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

David B. Sacks,[1] Mark Arnold,[2] George L. Bakris,[3] David E. Bruns,[4] Andrea R. Horvath,[5] Åke Lernmark,[6] Boyd E. Metzger,[7] David M. Nathan,[8] and M. Sue Kirkman[9]

Diabetes Care 2023;46:e151–e199 | https://doi.org/10.2337/dci23-0036

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.

APPROACH

An expert committee compiled evidence-based recommendations for laboratory analysis in screening, diagnosis, or monitoring of 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). The guidelines were reviewed by the Evidence Based Laboratory Medicine Committee and the Board of Directors of the American Association for Clinical Chemistry and by the Professional Practice Committee of the American Diabetes Association.

CONTENT

Diabetes can be diagnosed by demonstrating increased concentrations of glucose in venous plasma or increased hemoglobin A_{1c} (HbA_{1c}) in the blood. Glycemic control is monitored by the people with diabetes measuring their own blood glucose with meters and/or with continuous interstitial glucose monitoring (CGM) devices and also by laboratory analysis of HbA_{1c}. The potential roles of noninvasive glucose monitoring, genetic testing, and measurement of ketones, autoantibodies, urine albumin, insulin, proinsulin, and C-peptide are addressed.

SUMMARY

The guidelines provide specific recommendations based on published data or derived from expert consensus. Several analytes are found to have minimal clinical value at the present time, and measurement of them is not recommended.

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, although these are being reconsidered based on genetic, metabolomic, and other characteristics and

[1]*Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD*
[2]*Department of Chemistry, University of Iowa, Iowa City, IA*
[3]*Department of Medicine, American Heart Association Comprehensive Hypertension Center, Section of Endocrinology, Diabetes and Metabolism, University of Chicago Medicine, Chicago, IL*
[4]*Department of Pathology, University of Virginia Medical School, Charlottesville, VA*
[5]*New South Wales Health Pathology Department of Chemical Pathology, Prince of Wales Hospital, Sydney, New South Wales, Australia*
[6]*Department of Clinical Sciences, Lund University/CRC, Skane University Hospital Malmö, Malmö, Sweden*
[7]*Division of Endocrinology, Northwestern University, The Feinberg School of Medicine, Chicago, IL*
[8]*Massachusetts General Hospital Diabetes Center and Harvard Medical School, Boston, MA*
[9]*Department of Medicine, University of North Carolina, Chapel Hill, NC*

Corresponding author: David B. Sacks, sacksdb@mail.nih.gov

Received 19 April 2023 and accepted 11 May 2023

This article contains supplementary material online at https://doi.org/10.2337/figshare.23519148.

This document was approved by American Association for Clinical Chemistry's (AACC's) Evidence Based Laboratory Medicine Subcommittee in January 2023, the AACC Academy Council in

Table 1—Classification of diabetes mellitus[a]

1. Type 1 diabetes
A. Immune-mediated
B. Idiopathic
2. Type 2 diabetes
3. 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
4. GDM

[a]Data are from the ADA (2).

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% to 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 U.S. Centers for Disease Control and Prevention (CDC) estimated that there were 37.3 million people (11.3% of the U.S. 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 U.S. 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 U.K. 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 (CAD). 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 of 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 U.S., 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., health care 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)

February 2023, the AACC Board of Directors in March 2023, and the American Diabetes Association in March 2023.

A consensus report of a particular topic contains a comprehensive examination and is authored by an expert panel (i.e., consensus panel) and represents the panel's collective analysis, evaluation, and opinion. The need for a consensus report arises when clinicians, scientists, regulators, and/or policy makers desire guidance and/or clarity on a medical or scientific issue related to diabetes for which the evidence is contradictory, emerging, or incomplete. Consensus reports may also highlight gaps in evidence and propose areas of future research to address these gaps. A consensus report is not an American Diabetes Association (ADA) position but represents expert opinion only and is produced under the auspices of the ADA by invited experts. A consensus report may be developed after an ADA Clinical Conference or Research Symposium.

The articles are identical except for minor stylistic differences in keeping with each journal's style.

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 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 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.

was used and the key steps and evidence summaries are detailed in the guideline and in the Supplementary Material 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 health care 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 health care 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.

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 (≥126 mg/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 subjects at risk for retinopathy and nephropathy (19,20). The revised criteria comprised (a) fasting plasma glucose (FPG) $\geq$7.0 mmol/L (126 mg/dL), (b) 2-h postload 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 $\geq$11.1 mmol/L (200 mg/dL) (Table 4) (18). The WHO and International Diabetes Federation (IDF) recommend either FPG or 2-h postload 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} (HbA_{1c}), which reflects long-term blood glucose concentrations (see *Hemoglobin A_{1c}* section below). The ADA (23), EASD, IDF, and the WHO (24) have endorsed the use of HbA_{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 HbA_{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, HbA_{1c} can be the confirmatory test in the subsequent sample or HbA_{1c} initially, followed by glucose. A third option is to measure 2 different analytes, namely glucose and HbA_{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).

Table 3—Grading the strength of recommendations

A. STRONGLY RECOMMEND

(a) adoption when:

- There is high 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 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 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 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 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 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 a 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 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 low-quality evidence but strong or very strong agreement and a 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.

GPP. GOOD PRACTICE POINT

Good Practice Points (GPPs) are recommendations mostly driven by expert consensus and professional agreement and are based on the information listed below listed information and/or professional experience, or widely accepted standards of best practice. This category mostly applies to technical (e.g., preanalytical, analytical, postanalytical), 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, non–evidence-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.

Screening

Recommendation: Screening by HbA_{1c}, FPG, or 2-h OGTT is recommended for individuals who are at high risk of diabetes. If HbA_{1c} is <5.7% (<39 mmol/mol), FPG 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)

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 for 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 U.S. (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 health care setting. HbA_{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 HbA_{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

Table 4—Criteria for the diagnosis of diabetes[a]

1. HbA_{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]

Adapted from the ADA (2). [a]In the absence of unequivocal hyperglycemia, diagnosis requires abnormal results on two different tests (glucose and HbA_{1c}) on the same day or two 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.

individuals who are overweight or obese (BMI ≥25 kg/m^2) and who have one 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 youth (BMI >85th percentile) who have one 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).

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 HbA_{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 people 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). In 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-saving with metformin (41) and has been endorsed by the Centers for Medicare & Medicaid 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 with 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.

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.

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 in people 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 three other trials failed to demonstrate a significant difference in macrovascular disease outcomes between very intensive treatment strategies achieving HbA_{1c} concentrations of approximately 6.5% (48 mmol/mol) compared with the control groups who had HbA_{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 HbA_{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 HbA_{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 cut points 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 two 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 with 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 U.S. 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 [6,21].) 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 those in 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 HbA_{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 U.S. (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 coefficient of variation (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 6,000 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 with 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 2 to 4 weeks apart was 5.7% (95% CI, 5.3% to 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 6.2 to 7.8 mmol/L (112 to 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 to 7.9 mmol/L (110 to 142 mg/dL). Using assay imprecision of 3% (CV) only (excluding biological variability) would yield 95% CI of 6.6 to 7.4 mmol/L (118 to 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 to 6.3 mmol/L (87 to 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 $\leq$2.4%, bias $\leq$2.1%, and total error $\leq$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 $\geq$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 people (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 three 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 U.S. (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 HbA_{1c} $<$7% for many nonpregnant individuals), with multiple daily injections or an insulin pump,

and with selection of doses guided by BGM, CGM, or 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 multibillion-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 mellitus. B (moderate)

The glucose-based criteria for the diagnosis of diabetes (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 HbA_{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 measurements of glucose in plasma (or measurement of HbA_{1c}). In 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 fingerstick 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 health care 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 health care 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 of Care (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 four 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 diabetes, 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 toward 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 two- to threefold higher in the intensive group than in the conventional group in clinical 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 calibration 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 *Continuous Glucose Monitoring* section.

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 HbA_{1c} in people with type 2 diabetes who were not using insulin (111–114). The decreases of HbA_{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 HbA_{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 HbA_{1c} seen at earlier time

points were lost (111,113). There is insufficient evidence to conclude whether the observed small and transient differences in HbA_{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 diabetes (115). The study found no clinically or statistically significant differences at 1 year in glycemic control (as assessed by HbA_{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 recommend routine use of BGM for people with type 2 diabetes 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 multiple injections of insulin (105). These situations include sick days and stressful periods, and when altering diet, physical activity, and/or medications (particularly medications 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 U.S. 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 U.S. (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 health care facilities (122). In contrast, the ISO standard applies only to glucose meters used for home BGM and the CLSI document applies only to meters used in health care facilities.

These criteria serve as de facto minimal quality requirements 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 clinical 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 revealed that meters that achieve both an imprecision (as 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 two 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 fingersticks per day. Analytical errors of meters that meet the 2013 ISO standard have only limited impact on the three outcome measures or on HbA_{1c}. The second modeling study (128) demonstrated that meter inaccuracy increased the total cost of health care (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

Recommendation: 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 because 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 health care settings address this problem and offer additional

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.

features 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 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 accurate in the presence of the high hematocrits that are common in this population. High hematocrit will increase or decrease the measured glucose, or will have minimal effect, depending on the design of the measuring system (132,133). Analytical bias and/or imprecision at low concentrations 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 eliminate or greatly ameliorate most interferences, but interferences 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 metabolism of icodextrin that is used in some peritoneal dialysis 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 three to four times per day in adults and adolescents has been associated with less effective control of glycemia as measured by HbA_{1c} (150–152). In a study of individuals aged 1 to over 65 years and treated with insulin, HbA_{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 HbA_{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 HbA_{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 diabetes, the frequency of BGM was found to correlate inversely with HbA_{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 performance 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 health care provider's office. These devices are worn for the duration of the sensor and then returned to the health care 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 health care 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 health care professional office via the cloud.

Use/Rationale

Recommendation: Real-time CGM should be used in conjunction with insulin as a tool to lower HbA_{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 HbA_{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 HbA_{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 HbA_{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 HbA_{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 HbA_{1c} (163). Several observational studies have shown HbA_{1c} reduction (164) or reductions in hypoglycemia without change in HbA_{1c} (165). A systematic review of RCTs in adults with type 1 or type 2 diabetes suggested that is-CGM may reduce HbA_{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 HbA_{1c} (167). A meta-analysis of nonrandomized studies in adults suggested that HbA_{1c} was lowered by approximately 0.5% at 12 months with the technology (168).

Randomized controlled trials of the use of rt-CGM, compared with standard blood glucose monitoring, in adults with type 2 diabetes have generally shown reductions in HbA_{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 HbA_{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 HbA_{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 HbA_{1c} after 6 months compared with 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 HbA_{1c} (176,177), a single RCT in young children showed no impact on HbA_{1c} (178). An uncontrolled 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 women with type 1 diabetes showed a modest but statistically significant reduction of HbA_{1c} in women randomized to rt-CGM compared with those randomized to continuing to use blood glucose meters, with no differences 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 versus blood glucose monitoring in women with gestational diabetes mellitus showed no significant differences in HbA_{1c} or neonatal outcomes but less weight gain with CGM use (185).

Professional CGM, along with professional interpretation, 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 suspected 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 supratherapeutic 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 with 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 U.S. 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 (horizontal arrow) or is changing at progressively greater

rates (one, two, or in some systems three 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 HbA_{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 two 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 HbA_{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 with BGM) improves outcomes in people with type 2 diabetes, young children with type 1 diabetes, or pregnant women with preexisting diabetes or gestational diabetes mellitus.

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 coronavirus disease 2019 (COVID-19) pandemic, the FDA allowed use of CGM devices with remote monitoring in hospitals in the U.S. 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), multisensing 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 midinfrared (217,218) wavelengths, 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, midinfrared, 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 U.S.

The peer-reviewed literature contains numerous reports 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 concentrations accurately during subsequent OGTTs, thereby severely limiting clinical utility. Issues of concern remain (a) overmodeling 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 coworkers 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 (246). 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).

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 pregnancy 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 one-step or two-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 U.S., approximately 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 recommend that women with risk factors for type 2 diabetes 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 previously 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 U.S. 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 pregnant women) prospective multinational epidemiologic study to assess adverse outcomes as a function of maternal glycemia. The study revealed strong, graded, predominantly linear associations between maternal glycemia and primary study outcomes, namely frequency of birth weight >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 cut points for plasma glucose concentrations are indicated in Table 7, one-step strategy (2). Using the IADPSG criteria substantially increases the incidence of GDM, mainly because only one increased glucose value is required to diagnose GDM rather than two. 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 a National Institutes of Health (NIH) Consensus Development Conference Statement recommended that the two-step approach for detection and diagnosis of GDM, predominately used in the U.S., should continue to be used rather than the 1-step approach and criteria proposed by IADPSG (253,254). This continues to be the recommendation of ACOG (250); however, they indicate that only one 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

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

One-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)

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

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 prespecified 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 two sites. Significant limitations of this study have been identified (262,263).

RCT evidence that treatment of "mild" GDM improves 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 mothers with treated GDM and those with 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 GDM should perform 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 <6.7 mmol/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 four times daily compared with 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 third day instead of daily (271).

HbA_{1c}. HbA_{1c} concentrations decrease during normal pregnancy due to increased red cell turnover (272). Moreover, macrosomia results primarily from postprandial

hyperglycemia, which may not be adequately detected by HbA_{1c}. Therefore, while HbA_{1c} may provide valuable information, it should not replace BGM. An HbA_{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, HbA_{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 preexisting, 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. HbA_{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 HbA_{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 HbA_{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 people. 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 with samples collected in sodium fluoride–containing tubes (73).

Analytical

Analytical goals and methods of glucose analysis are addressed in the *Glucose* section. Based on the strict cutoffs used in the diagnosis 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 preexisting diabetes (281). However, there is no consensus on the glucose cutoff that should be used for diagnosis. The glycemic thresholds for the diagnosis of GDM in the second and third trimesters may not be appropriate for early pregnancy because FPG normally declines in early pregnancy (282,283). For example, in a large Chinese cohort, many women 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 HbA_{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.

Toward 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 (POC) fingerstick.

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 one-step and two-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 an 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 health care 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 replaced 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 (89). Semiquantitative test-strip methods that utilize specific reactions for glucose are recommended. Commercially available strips use the glucose oxidase reaction (89). The strip is moistened with freshly voided urine and after 10 s the color is compared with 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 determinations, 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 cotransporter 2 [SGLT2] 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)

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 emergency. 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 counterregulatory

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 derived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βOHB is shifted toward 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 with 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 department visits or hospitalizations (302). The ADA recommends 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 (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 settings, 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 typically reported as "positive at a 1/x dilution"), as with urine 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 interferes with some assay methods. AcAc interferes with some assay methods unless specimens are highly dilute. Specimen stability differs among methods, but in general, 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 U.S. 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 diagnosed

with diabetes who presents with typical symptoms 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 determinations 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). SGLT2 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 SGLT2 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 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 life span and turnover of the protein. Hemoglobin in the RBC has an average circulating life span 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, HbA_1, HbA_{1c}, and A1C have all been used; however, these terms are not interchangeable. The current acceptable term for glycation of hemoglobin in general is glycated hemoglobin (GHb). HbA_{1c} is the specific glycated species that is modified by glucose on the N-terminal valine of the hemoglobin β-chain. Assay methods that measure total glycated hemoglobins (e.g., boronate affinity methods) should be calibrated to report results equivalent to HbA_{1c} to harmonize results. HbA_1 is composed of HbA_{1a}, HbA_{1b}, and HbA_{1c} and should not be measured or reported. The term "A1C test" is commonly used and recommended by the ADA in place of HbA_{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 HbA_{1c}. In order to harmonize results, most clinical studies of glucose control recommend the use of HbA_{1c} assays that are traceable to the DCCT assay, as was done in the UKPDS. In this article, we use the abbreviation GHb to include all forms of glycated hemoglobin and HbA_{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 HbA_{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 HbA_{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 HbA_{1c} in the diagnosis of diabetes was first proposed and implemented

in 2009 (22), made possible by improved assay standardization through the NGSP (National Glycohemoglobin Standardization Program) and IFCC, and new data demonstrating the association between HbA_{1c} concentrations and risk for retinopathy (22). Guidelines have been updated over time (2). Several technical advantages of HbA_{1c} testing compared with glucose testing, such as its preanalytic stability and decreased biological variability (318), also played a role. Finally, the clinical convenience of the HbA_{1c} assay, which requires no fasting or glucose challenge, has led to increasing use of HbA_{1c} testing for diagnosis. An HbA_{1c} value of 6.5% (48 mmol/mol) or greater is considered diagnostic. Confirmation with a repeated HbA_{1c} test on a different sample or a glucose-based test is recommended (2,319). The frequency of HbA_{1c} testing for diagnosis has not been established, but guidelines similar to those for glucose-based testing seem appropriate (2). HbA_{1c} assays are not recommended for screening for or diagnosis of gestational diabetes mellitus (see *Gestational Diabetes Mellitus* section). Screening for diabetes will also identify populations with HbA_{1c} that are increased but not high enough to qualify as diabetes (≥6.5%). Although the risk for developing diabetes follows HbA_{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 HbA_{1c} levels from 6.0% to 6.4% and the ADA has recommended HbA_{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 HbA_{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 HbA_{1c} methods that are NGSP certified should be used to diagnose (or screen for) diabetes. The ADA has cautioned that POC testing (POCT) devices for HbA_{1c} should not be used for diagnosis (59). Although several point-of-care HbA_{1c} assays are NGSP certified, the test is CLIA waived in the U.S. and proficiency testing is not necessary. Therefore, minimal objective information is available concerning their performance in the hands of nonlaboratory personnel who often measure HbA_{1c} with POCT devices. Several published evaluations revealed that few POCT devices for HbA_{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 HbA_{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 three 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 HbA_{1c} devices should not be used for diagnosis of or screening for diabetes.

Monitoring

Recommendation: HbA_{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 HbA_{1c} is widely used for routine monitoring of long-term glycemic status in people with diabetes mellitus. HbA_{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 HbA_{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 HbA_{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 HbA_{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 HbA_{1c} levels, on long-term complications of diabetes (50,52).

Frequency of Measurement

There is no consensus on the optimal frequency of HbA_{1c} testing. The ADA (59) recommends "The frequency of A1C 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 HbA_{1c} testing "at least two times a year in patients who are meeting treatment goals (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 HbA_{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 HbA_{1c} are associated with significant reductions in HbA_{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 HbA_{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 comorbid illnesses, a history of severe hypoglycemia, and advanced complications. A (high)

The ADA recommends that in general an HbA_{1c} target <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. HbA_{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 an HbA_{1c} value <7% (53 mmol/mol) for many people with diabetes (59). Other endocrine specialty clinical organizations recommend HbA_{1c} targets similar to the ADA, ranging from 6.5% to 7% (48 to 53 mmol/mol), although higher levels have been suggested by nonspecialty organizations (330,331). These HbA_{1c} values apply only to assay methods that are certified as traceable to the DCCT reference, with nondiabetic reference interval of approximately 4% to 6% HbA_{1c} (20 to 42 mmol/mol). In the DCCT, each 10% reduction in HbA_{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 HbA_{1c} was associated with increased risk for severe hypoglycemia.

HbA_{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 include 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 semiautomated pumps that suspend insulin infusion as glucose concentrations decrease have facilitated achieving target HbA_{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 HbA_{1c} goals should be chosen (59).

Recommendation: During pregnancy and in preparation for pregnancy, women with diabetes should try to achieve HbA_{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, HbA_{1c} testing and maintenance of specified concentrations 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 HbA_{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 HbA_{1c} <6% (42 mmol/mol) during pregnancy in women with preexisting diabetes (recognizing that changes in RBC turnover during pregnancy in women without diabetes lower usual HbA_{1c} concentrations), if this can be achieved without significant hypoglycemia (251).

ANALYTICAL CONSIDERATIONS

Preanalytical

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

The effects of race on HbA_{1c} values remain controversial. Several studies have suggested a relatively higher HbA_{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 HbA_{1c} values 0.4% higher than White individuals (336). However, race did not modify the association between the HbA_{1c} concentration and adverse cardiovascular outcomes or death (336). In addition, a study among races showed that all measures of glycemia, including HbA_{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 with White populations suggests that higher HbA_{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 HbA_{1c} levels. The HbA_{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 HbA_{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 HbA_{1c} in Black compared with White participants (342). At the same average glucose values, HbA_{1c} was approximately 0.4% higher in the former compared with the latter.

Other Patient-Related Factors and Interfering Factors.

Recommendation: Laboratories should be aware of potential interferences,

including hemoglobin variants that may affect HbA_{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: HbA_{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 HbA_{1c} test results, although they reflect a shorter period of average glycemia than HbA_{1c}. GPP

Recommendation: HbA_{1c} cannot be measured and should not be reported in individuals who do not have HbA, e.g., those with homozygous hemoglobin variants, such as HbSS or HbEE; glycated proteins, such as fructosamine or glycated albumin, may be used. GPP

Any condition that shortens RBC survival or decreases mean RBC age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers HbA_{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 interindividual variability in the relationship between measured average glucose and HbA_{1c} levels (343).

Vitamins C and E are reported to lower HbA_{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 (independent of any effects due to shortened RBC survival) (348–350) (for a review, see reference 347). Depending on the particular hemoglobinopathy and assay method, results can be either falsely increased or decreased. Boronate affinity chromatographic assay methods are generally considered 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, HbA_{1c} can be measured accurately in most individuals heterozygous for hemoglobin variants (see https://ngsp.org/factors.asp for a summary of published studies). It is important to emphasize that HbA_{1c} cannot be measured in individuals with homozygous hemoglobin variants (e.g., HbSS, HbCC, HbEE) or 2 variant hemoglobins, like HbSC; they have no HbA and therefore do not have HbA_{1c}. In this situation, or if altered RBC turnover interferes with the relationship between mean blood glucose values and HbA_{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 HbA_{1c} assay method. A list of interfering factors for specific assays is maintained on the NGSP website (https://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 fingerstick capillary sampling. Blood tubes should contain anticoagulant as specified by the manufacturer of the HbA_{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 (353–355). 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 employed. The accuracy of such collection methods has been validated in several large research cohorts (353,354).

Analytical

Traceability of HbA_{1c} Methods.

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

There are >300 HbA_{1c} assay methods in current clinical use. Many of these use high-throughput automated systems dedicated to HbA_{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 glycated and nonglycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify HbA_{1c}, defined as hemoglobin A with glucose attached to the NH2-terminus valine of one or both β

chains. Other methods quantify "total glycated hemoglobin," which includes both HbA_{1c} and other hemoglobin-glucose adducts (i.e., internal glucose–lysine adducts, and terminal glucose–α-chain NH2-terminus valine adducts). Enzymatic methods to specifically measure HbA_{1c} are also commercially available. Generally, results of methods using different assay principles show excellent interassay 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 HbA_{1c} (295,312,330,356).

Recommendation: Laboratories that measure HbA_{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 standardized GHb test results among laboratories to DCCT-equivalent HbA_{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 HbA_{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: https://ngsp.org).

The IFCC has developed a higher order reference method and reference materials for HbA_{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 two methods use the same primary reference materials and the results are essentially identical. HbA_{1c} is measured as the ratio of glycated to nonglycated N-terminal peptide and is reported as mmol β N1-deoxyfructosylhemoglobin 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 HbA_{1c} measurement are intralaboratory CV <1.5% and interlaboratory CV <2.5% (using at least 2 control samples with different HbA_{1c} levels), and ideally no measurable bias. B (low)

Several expert groups have presented recommendations for assay performance. For example, intralaboratory CVs <3% (365) or <2% (14) and interlaboratory CV <5% (365) have been proposed. The prior version of these guidelines recommended intralaboratory CV <2% and interlaboratory CV <3.5% (14,15). Intraindividual CVs in healthy people are very small (<2%), and many current assay methods can achieve intralaboratory CVs <1.5% and interlaboratory 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% HbA_{1c} between successive patient samples is due to a significant change in glycemic control (when HbA_{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 HbA_{1c} result for an individual with a "true" HbA_{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 intralaboratory CV <1.5% and interlaboratory 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 of no more than 1.2% for HbA_{1c} (367). To minimize differences among laboratories in the diagnosis of diabetes in individuals whose HbA_{1c} concentrations are close to the diagnostic threshold value, we recommend that methods should be without measurable bias.

The laboratory should include two control materials with different mean values (high and low) at the beginning and end of each day's run. Frozen whole-blood controls 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 commercially available but, depending on the assay method, may show matrix effects when new reagents or columns are introduced. It is recommended that the laboratory consider using both commercial and in-house controls to optimize performance monitoring.

Removal of Labile GHb. Formation of HbA_{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 HbA_{1c} assay methods if not completely removed or separated. Currently available automated assays either remove the labile pre-HbA_{1c} during the assay process or do not measure the labile product.

Interpretation

Laboratory—Clinician Interactions

Laboratory professionals should work closely with clinicians who order HbA_{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 HbA_{1c} test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS (50,52). This information is available on the NGSP website. For example, each 1% (approximately 11 mmol/mol) change in HbA_{1c} is related to a change in mean plasma glucose of approximately 1.6 mmol/L (29 mg/dL). Reporting HbA_{1c} results with a calculated estimated average glucose (eAG) will eliminate the need for health care 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 HbA_{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 HbA_{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. HbA_{1c} values in people with diabetes are a continuum; they range from within the nondiabetic reference 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., two- to threefold higher levels than the nondiabetic mean of approximately 5%, in some individuals, reflecting extreme hyperglycemia. Proper interpretation of HbA_{1c} test results requires that clinicians understand the relationship between HbA_{1c} values and mean plasma glucose, the kinetics of HbA_{1c}, and specific assay limitations/interferences (295). Small changes in HbA_{1c} (e.g., ± 0.3% HbA_{1c}) over time may reflect assay variability rather than a true change in glycemic status (361).

Recommendation: HbA_{1c} should be reported as a percentage of total hemoglobin or as mmol/mol of total hemoglobin. GPP

HbA_{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 × IFCC%) + 2.152] (363). Clinical results reported in IFCC units (mmol/mol) correlate tightly with NGSP results reported in percent.

Recommendation: HbA_{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 HbA_{1c} more accessible to people with diabetes. GPP

Several studies have demonstrated a close mathematical relationship between the HbA_{1c} concentration and mean glycemia that should allow expression of HbA_{1c} as an eAG (341,373,374). The eAG is helpful in translating the HbA_{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 HbA_{1c} has not been adopted, with some countries, like the U.S., usually reporting HbA_{1c} as a percentage of total hemoglobin and eAG, while others, such as the U.K., 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 not have obesity and should 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, HbA_{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 HbA_{1c} results below the lower limit of the reference interval or greater than 15% (140 mmol/mol) HbA_{1c}. GPP

The laboratory should use repeat testing for all sample 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 HbA_{1c} should use a method based on an analytical principle different to the initial assay. In addition, sample results less than 4% (20 mmol/mol) or greater than 15% HbA_{1c} (140 mmol/mol) should be repeated 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 HbA_{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 HbA_{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 fingerstick 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 HbA_{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 HbA_{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 HbA_{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. Implementation of the reporting recommendations (375,376) needs to be carried out with education of health care providers and people with diabetes. Some believe that reporting eAG should complement the current reporting of HbA_{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 β-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-DQ2.5), 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 diabetes in the neonate (381–384) as well as in young individuals 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 diagnostic for type 1 diabetes. HLA-DQ A1 and B1 genotyping can be useful to signal absolute risk of diabetes. The HLA-DQA1*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 sufficient, for disease. The HLA DQ and DR genes are by far the most important determinants for the risk of developing a first β-cell autoantibody such as either insulin autoantibodies (IAA) or glutamic acid decarboxylase autoantibodies (GADA) following an environmental exposure by, for example, enterovirus (386). Once β-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 characterization 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

Table 8—Causes of maturity-onset diabetes of the young (MODY) and other types of monogenic diabetes[a]

	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

Adapted from the ADA (2). [a]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.

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 development of islet autoimmunity or type 1 diabetes (388, 390,392). Nevertheless, this strategy cannot be recommended 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 β-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 mutations 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 detection 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 (382,384,398).

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 inherent to recessive Seip–Berardinelli syndrome of generalized lipodystrophy mapping to chromosome 9q34 (18,399). There are over 60 distinct genetic disorders associated 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 mutation, 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 attempted 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 leukocytes. 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 involved. The heterodimeric proteins that are expressed on antigen presenting cells, such as macrophages and dendritic cells, B lymphocytes, platelets, and activated T lymphocytes (but not other somatic cells), are composed of cis- and sometimes trans-complemented α- and β-chain heterodimers. People at the highest genetic risk of type 1 diabetes are those in whom all four 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. In 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 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 nucleotide tandem repeat (VNTR) upstream from the insulin (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 β-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 for 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 cooccurrence 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 2 (IA-2A), or zinc transporter 8 (ZnT8A) and predict type 1 diabetes. In children with only one persistent islet autoantibody, the risk of diabetes within 10 years is 15% while two 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 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, symptoms. GPP

Recommendation: Standardized islet autoantibody tests are recommended in prospective research 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)

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 β-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) three 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 β-cells has multiple genetic predispositions and is thought to be initiated by environmental influences, such as certain enteroviruses. The ensuing autoimmunity may be present for months or years prior to the appearance of two 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 days before insulin antibodies are induced. People with type 1A diabetes have a significantly increased risk of other autoimmune disorders, 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 people 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 diagnosed 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 relatives 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 and being persistently 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) or from type 1 diabetes later in

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

Adapted from the ADA (2). [a]FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; 2-h PG, 2-h plasma glucose.

life. Because islet autoantibodies in these individuals have substantially reduced predictive significance, these subjects are often excluded from prevention trials.

Approximately 5% to 10% of White 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 four islet autoantibodies and modest hyperglycemia (HbA_{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 consideration for the use of immunosuppressive agents to try to halt recurrence of diabetes. Notwithstanding these theoretical 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 tests measure the degree of binding of immunoglobulin to islet sections and are compared with 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 two 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 people. 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 people 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 standardized radiobinding assays using coupled in vitro transcription translation to label the autoantigens (441) or with commercially available nonradiolabeled 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 associated 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 population. If one 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 two or more autoantibodies are positive (443).

The presence of islet autoantibodies suggests that insulin is the most appropriate therapeutic option, especially 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 one 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 -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 fingerstick 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 participants 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 two 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 distinctly 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 excretion 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 definitions have been adopted by the ADA. There are now three categories of albuminuria (Fig. 1, Table 10) which have been renamed. These are

- **A1—Normal to Mildly Increased Albuminuria:** urine albumin-to-creatinine ratio (uACR) <30 mg/g (<3 mg/mmol). This is equivalent to 24-h albumin excretion rate (AER) <30 mg/day and urine protein-to-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 >2,200 mg/day; uACR >2,200 mg/g (>220 mg/mmol); PER >3,500 mg/day, and uPCR >3,500 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 postpubertal 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 situations. 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

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 m^2) 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+

Figure 1—The KDIGO heatmap of staging and CKD/cardiovascular risk. 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 risk. Reprinted from the ADA (451).

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."

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 in Diabetic Kidney Disease (FIGARO-DKD) 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 cardiovascular 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 that albuminuria is a continuous variable for cardiovascular risk (466–468).

An estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, 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 associated 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-to-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-to-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-to-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 m^2 (59). SGLT2 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.

Frequency of Measurement

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

Recommendation: If eGFR 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)

The KDIGO and ADA recommend annual measurement of uACR if it is >30 mg/g. After documenting stage A2 albuminuria on two of three 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 albuminuria is accepted as a surrogate marker of slowed progression 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 U.S. 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 U.S. 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 creatinine-cystatin 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^2 (Fig. 1) have prognostic significance. In multiple epidemiological studies, moderately 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 developing 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 m^2. 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 of 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 albuminuria 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-to-creatinine 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 individual 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 albumin-to-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 albumin-to-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-to-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 (91). 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% to 13%, and total allowable error of ≤24% to 30% (499). The analytical measurement range for urine albumin should be 2 to 400 mg/L (499).

Semiquantitative or Qualitative.

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

Recommendation: Semiquantitative 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 semiquantitative tests should be confirmed by quantitative analysis in an accredited laboratory. GPP

Semiquantitative (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 semiquantitative 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 individuals) that evaluated semiquantitative 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 sensitivity and specificity estimates of 76% (95% CI, 63–86%) and 93% (CI, 84–97%), respectively, for the semiquantitative 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 semiquantitative POC test result does not rule out albuminuria, whereas quantitative POC testing meets required 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 the following: 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 with local laboratory methods, which, as indicated above, exhibit large biases (498).

A cost-effectiveness analysis of 1,881 individuals with diabetes published in 2020 evaluated medical costs of CKD and concluded that the semiquantitative 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 sensitivity (>80%). Clinical operators have a lower sensitivity 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 two or three 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 two or more of three tests define positive. Screening using two 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 semiquantitative 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 recommended unless they are able to specifically measure albumin at low concentrations and express the results as an albumin-to-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 standard for comparison as it has >95% sensitivity and specificity to detect moderately increased albuminuria (505). Semiquantitative 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 albumin testing (506), the diagnosis of moderately increased or severely increased albuminuria requires the demonstration of increased albumin excretion on two of three 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 one occasion is indicative of persistent albuminuria 50% to 75% of the time, while stage A3 albuminuria ($\geq$300 mg/g) even on one occasion is indicative of increased albuminuria ($>$30 mg/g) almost 100% of the time.

At least some of the semiquantitative POC methods 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 semiquantitative uACR dipstick testing as a useful approach when quantitative analysis is not possible. Advantages of semiquantitative 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

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, BMI, 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 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, BMI, degree of glucose tolerance, and plasma lipid/lipoprotein concentrations. It is these variables that are the focus of clinical 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 antihyperglycemic agent for initial therapy in an individual with type 2 diabetes is a question that arises from consideration of the pathophysiology of type 2 diabetes. In theory, the lower the pretreatment 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 efficacious treatment of people with type 2 diabetes.

In contrast to the above considerations, measurement 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 inappropriately increased plasma insulin concentrations in the face of a low glucose concentration. In addition, an increase 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 associated changes in glucose, insulin, and proinsulin concentrations from an individual with fasting hypoglycemia makes the diagnosis of an islet cell tumor most unlikely, 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 (5,511). However, even in this clinical situation, the response to drug therapy will provide useful information, and measurement of C-peptide may not be clinically necessary. 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 assays 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 metabolism; 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 (BMI, presence of acanthosis nigricans), and routine measurements of C-peptide or insulin are not recommended 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 standardized method available to measure serum insulin. Attempts to

harmonize insulin assays using commercial insulin reagent sets result in greatly discordant results (513). In 2009, an insulin standardization workgroup of the ADA, in conjunction with the NIDDK, CDC, and EASD, called for harmonization of insulin assay results through traceability to an isotope dilution liquid chromatography/tandem mass spectrometry reference (514). The Insulin Standardization 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 measurement of C-peptide. Stakeholders in the U.S., Japan, and elsewhere have worked on developing a reference standard and traceability schemes, but there is a need for further coordination to assure worldwide harmonization of C-peptide (516).

Measurement of proinsulin and C-peptide are accomplished by immunometric methods. Proinsulin reference intervals are dependent on methodology and each laboratory should establish its own reference interval. Although it has been suggested by some, insulin measurement should not be used in an OGTT to diagnose diabetes. In the case of C-peptide, there is a discrepancy in reliability because of variable specificity among antisera, lack of standardization of C-peptide calibration, and variable cross-reactivity with proinsulin. Of note is the requirement of the U.S. Centers for Medicare & Medicaid Services (CMS) that Medicare beneficiaries must have C-peptide measured in order to be eligible for coverage of insulin pumps. Initially, the requirement was that the C-peptide be $\leq$0.5 ng/mL; however, because of noncomparability 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 $\leq$110% of the lower limit of the reference interval of the laboratory'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 antibodies can be detected in any person being treated with exogenous insulin (518,519). In most of these individuals, the titer of insulin antibodies is low, particularly in those who were never treated with animal insulins, and their presence is of no clinical significance. 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 concentrations. This clinical situation is quite rare and 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 are unclear (519). There are several therapeutic approaches for treating these individuals, and a quantitative estimate of the concentration of circulating insulin antibodies does not appear to be of significant benefit.

Acknowledgments. We are grateful to the following individuals who coauthored the original document on which these guidelines are based: David Goldstein, Noel Maclaren, Jay McDonald, and Marian Parrott. The authors thank Randie Little for insightful suggestions, ADA staff Malaika Hill, Laura Mitchell, and Mindy Saraco for expert help in compiling the document, and Jacqueline Weild for compiling the evidence tables. We also thank all those who submitted comments on preliminary drafts of the document.

Funding. D.B.S., NIH Intramural Research Program. Å.L., Swedish Research Council.

Duality of Interest. D.B.S., Associate Editor for *Clinical Chemistry*, American Association for Clinical Chemistry, and Chair, Scientific Review Committee on Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. M.A.A., travel expenses paid by American Diabetes Association for attendance at the in-person manuscript review meeting, consulting for Rockley Photonics, a for-profit company working to develop wearable technology for fitness and wellness applications, including noninvasive glucose measurements, consulting for Foundation for Innovative New Diagnostics concerning the development of glucose sensing technologies for low- and middle-income countries, consulting for LifePlus concerning their interest in the development of a noninvasive glucose sensor for management of diabetes, and patent application filed (Olesberg, JT, Arnold, MA, Urea monitoring during dialysis for improved quality control and treatment guidance, U.S. Provisional Application 63/087,600 filed on 5 October 2020, International Patent Application No. PCT/US2021/053598 filed on 5 October 2021, U.S. Patent Application 18/030,339 filed on 5 April 2023). G.L.B., consultant or member of clinical trial steering committee for Bayer, KBP Biosciences, Ionis, Alnylam, AstraZeneca, Novo Nordisk, Janssen InREGEN and consulted for DiaMedica and Quantum Genomics. A.R.H., Elsevier, royalty for *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 6th edition, and *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, Chair of the Preanalytical glucose working party of the Australasian Association of Clinical Biochemistry and Laboratory Medicine, Chair of the Analytical Performance Specifications based on Outcomes Task Force Group of the European Federation of Clinical Chemistry and Laboratory Medicine. Å.L., DiaMyd AB, Stockholm, Sweden, consulting fee. No other potential conflicts of interest relevant to this article were reported.

The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Author Contributions. The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data, (b) drafting or revising the article for intellectual content, (c) final approval of the published article, and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.

David Sacks (Conceptualization-Equal, Funding acquisition-Equal, Investigation-Equal, Project administration-Lead, Writing—original draft-Lead, Writing—review & editing-Equal), Mark Arnold (Investigation-Equal, Writing—review & editing-Equal), George Bakris (Investigation-Equal, Writing—review & editing-Equal), David Bruns (Investigation-Equal, Writing—review & editing-Equal), Andrea Horvath (Investigation-Equal, Methodology-Lead, Writing—review & editing-Equal), Ake Lernmark (Investigation-Equal, Writing—review & editing-Equal), Boyd Metzger (Investigation-Equal, Writing—review & editing-Equal), David Nathan (Investigation-Equal, Writing—review & editing-Equal), and M. Sue Kirkman (Investigation-Equal, Writing—original draft-Equal, Writing—review & editing-Equal)

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37(Suppl. 1):S81–S90

2. American Diabetes Association Professional Practice Committee. 2. Classification and diagnosis of diabetes: *Standards of Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl 1):S17–S38

3. Castaño L, Eisenbarth GS. Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat. Annu Rev Immunol 1990;8:647–679

4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595–1607

5. Sacks DB, McDonald JM. The pathogenesis of type II diabetes mellitus. A polygenic disease. Am J Clin Pathol 1996;105:149–156

6. Balasubramanyam A, Garza G, Rodriguez L, et al. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. Diabetes Care 2006;29:2575–2579
7. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. 2021. Accessed 23 May 2022. Available from https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf
8. Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2022. Accessed 9 August 2022. Available from https://www.cdc.gov/diabetes/data/statistics-report/index.html
9. American Diabetes Association. Economic costs of diabetes in the U.S. in 2017. Diabetes Care 2018;41:917–928
10. American Diabetes Association. Economic costs of diabetes in the U.S. in 2007. Diabetes Care 2008;31:596–615
11. Roglic G, Unwin N, Bennett PH, et al. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes Care 2005;28:2130–2135
12. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2002;25:750–786
13. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002;48:436–472
14. Sacks DB, Arnold M, Bakris GL, et al.; National Academy of Clinical Biochemistry; Evidence-Based Laboratory Medicine Committee of the American Association for Clinical Chemistry. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34:e61–e99
15. Sacks DB, Arnold M, Bakris GL, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:e1–e47
16. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039–1057
17. WHO. WHO Expert Committee on Diabetes Mellitus: second report. World Health Organ Tech Rep Ser 1980;646:1–80
18. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197
19. Engelgau MM, Thompson TJ, Herman WH, et al. Comparison of fasting and 2-hour glucose and HbA_{1c} levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diabetes Care 1997;20:785–791
20. McCance DR, Hanson RL, Charles MA, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ 1994;308:1323–1328
21. WHO and International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia, 2006. Accessed 23 May 2022. Available from https://www.who.int/publications/i/item/definition-and-diagnosis-of-diabetes-mellitus-and-intermediate-hyperglycaemia
22. International Expert Committee. International Expert Committee report on the role of the A_{1c} assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–1334
23. WHO. Use of glycated haemoglobin (HbA_{1c}) in the diagnosis of diabetes mellitus: abbreviated report of a WHO consultation, 2011. Accessed 23 May 2022. Available from https://apps.who.int/iris/bitstream/handle/10665/70523/WHO_NMH_CHP_CPM_11.1_eng.pdf
24. Mbanya JC, Henry RR, Smith U. Presidents' statement on WHO recommendation on HbA_{1c} for diabetes diagnosis. Diabetes Res Clin Pract 2011;93:310–311
25. American Diabetes Association. Tests of glycemia in diabetes. Diabetes Care 2000;23(Suppl. 1):S80–S82
26. Harris MI. Undiagnosed NIDDM: clinical and public health issues. Diabetes Care 1993;16: 642–652
27. Nathan DM, Bennett PH, Crandall JP, et al.; Research Group. Does diabetes prevention translate into reduced long-term vascular complications of diabetes? Diabetologia 2019;62: 1319–1328
28. American Diabetes Association. Type 2 diabetes in children and adolescents. Diabetes Care 2000;23:381–389
29. Knowler WC, Barrett-Connor E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403
30. Tuomilehto J, Lindström J, Eriksson JG, et al.; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343–1350
31. Selph S, Dana T, Blazina I, Bougatsos C, Patel H, Chou R. Screening for type 2 diabetes mellitus: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 2015;162: 765–776
32. Icks A, Rathmann W, Haastert B, et al.; KORA Study Group. Cost-effectiveness of type 2 diabetes screening: results from recently published studies. Gesundheitswesen 2005;67(Suppl. 1):S167–S171
33. Perry RC, Shankar RR, Fineberg N, McGill J; Early Diabetes Intervention Program (EDIP). HbA_{1c} measurement improves the detection of type 2 diabetes in high-risk individuals with nondiagnostic levels of fasting plasma glucose: the Early Diabetes Intervention Program (EDIP). Diabetes Care 2001;24:465–471
34. Jesudason DR, Dunstan K, Leong D, Wittert GA. Macrovascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and $HbA(_{1c})$ for cost-effective screening. Diabetes Care 2003;26:485–490
35. CDC Diabetes Cost-effectiveness Study Group, Centers for Disease Control and Prevention. The cost-effectiveness of screening for type 2 diabetes. JAMA 1998;280:1757–1763
36. Hoerger TJ, Harris R, Hicks KA, Donahue K, Sorensen S, Engelgau M. Screening for type 2 diabetes mellitus: a cost-effectiveness analysis. Ann Intern Med 2004;140:689–699
37. Glümer C, Yuyun M, Griffin S, et al. What determines the cost-effectiveness of diabetes screening? Diabetologia 2006;49:1536–1544
38. Kahn R, Alperin P, Eddy D, et al. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. Lancet 2010;375:1365–1374
39. Zhou X, Siegel KR, Ng BP, et al. Cost-effectiveness of diabetes prevention interventions targeting high-risk individuals and whole populations: a systematic review. Diabetes Care 2020; 43:1593–1616
40. Greenberg RA, Sacks DB. Screening for diabetes: is it warranted? Clin Chim Acta 2002; 315:61–69
41. Herman WH, Hoerger TJ, Brandle M, et al.; Diabetes Prevention Program Research Group. The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. Ann Intern Med 2005;142:323–332
42. Genuth S, Alberti KGMM, Bennett P, et al.; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–3167
43. Forouhi NG, Balkau B, Borch-Johnsen K, et al.; EDEG. The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. Diabetologia 2006;49:822–827
44. Tai ES, Goh SY, Lee JJM, et al. Lowering the criterion for impaired fasting glucose: impact on disease prevalence and associated risk of diabetes and ischemic heart disease. Diabetes Care 2004; 27:1728–1734
45. Gabir MM, Hanson RL, Dabelea D, et al. The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 2000;23:1108–1112
46. Tirosh A, Shai I, Tekes-Manova D, et al.; Israeli Diabetes Research Group. Normal fasting plasma glucose levels and type 2 diabetes in young men. N Engl J Med 2005;353:1454–1462
47. Emanuelsson F, Marott S, Tybjærg-Hansen A, Nordestgaard BG, Benn M. Impact of glucose level on micro- and macrovascular disease in the general population: a Mendelian randomization study. Diabetes Care 2020;43:894–902
48. The Diabetes Control and Complications Trial Research Group. The relationship of glycemic exposure (HbA_{1c}) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995; 44:968–983
49. Stratton IM, Adler AI, Neil HAW, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405–412
50. Nathan DM, Genuth S, Lachin J, et al.; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986
51. Nathan DM, Cleary PA, Backlund JYC, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–2653
52. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with con-

ventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–853
53. Selvin E, Marinopoulos S, Berkenblit G, et al. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141:421–431
54. Ray KK, Seshasai SRK, Wijesuriya S, et al. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet 2009;373: 1765–1772
55. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-Year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–1589
56. Gerstein HC, Miller ME, Byington RP, et al.; Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008; 358:2545–2559
57. Patel A, MacMahon S, Chalmers J, et al.; ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008; 358:2560–2572
58. Duckworth W, Abraira C, Moritz T, et al.; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360:129–139
59. American Diabetes Association Professional Practice Committee. 6. Glycemic targets: *Standards of Medical Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl. 1):S83–S96
60. Howe-Davies S, Simpson RW, Turner RC. Control of maturity-onset diabetes by monitoring fasting blood glucose and body weight. Diabetes Care 1980;3:607–610
61. Troisi RJ, Cowie CC, Harris MI. Diurnal variation in fasting plasma glucose: implications for diagnosis of diabetes in patients examined in the afternoon. JAMA 2000;284:3157–3159
62. Bruns DE, Metzger BE, Sacks DB. Diagnosis of gestational diabetes mellitus will be flawed until we can measure glucose. Clin Chem 2020; 66:265–267
63. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. Clin Chem 2009;55:850–852
64. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315–317
65. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In Clinical Laboratory Methods and Diagnosis. St. Louis, C.V. Mosby Co., 1980, p. 149–192
66. Rifai N. Carbohydrates. In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. New York, Elsevier, 2017
67. Sacks DB. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. St. Louis, Elsevier Saunders, 2018, p. 518–538
68. Wilson SS, Guillan RA, Hocker EV. Studies of the stability of 18 chemical constituents of human serum. Clin Chem 1972;18:1498–1503
69. Lin YL, Smith CH, Dietzler DN. Stabilization of blood glucose by cooling with ice: an effective procedure for preservation of samples from adults and newborns. Clin Chem 1976;22:2031–2033
70. Gambino R, Piscitelli J, Ackattupathil TA, et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem 2009;55:1019–1021
71. Uchida K, Matuse R, Toyoda E, Okuda S, Tomita S. A new method of inhibiting glycolysis in blood samples. Clin Chim Acta 1988;172:101–108
72. Fobker M. Stability of glucose in plasma with different anticoagulants. Clin Chem Lab Med 2014;52:1057–1060
73. Daly N, Flynn I, Carroll C, Stapleton M, O'Kelly R, Turner MJ. Comparison of citrate-fluoride-EDTA with fluoride-EDTA additives to stabilize plasma glucose measurements in women being screened during pregnancy with an oral glucose tolerance test: a prospective observational study. Clin Chem 2016;62:886–887
74. van den Berg SAA, Thelen MHM, Salden LPW, van Thiel SW, Boonen KJM. It takes acid, rather than ice, to freeze glucose. Sci Rep 2015; 5:8875
75. Carey R, Lunt H, Heenan HF, Frampton CMA, Florkowski CM. Collection tubes containing citrate stabiliser over-estimate plasma glucose, when compared to other samples undergoing immediate plasma separation. Clin Biochem 2016;49:1406–1411
76. Fischer MM, Hannemann A, Winter T, Schäfer C, Petersmann A, Nauck M. Relative efficacy of different strategies for inhibition of in vitro glycolysis. Clin Chem 2021;67:1032–1034
77. Ridefelt P, Åkerfeldt T, Helmersson-Karlqvist J. Increased plasma glucose levels after change of recommendation from NaF to citrate blood collection tubes. Clin Biochem 2014;47:625–628
78. Ladenson JH, Tsai LM, Michael JM, Kessler G, Joist JH. Serum versus heparinized plasma for eighteen common chemistry tests: is serum the appropriate specimen? Am J Clin Pathol 1974;62: 545–552
79. Stahl M, Jørgensen LG, Hyltoft Petersen P, Brandslund I, de Fine Olivarius N, Borch-Johnsen K. Optimization of preanalytical conditions and analysis of plasma glucose. 1. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. Scand J Clin Lab Invest 2001; 61:169–179
80. Carstensen B, Lindström J, Sundvall J, Borch-Johnsen K; DPS Study Group. Measurement of blood glucose: comparison between different types of specimens. Ann Clin Biochem 2008;45: 140–148
81. Miles RR, Roberts RF, Putnam AR, Roberts WL. Comparison of serum and heparinized plasma samples for measurement of chemistry analytes. Clin Chem 2004;50:1704–1706
82. Boyanton BL Jr, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–2247
83. Larsson-Cohn U. Differences between capillary and venous blood glucose during oral glucose tolerance tests. Scand J Clin Lab Invest 1976;36: 805–808
84. Lind T, de Groot HAVC, Brown G, Cheyne GA. Observations on blood glucose and insulin determinations. BMJ 1972;3:320–323
85. Sacks DB. Carbohydrates. In Tietz Textbook of Laboratory Medicine. 7th ed. Rifai N, Chiu R, Young I, Burnham C-A, Wittwer C, Eds. St. Louis, Elsevier, 2023, p. 353.e1–353.e23
86. Miller WG, Myers GL, Ashwood ER, et al. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. Arch Pathol Lab Med 2008;132:838–846
87. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. Clin Chem 1999;45: 321–323
88. Stöckl D, Baadenhuijsen H, Fraser CG, Libeer JC, Petersen PH, Ricós C. Desirable routine analytical goals for quantities assayed in serum. Discussion paper from the members of the External Quality Assessment (EQA) Working Group A on Analytical Goals in Laboratory Medicine. Eur J Clin Chem Clin Biochem 1995; 33:157–169
89. Fraser CG. The necessity of achieving good laboratory performance. Diabet Med 1990;7: 490–493
90. Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE, Turner RC. Within- and between-subject variation in commonly measured anthropometric and biochemical variables. Clin Chem 1999;45:561–566
91. Mooy JM, Grootenhuis PA, de Vries H, et al. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia 1996; 39:298–305
92. Sebastián-Gámbaro MA, Lirón-Hernández FJ, Fuentes-Arderiu X. Intra- and inter-individual biological variability data bank. Eur J Clin Chem Clin Biochem 1997;35:845–852
93. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. Arch Intern Med 2007;167:1545–1551
94. Lacher DA, Hughes JP, Carroll MD. Estimate of biological variation of laboratory analytes based on the third national health and nutrition examination survey. Clin Chem 2005;51:450–452
95. Aarsand AK, Díaz-Garzón J, Fernandez-Calle P, et al. The EuBIVAS: within- and between-subject biological variation data for electrolytes, lipids, urea, uric acid, total protein, total bilirubin, direct bilirubin, and glucose. Clin Chem 2018;64:1380–1393
96. González-Lao E, Corte Z, Simón M, et al.; European Federation of Clinical Chemistry and Laboratory Medicine Working Group on Biological Variation and Task Group for the Biological Variation Database. Systematic review of the biological variation data for diabetes related analytes. Clin Chim Acta 2019;488:61–67
97. Tchobroutsky G. Blood glucose levels in diabetic and non-diabetic subjects. Diabetologia 1991;34:67–73
98. van den Beld AW, Kaufman JM, Zillikens MC, Lamberts SWJ, Egan JM, van der Lely AJ. The physiology of endocrine systems with ageing. Lancet Diabetes Endocrinol 2018;6:647–658
99. Blunt BA, Barrett-Connor E, Wingard DL. Evaluation of fasting plasma glucose as screening test for NIDDM in older adults. Rancho Bernardo Study. Diabetes Care 1991;14:989–993
100. Chia CW, Egan JM, Ferrucci L. Age-related changes in glucose metabolism, hyperglycemia, and cardiovascular risk. Circ Res 2018;123:886–904
101. Howanitz PJ, Cembrowski GS, Steindel SJ, Long TA. Physician goals and laboratory test turnaround times. A College of American Patho-

logists Q-Probes study of 2763 clinicians and 722 institutions. Arch Pathol Lab Med 1993;117: 22–28
102. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. N Engl J Med 2001;345:1359–1367
103. Allemann S, Houriet C, Diem P, Stettler C. Self-monitoring of blood glucose in non-insulin treated patients with type 2 diabetes: a systematic review and meta-analysis. Curr Med Res Opin 2009;25:2903–2913
104. Harris MI, Cowie CC, Howie LJ. Self-monitoring of blood glucose by adults with diabetes in the United States population. Diabetes Care 1993;16:1116–1123
105. American Diabetes Association Professional Practice Committee. 7. Diabetes technology: *Standards of Medical Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl. 1):S97–S112
106. Siu AL; U.S. Preventive Services Task Force. Screening for abnormal blood glucose and type 2 diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2015;163:861–868
107. American Diabetes Association. Self-monitoring of blood glucose. Diabetes Care 1996; 19(Suppl. 1):S62–S66
108. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. Endocr Rev 1991;12:356–371
109. American Diabetes Association. Standards of medical care in diabetes—2011. Diabetes Care 2011;34(Suppl. 1):S11–S61
110. Simon J, Gray A, Clarke P, Wade A, Neil A; Diabetes Glycaemic Education and Monitoring Trial Group. Cost effectiveness of self monitoring of blood glucose in patients with non-insulin treated type 2 diabetes: economic evaluation of data from the DiGEM trial. BMJ 2008;336: 1177–1180
111. Malanda UL, Welschen LMC, Riphagen II, Dekker JM, Nijpels G, Bot SDM. Self-monitoring of blood glucose in patients with type 2 diabetes mellitus who are not using insulin. Cochrane Database Syst Rev 2012;1:CD005060
112. Farmer AJ, Perera R, Ward A, et al. Meta-analysis of individual patient data in randomised trials of self monitoring of blood glucose in people with non-insulin treated type 2 diabetes. BMJ 2012;344:e486
113. Machry RV, Rados DV, Gregório GR, Rodrigues TC. Self-monitoring blood glucose improves glycemic control in type 2 diabetes without intensive treatment: a systematic review and meta-analysis. Diabetes Res Clin Pract 2018; 142:173–187
114. Mannucci E, Antenore A, Giorgino F, Scavini M. Effects of structured versus unstructured self-monitoring of blood glucose on glucose control in patients with non-insulin-treated type 2 diabetes: a meta-analysis of randomized controlled trials. J Diabetes Sci Technol 2018;12:183–189
115. Young LA, Buse JB, Weaver MA, et al. Glucose self-monitoring in non–insulin-treated patients with type 2 diabetes in primary care settings: a randomized trial. JAMA Intern Med 2017;177:920–929
116. Kabadi UM, O'Connell KM, Johnson J, Kabadi M. The effect of recurrent practice at home on the acceptability of capillary blood glucose readings. Accuracy of self blood glucose testing. Diabetes Care 1994;17:1110–1123
117. Ellison JM, Stegmann JM, Colner SL, et al. Rapid changes in postprandial blood glucose produce concentration differences at finger, forearm, and thigh sampling sites. Diabetes Care 2002;25:961–964
118. Burnett RW, D'Orazio P, Fogh-Andersen N, et al.; Scientific Division, Working Group on Selective Electrodes. IFCC recommendation on reporting results for blood glucose. Clin Chim Acta 2001;307:205–209
119. D'Orazio P, Burnett RW, Fogh-Andersen N, et al.; International Federation of Clinical Chemistry Scientific Division Working Group on Selective Electrodes and Point of Care Testing. Approved IFCC recommendation on reporting results for blood glucose (abbreviated). Clin Chem 2005;51:1573–1576
120. Steffes MW, Sacks DB. Measurement of circulating glucose concentrations: the time is now for consistency among methods and types of samples. Clin Chem 2005;51:1569–1570
121. U.S. Food and Drug Administration Center for Devices and Radiological Health. Self-Monitoring Blood Glucose Test Systems for Over-the-Counter Use, 2020. Accessed 23 May 2022. Available from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/self-monitoring-blood-glucose-test-systems-over-counter-use
122. U.S. Food and Drug Administration Center for Devices and Radiological Health. Blood Glucose Monitoring Test Systems for Prescription Point-of-Care Use, 2020. Accessed 23 May 2022. Available from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/blood-glucose-monitoring-test-systems-prescription-point-care-use
123. International Standards Organization. In vitro diagnostic test systems—requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus, 2013. Accessed 23 May 2022. Available from https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/49/54976.html
124. Clinical & Laboratory Standards Institute. POCT12A3E. Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities. 3rd Ed. Accessed 1 March 2022. Available from https://clsi.org/standards/products/point-of-care-testing/documents/poct12/
125. Ekhlaspour L, Mondesir D, Lautsch N, et al. Comparative accuracy of 17 point-of-care glucose meters. J Diabetes Sci Technol 2017;11:558–566
126. Boyd JC, Bruns DE. Quality specifications for glucose meters: assessment by simulation modeling of errors in insulin dose. Clin Chem 2001;47:209–214
127. Campos-Náñez E, Fortwaengler K, Breton MD. Clinical impact of blood glucose monitoring accuracy: an in-silico study. J Diabetes Sci Technol 2017;11:1187–1195
128. Fortwaengler K, Campos-Náñez E, Parkin CG, Breton MD. The financial impact of inaccurate blood glucose monitoring systems. J Diabetes Sci Technol 2018;12:318–324
129. Nichols JH, Brandler ES, Fantz CR, et al. A multicenter evaluation of a point-of-care blood glucose meter system in critically ill patients. J Appl Lab Med 2021;6:820–833
130. Mitsios JV, Ashby LA, Haverstick DM, Bruns DE, Scott MG. Analytic evaluation of a new glucose meter system in 15 different critical care settings. J Diabetes Sci Technol 2013;7:1282–1287
131. Karon BS, Meeusen JW, Bryant SC. Impact of glucose meter error on glycemic variability and time in target range during glycemic control after cardiovascular surgery. J Diabetes Sci Technol 2015;10:336–342
132. Warner JV, Wu JY, Buckingham N, McLeod DSA, Mottram B, Carter AC. Can one point-of-care glucose meter be used for all pediatric and adult hospital patients? Evaluation of three meters, including recently modified test strips. Diabetes Technol Ther 2011;13:55–62
133. Ceriotti F, Kaczmarek E, Guerra E, et al. Comparative performance assessment of point-of-care testing devices for measuring glucose and ketones at the patient bedside. J Diabetes Sci Technol 2015;9:268–277
134. Inman M, Kyle BD, Lyon ME. Contribution of glucose meter error to misclassification of neonatal glycemic status. JAMA Pediatr 2021;175:453–455
135. Erbach M, Freckmann G, Hinzmann R, Kulzer B, Ziegler R, Heinemann L, et al. Interferences and limitations in blood glucose self-testing: an overview of the current knowledge. J Diabetes Sci Technol 2016;10:1161–1168
136. Tirimacco R, Koumantakis G, Erasmus R, et al.; International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Glucose Point-of-Care Testing. Glucose meters—fit for clinical purpose. Clin Chem Lab Med 2013;51:943–952
137. Sai S, Urata M, Ogawa I. Evaluation of linearity and interference effect on SMBG and POCT devices, showing drastic high values, low values, or error messages. J Diabetes Sci Technol 2019;13:734–743
138. Vanavanan S, Santanirand P, Chaichanajarernkul U, et al. Performance of a new interference-resistant glucose meter. Clin Biochem 2010;43:186–192
139. Perera NJ, Stewart PM, Williams PF, Chua EL, Yue DK, Twigg SM. The danger of using inappropriate point-of-care glucose meters in patients on icodextrin dialysis. Diabet Med 2011; 28:1272–1276
140. Lv H, Zhang G-J, Kang X-X, et al. Factors interfering with the accuracy of five blood glucose meters used in Chinese hospitals. J Clin Lab Anal 2013;27:354–366
141. Pfützner A, Demircik F, Sachsenheimer D, Spatz J, Pfützner AH, Ramljak S. Impact of xylose on glucose-dehydrogenase-based blood glucose meters for patient self-testing. J Diabetes Sci Technol 2017;11:577–583
142. Macrury S, Srinivasan A, Mahoney JJ. Performance of a new meter designed for assisted monitoring of blood glucose and point-of-care testing. J Diabetes Sci Technol 2013;7: 389–398
143. Wada Y, Nakamura T, Kaneshige M, et al. Evaluation of two glucose meters and interference corrections for screening neonatal hypoglycemia. Pediatr Int 2015;57:603–607
144. Demircik F, Ramljak S, Hermanns I, Pfützner A, Pfützner A. Evaluation of hematocrit interference with MyStar extra and seven competitive devices. J Diabetes Sci Technol 2015;9:262–267
145. Teodorczyk M, Cardosi M, Setford S. Hematocrit compensation in electrochemical blood glucose monitoring systems. J Diabetes Sci Technol 2012;6:648–655
146. Tendl KA, Christoph J, Bohn A, Herkner KR, Pollak A, Prusa A-R. Two site evaluation of the

performance of a new generation point-of-care glucose meter for use in a neonatal intensive care unit. Clin Chem Lab Med 2013;51:1747–1754
147. Chenoweth JA, Dang LT, Gao G, Tran NK. Acetaminophen interference with Nova StatStrip glucose meter: case report with bench top confirmation. Clin Toxicol (Phila) 2020;58:1067–1070
148. Lyon ME, Lyon AW. N-Acetylcysteine interference with a glucose dehydrogenase linked glucose meter. J Diabetes Sci Technol 2022;16:1114–1119
149. Kelly BN, Haverstick DM, Bruns DE. Interference in a glucose dehydrogenase-based glucose meter revisited. Clin Chim Acta 2012; 413:829–830
150. Miller KM, Beck RW, Bergenstal RM, et al.; T1D Exchange Clinic Network. Evidence of a strong association between frequency of self-monitoring of blood glucose and hemoglobin A_{1c} levels in T1D exchange clinic registry participants. Diabetes Care 2013;36:2009–2014
151. Ziegler R, Heidtmann B, Hilgard D, Hofer S, Rosenbauer J; DPV-Wiss-Initiative. Frequency of SMBG correlates with HbA_{1c} and acute complications in children and adolescents with type 1 diabetes. Pediatr Diabetes 2011;12:11–17
152. Schütt M, Kern W, Krause U, et al.; DPV Initiative. Is the frequency of self-monitoring of blood glucose related to long-term metabolic control? Multicenter analysis including 24,500 patients from 191 centers in Germany and Austria. Exp Clin Endocrinol Diabetes 2006;114: 384–388
153. Yu-Fei W, Wei-Ping J, Ming-Hsun W, et al. Accuracy evaluation of 19 blood glucose monitoring systems manufactured in the Asia-Pacific region: a multicenter study. J Diabetes Sci Technol 2017;11:953–965
154. McQueen RB, Breton MD, Craig J, Holmes H, Whittington MD, Ott MA, et al. Economic value of improved accuracy for self-monitoring of blood glucose devices for type 1 and type 2 diabetes in England. J Diabetes Sci Technol 2018; 12:992–1001
155. McQueen RB, Breton MD, Ott M, Koa H, Beamer B, Campbell JD. Economic value of improved accuracy for self-monitoring of blood glucose devices for type 1 diabetes in Canada. J Diabetes Sci Technol 2015;10:366–377
156. Beck RW, Riddlesworth T, Ruedy K, et al. Effect of continuous glucose monitoring on glycemic control in adults with type 1 diabetes using insulin injections: the DIAMOND randomized clinical trial. JAMA 2017;317:371–378
157. Riddlesworth T, Price D, Cohen N, Beck RW. Hypoglycemic event frequency and the effect of continuous glucose monitoring in adults with type 1 diabetes using multiple daily insulin injections. Diabetes Ther 2017;8:947–951
158. Lind M, Polonsky W, Hirsch IB, et al. Continuous glucose monitoring vs conventional therapy for glycemic control in adults with type 1 diabetes treated with multiple daily insulin injections: the GOLD randomized clinical trial. JAMA 2017;317:379–387
159. Tamborlane WV, Beck RW, Bode BW, et al.; Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 2008;359:1464–1476
160. Hermanns N, Schumann B, Kulzer B, Haak T. The impact of continuous glucose monitoring on low interstitial glucose values and low blood glucose values assessed by point-of-care blood glucose meters: results of a crossover trial. J Diabetes Sci Technol 2014;8:516–522
161. van Beers CAJ, DeVries JH, Kleijer SJ, et al. Continuous glucose monitoring for patients with type 1 diabetes and impaired awareness of hypoglycaemia (IN CONTROL): a randomised, open-label, crossover trial. Lancet Diabetes Endocrinol 2016;4:893–902
162. Pratley RE, Kanapka LG, Rickels MR, et al. Effect of continuous glucose monitoring on hypoglycemia in older adults with type 1 diabetes: a randomized clinical trial. JAMA 2020;323:2397–2406
163. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kröger J, Weitgasser R. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. Lancet 2016;388:2254–2263
164. Paris I, Henry C, Pirard F, Gérard AC, Colin IM. The new FreeStyle libre flash glucose monitoring system improves the glycaemic control in a cohort of people with type 1 diabetes followed in real-life conditions over a period of one year. Endocrinol Diabetes Metab 2018; 1:e00023
165. Charleer S, De Block C, Van Huffel L, et al. Quality of life and glucose control after 1 year of nationwide reimbursement of intermittently scanned continuous glucose monitoring in adults living with type 1 diabetes (FUTURE): a prospective observational real-world cohort study. Diabetes Care 2020;43:389–397
166. Cowart K, Updike W, Bullers K. Systematic review of randomized controlled trials evaluating glycemic efficacy and patient satisfaction of intermittent-scanned continuous glucose monitoring in patients with diabetes. Diabetes Technol Ther 2020;22:337–345
167. Castellana M, Parisi C, Di Molfetta S, et al. Efficacy and safety of flash glucose monitoring in patients with type 1 and type 2 diabetes: a systematic review and meta-analysis. BMJ Open Diabetes Res Care 2020;8:e001092
168. Evans M, Welsh Z, Ells S, Seibold A. The impact of flash glucose monitoring on glycaemic control as measured by HbA_{1c}: a meta-analysis of clinical trials and real-world observational studies. Diabetes Ther 2020;11:83–95
169. Yoo HJ, An HG, Park SY, et al. Use of a real time continuous glucose monitoring system as a motivational device for poorly controlled type 2 diabetes. Diabetes Res Clin Pract 2008;82:73–79
170. Ehrhardt NM, Chellappa M, Walker MS, Fonda SJ, Vigersky RA. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. J Diabetes Sci Technol 2011;5:668–675
171. Beck RW, Riddlesworth TD, Ruedy K, et al. Continuous glucose monitoring versus usual care in patients with type 2 diabetes receiving multiple daily insulin injections: a randomized trial. Ann Intern Med 2017;167:365–374
172. Martens T, Beck RW, Bailey R, et al. Effect of continuous glucose monitoring on glycemic control in patients with type 2 diabetes treated with basal insulin: a randomized clinical trial. JAMA 2021;325:2262–2272
173. Haak T, Hanaire H, Ajjan R, Hermanns N, Riveline JP, Rayman G. Flash glucose-sensing technology as a replacement for blood glucose monitoring for the management of insulin-treated type 2 diabetes: a multicenter, open-label randomized controlled trial. Diabetes Ther 2017;8:55–73
174. Yaron M, Roitman E, Aharon-Hananel G, et al. Effect of flash glucose monitoring technology on glycemic control and treatment satisfaction in patients with type 2 diabetes. Diabetes Care 2019;42:1178–1184
175. Laffel LM, Kanapka LG, Beck RW, et al. Effect of continuous glucose monitoring on glycemic control in adolescents and young adults with type 1 diabetes: a randomized clinical trial. JAMA 2020;323:2388–2396
176. Wong JC, Foster NC, Maahs DM, et al.; T1D Exchange Clinic Network. Real-time continuous glucose monitoring among participants in the T1D Exchange clinic registry. Diabetes Care 2014; 37:2702–2709
177. Foster NC, Miller KM, Tamborlane WV, Bergenstal RM, Beck RW; T1D Exchange Clinic Network. Continuous glucose monitoring in patients with type 1 diabetes using insulin injections. Diabetes Care 2016;39:e81–e82
178. Mauras N, Beck R, Xing D, et al.; Diabetes Research in Children Network (DirecNet) Study Group. A randomized clinical trial to assess the efficacy and safety of real-time continuous glucose monitoring in the management of type 1 diabetes in young children aged 4 to $<$10 years. Diabetes Care 2012;35:204–210
179. Tsalikian E, Fox L, Weinzimer S, et al.; Diabetes Research in Children Network Study Group. Feasibility of prolonged continuous glucose monitoring in toddlers with type 1 diabetes. Pediatr Diabetes 2012;13:301–307
180. Pintus D, Ng SM. Freestyle libre flash glucose monitoring improves patient quality of life measures in children with type 1 diabetes mellitus (T1DM) with appropriate provision of education and support by healthcare professionals. Diabetes Metab Syndr 2019;13:2923–2926
181. Vergier J, Samper M, Dalla-Vale F, et al. Evaluation of flash glucose monitoring after long-term use: a pediatric survey. Prim Care Diabetes 2019;13:63–70
182. Landau Z, Abiri S, Gruber N, et al. Use of flash glucose-sensing technology (FreeStyle Libre) in youth with type 1 diabetes: aWeSoMe study group real-life observational experience. Acta Diabetol 2018;55:1303–1310
183. Deja G, Kłeczek M, Chumięcki M, Strzała-Kłeczek A, Deja R, Jarosz-Chobot P. The usefulness of the Flashtyle Libre system in glycemic control in children with type 1 diabetes during summer camp. Pediatr Endocrinol Diabetes Metab 2018; 24:11–19
184. Feig DS, Donovan LE, Corcoy R, et al.; CONCEPTT Collaborative Group. Continuous Glucose Monitoring in Pregnant Women with Type 1 Diabetes (CONCEPTT): a multicentre international randomised controlled trial. Lancet 2017;390:2347–2359
185. Wei Q, Sun Z, Yang Y, Yu H, Ding H, Wang S. Effect of a CGMS and SMBG on maternal and neonatal outcomes in gestational diabetes mellitus: a randomized controlled trial. Sci Rep 2016;6:19920

186. U.S. Food and Drug Administration. Meeting of the Clinical Chemistry and Clinical Toxicology Devices. Panel P160048. Eversense Continuous Glucose Monitoring System Senseonics, Inc. Accessed 5 September 2020. Available from https://www.fda.gov/media/112110/download
187. U.S. Food and Drug Administration. FDA Summary of Safety and Effectiveness Data. FreeStyle Libre 14 Day Flash Glucose Monitoring System. PMA P160030/S017. Abbott Diabetes Care, Inc. Accessed 12 May 2020. Available from https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160030S017B.pdf
188. U.S. Food and Drug Administration. FDA Summary of Safety and Effectiveness Data, 2018. PMA P160007. Accessed 12 May 2020. Available from https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160007b.pdf
189. U.S. Food and Drug Administration. Evaluation of Automatic Class III Designation for Dexcom G6 Continuous Glucose Monitoring System. Accessed 12 May 2020. Available from https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170088.pdf
190. Facchinetti A. Continuous glucose monitoring sensors: past, present and future algorithmic challenges. Sensors (Basel) 2016;16:E2093
191. Aleppo G, Ruedy KJ, Riddlesworth TD, et al. REPLACE-BG: a randomized trial comparing continuous glucose monitoring with and without routine blood glucose monitoring in adults with well-controlled type 1 diabetes. Diabetes Care 2017;40:538–545
192. Danne T, Nimri R, Battelino T, et al. International consensus on use of continuous glucose monitoring. Diabetes Care 2017;40:1631–1640
193. Battelino T, Danne T, Bergenstal RM, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus on time in range. Diabetes Care 2019;42:1593–1603
194. U.S. Food and Drug Administration. Enforcement Policy for Non-Invasive Remote Monitoring Devices Used to Support Patient Monitoring During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency (Revised), 2020. Accessed 2 September 2020. Available from https://www.fda.gov/media/136290/download
195. Zhang R, Liu S, Jin H, Luo Y, Zheng Z, Gao F, et al. Noninvasive electromagnetic wave sensing of glucose. Sensors (Basel) 2019;19:E1151
196. Li J, Igbe T, Liu Y, Nie Z, Qin W, Wang L, et al. An approach for noninvasive blood glucose monitoring based on bioimpedance difference considering blood volume pulsation. IEEE Access 2018;6:51119–51129
197. Lan YT, Kuang YP, Zhou LP, Wu GY, Gu PC, Wei HJ, et al. Noninvasive monitoring of blood glucose concentration in diabetic patients with optical coherence tomography. Laser Phys Lett 2017;14:035603
198. Chen T-L, Lo Y-L, Liao C-C, Phan Q-H. Noninvasive measurement of glucose concentration on human fingertip by optical coherence tomography. J Biomed Opt 2018;23:1–9
199. Yamakoshi Y, Matsumura K, Yamakoshi T, et al. Side-scattered finger-photoplethysmography: experimental investigations toward practical noninvasive measurement of blood glucose. J Biomed Opt 2017;22:67001
200. Mesch M, Zhang C, Braun PV, Giessen H. Functionalized hydrogel on plasmonic nanoantennas for noninvasive glucose sensing. ACS Photonics 2015;2:475–480
201. Huang S, Omkar, Yoshida Y, Inda A, Chia XX, Mu WC, et al. Microstrip line-based glucose sensor for noninvasive continuous monitoring using the main field for sensing and multivariable crosschecking. IEEE Sens J 2019;19:535–547
202. Omkar YW, Huang S. T-shaped patterned microstrip line for noninvasive continuous glucose sensing. IEEE Microw Wirel Compon Lett 2018;28:942–944
203. Li J, Yang D, Nie Z, Liu Y, Wang L. Investigation of resonant frequency and impedance for noninvasive blood glucose monitoring. In *2017 IEEE 7th Annual International Conference on CYBER Technology in Automation, Control, and Intelligent Systems (CYBER),* 2017. Washington, DC, IEEE, p. 1649–1652
204. Geng Z, Tang F, Ding Y, Li S, Wang X. Noninvasive continuous glucose monitoring using a multisensor-based glucometer and time series analysis. Sci Rep 2017;7:12650
205. Yadav J, Singh V, Murari BM. Investigations on multisensor-based noninvasive blood glucose measurement system. J Med Device 2017;11:031006
206. Chowdhury MK, Srivastava A, Sharma N, Sharma S. Noninvasive blood glucose measurement utilizing a newly designed system based on modulated ultrasound and infrared light. Int J Diabetes Dev Ctries 2015;36:439–448
207. Kitazaki T, Kawashima N, Yamamoto N, et al. Parametric standing wave generation of a shallow reflection plane in a nonrigid sample for use in a noninvasive blood glucose monitor. J Biomed Opt 2019;24:1–7
208. Zhai Q, Gong S, Wang Y, et al. Enokitake mushroom-like standing gold nanowires toward wearable noninvasive bimodal glucose and strain sensing. ACS Appl Mater Interfaces 2019;11:9724–9729
209. Karpova EV, Shcherbacheva EV, Galushin AA, Vokhmyanina DV, Karyakina EE, Karyakin AA. Noninvasive diabetes monitoring through continuous analysis of sweat using flow-through glucose biosensor. Anal Chem 2019;91:3778–3783
210. Liu J, Liu R, Xu K. Accuracy of noninvasive glucose sensing based on near-infrared spectroscopy. Appl Spectrosc 2015;69:1313–1318
211. Jintao X, Liming Y, Yufei L, Chunyan L, Han C. Noninvasive and fast measurement of blood glucose in vivo by near infrared (NIR) spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc 2017;179:250–254
212. Haxha S, Jhoja J. Optical based noninvasive glucose monitoring sensor prototype. IEEE Photonics J 2016;8:1–11
213. Ramasahayam S, Koppuravuri SH, Arora L, Chowdhury SR. Noninvasive blood glucose sensing using near infra-red spectroscopy and artificial neural networks based on inverse delayed function model of neuron. J Med Syst 2015;39:166
214. Hu Y-X, Liu R, Zhang W, Xu K-X. Application of two-dimensional near-infrared correlation spectroscopy in the specificity analysis of noninvasive blood glucose sensing. Guangpuxue Yu Guangpu Fenxi 2017;37:491–496
215. Han G, Yu X, Xia D, Liu R, Liu J, Xu K. Preliminary clinical validation of a differential correction method for improving measurement accuracy in noninvasive measurement of blood glucose using near-infrared spectroscopy. Appl Spectrosc 2017;71:2177–2186
216. Lü X-F, Zhang T-L, Xiao F, Li G, Wang Y. Noninvasive blood glucose analysis based on near-infrared reflectance spectroscopy. Guangpuxue Yu Guangpu Fenxi 2016;36:2312–2317
217. Kasahara R, Kino S, Soyama S, Matsuura Y. Noninvasive glucose monitoring using mid-infrared absorption spectroscopy based on a few wavenumbers. Biomed Opt Express 2017;9:289–302
218. Werth A, Liakat S, Dong A, Woods CM, Gmachl CF. Implementation of an integrating sphere for the enhancement of noninvasive glucose detection using quantum cascade laser spectroscopy. Appl Phys B 2018;124:75
219. Pandey R, Paidi SK, Valdez TA, et al. Noninvasive monitoring of blood glucose with Raman spectroscopy. Acc Chem Res 2017;50:264–272
220. Shih WC, Bechtel KL, Rebec MV. Noninvasive glucose sensing by transcutaneous Raman spectroscopy. J Biomed Opt 2015;20:051036
221. Zheng Y, Zheng Y, Zhu X, et al. Noninvasive blood glucose detection using a miniature wearable Raman spectroscopy system. Chin Opt Lett, COL 2017;15:083001
222. Shih WC. Constrained regularization for noninvasive glucose sensing using Raman spectroscopy. J Innov Opt Health Sci 2014;8:1550022
223. Li N, Zang H, Sun H, Jiao X, Wang K, Liu TC-Y, et al. A noninvasive accurate measurement of blood glucose levels with Raman spectroscopy of blood in microvessels. Molecules 2019;24:E1500
224. Choi H, Naylon J, Luzio S, Beutler J, Birchall J, Martin C, et al. Design and in vitro interference test of microwave noninvasive blood glucose monitoring sensor. IEEE Trans Microw Theory Tech 2015;63:3016–3025
225. Vrba J, Karch J, Vrba D. Phantoms for development of microwave sensors for noninvasive blood glucose monitoring. Int J Antennas Propag 2015;2015:1–5
226. Xiao X, Li Q. A noninvasive measurement of blood glucose concentration by UWB microwave spectrum. IEEE Antennas Wirel Propag Lett 2017;16:1040–1043
227. Turgul V, Kale I. Characterization of the complex permittivity of glucose/water solutions for noninvasive RF/mMicrowave blood glucose sensing. In *2016 IEEE International Instrumentation and Measurement Technology Conference Proceedings,* 2016. Washington, DC, IEEE
228. Choi H, Luzio S, Beutler J, Porch A. Microwave noninvasive blood glucose monitoring sensor: human clinical trial results. In *2017 IEEE MTT-S International Microwave Symposium (IMS),* 2017. Washington, DC, IEEE
229. Zhang R, Gao F, Feng X, et al. Noninvasive photoacoustic measurement of glucose by data fusion. Analyst (Lond) 2017;142:2892–2896
230. Pai PP, De A, Banerjee S. Accuracy enhancement for noninvasive glucose estimation using dual-wavelength photoacoustic measurements and kernel-based calibration. IEEE Trans Instrum Meas 2018;67:126–136

231. Zhang R, Gao F, Feng X, et al. "Guide star" assisted noninvasive photoacoustic measurement of glucose. ACS Sens 2018;3:2550–2557
232. Tanaka Y, Purtill C, Tajima T, Seyama M, Koizumi H. Sensitivity improvement on CW dual-wavelength photoacoustic spectroscopy using acoustic resonant mode for noninvasive glucose monitor. In *IEEE Sensors* 2016. Washington, DC, IEEE
233. Tachibana K, Okada K, Kobayashi R, Ishihara Y. Development of a high-sensitivity and portable cell using Helmholtz resonance for noninvasive blood glucose-level measurement based on photoacoustic spectroscopy. In *38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC),* 2016. Washington, DC, IEEE. p. 6477–6480
234. Tanaka Y, Higuchi Y, Camou S. Noninvasive measurement of aqueous glucose solution at physiologically relevant blood concentration levels with differential continuous-wave laser photoacoustic technique. In *IEEE Sensors* 2015. Washington, DC, IEEE. p. 1–4
235. Small GW. Chemometrics and near-infrared spectroscopy: avoiding the pitfalls. Trends Analyt Chem 2006;25:1057–1066
236. Rothberg LJ, Lees T, Clifton-Bligh R, Lal S. Association between heart rate variability measures and blood glucose levels: implications for noninvasive glucose monitoring for diabetes. Diabetes Technol Ther 2016;18:366–376
237. Segman YJ. Device and method for non-invasive glucose assessment. J Diabetes Sci Technol 2018;12:1159–1168
238. Pfützner A, Strobl S, Demircik F, Redert L, Pfützner J, Pfützner AH, et al. Evaluation of a new noninvasive glucose monitoring device by means of standardized meal experiments. J Diabetes Sci Technol 2018;12:1178–1183
239. Segman Y. Combination non-invasive and invasive bioparameter measuring device. US8948833B2, 2015. Accessed 3 March 2022. Available from https://patents.google.com/patent/US8948833B2/fi
240. Vahlsing T, Delbeck S, Leonhardt S, Heise HM. Noninvasive monitoring of blood glucose using color-coded photoplethysmographic images of the illuminated fingertip within the visible and near-infrared range: opportunities and questions. J Diabetes Sci Technol 2018;12:1169–1177
241. Ascaso FJ, Huerva V. Noninvasive continuous monitoring of tear glucose using glucose-sensing contact lenses. Optom Vis Sci 2016;93:426–434
242. Ruan J-L, Chen C, Shen J-H, Zhao X-L, Qian S-H, Zhu Z-G. A gelated colloidal crystal attached lens for noninvasive continuous monitoring of tear glucose. Polymers (Basel) 2017;9:E125
243. Baca JT, Finegold DN, Asher SA. Tear glucose analysis for the noninvasive detection and monitoring of diabetes mellitus. Ocul Surf 2007;5:280–293
244. Baca JT, Taormina CR, Feingold E, Finegold DN, Grabowski JJ, Asher SA. Mass spectral determination of fasting tear glucose concentrations in nondiabetic volunteers. Clin Chem 2007;53:1370–1372
245. Cha KH, Jensen GC, Balijepalli AS, Cohan BE, Meyerhoff ME. Evaluation of commercial glucometer test strips for potential measurement of glucose in tears. Anal Chem 2014;86:1902–1908
246. Peng B, Lu J, Balijepalli AS, Major TC, Cohan BE, Meyerhoff ME. Evaluation of enzyme-based tear glucose electrochemical sensors over a wide range of blood glucose concentrations. Biosens Bioelectron 2013;49:204–209
247. Yan Q, Peng B, Su G, Cohan BE, Major TC, Meyerhoff ME. Measurement of tear glucose levels with amperometric glucose biosensor/capillary tube configuration. Anal Chem 2011;83:8341–8346
248. Metzger BE, Lowe LP, Dyer AR, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002
249. Azeez O, Kulkarni A, Kuklina EV, Kim SY, Cox S. Hypertension and diabetes in non-pregnant women of reproductive age in the United States. Prev Chronic Dis 2019;16:E146
250. Committee on Practice Bulletins—Obstetrics. ACOG Practice Bulletin No. 190: gestational diabetes mellitus. Obstet Gynecol 2018;131:e49–e64
251. American Diabetes Association Professional Practice Committee. 15. Management of diabetes in pregnancy: *Standards of Medical Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl. 1):S232–S243
252. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964;13:278–285
253. Vandorsten JP, Dodson WC, Espeland MA, et al. NIH consensus development conference: diagnosing gestational diabetes mellitus. NIH Consens State Sci Statements 2013;29:1–31
254. Anonymous. NIH consensus development conference statement: diagnosing gestational diabetes mellitus, March 4-6, 2013. Obstet Gynecol 2013;122:358–369
255. Sacks DB. Diagnosis of gestational diabetes mellitus: it is time for international consensus. Clin Chem 2014;60:141–143
256. Metzger BE, Persson B, Lowe LP, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome study: neonatal glycemia. Pediatrics 2010;126:e1545–e1552
257. Yogev C, Chen, Hod, et al.; Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: preeclampsia. Am J Obstet Gynecol 2010;202:255.e1–255.e7
258. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA; International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676–682
259. Colagiuri S, Falavigna M, Agarwal MM, et al. Strategies for implementing the WHO diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Diabetes Res Clin Pract 2014;103:364–372
260. American Diabetes Association. Standards of medical care in diabetes—2014. Diabetes Care 2014;37(Suppl. 1):S14–S80
261. Hillier TA, Pedula KL, Ogasawara KK, Vesco KK, Oshiro CES, Lubarsky SL, et al. A pragmatic, randomized clinical trial of gestational diabetes screening. N Engl J Med 2021;384:895–904
262. Coustan DR, Dyer AR, Metzger BE. One-step or 2-step testing for gestational diabetes: which is better? Am J Obstet Gynecol 2021;225:634–644
263. McIntyre HD, Oats JJN, Kihara AB, et al. Update on diagnosis of hyperglycemia in pregnancy and gestational diabetes mellitus from FIGO's Pregnancy & Non-Communicable Diseases Committee. Int J Gynaecol Obstet 2021;154:189–194
264. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS; Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477–2486
265. Landon MB, Spong CY, Thom E, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med 2009;361:1339–1348
266. Gillman MW, Oakey H, Baghurst PA, Volkmer RE, Robinson JS, Crowther CA. Effect of treatment of gestational diabetes mellitus on obesity in the next generation. Diabetes Care 2010;33:964–968
267. Landon MB, Rice MM, Varner MW, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (MFMU) Network. Mild gestational diabetes mellitus and long-term child health. Diabetes Care 2015;38:445–452
268. Lowe WL Jr, Scholtens DM, Lowe LP, et al.; HAPO Follow-up Study Cooperative Research Group. Association of gestational diabetes with maternal disorders of glucose metabolism and childhood adiposity. JAMA 2018;320:1005–1016
269. Lowe WL Jr, Scholtens DM, Kuang A, et al.; HAPO Follow-up Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcome follow-up study (HAPO FUS): maternal gestational diabetes mellitus and childhood glucose metabolism. Diabetes Care 2019;42:372–380
270. Hawkins JS, Casey BM, Lo JY, Moss K, McIntire DD, Leveno KJ. Weekly compared with daily blood glucose monitoring in women with diet-treated gestational diabetes. Obstet Gynecol 2009;113:1307–1312
271. Mendez-Figueroa H, Daley J, Lopes VV, Coustan DR. Comparing daily versus less frequent blood glucose monitoring in patients with mild gestational diabetes. J Matern Fetal Neonatal Med 2013;26:1268–1272
272. Mosca A, Paleari R, Dalfrà MG, et al. Reference intervals for hemoglobin A_{1c} in pregnant women: data from an Italian multi-center study. Clin Chem 2006;52:1138–1143
273. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. BMJ 2020;369:m1361
274. Ratner RE, Christophi CA, Metzger BE, et al.; Diabetes Prevention Program Research Group. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. J Clin Endocrinol Metab 2008;93:4774–4779
275. Li Z, Cheng Y, Wang D, Chen H, Chen H, Ming W-K, et al. Incidence rate of type 2 diabetes mellitus after gestational diabetes mellitus: a

systematic review and meta-analysis of 170,139 women. J Diabetes Res 2020;2020:3076463
276. American Diabetes Association. Standardization of the oral glucose tolerance test. Report of the Committee on Statistics of the American Diabetes Association June 14, 1968. Diabetes 1969;18:299–307
277. Daly N, Flynn I, Carroll C, Farren M, McKeating A, Turner MJ. Impact of implementing preanalytical laboratory standards on the diagnosis of gestational diabetes Mmellitus: a prospective observational study. Clin Chem 2016;62:387–391
278. National Institute of Diabetes and Digestive and Kidney Diseases. Diabetes in America. 3rd Ed. Washington, DC, National Institute of Diabetes and Digestive and Kidney Diseases. Accessed 27 August 2019. Available from https://www.niddk.nih.gov/about-niddk/strategic-plans-reports/diabetes-in-america-3rd-edition
279. Zhu W-W, Yang H-X, Wei Y-M, et al. Evaluation of the value of fasting plasma glucose in the first prenatal visit to diagnose gestational diabetes mellitus in China. Diabetes Care 2013; 36:586–590
280. Wexler DJ, Powe CE, Barbour LA, et al. Research gaps in gestational diabetes mellitus: executive summary of a National Institute of Diabetes and Digestive and Kidney Diseases workshop. Obstet Gynecol 2018;132:496–505
281. Sweeting AN, Ross GP, Hyett J, Molyneaux L, Constantino M, Harding AJ, et al. Gestational diabetes mellitus in early pregnancy: evidence for poor pregnancy outcomes despite treatment. Diabetes Care 2016;39:75–81
282. Wei Y-M, Liu X-Y, Shou C, et al. Value of fasting plasma glucose to screen gestational diabetes mellitus before the 24th gestational week in women with different pre-pregnancy body mass index. Chin Med J (Engl) 2019;132: 883–888
283. Mills JL, Jovanovic L, Knopp R, et al. Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study. Metabolism 1998;47:1140–1144
284. Powe CE. Early pregnancy biochemical predictors of gestational diabetes mellitus. Curr Diab Rep 2017;17:12
285. Mañé L, Flores-Le Roux JA, Benaiges D, et al. Role of first-trimester Hb_{1c} as a predictor of adverse obstetric outcomes in a multiethnic cohort. J Clin Endocrinol Metab 2017;102:390–397
286. Fong A, Serra AE, Gabby L, Wing DA, Berkowitz KM. Use of hemoglobin A_{1c} as an early predictor of gestational diabetes mellitus. Am J Obstet Gynecol 2014;211:641.e1–641.e7
287. Ghosh P, Luque-Fernandez MA, Vaidya A, et al. Plasma glycated CD59, a novel biomarker for detection of pregnancy-induced glucose intolerance. Diabetes Care 2017;40:981–984
288. Oztas E, Ozler S, Ersoy E, et al. Prediction of gestational diabetes mellitus by first trimester serum secreted frizzle-related protein-5 levels. J Matern Fetal Neonatal Med 2016;29:1515–1519
289. McIntyre HD, Jensen DM, Jensen RC, Kyhl HB, Jensen TK, Glintborg D, et al. Gestational diabetes mellitus: does one size fit all? A challenge to uniform worldwide diagnostic thresholds. Diabetes Care 2018;41:1339–1342
290. Kyhl HB, Jensen TK, Barington T, et al. The Odense Child Cohort: aims, design, and cohort profile. Paediatr Perinat Epidemiol 2015;29:250–258
291. Agarwal MM, Dhatt GS, Shah SM. Gestational diabetes mellitus: simplifying the international association of diabetes and pregnancy diagnostic algorithm using fasting plasma glucose. Diabetes Care 2010;33:2018–2020
292. McIntyre HD, Gibbons KS, Ma RCW, et al. Testing for gestational diabetes during the COVID-19 pandemic. An evaluation of proposed protocols for the United Kingdom, Canada and Australia. Diabetes Res Clin Pract 2020;167: 108353
293. Hod M, Kapur A, Sacks DA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet 2015; 131(Suppl. 3):S173–S211
294. Bilous RW, Jacklin PB, Maresh MJ, Sacks DA. Resolving the gestational diabetes diagnosis conundrum: the need for a randomized controlled trial of treatment. Diabetes Care 2021 ;44:858–864
295. Goldstein DE, Little RR, Lorenz RA, et al. Tests of glycemia in diabetes. Diabetes Care 2004; 27:1761–1773
296. IDF Clinical Guidelines Task Force. Global guideline for type 2 diabetes: recommendations for standard, comprehensive, and minimal care. Diabet Med 2006;23:579–593
297. International Diabetes Federation Guideline Development Group. Global guideline for type 2 diabetes. Diabetes Res Clin Pract 2014;104:1–52
298. Dhatariya KK, Glaser NS, Codner E, Umpierrez GE. Diabetic ketoacidosis. Nat Rev Dis Primers 2020;6:40
299. Kilpatrick ES, Butler AE, Ostlundh L, Atkin SL, Sacks DB. Controversies around the measurement of blood ketones to diagnose and manage diabetic ketoacidosis. Diabetes Care 2022;45:267–272
300. Schwab TM, Hendey GW, Soliz TC. Screening for ketonemia in patients with diabetes. Ann Emerg Med 1999;34:342–346
301. Brooke J, Stiell M, Ojo O. Evaluation of the accuracy of capillary hydroxybutyrate measurement compared with other measurements in the diagnosis of diabetic ketoacidosis: a systematic review. Int J Environ Res Public Health 2016;13:E837
302. Laffel LMB, Wentzell K, Loughlin C, Tovar A, Moltz K, Brink S. Sick day management using blood 3-hydroxybutyrate (3-OHB) compared with urine ketone monitoring reduces hospital visits in young people with T1DM: a randomized clinical trial. Diabet Med 2006;23:278–284
303. American Diabetes Association Professional Practice Committee. 6. Glycemic targets: *Standards of Medical Care in Diabetes—2021*. Diabetes Care 2021;44(Suppl. 1):S73–S84
304. Sacks DB. Diabetes mellitus. In Tietz Textbook of Laboratory Medicine. 7th ed. Rifai N, Chiu R, Young I, Burnham C-A, Wittwer C, Eds. St. Louis, Elsevier, 2023, p. 502–543
305. Custer EM, Myers JL, Poffenbarger PL, Schoen I. The storage stability of 3-hydroxybutyrate in serum, plasma, and whole blood. Am J Clin Pathol 1983;80:375–380
306. Yu HYE, Agus M, Kellogg MD. Clinical utility of Abbott Precision Xceed Pro ketone meter in diabetic patients. Pediatr Diabetes 2011;12:649–655
307. Danne T, Garg S, Peters AL, et al. International consensus on risk management of diabetic ketoacidosis in patients with type 1 diabetes treated with sodium-glucose cotransporter (SGLT) inhibitors. Diabetes Care 2019;42: 1147–1154
308. Noyes KJ, Crofton P, Bath LE, et al. Hydroxybutyrate near-patient testing to evaluate a new end-point for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. Pediatr Diabetes 2007;8:150–156
309. Tran TTT, Pease A, Wood AJ, Zajac JD, Mårtensson J, Bellomo R, et al. Review of evidence for adult diabetic ketoacidosis management protocols. Front Endocrinol (Lausanne) 2017;8:106
310. Benoit SR, Hora I, Pasquel FJ, Gregg EW, Albright AL, Imperatore G. Trends in emergency department visits and inpatient admissions for hyperglycemic crises in adults with diabetes in the U.S., 2006–2015. Diabetes Care 2020;43: 1057–1064
311. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med 1981; 70:325–330
312. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Chem 1986;32(Suppl.):B64–B70
313. Svendsen PA, Lauritzen T, Søegaard U, Nerup J. Glycosylated haemoglobin and steady-state mean blood glucose concentration in type 1 (insulin-dependent) diabetes. Diabetologia 1982; 23:403–405
314. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin measured by automated affinity HPLC correlates with both short-term and long-term antecedent glycemia. Clin Chem 1994;40:1317–1321
315. Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. Br Med J (Clin Res Ed) 1984;288:1484–1486
316. Tahara Y, Shima K. Kinetics of HbA_{1c}, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995;18: 440–447
317. Nathan DM, McGee P, Steffes MW; DCCT/EDIC Research Group. Relationship of glycated albumin to blood glucose and HbA_{1c} values and to retinopathy, nephropathy, and cardiovascular outcomes in the DCCT/EDIC study. Diabetes 2014;63:282–290
318. Sacks DB. A_{1c} versus glucose testing: a comparison. Diabetes Care 2011;34:518–523
319. Selvin E, Wang D, Matsushita K, Grams ME, Coresh J. Prognostic implications of single-sample confirmatory testing for undiagnosed diabetes: a prospective cohort study. Ann Intern Med 2018; 169:156–164
320. Droumaguet C, Balkau B, Simon D, et al.; DESIR Study Group. Use of HbA_{1c} in predicting progression to diabetes in French men and women: data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care 2006;29:1619–1625
321. Edelman D, Olsen MK, Dudley TK, Harris AC, Oddone EZ. Utility of hemoglobin A_{1c} in

predicting diabetes risk. J Gen Intern Med 2004; 19:1175–1180
322. Little RR, England JD, Wiedmeyer HM, et al. Glycated haemoglobin predicts progression to diabetes mellitus in Pima Indians with impaired glucose tolerance. Diabetologia 1994;37:252–256
323. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A_{1c} point-of-care instruments do not meet the general accepted analytical performance criteria. Clin Chem 2010;56:44–52
324. Hirst JA, McLellan JH, Price CP, et al. Performance of point-of-care HbA_{1c} test devices: implications for use in clinical practice—a systematic review and meta-analysis. Clin Chem Lab Med 2017;55:167–180
325. Nathan DM, Griffin A, Perez FM, Basque E, Do L, Steiner B. Accuracy of a point-of-care hemoglobin A_{1c} assay. J Diabetes Sci Technol 2019;13:1149–1153
326. U.S. Centers for Medicare & Medicaid Services. CLIA brochures. Accessed 26 August 2022. Available from https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures
327. American Diabetes Association. Implications of the diabetes control and complications trial. Diabetes Care 2000;23(Suppl. 1):S24–S26
328. Nathan DM, Buse JB, Davidson MB, et al.; Professional Practice Committee, American Diabetes Association; European Association for the Study of Diabetes. Management of hyperglycaemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia 2006;49:1711–1721
329. Larsen ML, Hørder M, Mogensen EF. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. N Engl J Med 1990;323:1021–1025
330. Berg AH, Sacks DB. Haemoglobin A_{1c} analysis in the management of patients with diabetes: from chaos to harmony. J Clin Pathol 2008;61:983–987
331. Qaseem A, Vijan S, Snow V, Cross JT, Weiss KB; Clinical Efficacy Assessment Subcommittee of the American College of Physicians. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A_{1c} targets. A guidance statement from the American College of Physicians. Ann Intern Med 2007;147:417–422
332. Brown SA, Kovatchev BP, Raghinaru D, et al.; iDCL Trial Research Group. Six-month randomized, multicenter trial of closed-loop control in type 1 diabetes. N Engl J Med 2019; 381:1707–1717
333. Kitzmiller JL, Block JM, Brown FM, et al. Managing preexisting diabetes for pregnancy. Diabetes Care 2008;31:1060–1079
334. Pani LN, Korenda L, Meigs JB, et al. Effect of aging on A_{1c} levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care 2008;31:1991–1996
335. Ziemer DC, Kolm P, Weintraub WS, et al. Glucose-independent, black-white differences in hemoglobin A_{1c} levels: a cross-sectional analysis of 2 studies. Ann Intern Med 2010;152:770–777
336. Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010;362: 800–811
337. Little RR, Sacks DB. HbA_{1c}: how do we measure it and what does it mean? Curr Opin Endocrinol Diabetes Obes 2009;16:113–118
338. Herman WH, Ma Y, Uwaifo G, et al.; Diabetes Prevention Program Research Group. Differences in A_{1c} by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care 2007;30:2453–2457
339. Saaddine JB, Fagot-Campagna A, Rolka D, et al. Distribution of $HbA_{(1c)}$ levels for children and young adults in the U.S.: Third National Health and Nutrition Examination Survey. Diabetes Care 2002; 25:1326–1330
340. Parrinello CM, Sharrett AR, Maruthur NM, et al. Racial differences in and prognostic value of biomarkers of hyperglycemia. Diabetes Care 2016;39:589–595
341. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D; A_{1c}-Derived Average Glucose Study Group. Translating the A_{1c} assay into estimated average glucose values. Diabetes Care 2008;31:1473–1478
342. Bergenstal RM, Gal RL, Connor CG, et al.; T1D Exchange Racial Differences Study Group. Racial differences in the relationship of glucose concentrations and hemoglobin A_{1c} levels. Ann Intern Med 2017;167:95–102
343. Malka R, Nathan DM, Higgins JM. Mechanistic modeling of hemoglobin glycation and red blood cell kinetics enables personalized diabetes monitoring. Sci Transl Med 2016;8: 359ra130
344. Davie SJ, Gould BJ, Yudkin JS. Effect of vitamin C on glycosylation of proteins. Diabetes 1992;41:167–173
345. Ceriello A, Giugliano D, Quatraro A, Donzella C, Dipalo G, Lefebvre PJ. Vitamin E reduction of protein glycosylation in diabetes. New prospect for prevention of diabetic complications? Diabetes Care 1991;14:68–72
346. Tarim O, Küçükerdoğan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A_{1c} in type 1 diabetes mellitus. Pediatr Int 1999;41:357–362
347. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem 2001;47:153–163
348. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW, Krejs GJ. Evaluation of Hb_{1c} determination methods in patients with hemoglobinopathies. Diabetes Care 2000;23: 339–344
349. Rohlfing C, Hanson S, Estey MP, Bordeleau P, Little RR. Evaluation of interference from hemoglobin C, D, E and S traits on measurements of hemoglobin A_{1c} by fifteen methods. Clin Chim Acta 2021;522:31–35
350. Little RR, La'ulu SL, Hanson SE, Rohlfing CL, Schmidt RL. Effects of 49 different rare HhHb variants on HbA_{1c} measurement in eight methods. J Diabetes Sci Technol 2015;9:849–856
351. Rohlfing CL, Hanson S, Tennill AL, Little RR. Effects of whole blood storage on hemoglobin a_{1c} measurements with five current assay methods. Diabetes Technol Ther 2012;14:271–275
352. Little RR, Rohlfing CL, Tennill AL, Connolly S, Hanson S. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. Diabetes Technol Ther 2007;9: 36–42
353. Beck RW, Bocchino LE, Lum JW, et al. An evaluation of two capillary sample collection kits for laboratory measurement of HbA_{1c}. Diabetes Technol Ther 2021;23:537–545
354. Nathan DM, Krause-Steinrauf H, Braffett BH, et al.; GRADE Research Group; DCCT/EDIC Research Group. Comparison of central laboratory HbA_{1c} measurements obtained from a capillary collection versus a standard venous whole blood collection in the GRADE and EDIC studies. PLoS One 2021;16:e0257154
355. Little RR, Wiedmeyer HM, Huang DH, Goldstein DE, Parson RG, Kowal R, et al. A simple blood collection device for analysis of glycohemoglobin (GHB). Clin Chem 1998;44:A139
356. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Effect of calibration on dispersion of glycohemoglobin values determined by 111 laboratories using 21 methods. Clin Chem 1994; 40:138–144
357. Little RR, Rohlfing C, Sacks DB. The national glycohemoglobin standardization program: over 20 years of improving hemoglobin A_{1c} measurement. Clin Chem 2019;65:839–848
358. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB; NGSP Steering Committee. The national glycohemoglobin standardization program: a five-year progress report. Clin Chem 2001;47:1985–1992
359. Goldstein DE, Little RR. Bringing order to chaos: the National Glycohemoglobin Standardization Program. Contemp Intern Med 1997; 9:27–32
360. Steffes M, Cleary P, Goldstein D, et al. Hemoglobin A_{1c} measurements over nearly two decades: sustaining comparable values throughout the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications study. Clin Chem 2005;51:753–758
361. Little RR, Rohlfing CL; National Glycohemoglobin Standardization Program (NGSP) Steering Committee. Status of hemoglobin A_{1c} measurement and goals for improvement: from chaos to order for improving diabetes care. Clin Chem 2011;57:205–214
362. Jeppsson JO, Kobold U, Barr J, et al.; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Approved IFCC reference method for the measurement of HbA_{1c} in human blood. Clin Chem Lab Med 2002; 40:78–89
363. Hoelzel W, Weykamp C, Jeppsson JO, et al.; IFCC Working Group on HbA_{1c} Standardization. IFCC reference system for measurement of hemoglobin A_{1c} in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166–174
364. Weykamp C, John WG, Mosca A, et al. The IFCC Reference Measurement System for HbA_{1c}: a 6-year progress report. Clin Chem 2008;54: 240–248
365. Marshall SM, Barth JH. Standardization of HbA_{1c} measurements: a consensus statement. Ann Clin Biochem 2000;37:45–46
366. Sacks DB. Participant Summary for Hemoglobin A1c Survey 2020 Set GH5-C. Northfield, IL, College of American Pathologists, 2020

367. European Federation of Clinical Chemistry and Laboratory Medicine. EFLM Biological Variation. Biological Variation Database. Accessed 3 March 2022. Available from https://biologicalvariation.eu/
368. Goldstein DE, Peth SB, England JD, Hess RL, Da Costa J. Effects of acute changes in blood glucose on HbA_{1c}. Diabetes 1980;29:623–628
369. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA_{1c} levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. Diabetes Care 1999;22:1785–1789
370. Kennedy L, Herman WH, Strange P, Harris A; GOAL AIC Team. Impact of active versus usual algorithmic titration of basal insulin and point-of-care versus laboratory measurement of HbA_{1c} on glycemic control in patients with type 2 diabetes: the glycemic optimization with algorithms and labs at point of care (GOAL A_{1c}) trial. Diabetes Care 2006;29:1–8
371. Khunti K, Stone MA, Burden AC, et al. Randomised controlled trial of near-patient testing for glycated haemoglobin in people with type 2 diabetes mellitus. Br J Gen Pract 2006;56:511–517
372. Geistanger A, Arends S, Berding C, et al.; IFCC Working Group on Standardization of Hemoglobin A_{1c}. Statistical methods for monitoring the relationship between the IFCC reference measurement procedure for hemoglobin A_{1c} and the designated comparison methods in the United States, Japan, and Sweden. Clin Chem 2008;54:1379–1385
373. Murata GH, Hoffman RM, Duckworth WC, Wendel CS; Diabetes Outcomes in Veterans Study. Contributions of weekly mean blood glucose values to hemoglobin A_{1c} in insulin-treated type 2 diabetes: the Diabetes Outcomes in Veterans Study (DOVES). Am J Med Sci 2004;327:319–323
374. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50:2239–2244
375. Hanas R, John G; International $HbA_{(1c)}$ Consensus Committee. 2010 Consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. Clin Chem 2010;56:1362–1364
376. Sacks DB. 2011 Consensus meeting on the worldwide standardization of hemoglobin $A_{(1c)}$ measurement. Clin Chem 2013;59:857–858
377. Makita Z, Radoff S, Rayfield EJ, et al. Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med 1991;325:836–842
378. Monnier VM, Bautista O, Kenny D, et al. Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA_{1c} as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes 1999;48:870–880
379. Graham J, Hagopian WA, Kockum I, et al.; Diabetes Incidence in Sweden Study Group; Swedish Childhood Diabetes Study Group. Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. Diabetes 2002;51:1346–1355
380. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med 2000;343:702–709
381. Edghill EL, Flanagan SE, Patch A-M, et al.; Neonatal Diabetes International Collaborative Group. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes 2008;57:1034–1042
382. Støy J, Greeley SAW, Paz VP, et al.; United States Neonatal Diabetes Working Group. Diagnosis and treatment of neonatal diabetes: a United States experience. Pediatr Diabetes 2008;9:450–459
383. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab 2008;4:200–213
384. Carlsson A, Shepherd M, Ellard S, et al. Absence of islet autoantibodies and modestly raised glucose values at diabetes diagnosis should lead to testing for MODY: lessons from a 5-year pediatric Swedish National Cohort Study. Diabetes Care 2020;43:82–89
385. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345:971–980
386. Barrett JC, Clayton DG, Concannon P, et al.; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703–707
387. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009;360:1646–1654
388. Sharp SA, Rich SS, Wood AR, et al. Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis. Diabetes Care 2019;42:200–207
389. Robertson CC, Inshaw JRJ, Onengut-Gumuscu S, et al.; Type 1 Diabetes Genetics Consortium. Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. Nat Genet 2021;53:962–971
390. Ferrat LA, Vehik K, Sharp SA, et al.; TEDDY Study Group. A combined risk score enhances prediction of type 1 diabetes among susceptible children. Nat Med 2020;26:1247–1255
391. Hagopian WA, Lernmark A, Rewers MJ, et al. TEDDY—The Environmental Determinants of Diabetes in the Young: an observational clinical trial. Ann N Y Acad Sci 2006;1079:320–326
392. Bonifacio E, Beyerlein A, Hippich M, et al.; TEDDY Study Group. Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: a prospective study in children. PLoS Med 2018;15:e1002548
393. Barker JM, Goehrig SH, Barriga K, et al.; DAISY Study. Clinical characteristics of children diagnosed with type 1 diabetes through intensive screening and follow-up. Diabetes Care 2004;27:1399–1404
394. Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat Genet 2018;50:1505–1513
395. Mansour Aly D, Dwivedi OP, Prasad RB, et al.; Regeneron Genetics Center. Genome-wide association analyses highlight etiological differences underlying newly defined subtypes of diabetes. Nat Genet 2021;53:1534–1542
396. Viñuela A, Varshney A, van de Bunt M, et al. Genetic variant effects on gene expression in human pancreatic islets and their implications for T2D. Nat Commun 2020;11:4912
397. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208–2219
398. Pipatpolkai T, Usher S, Stansfeld PJ, Ashcroft FM. New insights into K_{ATP} channel gene mutations and neonatal diabetes mellitus. Nat Rev Endocrinol 2020;16:378–393
399. Taylor SI, Arioglu E. Genetically defined forms of diabetes in children. J Clin Endocrinol Metab 1999;84:4390–4396
400. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. Pediatr Diabetes 2018;19:346–353
401. Redondo MJ, Kawasaki E, Mulgrew CL, et al. DR- and DQ-associated protection from type 1A diabetes: comparison of DRB1*1401 and DQA1*0102-DQB1*0602*. J Clin Endocrinol Metab 2000;85:3793–3797
402. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221–229
403. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet 2016;387:2331–2339
404. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678
405. Todd JA, Walker NM, Cooper JD, et al.; Genetics of Type 1 Diabetes in Finland; Wellcome Trust Case Control Consortium. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864
406. Pucci M, Benati M, Lo Cascio C, Montagnana M, Lippi G. The challenges of diagnosing diabetes in childhood. Diagnosis 2021;8:310–316
407. Oram RA, Sharp SA, Pihoker C, Ferrat L, Imperatore G, Williams A, et al. Utility of diabetes type-specific genetic risk scores for the classification of diabetes type among multiethnic youth. Diabetes Care 2022;45:1124–1131
408. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA 2013;309:2473–2479
409. Vehik K, Bonifacio E, Lernmark Å, et al.; TEDDY Study Group. Hierarchical order of distinct autoantibody spreading and progression to type 1 diabetes in the TEDDY study. Diabetes Care 2020;43:2066–2073
410. Warshauer JT, Bluestone JA, Anderson MS. New frontiers in the treatment of type 1 diabetes. Cell Metab 2020;31:46–61
411. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553
412. Palmer JP, Asplin CM, Clemons P, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983;222:1337–1339
413. Baekkeskov S, Aanstoot H-J, Christgau S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-

synthesizing enzyme glutamic acid decarboxylase. Nature 1990;347:151–156
414. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 1992; 89:283–292
415. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 1993;92:1608–1616
416. Lu J, Li Q, Xie H, et al. Identification of a second transmembrane protein tyrosine phosphatase, IA-2beta, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. Proc Natl Acad Sci U S A 1996;93:2307–2311
417. Wenzlau JM, Juhl K, Yu L, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A 2007;104:17040–17045
418. Wenzlau JM, Liu Y, Yu L, et al. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes 2008;57:2693–2697
419. Nederstigt C, Uitbeijerse BS, Janssen LGM, Corssmit EPM, de Koning EJP, Dekkers OM. Associated auto-immune disease in type 1 diabetes patients: a systematic review and meta-analysis. Eur J Endocrinol 2019;180:135–144
420. Patterson CC, Dahlquist GG, Gyürüs E, Green A; EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009;373:2027–2033
421. Maclaren N, Lan M, Coutant R, et al. Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2beta predict immune-mediated (type 1) diabetes in relatives. J Autoimmun 1999;12:279–287
422. Jacobsen LM, Bocchino L, Evans-Molina C, et al. The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. Diabetologia 2020;63:588–596
423. Anand V, Li Y, Liu B, Ghalwash M, Koski E, Ng K, et al. Islet autoimmunity and HLA markers of presymptomatic and clinical type 1 diabetes: joint analyses of prospective cohort studies in Finland, Germany, Sweden, and the U.S. Diabetes Care 2021;44:2269–2276
424. Schott M, Schatz D, Atkinson M, et al. GAD65 autoantibodies increase the predictability but not the sensitivity of islet cell and insulin autoantibodies for developing insulin dependent diabetes mellitus. J Autoimmun 1994;7:865–872
425. Turner R, Stratton I, Horton V, et al.; UK Prospective Diabetes Study Group. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. Lancet 1997;350: 1288–1293
426. Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. Diabetes Care 2001;24:1460–1467
427. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM. Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? Diabetes 2005;54(Suppl. 2):S62–S67
428. Kobayashi T, Tanaka S, Harii N, et al. Immunopathological and genetic features in slowly progressive insulin-dependent diabetes mellitus and latent autoimmune diabetes in adults. Ann N Y Acad Sci 2006;1079:60–66
429. Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. N Engl J Med 2019;381: 603–613
430. Steck AK, Larsson HE, Liu X, et al.; TEDDY Study Group. Residual beta-cell function in diabetes children followed and diagnosed in the TEDDY study compared to community controls. Pediatr Diabetes 2017;18:794–802
431. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. Diabetes 2000;49:218–224
432. Ziegler AG, Kick K, Bonifacio E, et al.; Fr1da Study Group. Yield of a public health screening of children for islet autoantibodies in Bavaria, Germany. JAMA 2020;323:339–351
433. Zimmet P, Turner R, McCarty D, Rowley M, Mackay I. Crucial points at diagnosis. Type 2 diabetes or slow type 1 diabetes. Diabetes Care 1999;22(Suppl. 2):B59–B64
434. Petersen JS, Dyrberg T, Damm P, Kühl C, Mølsted-Pedersen L, Buschard K. GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. Diabetologia 1996;39:1329–1333
435. Füchtenbusch M, Ferber K, Standl E, Ziegler A-G. Prediction of type 1 diabetes postpartum in patients with gestational diabetes mellitus by combined islet cell autoantibody screening: a prospective multicenter study. Diabetes 1997;46: 1459–1467
436. Gleichmann H, Bottazzo GF. Progress toward standardization of cytoplasmic islet cell-antibody assay. Diabetes 1987;36:578–584
437. Mire-Sluis AR, Gaines Das R, Lernmark A. The World Health Organization International Collaborative Study for islet cell antibodies. Diabetologia 2000;43:1282–1292
438. Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA. A novel micro-assay for insulin autoantibodies. J Autoimmun 1997;10:473–478
439. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes 2003;52: 1128–1136
440. Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for National Institute of Diabetes and Digestive and Kidney Diseases consortia. J Clin Endocrinol Metab 2010;95:3360–3367
441. Grubin CE, Daniels T, Toivola B, et al. A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 1994;37:344–350
442. Lampasona V, Pittman DL, Williams AJ, et al.; Participating Laboratories. Islet Autoantibody Standardization Program 2018 workshop: inter-laboratory comparison of glutamic acid decarboxylase autoantibody assay performance. Clin Chem 2019;65:1141–1152
443. Diabetes Prevention Trial–Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;346:1685–1691
444. Verge CF, Gianani R, Kawasaki E, et al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes 1996; 45:926–933
445. Andersson C, Kolmodin M, Ivarsson SA, et al.; Better Diabetes Diagnosis Study Group. Islet cell antibodies (ICA) identify autoimmunity in children with new onset diabetes mellitus negative for other islet cell antibodies. Pediatr Diabetes 2014;15:336–344
446. Siljander H, Härkönen T, Hermann R, et al. Role of insulin autoantibody affinity as a predictive marker for type 1 diabetes in young children with HLA-conferred disease susceptibility. Diabetes Metab Res Rev 2009;25:615–622
447. Skyler JS, Krischer JP, Wolfsdorf J, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial—Type 1. Diabetes Care 2005;28:1068–1076
448. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015;38:1964–1974
449. Primavera M, Giannini C, Chiarelli F. Prediction and prevention of type 1 diabetes. Front Endocrinol (Lausanne) 2020;11:248
450. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2020 Clinical practice guideline for diabetes management in chronic kidney disease. Kidney Int 2020;98(4S): S1–S115
451. American Diabetes Association Professional Practice Committee. 11. Chronic kidney disease and risk management: *Standards of Medical Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl. 1):S175–S184
452. Thurlow JS, Joshi M, Yan G, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement therapy. Am J Nephrol 2021;52:98–107
453. Davidson MB, Bazargan M, Bakris G, Peters Harmel A, Campese V, Basta E. ImmunoDip: an improved screening method for microalbuminuria. Am J Nephrol 2004;24:284–288
454. Khosla N, Sarafidis PA, Bakris GL. Microalbuminuria. Clin Lab Med 2006;26:635–653
455. Sarafidis PA, Riehle J, Bogojevic Z, Basta E, Chugh A, Bakris GL. A comparative evaluation of various methods for microalbuminuria screening. Am J Nephrol 2008;28:324–329
456. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis 2007;49(Suppl. 2):S12–S154
457. Vassalotti JA, Stevens LA, Levey AS. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. Am J Kidney Dis 2007;50:169–180
458. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-Y. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004;351:1296–1305
459. Beddhu S, Boucher RE, Sun J, et al. Chronic kidney disease, atherosclerotic plaque characteristics on carotid magnetic resonance imaging,

and cardiovascular outcomes. BMC Nephrol 2021;22:69
460. Pitt B, Filippatos G, Agarwal R, et al.; FIGARO-DKD Investigators. Cardiovascular events with finerenone in kidney disease and type 2 diabetes. N Engl J Med 2021;385:2252–2263
461. Lepore G, Maglio ML, Nosari I, Dodesini AR, Trevisan R. Cost-effectiveness of two screening programs for microalbuminuria in type 2 diabetes. Diabetes Care 2002;25:2103–2104
462. Incerti J, Zelmanovitz T, Camargo JL, Gross JL, de Azevedo MJ. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial Transplant 2005;20:2402–2407
463. Klausen KP, Scharling H, Jensen JS. Very low level of microalbuminuria is associated with increased risk of death in subjects with cardiovascular or cerebrovascular diseases. J Intern Med 2006;260:231–237
464. Klausen KP, Scharling H, Jensen G, Jensen JS. New definition of microalbuminuria in hypertensive subjects: association with incident coronary heart disease and death. Hypertension 2005;46:33–37
465. Ratto E, Leoncini G, Viazzi F, Vaccaro V, Parodi A, Falqui V, et al. Microalbuminuria and cardiovascular risk assessment in primary hypertension: should threshold levels be revised? Am J Hypertens 2006;19:728–736
466. Pambianco G, Costacou T, Orchard TJ. The prediction of major outcomes of type 1 diabetes: a 12-year prospective evaluation of three separate definitions of the metabolic syndrome and their components and estimated glucose disposal rate: the Pittsburgh Epidemiology of Diabetes Complications Study experience. Diabetes Care 2007;30:1248–1254
467. Rachmani R, Levi Z, Lidar M, Slavachevski I, Half-Onn E, Ravid M. Considerations about the threshold value of microalbuminuria in patients with diabetes mellitus: lessons from an 8-year follow-up study of 599 patients. Diabetes Res Clin Pract 2000;49:187–194
468. Wachtell K, Ibsen H, Olsen MH, et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med 2003;139:901–906
469. Ibsen H, Olsen MH, Wachtell K, et al. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for endpoint reduction in hypertension study. Hypertension 2005;45:198–202
470. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206–1252
471. Perkovic V, Jardine MJ, Neal B, et al.; CREDENCE Trial Investigators. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med 2019;380:2295–2306
472. Heerspink HJL, Stefánsson BV, Correa-Rotter R, et al.; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in patients with chronic kidney disease. N Engl J Med 2020;383:1436–1446
473. Bakris GL, Agarwal R, Anker SD, et al.; FIDELIO-DKD Investigators. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. N Engl J Med 2020;383:2219–2229
474. Tangri N, Grams ME, Levey AS, et al.; CKD Prognosis Consortium. Multinational assessment of accuracy of equations for predicting risk of kidney failure: a meta-analysis. JAMA 2016;315:164–174
475. Holl RW, Grabert M, Thon A, Heinze E. Urinary excretion of albumin in adolescents with type 1 diabetes: persistent versus intermittent microalbuminuria and relationship to duration of diabetes, sex, and metabolic control. Diabetes Care 1999;22:1555–1560
476. Inker LA, Eneanya ND, Coresh J, et al.; Chronic Kidney Disease Epidemiology Collaboration. New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med 2021;385:1737–1749
477. Inker LA, Schmid CH, Tighiouart H, et al.; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20–29
478. Delgado C, Baweja M, Crews DC, et al. A unifying approach for GFR estimation: recommendations of the NKF-ASN Task Force on reassessing the inclusion of race in diagnosing kidney disease. Am J Kidney Dis 2022;79:268–288.e1
479. Miller WG, Kaufman HW, Levey AS, Straseski JA, Wilhelms KW, Yu H-YE; National Kidney Foundation Laboratory Engagement Working Group. Recommendations for implementing the CKD-EPI 2021 race-free equations for estimated glomerular filtration rate: practical guidance for clinical laboratories. Clin Chem 2021;68:511–520
480. Asmamaw T, Genet S, Menon M, et al. Early detection of renal impairment among patients with type 2 diabetes mellitus through evaluation of serum cystatin C in comparison with serum creatinine levels: a cross-sectional study. Diabetes Metab Syndr Obes 2020;13:4727–4735
481. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011;80:17–28
482. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. JAMA 2005;293:1609–1616
483. Yuyun MF, Khaw KT, Luben R, et al.; European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) Population Study. Microalbuminuria independently predicts all-cause and cardiovascular mortality in a British population: The European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) population study. Int J Epidemiol 2004;33:189–198
484. Duka I, Bakris G. Influence of microalbuminuria in achieving blood pressure goals. Curr Opin Nephrol Hypertens 2008;17:457–463
485. Weir MR, Bakris GL. Editorial perspective. Should microalbuminuria ever be considered as a renal endpoint in any clinical trial? Am J Nephrol 2010;31:469–470
486. Mok Y, Ballew SH, Stacey RB, et al. Albuminuria and prognosis among individuals with atherosclerotic cardiovascular disease: the ARIC study. J Am Coll Cardiol 2021;78:87–89
487. Mok Y, Ballew SH, Sang Y, et al. Albuminuria as a predictor of cardiovascular outcomes in patients with acute myocardial infarction. J Am Heart Assoc 2019;8:e010546
488. Steinke JM, Sinaiko AR, Kramer MS, Suissa S, Chavers BM, Mauer M; International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes: III. Predictors of 5–year urinary albumin excretion rate patterns in initially normoalbuminuric patients. Diabetes 2005;54:2164–2171
489. Miller WG, Bruns DE, Hortin GL, et al.; National Kidney Disease Education Program-IFCC Working Group on Standardization of Albumin in Urine. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem 2009;55:24–38
490. Lambers Heerspink HJ, Gansevoort RT, Brenner BM, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol 2010;21:1355–1360
491. Howey JE, Browning MC, Fraser CG. Biologic variation of urinary albumin: consequences for analysis, specimen collection, interpretation of results, and screening programs. Am J Kidney Dis 1989;13:35–37
492. Gansevoort RT, Verhave JC, Hillege HL, et al.; PREVEND Study Group. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int Suppl 2005;94:S28–S35
493. Meinhardt U, Ammann RA, Flück C, Diem P, Mullis PE. Microalbuminuria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collection. J Diabetes Complications 2003;17:254–257
494. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJL, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am Soc Nephrol 2009;20:436–443
495. Collins AC, Sethi M, MacDonald FA, Brown D, Viberti GC. Storage temperature and differing methods of sample preparation in the measurement of urinary albumin. Diabetologia 1993;36:993–997
496. MacNeil ML, Mueller PW, Caudill SP, Steinberg KK. Considerations when measuring urinary albumin: precision, substances that may interfere, and conditions for sample storage. Clin Chem 1991;37:2120–2123
497. Hishiki S, Tochikubo O, Miyajima E, Ishii M. Circadian variation of urinary microalbumin excretion and ambulatory blood pressure in patients with essential hypertension. J Hypertens 1998;16:2101–2108
498. Bachmann LM, Nilsson G, Bruns DE, et al. State of the art for measurement of urine albumin: comparison of routine measurement procedures to isotope dilution tandem mass spectrometry. Clin Chem 2014;60:471–480
499. Miller WG, Seegmiller JC, Lieske JC, Narva AS, Bachmann LM. Standardization of urine albumin measurements: status and performance goals. J Appl Lab Med 2017;2:423–429
500. McTaggart MP, Newall RG, Hirst JA, et al. Diagnostic accuracy of point-of-care tests for detecting albuminuria: a systematic review and meta-analysis. Ann Intern Med 2014;160:550–557
501. Shin JI, Chang AR, Grams ME, et al.; CKD Prognosis Consortium. Albuminuria testing in

hypertension and diabetes: an individual-participant data meta-analysis in a global consortium. Hypertension 2021;78:1042–1052
502. Mejia JR, Fernandez-Chinguel JE, Dolores-Maldonado G, et al. Diagnostic accuracy of urine dipstick testing for albumin-to-creatinine ratio and albuminuria: a systematic review and meta-analysis. Heliyon 2021;7:e08253
503. Kim Y, Park S, Kim M-H, et al. Can a semi-quantitative method replace the current quantitative method for the annual screening of microalbuminuria in patients with diabetes? Diagnostic accuracy and cost-saving analysis considering the potential health burden. PLoS One 2020;15:e0227694
504. National Institute for Health and Care Excellence. Chronic kidney disease: assessment and management, 2021. Accessed 7 March 2022. Available from https://www.nice.org.uk/guidance/ng203
505. Shaikh A, Seegmiller JC, Borland TM, et al. Comparison between immunoturbidimetry, size-exclusion chromatography, and LC-MS to quantify urinary albumin. Clin Chem 2008;54:1504–1510
506. American Diabetes Association Professional Practice Committee. 12. Retinopathy, neuropathy, and foot care: *Standards of Medical Care in Diabetes—2022*. Diabetes Care 2021;45(Suppl. 1):S185–S194
507. Després JP, Lamarche B, Mauriège P, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996; 334:952–957
508. Wilson PWF, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007;167:1068–1074
509. Rutter MK, Wilson PWF, Sullivan LM, Fox CS, D'Agostino RB Sr, Meigs JB. Use of alternative thresholds defining insulin resistance to predict incident type 2 diabetes mellitus and cardiovascular disease. Circulation 2008;117:1003–1009
510. Wexler DJ, Macias-Konstantopoulos W, Forcione DG, Xiong L, Cauley CE, Pierce KJ. Case 23-2018: a 36-year-old man with episodes of confusion and hypoglycemia. N Engl J Med 2018;379:376–385
511. Leighton E, Sainsbury CA, Jones GC. A practical review of C-peptide testing in diabetes. Diabetes Ther 2017;8:475–487
512. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology. ACOG Practice Bulletin No. 194: polycystic ovary syndrome. Obstet Gynecol 2018;131:e157–e171
513. Marcovina S, Bowsher RR, Miller WG, et al.; Insulin Standardization Workgroup. Standardization of insulin immunoassays: report of the American Diabetes Association Workgroup. Clin Chem 2007; 53:711–716
514. Miller WG, Thienpont LM, Van Uytfanghe K, et al.; Insulin Standardization Work Group. Toward standardization of insulin immunoassays. Clin Chem 2009;55:1011–1018
515. Staten MA, Stern MP, Miller WG, Steffes MW; Insulin Standardization Workgroup. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diabetes Care 2010;33:205–206
516. Little RR, Wielgosz RI, Josephs R, et al. Implementing a reference measurement system for C-peptide: successes and lessons learned. Clin Chem 2017;63:1447–1456
517. U.S. Centers for Medicare & Medicaid Services. C-peptide levels as a criterion for use, 2005. Accessed 7 March 2022. Available from https://www.hhs.gov/guidance/document/infusion-pumps-c-peptide-levels-criterion-use
518. Censi S, Mian C, Betterle C. Insulin auto-immune syndrome: from diagnosis to clinical management. Ann Transl Med 2018;6:335
519. Hu X, Chen F. Exogenous insulin antibody syndrome (EIAS): a clinical syndrome associated with insulin antibodies induced by exogenous insulin in diabetic patients. Endocr Connect 2018; 7:R47–R55