals whose Hb A_{1c} concentrations are close to the diagnostic threshold value, we recommend that methods should be without measurable bias.

The laboratory should include 2 control materials with different mean values (high and low) at the begin-ning and end of each day's run. Frozen whole blood con-trols stored at -70 °C or colder in single-use aliquots are ideal and are stable for months or even years depending on the assay method. Lyophilized controls are commer-cially available, but depending on the assay method, may show matrix effects when new reagents or columns are introduced. It is recommended that the laboratory con-sider using both commercial and in-house controls to optimize performance monitoring.

Removal of labile GHb

Formation of Hb A_{1c} includes an intermediate Schiff base which is called "pre-A1c" or labile A1c (368). This material is formed rapidly with hyperglycemia and could interfere with some Hb A_{1c} assay methods if not completely removed or separated. Currently available automated assays either remove the labile pre-Hb A_{1c} during the assay process or they do not measure the labile product.

INTERPRETATION

Laboratory—clinician interactions.

Laboratory professionals should work closely with clinicians who order Hb A_{1c} testing. Proper interpretation of test results requires an understanding of the assay method, including its known interferences. For example, if the assay method is affected by hemoglobin variants, the clinician should be made aware of this.

An important advantage of using an NGSP-certified assay method is that the laboratory can provide specific information relating Hb A_{1c} test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS (50, 52). This information is avail-able on the NGSP website. For example, each 1% (approximately 11 mmol/mol) change in Hb A_{1c} is re-lated to a change in mean plasma glucose of approxi-mately 1.6 mmol/L (29 mg/dL). Reporting Hb A_{1c} results with a calculated estimated average glucose (eAG) will eliminate the need for healthcare providers or people with diabetes to perform these calculations themselves. The equation generated by the ADAG study is generally considered the most reliable one to date (341).

There is some evidence to suggest that immediate feedback of Hb A_{1c} test results to patients at the time of the clinic visit improves long-term glycemic control (369, 370). However, not all publications support this observation (371) and additional studies are needed to resolve this question before the strategy can be strongly recommended. It is possible to have Hb A_{1c} test results available at the time of the clinic visit by either having the individual go to the laboratory shortly before the scheduled clinic visit or by having a rapid assay system convenient to the clinic.

Clinical application Reporting.

Hb A_{1c} values in people with diabetes are a continuum; they range from within the nondiabetic refer-ence interval in a small percentage of people whose mean plasma glucose concentrations are close to those of individuals without diabetes, to markedly increased values, e.g., 2- to 3-fold higher levels than the nondiabetic mean of approximately 5%, in some individuals, reflecting extreme hyperglycemia. Proper interpretation of Hb A_{1c} test results requires that clinicians understand the relationship between Hb A_{1c} values and mean plasma glucose, the kinetics of Hb A_{1c}, and specific assay limitations/ interferences (295). Small changes in Hb A_{1c} (e.g., ±0.3% Hb A_{1c}) over time may reflect assay variability rather than a true change in glycemic status (361).

RECOMMENDATION: *Hb A_{1c} should be reported as a percentage of total hemoglobin or as mmol/mol of total hemoglobin.* ***GPP***

Hb A_{1c} can be reported as a percentage (glycated hemoglobin as a fraction of total hemoglobin) or as mmol/mol [based on the IFCC standardization that uses synthetic glycated hemoglobin fragments (372)]. Comparison of pooled blood samples between the IFCC and the NGSP (DCCT-aligned) networks has revealed a linear relationship (termed the master equation): [NGSP% = (0.915 X IFCC%) + 2.152] (363). Clinical results reported in IFCC units (mmol/mol) correlate tightly with NGSP results reported in percent.

RECOMMENDATION: *Hb A_{1c} may also be reported as estimated average glucose (eAG) to facilitate comparison with the home glucose monitoring results and make the interpretation of the Hb AIc more accessible to people with diabetes. .* ***GPP***

Several studies have demonstrated a close mathematical relationship between the Hb A_{1c} concentration and mean glycemia that should allow expression of Hb A_{1c} as an eAG (341, 373, 374). The eAG is helpful in translating the Hb A_{1c} results into the same glucose levels as BGM and CGM for the purposes of clinical management and therapeutic adjustments.

An international agreement recommended that both NGSP and IFCC units be reported (375, 376), with reporting of eAG left to the discretion of individual countries; however, universal reporting of Hb A_{1c} has not been adopted, with some countries, like the US, usually reporting Hb A_{1c} as a % of total hemoglobin and eAG, while others, such as the UK, report results in IFCC mmol/mol units with or without eAG.

Reference intervals.

Laboratories should ideally determine their own reference interval according to CLSI guidelines (CLSI Document C28A) even if the manufacturer has provided one. If a laboratory chooses to establish its own reference interval, test subjects without known diabetes should be non-obese and have FPG <5.6 mmol/L (100 mg/dL) and, ideally, a 2-h glucose <11.1 mmol/L (200 mg/dL) during an OGTT. For many years, Hb A_{1c} reference intervals were 4% to 6% (20 to 42 mmol/mol). This reflected mean ± 2 SD. Improvements in assay accuracy now allow a narrower range. For assay methods that are NGSP-certified, reference intervals should not deviate substantially (e.g., > 0.5%) from a mean of 5% (31 mmol/mol) i.e., 4.5% to 5.5% (26 to 37 mmol/mol). Many organizations and laboratories have lowered the upper limit of the reference interval to 5.6% (31 mmol/mol). Note that treatment target values recommended by the ADA and other clinical organizations, not the reference intervals, are used to evaluate metabolic control and diagnostic cutoffs.

Out-of-range specimens.

RECOMMENDATION: *Laboratories should verify by repeat testing specimens with Hb A_{1c} results below the lower limit of the reference interval or greater than 15% (140 mmol/mol) Hb A_{1c}.* ***GPP***

The laboratory should use repeat testing for all sam-ple results below the lower limit of the reference interval and, if confirmed, notify the ordering clinician to see whether the person tested has a variant hemoglobin or evidence of red cell destruction. If possible, the repeat measurement of Hb A_{1c} should use a method based on an analytical principle different to the initial assay. In add-ition, sample results less than 4% (20 mmol/mol) or greater than 15% Hb A_{1c} (140 mmol/mol) should be re-peated and, if confirmed, the possibility of a hemoglobin variant should be considered (347). Any result that does not correlate with the clinical impression should also be investigated. Comparison of suspicious Hb A_{1c} results with other glycated protein assays (e.g., fructosamine, glycated albumin) may be informative.

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

Capillary kits for measurement of Hb A_{1c}.

Capillary blood sample kits have been used in research studies and shown to perform well compared with venous whole blood samples when assayed with a high-performance chromatography method (353, 354). The capillary tubes are filled with a finger-stick sample and can be mailed to a central laboratory. Although the capillary tubes are not currently approved by the FDA, they may prove to be useful when in-person clinical visits are not possible.

Use of other glycated proteins including advanced glycation end-products for routine management of diabetes.

Further studies are needed to determine whether other glycated proteins such as fructosamine or glycated albumin are clinically useful for routine monitoring of glycemic status. The limited period of glycemia that they reflect limits their clinical utility. Similarly, the limited data that support their relationship with risk of complications makes them less useful than Hb A_{1c}. Moreover, treatment goals have not been established. While efforts are underway to develop a reference method for glycated albumin, neither fructosamine nor glycated albumin is standardized. Further studies are also needed to determine whether measurements of advanced glycation end-products (AGEs) are clinically useful as predictors of risk for chronic diabetes complications (377). Only one study in a subset of DCCT participants evaluated AGEs measured in dermal collagen obtained with skin biopsies. Interestingly, the concentration of AGEs in dermal collagen correlated more strongly with the presence of complications than the mean Hb A_{1c} values over time (378). The clinical role of such measurements remains undefined. Similarly, the role of noninvasive methods using light to measure tissue glycation transdermally is undefined.

Global harmonization of Hb A_{1c} testing and uniform reporting of results.

As noted above, the NGSP has largely succeeded in standardizing the GHb assay across methods and laboratories. Furthermore, the IFCC reference method, which provides reference materials for manufacturers, is being implemented worldwide. Implemen-

tation of the reporting recommendations (375, 376) needs to be carried out with education of healthcare providers and people with diabetes. Some believe that reporting eAG should complement the current reporting of Hb A_{1c} in NGSP-DCCT aligned units (%) and the IFCC results (mmol/mol), since the eAG results will be in the same units (mmol/L or mg/dL) as home BGM results. Educational campaigns will be necessary to ensure clear understanding of this assay (and the reported units) that is central to diabetes management.

Genetic Markers

DESCRIPTION/INTRODUCTION/TERMINOLOGY

Type 1 diabetes results from a selective autoimmune destruction of the pancreatic beta cell functional mass, eventually leading to an absolute lack of insulin and consequent hyperglycemia. The mode of inheritance is complex, and around 80% to 85% of newly diagnosed cases occur sporadically without familial aggregation. Among identical twins or HLA-identical siblings of type 1 diabetes, about 20% to 30% eventually manifest the disease. Type 1 diabetes is genetically linked to HLA of the major histocompatibility complex (MHC) on chromosome 6. Up to 90% of individuals with type 1 diabetes diagnosed before age 30 years have the HLA haplotypes *DRB1*04-DQA1*03:01-DQB1*03:02(DR4 -DQ8), DRB1*03-DQA1*05:01-DQB1*02:01 (DR3-D Q2.5)*, or both (379). These haplotypes are common in the general population and confer increased risk but are not causative for type 1 diabetes.

USE/RATIONALE

Diagnosis/screening

Type 1 diabetes.

RECOMMENDATION: *Routine determination of genetic markers such as HLA genes or single nucleotide polymorphisms (SNP) is of no value at this time for the diagnosis or management of type 1 diabetes. Typing for genetic markers and the use of genetic risk scores are recommended for individuals who cannot be clearly classified as having type 1 or type 2 diabetes.* ***A (moderate)***

RECOMMENDATION: *For selected diabetes syndromes, including neonatal diabetes and MODY (maturity onset diabetes of the young), valuable information including treatment options can be obtained with definition of diabetes-associated mutations.* ***A (moderate)***

The HLA system, which has a fundamental role in the adaptive immune response, exhibits considerable genetic complexity. HLA molecules present short peptides, derived from pathogens or autoantigens, to T cells to initiate the adaptive immune response (380). Therefore, HLA molecules are genetic etiological factors in the initiation phase of autoimmune diabetes, but not during pathogenesis. HLA typing thus has limited value in the diagnosis or management of type 1 diabetes. However, HLA typing is useful for clinical research studies, either in subjects followed from birth or children identified by autoantibody screening of relatives of individuals with type 1 diabetes. Subjects with the HLA DQB1*06:02 allele, which protects against progression to diabetes onset in children, are excluded.

Genetic markers are in general of limited clinical value in the diagnosis, classification, and management of children with diabetes. However, an exception is the mutational analyses established for classification of dia-betes in the neonate (381-384) as well as in young indi-viduals with a dominant family history of diabetes, often referred to as maturity-onset diabetes of the young (MODY) (384, 385) (Table 8). Type 1 or autoimmune diabetes is strongly associated with HLA DR and DQ genes. Typing of the class II major histocompatibility antigens or HLA DRB1, DQA1, and DQB1 is not diag-nostic for type 1 diabetes. HLA-DQ A1 and B1 genotyping can be useful to signal absolute risk of diabetes. The HLA-DQA-1*03:01-B1*03:02 (DQ8) and HLA-DQA1*05:01-B1*02:01 (DQ2) haplotypes, alone or in combination, may account for up to 90% of children and young adults with type 1 diabetes (379). Both haplotypes may be present in 30% to 40% of the White population; HLA is therefore necessary, but not suffi-cient, for disease. The HLA DQ and DR genes are by far the most important determinants for the risk of developing a first beta cell autoantibody such as either insulin autoantibodies (IAA) or glutamic acid decarboxylase autoantibodies (GADA) following an environ-mental exposure by, for example, enterovirus (386). Once beta cell autoimmunity has developed, HLA genes do not seem to contribute to the risk of progression to clinical onset of type 1 diabetes (387).

Thus, HLA-DR-DQ typing can be used only to increase or decrease the probability of type 1 diabetes and cannot be recommended for routine clinical diagnosis or classification (388). Precision in the genetic characteriza-tion of type 1 diabetes may be extended by typing for polymorphisms in several genetic loci identified in genome-wide association studies (386, 389). Non-HLA genetic factors include the genes for insulin (INS), PTPN22, CTLA-4 and several others (386, 387). These additional genetic factors may assist in assigning a probability of the diagnosis of type 1 diabetes of uncertain etiology, and genetic risk scores for type 1 diabetes have been developed (390).

It is possible to screen newborn children to identify those at increased risk of developing type 1 diabetes (391). A genetic risk score may be used at birth to identify children with a particularly high genetic risk of de-velopment of islet autoimmunity or type 1 diabetes (388, 390, 392). Nevertheless, this strategy cannot be re-commended until there is a proven intervention available to delay or prevent the disease (393). There is some evidence that early diagnosis may prevent hospitalization with ketoacidosis and preserve residual beta cells (393). The rationale for the approach is thus placed below under emerging considerations.

Type 2 diabetes and MODY.

RECOMMENDATION: *There is no role for routine genetic testing in people with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes.* ***A (moderate)***

Type 2 diabetes.

Type 2 diabetes is a heterogenous polygenic disease with both resistance to the action of insulin and defective insulin secretion (4, 5). Multiple genetic factors interact with exogenous influences (e.g., environmental factors such as obesity) to produce the phenotype. Identification of the genetic factors involved is therefore highly complex. Genome-wide association studies have identified more than 30 genetic factors, which increase the risk for type 2 diabetes (394, 395). However, the risk alleles in these loci all have relatively small effects and do not significantly enhance our ability to predict the risk of type 2 diabetes (396, 397).

Neonatal diabetes.

Neonatal diabetes is diagnosed at <6 months of age. Seven different genes affected by mu-tations may lead to transient or permanent diabetes (Table 8). Genetic analysis should be performed on all infants with diabetes diagnosed at <6 months of age.

MODY.

Mutation detection for MODY is technically feasible. The reduced cost of sequencing and emerging new technologies make it possible to identify mutations and properly classify individuals with MODY based on their specific mutations (Table 8). As direct automated sequencing of genes becomes standard, it is likely that de-tection of specific diabetes mutations will become routine.

Monitoring/prognosis.

Although genetic screening may provide prognostic information and could be useful for genetic counseling, the phenotype may not correlate with the genotype. In addition to environmental factors, interactions among expression of multiple quantitative trait loci may be involved. Genetic identification of a defined MODY will have value for anticipating the prognosis. For example, infants with neonatal diabetes due to a mutation in the KCNJ11 (Kir6.2) gene may be treated with a sulfonylurea rather than with insulin (381, 383, 398).

ANALYTICAL CONSIDERATIONS

The rationale for genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself (2). Such diabetes may be secondary to the obesity associated with Prader-Willi syndrome, which maps to chromosome 15q, or to the absence of adipose tissue in-herent to recessive Seip-Berardinelli syndrome of general-ized lipodystrophy mapping to chromosome 9q34 (18, 399). There are over 60 distinct genetic disorders asso-ciated with glucose intolerance or frank diabetes. The genetic factors that contribute to type 2 diabetes risk are complex (394, 395). Four major genetic forms of MODY have been identified (Table 8) and individuals at risk within MODY pedigrees can be identified through genetic means. Depending on the specific MODY muta-tion, the disease can be mild (e.g., glucokinase mutation) and not usually associated with long term complications of diabetes or as severe as typical type 1 diabetes (e.g., hepatocyte nuclear factor [HNF] mutations) (2).

A detailed review of analytical issues will not be at-tempted here, since genetic testing for diabetes outside of a research setting is currently not recommended for clinical care. Molecular HLA typing methods, replacing serological HLA typing, are commercially available.

Preanalytical.

Detection of mutations is performed using genomic DNA extracted from peripheral blood leu-kocytes. Blood samples should be drawn into test tubes containing EDTA and the DNA preparations should be harvested within 3 days; longer periods both lower the yield and degrade the quality of the DNA obtained. Genomic DNA can be isolated from fresh or frozen whole blood by lysis, digestion with proteinase K, extraction with phenol, and then dialysis. The average yield is 30 to 40 µg DNA from 1 mL of whole blood. DNA samples are best kept at -80 °C in Tris-EDTA solution, where the integrity of the sample lasts virtually indefinitely.

Analytical.

Methods for the detection of mutations differ for different types of mutation. MODY may be due to substitution, deletion, or insertion of nucleotides in the coding region of the genes. These are detected by PCR. Detailed protocols for the detection of specific mutations are beyond the scope of this guideline.

INTERPRETATION

The risk of type 1 diabetes in the general population may be determined by HLA-DQ typing, which contribute as much as 50% of familial susceptibility (400). HLA-DQ genes appear to be central to the HLA-associated risk of type 1 diabetes, albeit DR genes may be independently in-volved. The heterodimeric proteins that are expressed on antigen presenting cells, such as macrophages and den-dritic cells, B lymphocytes, platelets and activated T lymphocytes (but not other somatic cells) are composed of cis and sometimes trans complemented alpha and beta chain heterodimers. Persons at the highest genetic risk of type 1 diabetes are those in whom all 4 DQ combinations meet this criterion. Individuals heterozygous for HLA-DRB1*04:01-DQA1*03:01-DQB1*03:02 and DRB1*03-DQA1*05:01-DQB1*02:01 are the most susceptible. By contrast, individuals with the DRB1*15-DQA1*02:01-DQB1*06:02 haplotype are protected from type 1 diabetes at a young age (401). Individuals with the DRB1*11 or *04 who also have DQB1*03:01 are not likely to develop type 1 diabetes at a young age. HLA-DR4 subtypes contribute to type 1 diabetes risk in that HLA-DR B1*04:01,04:04 and 04:07 are susceptible, while

Table 8. Causes of maturity onset diabetes of the young (MODY) and other types of monogenic diabetes.[a, b]

	Gene	Inheritance	Clinical features
MODY	GCK	AD	GCK-MODY: higher glucose threshold (set point) for glucose-stimulated insulin secretion, causing stable, nonprogressive elevated fasting blood glucose; typically does not require treatment; microvascular complications are rare; small rise in 2-h PG level on OGTT (<54 mg/dL [3 mmol/L])
	HNF1A	AD	HNF1A-MODY: progressive insulin secretory defect with presentation in adolescence or early adulthood; lowered renal threshold for glucosuria; large rise in 2-h PG level on OGTT (>90 mg/dL [5 mmol/L]); sensitive to sulfonylureas
	HNF4A	AD	HNF4A-MODY: progressive insulin secretory defect with presentation in adolescence or early adulthood; may have large birth weight and transient neonatal hypoglycemia; sensitive to sulfonylureas
	HNF1B	AD	HNF1B-MODY: developmental renal disease (typically cystic); genitourinary abnormalities; atrophy of the pancreas; hyperuricemia; gout
Neonatal diabetes	KCNJ11	AD	Permanent or transient: IUGR; possible developmental delay and seizures; responsive to sulfonylureas
	INS	AD	Permanent: IUGR; insulin requiring
	ABCC8	AD	Permanent or transient: IUGR; rarely developmental delay; responsive to sulfonylureas
	6q24 (PLAGL1, HYMA1)	AD for paternal duplications	Transient: IUGR; macroglossia; umbilical hernia; mechanisms include UPD6, paternal duplication, or maternal methylation defect; may be treatable with medications other than insulin
	GATA6	AD	Permanent: pancreatic hypoplasia; cardiac malformations; pancreatic exocrine insufficiency; insulin requiring
	EIF2AK3	AR	Permanent: Wolcott–Rallison syndrome: epiphyseal dysplasia; pancreatic exocrine insufficiency; insulin requiring
	EIF2B1	AD	Permanent diabetes: can be associated with fluctuating liver function
	FOXP3	X-linked	Permanent: immunodysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome: autoimmune diabetes, autoimmune thyroid disease, exfoliative dermatitis; insulin requiring

[a] From the American Diabetes Association (2)

[b] Abbreviations: AD, autosomal dominant; AR, autosomal recessive; IUGR, intrauterine growth restriction; OGTT, oral glucose tolerance test; UPD6, uniparental disomy of chromosome 6; 2-h PG, 2-h plasma glucose.

the 04:03 and 04:06 subtypes are negatively associated with the disease, even when found in HLA genotypes with the susceptible HLA DQA1*03:01-B1*03:02 haplotype.

Multiple non-HLA loci also contribute to type 1 diabetes risk (387, 402). For example, the variable nu-cleotide tandem repeat (VNTR) upstream from the in-sulin (INS) gene on chromosome 11q may be useful for predicting IAA as the first appearing autoantibody and thereby increasing the risk of type 1 diabetes. Typing newborns for HLA-DR-DQ and to a lesser degree the INS gene results in prediction of type 1 diabetes to better than 1 in 10 in the general population. The risk of type 1 diabetes in HLA-identical siblings of a proband with type 1 diabetes is 1 in 4, while siblings who have HLA-haplotype identity have a 1 in 12 risk and those with no shared haplotype a 1 in 100 risk (403). Genome-wide association studies have confirmed a number of non-HLA genetic factors that increase the risk of a first appearing beta-cell autoantibody or type 1 diabetes, both in first degree relatives of individuals with type 1 diabetes and in the general population (386, 387, 404, 405). Combining HLA and non-HLA polymorphisms in genetic risk scores has improved the selection of individuals at risk of type 1 diabetes into prevention clinical trials.

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

The sequencing of the human genome and the formation of consortia demonstrate advances in the identification of the genetic bases for monogenic type 1 as well as type 2 diabetes. This progress should ultimately result in family counseling, prognostic information, and the selection of optimal treatment (403, 406, 407). The prospect of genotyping is to identify pathophysiological variants and provide personalized medicine.

Autoimmune Markers

DESCRIPTION/INTRODUCTION/TERMINOLOGY

The pathogenesis of type 1 diabetes is strongly associated with several immune abnormalities most prominently islet autoantibodies, but also co-occurrence of other organ-specific autoimmune diseases such as autoimmune thyroid disease and celiac disease. The islet autoantibodies are directed against insulin (IAA), GAD65 (GADA), insulinoma-associated antigen (IA-2A) or zinc transporter ZnT8 (ZnT8A) and predict type 1 diabetes. In children with only 1 persistent islet autoantibody, the risk of diabetes within 10 years is 15% while 2 or more islet autoantibodies predict type 1 diabetes in 70% within 10 years (408, 409). The islet autoantibody biomarkers are useful to predict and classify type 1 diabetes.

USE/RATIONALE

RECOMMENDATION: *Standardized islet autoantibody tests are recommended for classification of diabetes in adults in whom there is phenotypic overlap between type 1 and type 2 diabetes and uncertainty as to the type of diabetes.* ***GPP***

RECOMMENDATION: *Islet autoantibodies are not recommended for routine diagnosis of diabetes.* ***B (low)***

RECOMMENDATION: *Longitudinal follow-up of subjects with 2 or more islet autoantibodies is recommended to stage diabetes into stage 1: two or more islet autoantibodies, normoglycemia, no symptoms; stage 2: two or more islet autoantibodies, dysglycemia, no symptoms; and stage 3: two or more islet autoantibodies, diabetes and symptoms.* ***GPP***

RECOMMENDATION: *Standardized islet autoantibody tests are recommended in prospective research studies ofchildren at increased genetic risk of type 1 diabetes following HLA typing at birth or in first degree relatives of individuals with type 1 diabetes.* ***B (low)***

Although several islet autoantibodies have been detected in individuals with type 1 diabetes, and these have prognostic value in individuals at high risk of type 1 diabetes, routine measurement of islet autoantibodies has limited use outside of clinical studies (410). Currently islet autoantibodies are not used in routine management of diabetes. This section will focus on the pragmatic aspects of clinical laboratory testing for islet autoantibodies.

Diagnosis.

The clinical onset of type 1 diabetes is related to the loss of the functional beta-cell mass. In most people with type 1 diabetes, the loss of function is associated with an autoimmune attack (411). This is termed type 1A or immune-mediated diabetes. As further discussed under Analytical Considerations, quantitative assays of specific autoantibodies have generally replaced the islet cell antibody (ICA) test, which is indirect immunofluorescence on frozen sections of human pancreas. Islet autoantibodies comprise autoantibodies to (a) islet cell cytoplasm (ICA), (b) native insulin, termed IAA (412), (c) GADA (413-415), (d) islet antigen-2, IA-2A (414) and IA-2βA (also known as phogrin) (416), and (e) 3 variants of the ZnT8 transporter (ZnT8A) (417, 418). Autoantibody markers are usually present in 85% to 90% of individuals with type 1 diabetes when fasting hyperglycemia is initially detected (2). Autoimmune destruction of the islet beta cells has multiple genetic predispositions and is thought to be initiated by environmental influences, such as cer-tain enteroviruses. The ensuing autoimmunity may be present for months or years prior to the appearance of 2 or more islet autoantibodies without either dysglycemia or symptoms (Stage 1) and the subsequent development of dysglycemia (Stage 2), followed by the onset of hyperglycemia and symptoms of diabetes (Stage 3) (see Table 9). After years of type 1 diabetes, the autoantibodies tend to fall below detection limits, but GADA usually remains increased. Insulin treatment precludes the analysis of IAA as it takes only about 11

Table 9. Staging of type 1 diabetes.[a]

	Stage 1	Stage 2	Stage 3
Characteristics	• Autoimmunity • Normoglycemia • Presymptomatic	• Autoimmunity • Dysglycemia • Presymptomatic	• Autoimmunity • Overt hyperglycemia • Symptomatic
Diagnostic criteria	• Multiple islet autoantibodies • No IGT or IFG	• Islet autoantibodies (usually multiple) • Dysglycemia: IFG and/or IGT • FPG 100 to 125 mg/dL (5.6 to 6.9 mmol/L) • 2-h PG 140 to 199 mg/dL (7.8 to 11.0 mmol/L) • HbA_{1c} 5.7% to 6.4% (39 to 47 mmol/mol) or ≥10% increase in A_{1C}	• Autoantibodies may become absent • Diabetes by standard criteria

[a] Adapted from the American Diabetes Association (2)

[b] Abbreviations: FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; 2-h PG, 2-h plasma glucose; Hb A_{1c}, hemoglobin A_{1c}.

days before insulin anti-bodies are induced. People with type 1A diabetes have a significantly increased risk of other autoimmune disor-ders, including celiac disease, Graves disease, thyroiditis, Addison disease, and atrophic gastritis along with pernicious anemia. As many as 1 in 4 females with type 1 diabetes have autoimmune thyroid disease while 1 in 280 individuals develop adrenal autoantibodies and adrenal insufficiency (419). A small subset of people with type 1 diabetes (type 1B, idiopathic) have no known etiology and no evidence of autoimmunity. Many of these individuals are of African or Asian origin (2).

Screening.

RECOMMENDATION: *Screening for islet autoantibodies in relatives of individuals with type 1 diabetes or in persons in the general population is recommended in the setting of a research study or can be offered as an option for first degree relatives of a proband with type 1 diabetes.* ***B (low)***

RECOMMENDATION: *Routine screening for islet autoantibodies in people with type 2 diabetes is not recommended at present.* ***B (low)***

Only about 15% of individuals with newly diag-nosed type 1 diabetes have a first-degree relative with the disease (420). The risk of developing type 1 diabetes in relatives of someone with the disease is approximately 5%, which is 15-fold higher than the risk in the general population (1 in 250 to 300 lifetime risk). Screening re-latives of individuals with type 1 diabetes for islet autoantibodies can identify those at high risk of the disease. However, as many as 1% to 2% of healthy individuals may have either IAA, GADA, IA-2A, or ZnT8A alone and are at low risk of type 1 diabetes (421). Children with only one autoantibody may revert to negativity, but their risk of type 1 diabetes remains between not having an islet autoantibody to being persistent single-autoantibody positive. Because of the low prevalence of type 1 diabetes (approximately 0.3% in the general population), the positive predictive value of a single islet autoantibody is low (408). The presence of multiple islet autoantibodies (IAA, GADA, IA-2A/IA-2βA or ZnT8A) is associated with a risk of type 1 diabetes of >90% (408, 422, 423). However, until cost-effective screening strategies can be developed for young children and effective intervention therapies to prevent or delay the clinical onset of the disease become available, such testing cannot be recommended outside of a research setting.

Children with certain HLA-DQB1 alleles such as B1*06:02, B1*06:03 or B1*03:01 are mostly protected from type 1 diabetes, but not from developing islet autoantibodies (424) nor from type 1 diabetes later in life. Because islet autoantibodies in these individuals have substantially reduced predictive significance, these subjects are often excluded from prevention trials.

Approximately 5% to 10% ofWhite adults who present with a type 2 diabetes phenotype have islet autoantibodies (425), particularly GADA, which predict insulin dependency. This has been termed latent autoimmune diabetes of adults (LADA) (426), type 1.5 diabetes (427), or slowly progressive insulin-dependent diabetes (SPIDDM) (428). Although individuals who are GADA-positive progress to absolute insulinopenia faster than do those who are autoantibody-negative, some autoantibody-negative adults with type 2 diabetes also progress (albeit more slowly) to insulin dependence with time. Some of these individuals show T cell reactivity to islet cell components (427). There is limited utility for islet autoantibody testing in individuals with type 2 diabetes because the institution of insulin therapy is based on glucose control. At diagnosis of diabetes in children, absence of all 4 islet autoantibodies and modest hypergly-cemia (Hb A_{1c} < 7.5% [58 mmol/mol]) proved useful for the detection of MODY (384). Routine testing for GADA in adults with newly diagnosed diabetes could better define autoimmune diabetes.

Monitoring/prognosis.

RECOMMENDATION: *There is currently no role for measurement of islet autoantibodies in the monitoring of individuals with established type 1 diabetes.* ***B (low)***

The CD3 monoclonal antibody teplizumab has been shown to delay progression to type 1 diabetes in high-risk individuals (429). Despite a theoretical rationale to assess islet autoantibodies in those at risk for type 1 diabetes who might be eligible for this intervention, there is no clear rationale for following titers of islet autoantibodies in those with established type 1 diabetes. Repeated testing for islet autoantibodies to monitor islet autoimmunity is not clinically useful outside of research protocols. However, high-risk individuals identified within such protocols are less likely to present in DKA (430). In islet cell or pancreas transplantation, the presence or absence of islet autoantibodies may indicate whether a subsequent failure of the transplanted islets is due to recurrent autoimmune disease or to rejection (431). When a partial pancreas has been transplanted from an identical twin or HLA-identical sibling, appearance of islet autoantibodies may raise consider-ation for the use of immunosuppressive agents to try to halt recurrence of diabetes. Notwithstanding these theor-etical advantages, the value of this therapeutic strategy has not been established.

Some experts have proposed that testing for islet autoantibodies may be useful in the following situations: (a) public health screening for type 1 diabetes (432); (b) to identify a subset of adults initially thought to have type 2 diabetes, but have islet autoantibody markers of type 1 diabetes and progress to insulin dependency (433); (c) to screen family members without a diabetes diagnosis who wish to donate a kidney or part of their pancreas for transplantation; (d) to screen women with GDM to identify those at high risk of progression to type 1 diabetes; and (e) to distinguish type 1 from type 2 diabetes in children to institute insulin therapy at the time of diagnosis (434, 435). For example, some pediatric diabetologists treat children thought to have type 2 diabetes with oral medications, but treat islet autoantibody-positive children immediately with insulin. Nevertheless, it is possible to follow children who are islet autoantibody positive to the point of metabolic decompensation and then institute insulin therapy.

ANALYTICAL CONSIDERATIONS

RECOMMENDATION: *It is important that islet autoantibodies be measured only in an accredited laboratory with an established quality control program and participation in a proficiency testing program.* ***GPP***

ICA is determined by indirect immunofluorescence on frozen sections of human pancreas (436). ICA measure the degree of binding of immunoglobulin to islet sections and are compared to a WHO standard serum available from the National Institute of Biological Standards and Control (437). The results are reported in Juvenile Diabetes Foundation (JDF) units. Positive results depend upon the study or context in which they are used, but many laboratories use 10 JDF units determined on 2 separate occasions, or a single result ≥20 JDF units, as significant titers which may convey an increased risk of type 1 diabetes. The ICA test has been largely replaced by quantitative analytical methods.

For IAA, a radioisotopic method that calculates the displaceable insulin radioligand binding after the addition of excess nonradiolabeled insulin (438) is recommended. Results are reported as positive when the specific antibody binding exceeds the 99th percentile or possibly the mean + 2 (or 3) SD for healthy persons. IAA binding is not normally distributed. Each laboratory needs to assay at least 100 to 200 healthy individuals to determine the distribution of binding. An important caveat concerning IAA determination is that insulin antibodies develop following insulin therapy even in those persons who use human insulin. Data from the Diabetes Autoantibody Standardization Program (DASP) (439) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop (440) demonstrate that the interlaboratory variability for IAA is inappropriately large.

GADA, IA-2A, and ZnT8A are determined in stan-dardized radiobinding assays using coupled in vitro transcription translation to label the autoantigens (441) or with commercially available nonradiolabelled enzyme-linked immunosorbent assays (ELISAs) or chemiluminescence assays. The performance of GADA and IA-2A assays is improving, as demonstrated by the Islet Autoantibody Standardization Program (440, 442).

INTERPRETATION

GADA may be present in 60% to 80% of people newly diagnosed with type 1 diabetes, but the frequency varies with gender and age. GADA in those with and without type 1 diabetes is associated with HLA DR3-DQA1 *05:01-B1*02:01. IA-2A may be present in about 40% to 80% of those newly diagnosed with type 1 diabetes, but the frequency is highest in the young and decreases with increasing age at onset. IA-2A is associated with HLA DR4-DQA1*03:01-B1*03:02 and negatively asso-ciated with HLA DR3-DQA1*05:01-B1*02:01. IAAs are positive in more than 70% to 80% of children who develop type 1 diabetes before age 5 years, but in fewer than 40% of individuals developing diabetes after age 12. IAAs are associated with HLA DR4-DQA1* 03:01-B1*03:02 and with INS VNTR (379). ICA is found in about 75% to 85% of people with new-onset type 1 diabetes.

Islet autoantibodies are found in the general popu-lation. If 1 islet autoantibody is found, the test should be repeated and the other autoantibodies should be assayed because the risk of type 1 diabetes increases if 2 or more autoantibodies are positive (443).

The presence of islet autoantibodies suggests that insulin is the most appropriate therapeutic option, espe-cially in a child or young adult. Conversely, in children or young people without islet autoantibodies, consideration may be given to oral agents and lifestyle changes. There is not unanimity of opinion, but the presence of islet autoantibodies may alter therapy for some individuals, including Hispanic and Black children with a potential diagnosis of nonautoimmune diabetes, adults with islet autoantibodies but clinically classified with type 2 diabetes, and children with transient hyperglycemia. Most individuals without diabetes who have only 1 autoantibody will not develop type 1 diabetes, as the 10 year risk is about 15% (408). Although expression of multiple islet autoantibodies is associated with greatly increased risk of diabetes (421, 444), approximately 10% of individuals presenting with new-onset diabetes express only a single autoantibody (445). Prospective studies of children reveal that islet autoantibodies may be transient, suggesting that an islet autoantibody may have disappeared prior to the onset of hyperglycemia or diabetes symptoms (446).

The following suggestions have been proposed (402) as a rational approach to the use of autoantibodies in diabetes: (a) autoantibody assays should have specificity >99%; (b) proficiency testing should be documented; (c) multiple autoantibodies should be assayed; and (d) sequential measurement should be performed. Since immunoassays for IAA, GADA, IA-2A/IA-2βA, and ZnT8A are available, a panel of these autoantibodies can be used in screening studies (447). These strategies will reduce both false positive and false negative results.

EMERGING CONSIDERATIONS AND KNOWLEDGE GAPS/ RESEARCH NEEDS

It is likely that other islet autoantigens will be discovered, which could lead to additional diagnostic and predictive tests for type 1 diabetes. Autoantibody screening on finger-stick blood samples as dried blood spots appears feasible. In those individuals who are islet autoantibody positive, HLA-DR-DQ genotyping or an analysis of Genetic Risk Score (388, 392) will help define the risk of type 1 diabetes.

Many relatives of individuals with type 1 diabetes have been screened for IAA, GADA, IA-2A, and ZnT8A to enroll double-autoantibody-positive partici-pants in prevention trials (448). After

many years of negative studies of various immune interventions, there is now evidence that the anti-CD3 monoclonal antibody teplizumab delays progression to type 1 diabetes in high-risk individuals (429).

Several clinical trials to prevent or intervene in type 1 diabetes are being actively pursued, either in relatives of individuals with type 1 diabetes or in the general population based on islet autoantibodies and HLA-DR-DQ genotypes or genetic risk scores. Individuals with 2 or more islet autoantibodies undergo an OGTT, allowing classification to Stage 1 (normoglycemia and no symptoms) or Stage 2 (dysglycemia and no symptoms). Islet autoantibody positivity rates are dis-tinctly lower in the general population than in relatives of individuals with type 1 diabetes, so that trials in the latter group are more economical. Additional trials of antigen-based immunotherapies, adjuvants, cytokines, and T cell accessory molecule blocking agents are likely in the future (449). Decreased islet autoimmunity, along with glycemic status, will be an important outcome measure of these therapies.

Urine Albumin

DESCRIPTION/INTRODUCTION/TERMINOLOGY

Albuminuria is directly related to the filtration rate of the kidney and it is well known that excessive albumin excre-tion in the urine is directly related to future loss of kidney function and increased cardiovascular risk. The Kidney Disease Improving Global Outcomes (KDIGO) group, representing international guidelines for kidney disease, reclassified albuminuria in 2020 (450), and these defini-tions have been adopted by the ADA. There are now 3 categories of albuminuria (Fig. 1, Table 10) which have been renamed. These are:

- **A1— Normal to Mildly Increased Albuminuria:** urine albumin:creatinine ratio (uACR) < 30 mg/g (<3 mg/mmol). This is equivalent to 24-h albumin ex-cretion rate (AER) < 30 mg/day and urine protein:creatinine ratio (uPCR) < 150 mg/g (<15 mg/mmol).

- **A2—Moderately Increased Albuminuria:** uACR 30 to 299 mg/g (3 to 29 mg/mmol). This is equivalent to AER 30 to 299 mg/day and uPCR 150 to 499 mg/g (15 to 49 mg/mmol).

- **A3—Severely Increased Albuminuria** uACR ≥300 mg/g (≥30 mg/mmol). This is equivalent to AER ≥300 mg/day, protein excretion rate (PER ≥500 mg/day), and uPCR ≥500 mg/g (>50 mg/mmol).

The old nomenclature of "nephrotic-range", i.e., AER >2200 mg/day; uACR >2200 mg/g (>220 mg/mmol); PER >3500 mg/day, and uPCR >3500 mg/g (>350 mg/mmol), is no longer used for staging. Note that nephrotic syndrome would typically have hypoalbuminemia (with edema and hyperlipidemia in most cases) along with high urine albumin loss. The albumin to creatinine ratio is a continuous marker for cardiovascular event risk at all levels of kidney function and the risk starts at values that are consistently above 30 mg/g.

USE/RATIONALE

Diagnosis/screening.

RECOMMENDATION: *Annual testing for albuminuria should begin in pubertal or post-pubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment.* ***A (high)***

Diabetes is associated with a high rate of cardiovascular events and is also the leading cause of end-stage renal disease in the Western world (452). Early detection of risk markers, such as moderately increased albuminuria (formerly termed "microalbuminuria"), relies upon measurement of urine albumin concentration divided by urine creatinine concentration (the ratio accounts for the dilution or concentration of the urine specimen). Conventional qualitative tests (chemical strips or "dipsticks") for proteinuria do not detect small increases in urine albumin excretion. For the latter, tests to detect low concentrations of albumin are used (453—455).

Moderately increased albuminuria (stage A2, Fig. 1) rarely occurs with short duration of type 1 diabetes or before puberty. Thus, testing can be delayed in these si-tuations. Albuminuria testing is recommended 5 years after diagnosis of type 1 diabetes, although a baseline reading at the time of diagnosis may be appropriate. Most longitudinal cohort studies report significant increases in the prevalence of moderately increased albuminuria only after type 1 diabetes has been present for 5 years (456, 457).

In contrast, the difficulty in precisely dating the onset of type 2 diabetes warrants initiation of annual albuminuria testing at the time of diabetes diagnosis. While older individuals (age > 75 years) or with life expectancy <20 years may not be at increased risk of kidney failure requiring replacement therapy during their lifetimes, they will be at moderately increased risk of cardiovascular mortality, with severity of chronic kidney disease (CKD) acting as a risk multiplier (458, 459). In people with type 2 diabetes and CKD, the predictive role of reducing moderately increased albuminuria in the context of cardiovascular outcomes has become clearer over the last 5 years. The Finerenone in Reducing Cardiovascular Mortality and Morbidity (FIGARO) outcome trial (460) demonstrates a significant relationship between reduction in moderately increased albuminuria and reduction in cardiovascular risk. Decreasing albuminuria by at least 30% lowers cardiovas-cular risk and events, and slows CKD progression. Published studies have also demonstrated that it is cost-effective to screen all people with diabetes and/or kidney disease for albuminuria (461, 462). Moreover, cardiovascular risk may extend below the lower limit of 30 mg/day (463-465), reinforcing the notion

Fig. 1. The KDIGO HeatMap of staging and CKD/CV risk.[a] Both eGFR and albuminuria are needed to properly stage kidney disease. The colors signify both risk of progression to dialysis as well as cardiovascular risk. Green, very low or no risk; yellow, moderate risk; orange, moderate to high risk and red, highest

CKD is classified based on: • Cause (C) • GFR (G) • Albuminuria (A)				Albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-299 mg/g 3-29 mg/mmol	≥300 mg/g ≥30 mg/mmol
GFR categories (mL/min/1.73 m²) Description and range	G1	Normal to high	≥90	1 if CKD	Treat 1	Refer* 2
	G2	Mildly decreased	60-89	1 if CKD	Treat 1	Refer* 2
	G3a	Mildly to moderately decreased	45-59	Treat 1	Treat 2	Refer 3
	G3b	Moderately to severely decreased	30-44	Treat 2	Treat 3	Refer 3
	G4	Severely decreased	15-29	Refer* 3	Refer* 3	Refer 4+
	G5	Kidney failure	<15	Refer 4+	Refer 4+	Refer 4+

[a] From the ADA (451)

that albuminuria is a continuous variable for cardiovascular risk (466-468).

An estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m², regardless of the presence of moderately increased albuminuria, is an independent cardiovascular risk marker (450). Similarly, urine albumin >30 mg/g creatinine, especially if confirmed, is as-sociated with increased cardiovascular risk and assessed in the context of other cardiovascular risk factors and markers. Urine albumin should be reassessed annually, regardless of whether the person with diabetes is receiving antihypertensive therapy or is normotensive (456).

Monitoring.

Although the urine albumin:creatinine ratio appears entirely acceptable for screening, limited data are available for its use in monitoring the response to therapy. Post hoc analyses of clinical trials indicate that the albumin:creatinine ratio is a reasonable method to assess change over time (469). The KDIGO and ADA guidelines recommend annual quantitative testing for urine albumin in adults with diabetes, using morning spot (vs timed) albumin:creatinine ratio measurement (451, 456, 470).

Some experts have advocated urine albumin testing to monitor treatment, which includes reducing blood pressure (with a blocker of the renin angiotensin-aldosterone system as part of a blood pressure–lowering regimen), improving glycemic control and lipid lowering therapy in people with an eGFR >45 mL/min/1.73 m² (59). SGLT-2 inhibitors and finerenone, a nonsteroidal mineralocorticoid receptor antagonist, also reduce albuminuria in clinical trials of advanced diabetic kidney disease (471-473). These agents slow the rate of urine albumin excretion or prevent its development by reducing inflammation and decreasing intraglomerular pressure, reflected in a small reduction in eGFR.

Table 10. Definitions of albuminuria.[a]			
	Unit of measure		
	mg/24 h	µg/min	mg/g creatinine
Normal to mildly increased	<30	<20	<30
Moderately increased albuminuria (formerly microalbuminuria)	30 to 299	20 to 199	30 to 299
Severely increased albuminuria[b]	≥300	≥200	≥300

[a]Adapted from the ADA (451).
[b]Also called "overt nephropathy."

Frequency of measurement.

RECOMMENDATION: *Urine albumin should be measured annually in adults with diabetes using morning spot urine albumin:creatinine ratio (uACR).* ***A (high)***

RECOMMENDATION: *RIf eGFR is <60 mL/min/1.73 m² and/or albuminuria is >30 mg/g creatinine in a spot urine sample, the uACR should be repeated every 6 months to assess change among people with diabetes and hypertension.* ***A (moderate)***

The KDIGO and ADA recommend annual meas-urement of uACR if it is >30 mg/g. After documenting stage A2 albuminuria on 2 of 3 tests performed within a period of 3 to 6 months, repeat testing is reasonable to determine whether a chosen therapy is effective. The uACR may also be useful in determining the rate of progression of disease and thus support planning for care of end-stage renal disease using the Kidney Failure Risk Equation (474). Although the ADA recommendations suggest that uACR measurement is not generally needed before puberty, it may be considered on an individual basis if there is early onset of diabetes, poor control, or family history of diabetic kidney disease. The duration of diabetes prior to puberty was reported to be an important risk factor in adolescents with type 1 diabetes and could be used to support testing prior to puberty in some individuals (475).

Additionally, a >30% sustained reduction in albu-minuria is accepted as a surrogate marker of slowed pro-gression of kidney disease at the group level, e.g., in a clinical trial. Uncommonly, an individual can have as much as 40% to 50% variability in albumin excretion. Thus, the focus in an individual is not only the baseline value, but the goal should be to drop uACR by at least 30% to 50% and ideally try to achieve a uACR of <30 mg/g. This is difficult in many cases, but annual measurement of albuminuria is useful to assess risk and treatment.

Changes in eGFR measurement.

At the time of publication of this guideline, new recommendations had emerged from nephrology associations to use an equation for estimating GFR that, unlike prior equations, does not include a race adjustment. The rationale is that race is a social, not biologic, construct and that clear inequities occur with use of race-based equations for eGFR. Adding cystatin C to serum creatinine improves the accuracy of race-neutral eGFR equations (476, 477), but cystatin C assays are not widely available in the US. In 2021, the National Kidney Foundation and the American Society of Nephrology created the Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Diseases to examine the issue and provide recommendations (478, 479). Their recommendations included:

1. For US adults (>85% of whom have normal kidney function), we recommend immediate implementation of the 2021 CKD-EPI (Epidemiology) creatinine equation refit without the race variable in all laboratories in the United States because it does not include race in the calculation and reporting, included diversity in its development, is immediately available to all laboratories in the United States, and has acceptable performance characteristics and potential consequences that do not disproportionately affect any one group of individuals.

2. We recommend national efforts to facilitate increased, routine, and timely use of cystatin C, especially to confirm eGFR in adults who are at risk for or have chronic kidney disease, because combining filtration markers (creatinine and cystatin C) is more accurate and would support better clinical decisions than either marker alone. If ongoing evidence supports acceptable performance, the CKD-EPI eGFR-cystatin C (eGFRcys) and eGFR creatininecystatin C (eGFRcr−cys_R) refit without the race variables should be adopted to provide another first-line test, in addition to confirmatory testing.

Cystatin C is recommended for confirmatory testing in specific circumstances when eGFR based on serum creatinine is less accurate, such as in individuals with low muscle mass (477). Cystatin C may also detect kidney dysfunction at an earlier stage than creatinine in people with diabetes (480).

Prognosis.

Albuminuria above 30 mg/g creatinine and eGFR <60 mL/min/1.73 m (Fig. 1) have prognostic significance. In multiple epidemiological studies, mod-erately increased albuminuria is an independent risk marker for cardiovascular death (481-483). In 80% of people with type 1 diabetes and moderately increased albuminuria, urine albumin excretion can increase by as much as 10% to 20% per year, with more than half de-veloping severely increased albuminuria (>300 mg albumin/day) in 10 to 15 years. Once this occurs, most of these individuals will have a progressive decline in GFR and a moderately increased risk of complications, including end-stage kidney disease, cardiovascular disease, and mortality.

The magnitude of complications will vary depending on glycemic and blood pressure control as well as other predisposing factors, such as episodes of acute kidney injury and concomitant presence of heart failure. The level of risk may be assessed with calculators for earlier and later stage CKD (www.ckdpcrisk.org). In type 2 diabetes, 20% to 40% of those with Stage A2 albuminuria (Fig. 1) progress to an eGFR <60 mL/min/ 1.73 m². This will occur at a variable rate as the normal rate of GFR loss is about 0.8 mL/min/year in diabetes, depending on glycemic and blood pressure control, and may be as high as 10 mL/min/year without treatment. After 20 years (if the individual does not die from a cardiovascular event) kidney disease usually progresses to stage 4 and even stage 5. Approximately 20% develop end-stage kidney disease and almost all will have severely increased albuminuria despite achievement of blood pressure goals (484). Moderately increased albu- minuria without hypertension indicates increased relative risk of CKD progression, but absolute risks of end-stage kidney disease are higher with concomitant hypertension (485-487). Moreover, approximately 20% of people with diabetes progress to end-stage kidney disease without an increase in moderately increased albuminuria (488).

ANALYTICAL CONSIDERATIONS

Preanalytical.

RECOMMENDATION: *First morning void urine sample should be used for measurement of albumin:creatin- ine ratio.* ***A (moderate)***

RECOMMENDATION: *If first morning void sample is difficult to obtain, to minimize variability in test results, all urine collections should be at the same time of day. The individ-ual should be well hydrated and should not have ingested food within the preceding 2 h or have exercised.* ***GPP***

RECOMMENDATION: *Timed collection for urine albumin should be done only in research settings and should not be used to guide clinical practice.* ***GPP***

The within-individual variation (CVi) of albumin excretion is large in people without diabetes and is moderately increased in people with diabetes (489). The albu- min:creatinine ratio is the best method to predict renal events in people with type 2 diabetes (490). The ratio correlates well with both timed excretion and albumin concentration in a first morning void of urine (489, 491). Howey et al. (491) studied day-to-day CVi of 24-h albumin excretion, the albumin concentration, and the albu- min:creatinine ratio over 3 to 4 weeks. The last two were measured in the 24-h urine sample, the first morning void, and random untimed urine. In healthy volunteers, the lowest CVi was observed for the albumin concentration in the first morning void (36%) and for the albumin: creatinine ratio in that sample (31%) (491). Others have validated the reliability of a first morning void sample (462, 492, 493). To minimize variability, all collections should be at the same time of day and the person should not have ingested food for at least 2 h (494).

Transient increases in urine albumin excretion are reported with short-term hyperglycemia, exercise, urine tract infections, sustained blood pressure elevation, heart failure, fever, and hyperlipidemia (451).

Albumin is stable in untreated urine stored at 4 °C or 20 °C for at least a week (495). Neither centrifugation nor filtration appears necessary before storage at -20 °C or -80 °C (496). Whether centrifuged, filtered, or not treated, albumin concentration decreased by 0.27% per day at -20 °C, but showed no decrease over 160 days at -80 °C (496). Urine albumin excretion rate reportedly has no marked diurnal variation in diabetes, but does in essential hypertension (497).

Analytical
Quantitative.

RECOMMENDATION: *The analytical performance goals for urine albumin measurement should be between- day precision ≤6%, bias ≤7% to 13%, and total allowable error of ≤24% to 30%.* ***GPP***

Analytical goals can be based on biological variation, expert opinion, opinion of clinicians, or state of the art (87). A 2014 study compared 17 commercially available urine albumin measurement procedures to an isotope dilution mass spectrometry reference measurement procedure (498). Mean biases were large and ranged from -35% to 34% at 15 mg/L. The authors concluded that calibration bias was the main source of error for differences among methods and precision was adequate for most assays. Based on the performance of measurement procedures, the National Kidney Disease Education Program (NKDEP) Laboratory Working Group in 2017 recommended the following analytical performance goals for measurement of urine albumin: between-day precision ≤6%, bias ≤7%-13%, and total allowable error of ≤24%-30% (499). The analytical measurement range for urine albumin should be 2 to 400 mg/L (499).

Semi-quantitative or qualitative.

RECOMMENDATION: *Semi-quantitative uACR dipsticks can be used to detect early kidney disease and assess cardiovascular risk when quantitative tests are not available.* ***B (moderate)***

RECOMMENDATION: *Semi-quantitative or qualitative screening tests should be positive in >85% of individuals with moderately increased albuminuria to be useful for patient screening.* ***B (moderate)***

RECOMMENDATION: *Practitioners should strictly adhere to manufacturer's instructions when using the semiquantitative uACR dipstick test and repeat it for confirmation to achieve adequate sensitivity for detecting moderately increased albuminuria.* ***B (moderate)***

RECOMMENDATION: *Positive urine albumin screening results by semi-quantitative tests should be confirmed by quantitative analysis in an accredited laboratory.* ***GPP***

Semi-quantitative (or qualitative) assays have been proposed to screen for moderately increased albuminuria. To be useful, screening tests must have high detection rates, i.e., high clinical sensitivity. Although many studies have assessed the ability of reagent strips ("dipstick" methods) to detect increased urine albumin concentrations, the important question is whether the method can detect moderately increased albuminuria.

Numerous studies have compared the performance of semi-quantitative or quantitative POC methods with assays performed in an accredited laboratory. Systematic reviews and meta-analyses have been published. The first, published in 2014, identified 16 studies (3356 in-diduals) that evaluated semi-quantitative or quantitative POC tests of albuminuria and used random urine samples collected in primary or secondary ambulatory care settings that met inclusion criteria (500). Pooling results from a bivariate random-effects model gave sen-sitivity and specificity estimates of 76% (95% CI, 63%-86%) and 93% (CI, 84%-97%), respectively, for the semi-quantitative test (501). Sensitivity and specificity estimates for the quantitative test were 96% (95% CI, 78%-99%) and 98% (95% CI, 93%-99%), respectively. The authors concluded that a negative semi-quantitative POC test result does not rule out albuminuria, whereas quantitative POC testing meets re-quired performance standards and can be used to rule out albuminuria.

A second systematic review and meta-analysis, published in 2021, assessed the diagnostic accuracy of urine dipstick testing for detecting albuminuria (502). The authors identified 14 studies, 5 of which were in the 2014 review, and evaluated the performance of uACR. The pooled sensitivity and specificity at each cutoff point were as follows: uACR >30 mg/g, 0.82 (95% CI, 0.76-0.87) and 0.88 (95% CI, 0.83-0.91); uACR 30 to 300 mg/g, 0.72 (95% CI, 0.68-0.77) and 0.82 (95% CI, 0.76-0.89); and uACR >300 mg/g, 0.84 (95% CI, 0.71-0.90) and 0.97 (95% CI, 0.95-0.99), respectively. An important limitation of all these data is that the dipstick methods were compared to local laboratory methods, which, as indicated above, exhibit large biases (498).

A cost-effectiveness analysis of 1881 individuals with diabetes published in 2020 evaluated medical costs of CKD and concluded that the semi-quantitative uACR dipstick method could be an appropriate screening tool for albuminuria in people with diabetes. Moreover, the authors point out that it can minimize the testing time and inconvenience and significantly reduce national health costs (503).

There is heterogeneity among studies, but later studies generally show more uniformity and better sen-sitivity (>80%). Clinical operators have a lower sensitiv-ity, but better specificity, than laboratory technologists (500), perhaps because they do not wait the full time (usually 60 s) between dipping and scanning, which can result in an incomplete reaction. It is therefore critical that manufacturers' instructions for testing and quality control be followed. Another way to improve assay performance is to do 2 or 3 tests at different times. If tests are independent, a sensitivity of 83% and specificity of 91% improve to a sensitivity of 92% and specificity of 98% if 2 or more of 3 tests define positive. Screening using 2 tests with either being positive interpreted as a positive (leading to subsequent quantitative testing) increases the sensitivity to 97%, but reduces the specificity to 83% (500, 501).

RECOMMENDATION: *Currently available proteinuria dipstick tests should not be used to assess albuminuria.* ***B (moderate****)*

It is important to distinguish semi-quantitative uACR dipsticks from proteinuria dipsticks. Chemical strip methods for total protein are not sensitive when the urine albumin concentration is 20 to 50 mg/L. Thus, reagent strips to identify proteinuria cannot be re-commended unless they are able to specifically measure albumin at low concentrations and express the results as an albumin:creatinine ratio (504). Effective screening tests (e.g., for phenylketonuria) have low false-negative rates. Therefore, only positive results require confirmation by a quantitative method. If a screening test has low sensitivity, negative results must also be confirmed, a completely untenable approach.

INTERPRETATION

The most reliable method is the immunoturbidimetric laboratory assay, which should be considered the stand-ard for comparison as it has >95% sensitivity and specificity to detect moderately increased albuminuria (505). Semi-quantitative or qualitative screening tests should be positive in >85% of individuals with moderately increased albuminuria to be useful for assessment of cardiovascular risk and progression of kidney disease. Positive results using such methodologies must be confirmed by an immunoturbidimetric assay in an accredited laboratory (505).

In the KDIGO and ADA algorithms for urine al-bumin testing (506), the diagnosis of moderately in-creased or severely increased albuminuria requires the demonstration of increased albumin excretion on 2 of 3 tests repeated at intervals over a period of 3 to 6 months, and exclusion of conditions that "invalidate" the test. This is helpful to correctly stage CKD despite the moderately increased variability of albuminuria. Stage A2 albuminuria (30 to 299 mg/g) on 1 occasion is indicative of persistent albuminuria 50% to 75% of the time, while stage A3 albuminuria (≥300 mg/g) even on 1 occasion is indicative of increased albuminuria (>30 mg/g) almost 100% of the time.

At least some of the semi-quantitative POC meth-ods have the wrong characteristics for screening because they exhibit low sensitivity and positive results must be confirmed by a laboratory method. Taken together, these data support semi-quantitative uACR dipstick testing as a useful approach when quantitative analysis is not possible. Advantages of semi-quantitative testing include relatively high specificity and use as point-of-care testing which, if appropriately implemented, can improve access (particularly in resource-limited settings) and eliminate the need for shipping samples and delays in getting a test result.

Miscellaneous Potentially Important Analytes

INSULIN AND PRECURSORS

USE/RATIONALE

Diagnosis.

RECOMMENDATION: *In most people with diabetes or risk for diabetes or cardiovascular disease, routine testing for insulin or proinsulin is not recommended. These assays are useful primarily for research purposes.* ***B (moderate****)*

RECOMMENDATION: *Although differentiation between type 1 and type 2 diabetes can usually be made based on the clinical presentation and subsequent course, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as individuals who have a type 2 phenotype but present in ketoacidosis.* ***B (moderate)***

RECOMMENDATION: *If required by the payer for coverage of insulin pump therapy, measure fasting C-peptide level when simultaneous fasting plasma glucose is ≤220 mg/dL (12.5 mmol/L).* ***GPP***

For many years, there have been investigations into whether measurements of the concentration of plasma insulin and its precursors might be of clinical benefit. Population studies have shown that fasting insulin concentration predicts future risk of ischemic heart disease events (507). Increased insulin concentration is a surrogate marker for insulin resistance. However, accurate measurement of insulin resistance requires the use of complex methods, such as the hyperinsulinemic euglycemic clamp technique, which are generally confined to research laboratories. Due to the critical role of insulin resistance in the pathogenesis of type 2 diabetes, hyperinsulinemia would also appear to be a logical risk predictor for incident type 2 diabetes.

Earlier studies may not have controlled well for undiagnosed diabetes, glycemic measures, body mass in-dex, or other confounders (507). Subsequent analyses suggest that insulin values do not add significantly to diabetes risk prediction carried out using more traditional clinical and laboratory measurements (508), and that measures of insulin resistance (which include insulin measurements) predicted risk of diabetes or coronary artery disease (CAD) only moderately, with no threshold effects (509). Consequently, it seems of greater clinical importance to quantify the consequences of the insulin resistance and hyperinsulinemia (or hyperproinsulinemia) rather than the hormone values themselves, i.e., by measuring blood pressure, body mass index, degree of glucose tolerance, and plasma lipid/lipoprotein con-centrations. It is these variables that are the focus of clin-ical interventions, not plasma insulin or proinsulin concentrations (508, 509).

The clinical utility of measuring insulin, C-peptide, or proinsulin concentrations to help select the best anti–hyperglycemic agent for initial therapy in an individual with type 2 diabetes is a question that arises from consid-eration of the pathophysiology of type 2 diabetes. In the-ory, the lower the pre-treatment insulin concentration, the more appropriate might be insulin, or an insulin secretagogue, as the drug of choice to initiate treatment. While this line of reasoning may have some intellectual appeal, there is no evidence that measurement of plasma insulin or proinsulin concentrations will lead to more ef-ficacious treatment of people with type 2 diabetes.

In contrast to the above considerations, measure-ment of plasma insulin and proinsulin concentrations is necessary to establish the pathogenesis of non–diabetes-related hypoglycemia (510). The diagnosis of an islet cell tumor is based on the persistence of inappro-priately increased plasma insulin concentrations in the face of a low glucose concentration. In addition, an in-crease in the ratio of fasting proinsulin to insulin in an individual with hypoglycemia strongly suggests the presence of an islet cell tumor. The absence of these asso-ciated changes in glucose, insulin, and proinsulin concentrations from an individual with fasting hypoglycemia makes the diagnosis of an islet cell tumor most un-likely, and alternative explanations should be sought for the inability to maintain fasting euglycemia.

Measurement of the C-peptide, in the fasting state or in response to intravenous glucagon, can aid in instances in which it is difficult to differentiate between the diagnosis of type 1 and type 2 diabetes (6, 511). However, even in this clinical situation, the response to drug therapy will provide useful information, and measurement of C-peptide may not be clinically neces-sary. Measurement of C-peptide is essential in the investigation of nondiabetic hypoglycemia to rule out hypoglycemia due to surreptitious insulin administration (510).

In the past, some advocated insulin or C-peptide as-says in the evaluation and management of women with polycystic ovary syndrome. Women with this syndrome manifest insulin resistance triggered by androgen excess, and often have abnormalities of carbohydrate metabol-ism; both abnormalities may respond to treatment with insulin-sensitizing drugs such as metformin

or thiazolidinediones. However, it is unclear whether assessing insulin resistance through insulin or C-peptide measurement has any advantage over assessment of physical signs of insulin resistance (body mass index, presence of acanthosis nigricans) and routine measure-ments of C-peptide or insulin are not rec-ommended by ACOG (512).

ANALYTICAL CONSIDERATIONS

RECOMMENDATION: *Insulin and C-peptide assays should be standardized to facilitate measures of insulin secretion and sensitivity that will be comparable across research studies.* ***GPP***

Although assayed for over 60 years, there is no stan-dardized method available to measure serum insulin. Attempts to harmo-nize insulin assays using commercial insulin reagent sets result in greatly discordant results (513). In 2009, an insulin standardiza-tion workgroup of the ADA, in conjunction with NIDDK, CDC, and EASD, called for harmonization of insulin assay results through traceability to an isotope dilution liquid chromatography/tan-dem mass spectrometry reference (514). The Insulin Standard-ization Workgroup called for harmonization of the insulin assay to encourage the development of measures of insulin sensitivity and secretion that will be practical for clinical care (515), yet the usefulness of a harmonized assay would probably be greater to compare research studies. Analogous to insulin, considerable imprecision among laboratories is also observed for measure-ment of C-peptide. Stakeholders in the US, Japan, and elsewhere have worked on developing a reference standard and traceability schemes, but there is a need for further coordin-ation to assure worldwide harmonization of C-peptide (516).

Measurement of proinsulin and C-peptide are ac-complished by immunometric methods. Proinsulin ref-erence intervals are dependent on methodology and each laboratory should estab-lish its own reference interval. Although it has been suggested by some, insulin measurement should not be used in an OGTT to di-ag-nose diabetes. In the case of C-peptide, there is a dis-crepancy in reliability because of variable specificity among antisera, lack of standardization of C-peptide calibration, and variable cross-re-activity with proinsulin. Of note is the requirement of the United States Centers for Medicare and Medicaid Services (CMS) that Medicare beneficiaries must have C-peptide measured in order to be eligible for coverage of insulin pumps. Initially, the require-ment was that the C-peptide be ≤0.5 ng/mL; however, because of non–comparability of results from different assays resulting in denial of payment for some patients with values above 0.5 ng/mL, the requirement now states that the C-peptide should be ≤110% of the lower limit of the reference interval of the labora-tory's measurement method (517).

INSULIN ANTIBODIES

RECOMMENDATION: *There is no published evidence to support the use of insulin antibody testing for routine care of people with diabetes.*
C (very low)

Given sufficiently sensitive techniques, insulin anti-bodies can be detected in any person being treated with exogenous in-sulin (518, 519). In most of these indivi-duals, the titer of insulin antibodies is low, particularly in those who were never treated with animal insulins, and their presence is of no clinical signif-icance. However, on occasion high titers of insulin antibodies in the circulation can be associated with dramatic resistance to the ability of exogenous insulin to lower plasma glucose con-centrations. This clinical situation is quite rare, usually occurs in individuals with insulin-treated type 2 diabetes, and the cause-and-effect relationships between the magnitude of the increase in insulin antibodies and the degree of insulin resistance is un-clear (519). There are several therapeutic approaches for treat-ing these individuals and a quantitative estimate of the concen-tration of circulating insulin antibodies does not appear to be of significant benefit.

Supplemental Material

Supplemental material is available at Clinical Chemistry online.

Nonstandard Abbreviations: CGM, continuous glucose moni-toring; Hb A_{1c}, hemoglobin A1c; GDM, gestaional diabetes mel-litus; NHANES, National Health and Nutrition Examination Sur-vey; ADA, American Diabetes Association; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; IDF, International Diabetes Federation; GPP, good practice point; DCCT, Diabetes Control and Complications Trial; UKPDS, United Kingdom Pro-spective Diabetes Study; RBC, red blood cell; DKA, diabetic keto-acidosis; BGM, blood glucose monitoring; ISO, International Or-ganization for Standardization; rt-CGM, real-time CGM; is-CGM, intermittently scanned CGM; RCT, randomized controlled trial; ACOG, American College of Obstetrics and Gynecology; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; IADPSG, Inter-national Association of Diabetes and Pregnancy Study Groups; AcAc, acetoacetate; βOHB, β-hydroxybutyrate; GHb, glycated hemoglobin; POCT, point-of-care testing; eAG, estimated aver-age glucose; MODY, maturity onset diabetes of the young; IAA, insulin autoantibodies; GADA, glutamic acid decarboxylase auto-antibodies; IA-2A, islet antigen-2; ZnT8A, ZnT8 transporter; ICA, islet-cell cytoplasm antibodies; KDIGO, Kidney Disease Improv-ing Global Outcomes; uACR, urine albumin:creatinine ratio; AER, albumin excretion rate; uPCR, urine protein:creatinine ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtra-tion rate; GFR, glomerular filtration rate; SGLT-2, sodium-glucose co-transporter-2.

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