Title: Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus

Authors: 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]

Addresses:

[1]Department of Laboratory Medicine
National Institutes of Health
10 Center Drive
Building 10, Room 2C306
Bethesda, MD 20892-1508

[2]Department of Chemistry
University of Iowa
Iowa City, Iowa 52242

[3]Department of Medicine
Am. Heart Assoc. Comprehensive Hypertension Center
Section of Endocrinology, Diabetes and Metabolism
University of Chicago Medicine
5841 South Maryland Avenue, MC1027
Chicago, IL 60637

[4]Department of Pathology
University of Virginia Medical School
Box 800168
Charlottesville, VA 22908-0001

[5]New South Wales Health Pathology Department of Chemical Pathology
Level 4, Campus Centre, Prince of Wales Hospital
Barker Street, Randwick, NSW 2031
Sydney, Australia

[6]Department of Clinical Sciences
Lund University/CRC
Skane University Hospital Malmö
Se-205 02 Malmö, Sweden

[7]Division of Endocrinology
Northwestern University
The Feinberg School of Medicine
303 E. Chicago Ave., Tarry 15-735
Chicago, IL 60611

[8]Massachusetts General Hospital Diabetes Center and Harvard Medical School
50 Staniford Street, Suite 340
Boston, MA 02114

[9]Department of Medicine
University of North Carolina
Chapel Hill, NC 27599

[†]Corresponding author
David B. Sacks, MB, ChB
National Institutes of Health
Department of Laboratory Medicine
10 Center Drive
Building 10, Room 2C306
Bethesda, MD 20892-1508
301-496-3386 - phone
301-402-1885- fax
email: sacksdb@mail.nih.gov

ABSTRACT

THE ABSTRACT WILL BE COMPLETED PRIOR TO SUBMISSION

Nonstandard abbreviations: AACC, American Association for Clinical Chemistry; AcAc, acetoacetate; ACOG, American College of Obstetrics and Gynecology; ADA, American Diabetes Association; AER, albumin excretion rate; CAD, coronary artery disease; CAP, College of American Pathologists; CDC, Centers for Disease Control and Prevention; CGM, continuous glucose monitoring; CKD, chronic kidney disease; CI, confidence intervals; CLSI, Clinical and Laboratory Standards Institute; DCCT, Diabetes Control and Complications Trial; DKA, diabetic ketoacidosis; eGFR, estimated glomerular filtration rate; FDA, Food and Drug Administration; FPG, fasting plasma glucose; GAD_{65}, 65-kDa isoform of glutamic acid decarboxylase; GDM, gestational diabetes mellitus; GHb, glycated hemoglobin; GPP, good practice point; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; βOHB, β-hydroxybutyrate; HPLC, high-performance liquid chromatography; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; IAA, insulin autoantibodies; IADPSG, International Association of Diabetes and Pregnancy Study Groups; ICA, islet-cell cytoplasm antibodies; IDF, International Diabetes Federation; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IMD, immune-mediated diabetes; is-CGM, intermittently scanned CGM; JDF, Juvenile Diabetes Foundation; KDIGO, Kidney Disease Improving Global Outcomes; LDL, low density lipoprotein; MODY, maturity onset diabetes of the young; NGSP, National Glycohemoglobin Standardization Program; NHANES, National Health and Nutrition Examination Survey; NHIS, National Health Interview Survey; OGTT, oral glucose tolerance test; RCT, randomized controlled trial; rt-CGM, real-time CGM; SGLT, sodium-glucose transport; SMBG, self-monitoring of blood glucose; uACR, urine albumin/creatinine ration; UKPDS, United Kingdom Prospective Diabetes Study; WHO, World Health Organization.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is both underutilized and over-produced, resulting in hyperglycemia. The disease is classified conventionally into several clinical categories, although these are being reconsidered based on genetic, metabolomic and other characteristics and underlying pathophysiology. The revised classification published in 2014 (1) is indicated in Table 1. Type 1 diabetes mellitus is usually caused by autoimmune destruction of the pancreatic islet β-cells, rendering the pancreas unable to synthesize and secrete insulin (2). Type 2 diabetes mellitus results from a combination of insulin resistance and inadequate insulin secretion (3,4). Gestational diabetes mellitus (GDM), which resembles type 2 diabetes more than type 1, develops during ~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 is the most common form, accounting for 85-95% of diabetes in developed countries. Monogenic subtypes of type 2 diabetes have been identified but are rare. Some patients cannot be clearly classified as type 1 or type 2 diabetes (5) 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 ~537 million and is forecast to reach 783 million by 2045 (6). Based on 2017-2020 NHANES data and 2018-2019 NHIS data, the US Centers for Disease Control and Prevention (CDC) estimated that there were 37.3 million people (11.3% of the US population) with diabetes (7). The prevalence of

diabetes has also increased in other parts of the world. For example, estimates suggested 110 million diabetic individuals in Asia in 2007 (8). The true number is likely to be substantially greater as China alone was thought to have 92.4 million adults with diabetes in 2008 (9) and 141 million in 2021 (6). Approximately 50% of people with diabetes worldwide are thought to be undiagnosed (6).

The cost of diabetes in the US in 2012 was approximately $245 billion and increased to $327 billion by 2017 (10). 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 (11). Similarly, in the UK diabetes accounts for roughly 10% of the National Health Service budget (equivalent in 2014 to $14 billion per year), while worldwide spending in 2021 was thought to be $966 billion. The high costs of diabetes are attributable primarily to treating the debilitating complications (10), which can be divided into microvascular complications – predominantly retinopathy, nephropathy and neuropathy – and macrovascular complications, particularly stroke and coronary artery disease. Together these result in diabetes being the fourth most common cause of death in the developed world (12). About 6.7 million adults worldwide were thought to have died from diabetes-related causes in 2021 (6).

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 (13,13) and 2011 (14,15). Here we review and update these recommendations using an evidence-based approach, especially in key areas where new evidence has emerged since the 2011 publications. The process of updating guideline recommendations followed the standard operating procedures for preparing, publishing, and editing AACC Academy laboratory medicine practice guidelines. The key steps are detailed

in the Supplement accompanying this paper. The system developed in 2011 to grade both the overall quality of the evidence (Table 2) and the strength of recommendations (Table 3) was used.

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 patients with diabetes. Additional details concerning the scope, purpose, key topics and targets of this guideline are described in the accompanying Supplement.

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

GLUCOSE

1. 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.

2. Use/rationale

A. Diagnosis

Recommendation: 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 of retinopathy and nephropathy (19,20). The revised criteria comprised: (a) fasting plasma glucose (FPG) ≥ 7.0 mmol/L (126 mg/dL), (b) 2-h post load glucose > 11.1 mmol/L (200 mg/dL) during an OGTT or (c) symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose ≥11.1 mmol/L (200 mg/dL) (Table 4) (18). The WHO and International Diabetes Federation (IDF) recommend either FPG or 2-h post load glucose using the same cutoffs as the ADA (21) (Table 5). In 2009 an International Expert Committee (22), with members appointed by the ADA, European Association for the Study of Diabetes (EASD) and IDF, recommended that diabetes could also be diagnosed by measurement of hemoglobin A1c (HbA1c), which reflects long-term blood glucose concentrations (see HbA1c section below). The

ADA (23), EASD, IDF and the WHO (24) have endorsed the use of HbA1c 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 HbA1c) 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, HbA1c can be the confirmatory test in the subsequent sample or HbA1c initially, followed by glucose. A third option is to measure two different analytes, namely glucose and HbA1c, in samples obtained on the same day. Note that repeat testing is not required in patients who have unequivocal hyperglycemia i.e., >11.1 mmol/L (200 mg/dL).

B. Screening

Recommendation: Screening by HbA_{1c}, FPG or 2-h OGTT is recommended for individuals who are at high risk of diabetes. If HbA1c 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

Testing to detect type 2 diabetes in asymptomatic people, previously controversial, is now recommended for those at risk of developing the disease (25). Screening is recommended for several reasons. In the past, the onset of type 2 diabetes has been estimated to occur ~4-7 (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 ~25% of people in the U.S. (and nearly half of Asian and Hispanic Americans) with type 2 diabetes are undiagnosed (27). Global estimates are that ~50% of people with diabetes are undiagnosed (6). Notwithstanding this recommendation, the evidence that population screening for hyperglycemia and subsequent prevention efforts will provide long-term benefit is inconsistent (28).

The ADA proposes that all asymptomatic people aged 35 years or more should be screened in a health care setting. HbA_{1c}, FPG or 2-h OGTT are appropriate for screening (27). If FPG is <5.6 mmol/L (100 mg/dL), 2-h plasma glucose is <7.8 mmol/L (140 mg/dL) and/or HbA1c is <5.7% (39 mmol/mol), testing should be repeated at 3-year intervals. Screening should be considered at a younger age or be carried out more frequently in individuals who are at increased risk for diabetes; overweight (BMI $\geq$ 25 kg/m^2) or obese or who have a risk factor for diabetes (see Ref (27) for conditions associated with increased risk). Individuals with prediabetes (i.e., glucose concentration that do not meet the criteria for diabetes, but have abnormal carbohydrate metabolism) should be tested annually (27).

Because of the increasing prevalence of type 2 diabetes in children, screening of children is now advocated (27,29) . Starting at age 10 years (or at onset of puberty if puberty occurs at a younger age), testing should be performed every 3 years in overweight youths (BMI >85th percentile) who have 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 (27).

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) (30–32), 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 HbA1c is the most appropriate (22,33–35). Based on evaluation of NHANES III data, a strategy to screen whites who are ≥40 years and other populations ≥30 years of age with FPG has been proposed (36).

The cost-effectiveness of screening for type 2 diabetes has been estimated. The incremental cost of screening all persons aged 25 years or older was estimated to be $236,449 per life-year gained and $56,649 per quality-adjusted life-year (QALY) gained (37). Interestingly, screening was more cost-effective at ages younger than 45 years. In contrast, screening targeted to individuals with hypertension reduces the QALY from $360,966 to $34,375, with ages 55 to 75 years being most cost-effective (38). Modeling run on one million individuals suggests there is considerable uncertainty as to whether screening for diabetes would be cost effective (39). By contrast, a subsequent modeling study implies that screening commencing at age 30 or age 45 is highly cost-effective (<$11,000 per QALY gained) (40). Cohort studies support cost-effectiveness of screening (41). Long-term outcome studies are necessary to provide evidence to resolve the

question of the effectiveness of screening for diabetes (42). Screening and prevention of diabetes based on the Diabetes Prevention Program has been shown to be cost-effective and even cost-saving with metformin (43) and has been endorsed by the Center for Medicaid/Medicare Services based on independent cost-effective analyses.

In 2003 the ADA lowered the threshold for "normal" FPG from <6.1 mmol/L (110 mg/dL) to <5.6 mmol/L (100 mg/dL) (44). This change remains contentious and has not been accepted by all organizations (21,45). 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 (46,47). 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 (48). Data were obtained from 13,163 men aged 26-45 years with FPG <5.55 mmol/L (100 mg/dL) who were followed for a mean of 5.7 years. Men with FPG 4.83-5.05 mmol/L (87-91 mg/dL) have a significantly increased risk of type 2 diabetes compared to those with FPG <4.5 mmol/L (81 mg/dL). Although the prevalence of diabetes is low at these glucose concentrations, the data support the concept of a continuum between FPG and the risk of diabetes. In a population of 117,193 Danish individuals without diagnosed diabetes, random (nonfasting) glucose concentrations in the normoglycemic range and higher were associated with high risks of retinopathy, neuropathy, diabetic nephropathy and myocardial infarction (49). 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. These findings suggest that increased glucose concentration below the diabetes cutoff is a risk factor for microvascular and macrovascular disease.

C. Monitoring/Prognosis

Recommendation: Routine measurement of plasma glucose concentrations 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 (50) and type 2 (51) diabetes. Persons with type 1 diabetes who maintain lower average blood glucose concentrations exhibit a significantly lower incidence of microvascular complications, namely diabetic retinopathy, nephropathy and neuropathy (52). 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 (52). Longer follow up documented a significant reduction in cardiovascular disease in patients with type 1 diabetes treated with intensive glycemic control (53). The effects of tight glycemic control on microvascular complications in patients with type 2 diabetes (54) are similar to those with type 1 diabetes, considering the differences in glycemia achieved between the active intervention and control groups in the various trials. The United Kingdom Prospective Diabetes Study (UKPDS) showed that intensive blood glucose control significantly reduced microvascular complications in patients with short-duration type 2 diabetes. While meta-analyses suggest that intensive glycemic control in individuals with type 2 diabetes reduces cardiovascular disease (55,56), 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 follow up of the UKPDS

population supported a benefit of intensive therapy on macrovascular disease (57), 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 (58–60). One study even observed higher cardiovascular mortality in the intensive treatment arm (58). In both the Diabetes Control and Complications Trial (DCCT) and UKPDS, patients 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 (25,61) .

Laboratory measurements of random or fasting glucose concentrations should not be measured as the primary means of routine outpatient monitoring of patients with diabetes. Laboratory plasma glucose testing can be used to supplement information from other testing or to assess the accuracy of self-monitoring (see below) (62). In addition, individuals with well-controlled type 2 diabetes who are not on insulin therapy can be monitored with periodic measurement of FPG, although analysis need not be done in an accredited laboratory (62,63).

3. **Analytical Considerations**

A. 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 15-30 minutes. 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 hours) 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 patients seen in the afternoon (64).

Loss of glucose from sample containers is a serious and underappreciated problem (65,66). 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%-7% (~0.6 mmol/L; 10 mg/dL) per hour (67) —varies with the glucose concentration, temperature, white blood cell count and other factors (65,68). 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 reagents 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 (67). After 4 h, the glucose concentration is stable in whole blood for 72 h at room temperature in the presence of fluoride (67). (Leukocytosis will increase glycolysis even in the presence of fluoride if the white cell count is very high.)

Few effective and practical methods have been available for prompt stabilization of glucose in whole blood specimens. Loss of glucose can be minimized in two classical ways: (1) Immediate separation of blood cells after blood collection (69) (in separated, nonhemolyzed, sterile serum without fluoride the glucose concentration is stable for 8 h at 25 °C and 72 h at 4 °C (69–71) and (2) placing the blood tube in an ice-water slurry immediately after blood collection followed by separation of plasma from the cells within 30 minutes (72,73). 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 (73). 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 (74). Subsequently, several other studies also

demonstrated the effectiveness of tubes containing citrate/fluoride/EDTA (CFE) to inhibit glycolysis (75,76).

A few studies noted that glucose concentrations were higher in samples collected in tubes containing citrate than in control samples (77,78). While some suggest the increase is spurious (77,78), others state that the difference is likely due to glycolysis in the samples without citrate (73,79). In contrast, other studies observe no difference in glucose concentrations between samples collected in tubes containing citrate compared to those with stringent sample handling to prevent glycolysis (73,79). 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 (80). Importantly, elimination of glycolysis will 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 these tubes were not available in the US. We strongly encourage manufacturers of blood collection tubes to make these available worldwide.

Glucose can be measured in whole blood, serum or plasma, but plasma is recommended for diagnosis. [Note that while both the ADA and WHO recommend venous plasma, the WHO also accepts measurement of glucose in capillary (skin-puncture or "fingerstick") blood (21,27)] 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 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 whole blood if the hematocrit is normal. Glucose concentrations in heparinized

plasma were reported in 1974 to be 5% lower than in serum (81). (The reasons for the difference are not apparent but have been attributed to the shift in fluid from erythrocytes to plasma caused by anticoagulants.) In contrast, some subsequent studies found that glucose concentrations in plasma are slightly higher than serum. The differences observed were ~0.2 mmol/L (3.6 mg/dL) (82), ~2% (83) or 0.9% (73). Other studies indicate that glucose values measured in serum and plasma are essentially the same (84,85) 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 (21,27) 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 (30 mg/dL), equivalent to 20-25% (86,87), 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) (86,87).

Reference values: Glucose concentrations in healthy individuals vary with age. Reference intervals in children are 3.3 – 5.6 mmol/L (60-100 mg/dL), similar to the adult range of 4.1–5.5 mmol/L (74-99 mg/dL) (69). Note that the ADA and WHO criteria (21,27), not the reference values, are used for the diagnosis of diabetes.

The ADA classifies hypoglycemia in diabetes into three levels: Level 1, glucose <70 mg/dL (3.9 mmol/L) and ≥54 mg/dL (3.0 mmol/L); Level 2, glucose <54 mg/dL (3.0 mmol/L) and Level 3, severe event with altered mental/physical status that requires assistance for treatment of

hypoglycemia (61). 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 (88,89). FPG does not increase significantly after age 60, but glucose concentrations after a glucose challenge are considerably higher in older persons (89,90). Many factors participate in the metabolic dysregulation that develops with increasing age, and changes in body composition make an important contribution (91).

B. 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 patients, 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. (92). 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 ~7.1 mmol/L (128 mg/dL), imprecision among laboratories using the same method had a CV ≤2.7% (92). Similar findings have been reported for glucose analysis in samples from patients. The method of glucose measurement does not influence the result. Comparison of results from ~6000 clinical laboratories reveals that the mean glucose concentrations measured in serum samples by

436 the hexokinase and glucose oxidase methods are essentially the same (93). However, compared to
437 a reference measurement procedure, significant ($p<0.001$) bias (up to 13%) was observed for
438 40.6% of the peer groups (93). If, as is likely, similar biases occur with plasma, patients near the
439 diagnostic threshold could be misclassified.

440 No consensus has been achieved on the goals for glucose analysis. Numerous criteria have
441 been proposed to establish analytic goals. These include expert opinion (consensus conferences),
442 opinion of clinicians, regulation, state of the art and biological variation (94). A rational and
443 realistic recommendation that has received some support is to use biological criteria as the basis
444 for analytic goals. It has been suggested that imprecision should not exceed one half of the within-
445 subject biological CV (95,96). For plasma glucose, a CV $\leq$ 2.2% has been suggested as a target for
446 imprecision, with 0% bias (96). Although this recommendation was proposed for within-laboratory
447 error, it would be desirable to achieve this goal for inter-laboratory imprecision to minimize
448 differences among laboratories in the diagnosis of diabetes in individuals whose glucose
449 concentrations are close to the threshold value. Therefore, the goal for glucose analysis should be
450 to minimize total analytical error and methods should be without measurable bias. A national or
451 international program using commutable samples (e.g., fresh frozen plasma) that eliminate matrix
452 effects, with accuracy-based grading using values derived with a reference measurement
453 procedure, should be developed to assist in the achievement of this objective.
454
455 **4. Interpretation**
456
457 Despite the low analytical imprecision at the diagnostic decision limits of 7.0 mmol/L (126 mg/dL)
458 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. (Although total biological variation includes within-person and between-person variation, most discussions focus on the within-person variation.) Careful evaluation over several consecutive days in healthy individuals revealed that biological variation of FPG [mean glucose of 4.9 mmol/L (88 mg/dL)] exhibited within- and between-subject CVs of 4.8-6.1% and 7.5-7.8%, respectively (97–99). Measurement of FPG in 246 normal and 80 previously undiagnosed individuals with diabetes revealed mean intraindividual CVs of 4.8 and 7.1%, respectively (98). Similar findings were obtained with analysis of 685 adults from NHANES III where mean within-person variability of FPG measured 2-4 weeks apart was 5.7% (95% CI of 5.3-6.1%) (100). 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 ~5.1 mmol/L (92 mg/dL) (101). 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 ~4.6 mmol/L (83 mg/dL) (102). A meta-analysis published in 2019 (103) identified 23 publications that delivered 46 different estimates of glucose biological variation. Estimates for 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 6.2-7.8 mmol/L (112-140 mg/dL). If the CV (analytical) of the glucose assay (~3%) is included, the 95% CI is ~ ±12.88%. Thus, the 95% CI for a fasting glucose concentration of 7.0 mmol/L (126 mg/dL) would be 7.0 mmol/L ± 6.4% (126 mg/dL ± 6.4%), namely 6.1-7.9

mmol/L (110-142 mg/dL). Using assay imprecision of 3% (CV) only (excluding biological variability), would yield 95% CI of 6.6 – 7.4 mmol/L (118-134 mg/dL) among laboratories for a true glucose concentration of 7.0 mmol/L (126 mg/dL). Performing the same calculations at the cutoff for impaired fasting glucose (IFG) yields 95% CI of 5.6 ± 6.4% (100 ± 6.4%), namely 4.9-6.3 mmol/L (87-113 mg/dL). One should bear in mind that these ranges include 95% of results and the remaining 5% will be outside this range. Thus, the biological variability within an individual is substantially greater than analytic variability; analytic imprecision makes a negligible contribution to variation in patient results. Using biological variation as the basis for deriving analytical performance characteristics (94), the following desirable specifications for glucose have been proposed (102,103): analytical imprecision ≤2.4%, bias ≤2.1% and total error ≤6.1%.

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 (104). However, this value is based on suggestions of clinicians and no outcome data have been published that validate this figure. Inpatient management of diabetes patients may on occasion require a rapid turnaround time (minutes, not hours). Similarly, for protocols with intensive glucose control in critically ill patients (105), 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.

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 (21,27). 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 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., patients in hospital, patients with DKA, neonatal hypoglycemia, etc.) are highly variable and may range from 30 min to 24 hours or more.

5. Emerging considerations & knowledge gaps/research needs

Continuous glucose monitoring (CGM) and noninvasive analysis of glucose are addressed below.

GLUCOSE METERS

1. Description/introduction/terminology

Portable meters for measurement of blood glucose concentrations are used in three major settings: i) by patients in everyday activities; ii) in physicians' offices; and iii) in acute and chronic care facilities. The blood ("capillary") samples used with glucose meters typically are obtained by skin puncture, usually of a fingertip. Use of glucose meters by patients is referred to as self-monitoring of blood glucose (SMBG). The glucose-meter's results are used to guide therapy, especially adjustments of insulin dosing.

The ADA summarized uses of SMBG as early as 1987 (see reference (106) and references therein), and by 1993 SMBG was being performed at least once a day by 40% and 26% of individuals with type 1 and 2 diabetes, respectively, in the US (107). The ADA currently recommends that most patients with type 1 diabetes use intensive insulin regimens, aiming for

glycemia as close to the non-diabetic range as safely possible (usually a HbA1c <7% for many non-pregnant patients), with multiple daily injections or an insulin pump, and with selection of doses guided by SMBG, continuous glucose monitoring, or by both (108).

The benefit of SMBG is less clear for patients who are not using intensive insulin therapy, although the financial costs are large and real. Glucose meters and their associated supplies are thought to represent a multi-billion-dollar expense for diabetes care worldwide.

2. Use/Rationale

A. Diagnosis/Screening

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

The glucose-based criteria for the diagnosis of diabetes (Table 4) (27) are informed by studies that defined the relationship between risk of long-term complications (retinopathy) and premorbid venous plasma glucose concentrations (or HbA1c. Application of the diagnostic criteria in clinical practice relies on measurements of glucose in the same sample type (venous plasma) in an accredited laboratory (27). Similarly, the recommendations of the ADA (27) and of the U.S. Preventive Task Force on screening for diabetes (109,110) rely on measurements of glucose in plasma (or measurement of HbA1c). By contrast, portable meters typically use skin-puncture (capillary) samples (not venous samples) of whole blood (not plasma). Most portable meters have been programmed to report an estimated plasma glucose concentration, but the estimate depends on factors in addition to the glucose concentration in the plasma portion of the

finger-stick samples of whole blood. Moreover, the variability among meters (see Analytical Considerations below) precludes recommending their use in the diagnosis of diabetes.

Glucose meters have limited if any documented role in screening for diabetes in healthcare settings. The ADA Standards of Medical Care in Diabetes— 2022 (27) recommends that screening, typically by risk assessment with or without use of a questionnaire, be performed in a healthcare setting. This approach allows for follow-up and treatment, and it typically assures that measurements of glucose can be made by methods that are appropriate for diagnosis of diabetes

Community screening outside a health care setting is generally not recommended because of the risk that people with positive tests will be lost to follow-up (27). The ADA Standards (27) 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 of patients 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 (74) may provide another option for screening in remote areas when venipuncture is available.

B. Monitoring/Prognosis

Recommendation: Frequent self-monitoring of blood glucose (SMBG) is recommended for all insulin-treated patients with diabetes who use intensive insulin regimens (with multiple daily injections or insulin pump therapy) and who are not using continuous glucose monitors (CGMs). A (high)

Recommendation: Routine use of SMBG is not recommended for patients 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 (52) diabetes and by the UKPDS for type 2 (54) diabetes. In the DCCT, patients with type 1 achieved glycemic control by performing SMBG at least four times per day to guide insulin therapy (52). Therapy in patients with type 2 diabetes in the UKPDS (54) was adjusted according to FPG concentrations – SMBG was not utilized.

Insulin-requiring patients, particularly those with type 1 diabetes, use knowledge of ambient capillary (with SMBG) 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 (111). Frequent use of SMBG (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 SMBG or CGM may help to detect and avoid this potentially life-threatening complication. The risk of hypoglycemia is seen primarily in patients treated with insulin or insulin secretagogues, and risk increases significantly when pharmacologic therapy is directed towards maintaining glucose concentrations as close to those found in non-diabetic individuals as is safely possible (54). The incidence of major hypoglycemic episodes—requiring third-party help or medical intervention—was 2- to 3-fold higher in the intensive group than in the conventional group in clinical trials of patients with type 1 and type 2 diabetes, with the absolute rate far higher in type 1 diabetes than in type 2 (54). Furthermore, many patients with diabetes, particularly those with type 1, lose the autonomic warning symptoms that normally precede neuroglycopenia ("hypoglycemia unawareness") (112), increasing the risk of hypoglycemia. SMBG and CGM can be useful for detecting

asymptomatic hypoglycemia and allowing patients to avoid severe hypoglycemic episodes, especially when insulin is used in treatment.

For patients using CGMs that require calibration by users, SMBG should be used to calibrate the CGM. For all patients using CGM, SMBG should be done during periods when CGM results are not available or when the CGM results are inconsistent with the clinical state or suspected to be inaccurate. For discussion of these topics, see the section on CGM.

The role of SMBG in individuals with type 2 diabetes who are treated with only basal insulin or no insulin has generated considerable controversy (113). Intensive glycemic control is well established as beneficial in reducing the risk for microvascular complications. However, except for the potential use of SMBG in insulin-treated patients with type 2 diabetes and especially for those who use multiple daily injection regimens or, more rarely, for pump-treated patients, SMBG likely adds cost without benefit (114). Four meta-analyses have reported the effects of SMBG on HbA1c in patients with type 2 diabetes who were not using insulin (115–118). The decreases of HbA1c in those using SMBG were similar to the decreases in comparably treated patients who did not use SMBG. For example, the meta-analysis by Farmer et al (116) found that the mean pooled reduction in HbA1c was 0.88% in SMBG-assigned groups and 0.69% in the usual care groups. Meta-analyses also reported that, by one year of use of SMBG, the improvements in HbA1c seen at earlier time points were lost (115,117). There is insufficient evidence to conclude whether the observed small and transient differences in HbA1c lowering associated with SMBG improved clinically important outcomes for patients.

A pragmatic, open-label randomized trial, conducted in 15 primary care practices, evaluated use of once-daily SMBG in patients with non-insulin-treated type 2 diabetes (119). The study found no clinically or statistically significant differences at 1 year in glycemic control

(as assessed by HbA1c) or health-related quality of life between patients who performed SMBG, with or without enhanced feedback, and those who did not.

In summary, the evidence is insufficient to recommend routine use of SMBG for patients with type 2 diabetes whose diabetes is treated without use of insulin.

The ADA Standards of Care suggests that nonroutine use of SMBG is beneficial in specific situations for some patients with diabetes who are not using multiple injections of insulin (108). 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.

3. Analytical Considerations

A. Preanalytical

Recommendation: Patients 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 SMBG with concurrent laboratory glucose analysis have been shown to improve the accuracy of patients' blood glucose readings (120). It is important to evaluate the patient's technique at regular intervals (108).

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 (121).

B. 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.A. or comparable analytical performance specifications in other locations. GPP

Meters can be calibrated to report glucose concentrations in plasma or whole blood. An IFCC working group recommended that glucose meters report concentrations of glucose in plasma, irrespective of the sample type or technology (122,123); this approach can improve harmonization and allows comparison with laboratory-generated results (124).

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 (FDA) in the U.S. (125,126) and the similar standards of the International Organization for Standardization (ISO) (127) and the Clinical Laboratory Standards Institute (CLSI) (128). The accuracy standards of these organizations are summarized in Table 6. The FDA has separate standards for meters used for SMBG (125) and meters used in health care facilities (126). By contrast, the ISO standard applies only to glucose meters used for SMBG 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 SMBG use met the ISO standard (129).

The FDA and ISO standards agree on an allowable error of approximately 15% for SMBG 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 SMBG. An early 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 in the patient (130). That study revealed that meters that achieve both an imprecision (as coefficient of variation, CV) <5% and a bias <5% rarely lead to major errors in insulin dosing. With such a meter (CV <5% and bias <5%) approximately 95% of results fall within 15% of laboratory results, which corresponds to the 15% allowable error in the FDA and ISO standards for SMBG meters (Table 6).

In subsequent studies of meters for SMBG, Breton and colleagues used the UVA-PADOVA Type 1 Diabetes Simulator in 2 studies (131,132) to assess the effects of meter inaccuracy on patient outcomes and costs. The first study (131) addressed use of blood glucose meters for twice-daily calibration of continuous glucose monitors. The modeling demonstrated that increasing inaccuracy of the glucose measurements progressively increased (a) the number of severe hypoglycemic episodes over 30 days, (b) the total daily insulin use, and (c) the number of finger-sticks per day. Analytical errors of meters that meet the 2013 ISO standard have only limited impact on the three outcome measures, or on HbA1c. The second modeling study (132) 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 patients during routine use, not to

689 a meter's performance in the hands of trained workers or the performance reported by
690 manufacturers.

691 ***Recommendations: In hospitals and acute-care facilities, point-of-care testing personnel,***
692 ***including nurses, should use glucose meters that are intended for professional use.***
693 ***GPP***
694
695 ***When testing newborns, personnel should use only meters that are intended for use in***
696 ***newborns.***
697 ***GPP***

698 Meters that are designed for SMBG often do not meet the needs of testing in hospitals,
699 especially because of the danger of transmission of pathogens from one patient to another via the
700 meters. Professional-use meters that are cleared by the U.S. FDA for use in health-care settings,
701 address this problem and offer additional features such as the ability to communicate the results
702 to an electronic medical record. Moreover, these meters are held to a higher standard for
703 accuracy. Accuracy standards (analytical performance specifications) of the U.S. FDA and of
704 CLSI for professional-use meters are shown in Table 6. Meters that are designed for professional
705 use have been shown in published studies to have impressive accuracy on samples of whole
706 blood (133–135). Changing from one meter to a meter with less meter error (bias) was associated
707 with decreased glycemic variability and increased percentage of values in target glucose range in
708 patients following cardiovascular surgery (135).

709 For use in newborns, glucose meters must be accurate in the presence of the high
710 hematocrits that are common in this population. High hematocrit will increase or decrease the

711 measured glucose, or will have minimal effect, depending on the design of the measuring system
712 (136,137). Analytical bias and/or imprecision at low concentrations can lead to frequent false
713 alarms of neonatal hypoglycemia or missed cases of true hypoglycemia (138). Professional-use
714 meters that are selected on the basis of their performance in a population outside the newborn
715 nursery and newborn ICU are not necessarily the optimal choice for use in newborns (136).

716 **4. Interpretation**

717 *A. Interferences*

718 Numerous interfering factors have been reported to influence the results of blood glucose
719 meters (139,140). Many meters incorporate changes that eliminate or greatly ameliorate most
720 interferences, but interferences persist (141,142).

721 Several sugars— notably maltose, galactose and xylose— falsely increase results of some
722 glucose meters. Maltose interferes with measurements by some glucose meters that use glucose
723 dehydrogenase (143). Maltose is present in some medications; and it, along with maltotriose and
724 maltotetraose, is produced in vivo by metabolism of icodextrin that is used in some peritoneal
725 dialysis solutions (143). Interference from these sugars has been essentially eliminated as a threat
726 in meters that use a modified glucose dehydrogenase (137). Galactose (137,144) and xylose
727 (145,146) have been reported to falsely increase results of some glucose meters.

728 Hematocrit affects the glucose results of some meters, with falsely high glucose results at
729 low hematocrits and falsely low results at high hematocrits (147,148) . Various methods have
730 been developed to minimize the hematocrit effect (149) and numerous glucose meters have
731 minimal hematocrit interference (141,147,150). Nonetheless, hematocrit interference persists in
732 other meters (141).

Numerous additional factors have been reported as interferences for some meters and not others. These interfering factors include vitamin C (141), acetaminophen (paracetamol) (144,147,151), N-acetylcysteine (152), 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 (140). 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 (153).

B. *Frequency of measurement*

Recommendation: Unless CGM is used, patients using multiple daily injections of insulin should be encouraged to perform SMBG 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 patients with type 1 diabetes (108). Monitoring of blood glucose less frequently than 3-4 times per day in adults and adolescents has been associated with less-effective control of glycemia as measured by HbA1c (154–156). In a study of patients age 1 to over 65 years and treated with insulin, HbA1c showed greater improvement with SMBG performed 4 or more times per day than with SMBG performed less frequently (156). (This association was not found in the patients who were treated with diet or with oral drugs alone.) A later study found a strong, continuous association of SMBG frequency with improved glycemic control as measured by HbA1c (154). This association was seen in all age groups including in infants and children

younger than 6 years and children 6-12 years old. Testing more frequently than 10 times per day was not associated with greater control of glycemia as HbA1c levels were similar in participants testing 10–12 times per day and in those testing 13 or more times per day (7.8% and 7.7%, respectively). In a study of patients under 18 years of age with type 1 diabetes, the frequency of SMBG was found to correlate inversely with HbA1c and with the incidence of diabetic ketoacidosis (155).

The ADA recommends that most patients using intensive insulin regimens (multiple daily injections or insulin pump therapy) should be encouraged to assess glucose concentrations using SMBG (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 (108).

5. Emerging considerations & 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 (129,157). 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 (158,159). Further research to identify and address barriers to achieving optimal performance of SMBG

meters has potential to improve the glycemic control achieved by people using insulin to treat diabetes.

CONTINUOUS GLUCOSE MONITORING

1. Description/introduction/terminology

In type 1 diabetes, as well as insulin-treated type 2 diabetes, frequent assessments of blood glucose are needed to adjust insulin and detect impending or current hyper- or hypoglycemia. Devices that measure interstitial glucose (which correlates highly with blood glucose) every 5-15 minutes (herein called continuously) provide glucose measurements in a more feasible manner than hypothetical continuous blood glucose monitors. Continuous glucose monitors (CGM) 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 CGMs consist of a glucose sensor placed under the skin (either through a catheter that remains in place for 1-2 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 CGMs are available for clinical use. These include real-time CGMs (rt-CGM), which provide the user with glucose measurements and trends in real time. Such devices also provide alerts and alarms to notify the user that glucose 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 CGMs (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 is-CGM currently on the market initially did not have alerts for hyper- or hypoglycemia, but the second version has the option of turning on such alerts. The final type of available CGM is 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 healthcare provider's office, where data can be downloaded and analyzed after the fact (108). Some continuous glucose monitors require calibration with a blood glucose meter at least every 12 hours, while others are "factory calibrated" and do not. Confirmation of the CGM reading by blood glucose meter is advised when CGM results are not available, or when results reported do not correlate with the clinical scenario. Most CGMs for home use include the ability to "share" data with a caregiver and/or the health care professional office via the cloud.

2. Use/rationale

Recommendation: Use real-time CGM in conjunction with insulin as a tool to lower HbA1c 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 HbA1c 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 HbA1c levels, time in range, and neonatal outcomes in pregnant women with type 1 diabetes. B (moderate)

Recommendation: Consider using real-time CGM and intermittently scanned-CGM to lower HbA1c 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 (less than 14 years old) 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 HbA1c (160–163) and reduced time in the hypoglycemic range (164,165). 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 (166).

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 HbA1c (167). Several observational studies have shown HbA1c reduction (168), or reductions in hypoglycemia without change in HbA1c (169). A systematic review of randomized controlled trials in adults with type 1 or type 2 diabetes suggested that is-CGM may reduce HbA1c in those with type 1 diabetes or insulin-treated type 2 diabetes (170), 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 HbA1c (171). A meta-analysis of non-randomized studies in adults suggested that HbA1c was lowered by approximately 0.5% at 12 months with the technology (172).

Randomized controlled trials of use of rt-CGM, compared to standard blood glucose monitoring, in adults with type 2 diabetes have generally shown reductions in HbA1c with no significant change in time in hypoglycemia (173–176). These studies have typically been done in people taking insulin, and the interventions often included substantial patient education. Studies of is-CGM use in patients with type 2 diabetes have shown mixed results for both outcomes (171,177,178).

In a large trial of rt-CGM in people with type 1 diabetes showing significant reductions in HbA1c in adults (163), improved glucose control was not seen in children (ages 8-14 years) or adolescents and young adults (ages 15-24 years). These younger participants wore the CGM significantly less than adults aged 25 years and up, and consistency of CGM use was highly correlated with lower HbA1c in all participants. A subsequent RCT specifically targeting adolescents and young adults, which included significant education and support, showed that

those randomized to rt-CGM had significantly reduced HbA1c after six months compared to those randomized to SMBG (179).

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 HbA1c (180,181), a single RCT in young children showed no impact on HbA1c (182). An uncontrolled study in toddlers with type 1 diabetes showed no evidence of glycemic improvement over six months, but high levels of parental satisfaction (183). There are no RCTs of is-CGM use in children, although observational studies suggest higher quality of life and/or treatment satisfaction in children or their caregivers (184–187).

One RCT of rt-CGM use during pregnancy in women with type 1 diabetes showed a modest but significant reduction of HbA1c in women randomized to rt-CGM compared to 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 (188). One RCT of rt-CGM vs blood glucose monitoring in women with gestational diabetes showed no significant differences in HbA1c or neonatal outcomes, but less weight gain with CGM use (189).

Professional CGM, along with professional interpretation, patient education, and therapy adjustments, may help reduce hyper- and/or hypoglycemia, but rigorous data are lacking (108).

3. Analytical considerations

Recommendation: For patients using CGMs that require calibration by users, SMBG should be used to calibrate the CGM. Calibration should be done at a time when glucose is not rising or falling rapidly. For all patients using CGM, SMBG should be done during periods when CGM results are not available or when the CGM results are inconsistent with the clinical state or suspected to be inaccurate. GPP

Most CGMs 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 non-enzymatic 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 (2.2-22 mmol/L), while the range for the current is-CGM system is 40-500 mg/dL (2.2-27.8 mmol/L). Acetaminophen in therapeutic doses caused positive bias in several older, and one current, CGM systems. Other current systems have positive bias only with supra-therapeutic blood concentrations of acetaminophen (one system) or have no significant bias with acetaminophen (190–193).

The accuracy of CGMs has improved significantly over time, with manufacturers of current devices reporting mean absolute relative deviation (MARD) proportions of 8.1-12.3%, compared to 5-10% for current SMBG devices (and 22% for the first intermittently-read interstitial glucose monitor brought to market in 2001) (194). Concerns about accuracy resulted in early versions of CGM being approved only for adjunctive use (e.g., glucose was to be measured by SMBG 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 non-adjunctive to adjunctive use (195) has led the FDA to approve most current CGMs for non-adjunctive use in the US. Additionally,

several rt-CGM devices are approved for use in hybrid closed-loop systems, wherein CGM data are fed into an algorithm that controls insulin doses via a linked insulin pump.

Early CGMs required calibration with SMBG 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 patients using CGM should be advised to verify CGM readings that appear to be spurious or not consistent with the clinical scenario (108).

4. 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/minute (horizontal arrow), changing by 1-2 mg/dL/minute (one arrow up or down), or changing by > 2 mg/dL/min (two 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). Patients 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.

CGMs can be downloaded at the time of clinic visits (or by patients at home) to obtain useful data about the patient's 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 HbA1c based on the CGM data, glucose variability (%CV or SD), time spent in the hyperglycemic range (> 250 mg/dL (13.9 mmol/L) and > 180 mg/dL (10.0 mmol/L)), time in the normoglycemic range (70-180 mg/dL or 3.9-10.0 mmol/L), and time in the hypoglycemic range (<70 mg/dL or 3.9 mmol/L, and <54 mg/dL or 3.0 mmol/L) (61,196). A subsequent international consensus defined targets for most of the measures on the AGP that would correspond to individualized HbA1c targets (197).

5. Emerging considerations & knowledge gaps/research needs

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

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

CGMs have not been approved for use in hospitalized patients, in part due to concerns about accuracy, concomitant medication use, or theoretical alterations in the usually high correlation between interstitial and blood glucose concentrations caused by serious illness. However, during the COVID-19 pandemic, the FDA allowed use of CGMs with remote monitoring in hospitals in the US to potentially reduce transmission of the virus (198). 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 SMBG or CGM technologies at this time. C (very low)

1. 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 (199), bio-impedance (200), optical coherence tomography (201,202), photoplethysmography (203), plasmonic devices (204–207), multi-sensing devices (208–211) , and direct glucose measurements in noninvasively accessible fluids, such as tears or sweat (212,213).

2. Rationale

Spectroscopy is the predominant approach and includes techniques associated with absorption spectroscopy over near-infrared (214–220) and mid-infrared (221,222) wavelengths, Raman scattering spectroscopy (223–227), and microwave spectroscopy (228–232). Exploration

of the photoacoustic spectroscopic technique has received considerable attention since 2015 (233–238). For these spectroscopic approaches, noninvasive measurements involve passing non-ionizing electromagnetic radiation through the skin and then extracting the concentration of glucose from the resulting spectrum by using multivariate chemometric methods (239). Glucose information for near-infrared, mid-infrared and Raman measurements originates from unique vibrational modes within the chemical structure of the glucose molecule.

3. **Analytical Considerations**

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

The peer-reviewed literature contains numerous 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 1) over-modelling of the calibration data, 2) uncontrolled variations associated with skin, and 3) 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 (240).

A technology described in both the peer-reviewed (241,242) and patient (243) literature over the last 5 years purports successful noninvasive glucose measurements from color bands measured over visible wavelengths from human fingers, described by the authors as “real-time

color photography related to glucose levels in capillary tissues." However, Heise and co-workers provide a complete analysis of these measurements and conclude that direct measurement of glucose is not possible at the measured wavelength bands and that the system, as described, lacks the ability to produce stable calibration functions required for practical clinical operation (244).

Considerable attention has been given over the last few years to noninvasive glucose measurements in tear fluid (245,246). 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 (247–249) and animal studies (250). Variability is reported in the ratio between glucose concentrations in blood and tear fluid for individual rabbits (251). 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 (251).

GESTATIONAL DIABETES MELLITUS

1. 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 non-pregnancy 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 ~21 million live births worldwide (6). The interest in GDM is motivated by the adverse effects on both the mother and baby (252).

2. Use/rationale

A. 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 24-28 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 (253). Prevalence of undiagnosed diabetes is markedly increased in women aged 35-44 years, in those with race/ethnicity other than Non-Hispanic White, and those with obesity (253). 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

(27,254). 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 (255). Other women should be rescreened for GDM at 24-28 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 (256). A few years later a 2-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 (254). Different screening and diagnostic approaches have been proposed over the years by other organizations (257–259).

Because of the risks to the mother and the neonate, for many years the ADA has endorsed screening for GDM at 24-28 weeks gestation in all women not previously known to have diabetes (255). The American College of Obstetricians and Gynecologists (ACOG) recommends GDM screening in women with risk factors for diabetes (254). Since the vast majority of pregnant women in the US have one or more risk factors for diabetes, universal screening is now considered the norm.

In 2008, results of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study were published (252). HAPO was a large (~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 birthweight >90th percentile, delivery by Cesarean section, clinically identified neonatal hypoglycemia and cord serum insulin (assessed by

measuring C-peptide) concentrations >90th percentile of values in the HAPO study population. Associations remained strong after adjustments for multiple, potentially confounding factors. Strong associations were also found with infant adiposity (252). Neonatal hypoglycemia (detected clinically or biochemically) was also significantly associated with maternal glycemia (260). Some secondary outcomes, including risks of shoulder dystocia and/or birth injury and preeclampsia, were also associated with maternal glycemia (261).

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 (262). These were adopted by the ADA in 2011 (113), WHO, IDF (263) 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 (27). 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 an NIH Consensus Development Conference Statement recommended that the two-step approach for detection and diagnosis of GDM, predominately used in the US, should continue to be used rather than the one-step approach and criteria proposed by IADPSG (257,258). This continues to be the recommendation of ACOG (254); however, they indicate that 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 one-step or the two-step approach (264).

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 (265). Treatment and self monitoring of blood glucose were the same in both groups. The objective was to compare the frequency of GDM detected in the 1-step and 2-step groups and frequencies of some specific outcomes such as macrosomia and large for gestational age births as well as a composite outcome in the entire groups, not specifically among those with GDM. The frequency of GDM detected with the 1-step process was approximately twice that found with the 2-step process, but no significant differences in pre-specified single or the composite outcomes were found between the two groups. Unfortunately, ~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 (266,267).

Randomized controlled trial evidence that treatment of "mild" GDM improves perinatal outcome was not provided until the 21st century (268,269). Although two RCTs found that treatment of GDM can reduce perinatal morbidity (268,269), it is not known whether treatment reduces long-term risks in children. Follow-up of the children in both these studies at 4-5 (268–270) and 7 years of age (271), respectively, failed to observe differences in limited indicators of child adiposity between children of treated and untreated GDM. Thus, more information on the metabolic health of children of mothers with GDM is needed. A HAPO Follow Up Study (HAPO FUS) was carried out in a subset of the HAPO cohort (2013-2016) when the children were on

1112 average 11.4 years of age. The results clearly demonstrate that maternal glycemia is associated
1113 with immediate and long-term outcomes for both mother and offspring. The HAPO FUS
1114 documented in both groups that risk of disorders of glucose metabolism at follow up were
1115 associated with GDM and continuously with maternal glucose concentrations (272,273).

1116

1117 **B. Monitoring/Prognosis**

1118 a. Blood glucose

1119 ***Recommendation: Women with GDM should perform fasting and postprandial SMBG for***
1120 ***optimal glucose control. B (low)***

1121 ***Recommendation: Target glucose values are FPG <95 mg/dL (<5.3 mmol/L) and either 1-h***
1122 ***postprandial <140 mg/dL (<7.8 mmol/L) or 2-h postprandial <120 mg/dL (<6.7 mmol/L). B (low)***

1123 Glucose homeostasis in pregnancy differs from the nonpregnant state. Insulin-independent
1124 glucose uptake by the fetus and placenta leads to lower fasting glucose values, while diabetogenic
1125 placental hormones produce postprandial hyperglycemia and carbohydrate intolerance. Therefore,
1126 the ADA recommends that in GDM glucose be measured both fasting and postprandially by
1127 SMBG (255). Women with GDM should try to achieve the following glucose targets: FPG <95
1128 mg/dL (<5.3 mmol/L) and either 1-h postprandial <140 mg/dL (<7.8 mmol/L) or 2-h postprandial
1129 <120 mg/dL (<6.7 mmol/L). These target values are stricter than in nonpregnant individuals.
1130 ACOG advises that on commencing nutrition therapy, women with GDM should measure blood
1131 glucose concentrations to confirm that glycemic control has been established (254). The vast
1132 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 SMBG 4 times daily compared to a group with measurement of plasma glucose in the laboratory at the time of an office visit every 1-2 weeks (274). Another study observed that the decision whether to add pharmacological therapy in GDM could be made with SMBG every other or every 3rd day instead of daily (275).

b. HbA1c

HbA1c concentrations decrease during normal pregnancy due to increased red cell turnover (276). Moreover, macrosomia results primarily from postprandial hyperglycemia, which may not be adequately detected by HbA1c. Therefore, while HbA1c may provide valuable information, it should not replace SMBG. An HbA1c value <6% (<42 mmol/mol) is optimal in pregnancy, if it can be achieved without significant hypoglycemia (255). Due to the altered red cell turnover in pregnancy, HbA1c should be measured monthly.

c. Postpartum testing

Recommendation: Women with GDM should be tested for prediabetes or diabetes 4-12 weeks postpartum using non-pregnant OGTT criteria. A (moderate)

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

Although most cases of GDM resolve after delivery, some do not. Moreover, some cases of GDM may represent pre-existing, but undiagnosed, type 2 diabetes. In addition, women with GDM have a considerably increased risk of developing type 2 diabetes after pregnancy (277) and the Diabetes Prevention Program (DPP) found that progression to diabetes can be delayed or prevented by intervention (278); thus, long-term follow-up is important. A 75-g OGTT, interpreted by nonpregnant criteria, is recommended to find persistent hyperglycemia at 4-12 weeks postpartum. HbA1c is not recommended at this visit because the concentration may still be influenced by changes during pregnancy and/or peripartum blood loss. Since the risk of progression to diabetes after GDM is linear over time (reaching 50-60% (277,279)), women should be evaluated every 1-3 years with any recommended test of glycemia, e.g., annual HbA1c, annual FPG or triennial 75-g OGTT (with nonpregnant cutoffs) (255).

Many women with GDM will have subsequent pregnancies. If possible, preconception evaluation should be done and include measurement of glucose or HbA1c because of the risks of pre-diabetes or diabetes in women with prior GDM (254,255).

3. Analytical considerations

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

A. Preanalytical

The diagnosis of GDM is totally dependent on accurate measurement of glucose. The diagnostic thresholds for GDM, especially for FPG, are substantially lower than those for diabetes i.e., 92 mg/dL (5.1 mmol/L) or 95 mg/dL (5.3 mmol/L) by IADPSG or Carpenter-Coustan criteria, respectively (Table 7). Furthermore, in view of the relatively short interval between diagnosis of GDM and delivery, confirmatory diagnostic testing is not routinely recommended as it is in nonpregnant individuals. Therefore, preparation and timing of testing and analytical accuracy of glucose measurements are important for correct classification of GDM.

Screening and diagnostic testing should not be done in febrile or recently ill persons. Individuals should have normal, unrestricted meals for at least 3 consecutive days before testing. An 8-10 hour period of fasting must precede an OGTT which must be conducted during the morning because of circadian influences on circulating glucose (280).

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 15-30 min), as was done in the HAPO Study (273). 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 (113), with those kept at room temperature increased the rate of diagnosis of GDM by 2.7-fold (281), entirely due to control of glycolysis. Similarly, in 121 women screened for GDM

with OGTTs, collecting samples in tubes containing citrate buffer doubled the diagnostic sensitivity for GDM compared to samples collected in sodium fluoride-containing tubes (76).

B. 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.

4. Emerging considerations & knowledge gaps/research needs

A. 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 (282) and limited population surveys in early pregnancy (283), 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 (284). For example,

1217 the NIH has funded a study, termed “Go Moms”, to address this issue. Several other studies are
1218 also underway to explore screening, diagnosis and treatment of GDM before 20 weeks gestation.

1219 There is evidence that women diagnosed with GDM early are more likely to have adverse
1220 outcomes. For example, outcomes for women with GDM diagnosed before 12 weeks of gestation
1221 are similar to those in women with pre-existing diabetes (285). However, there is no consensus
1222 on the glucose cutoff that should be used for diagnosis. The glycemic thresholds for the
1223 diagnosis of GDM in the second and third trimester may not be appropriate for early pregnancy
1224 because FPG normally declines in early pregnancy (286,287). For example, in a large Chinese
1225 cohort many women with FPG in the first trimester above the IADPSG threshold for GDM did
1226 not have GDM when tested later in gestation (283).

1227 Efforts to detect GDM earlier than 24 weeks gestation by methods other than glucose
1228 have been reported (288). For example, the HbA1c concentration at the first prenatal visit
1229 identifies risk of adverse pregnancy outcomes and diabetes during pregnancy, but is less effective
1230 for ascertainment of GDM (289,290). Other studies suggest that biomarkers such as CD59 (291)
1231 or serum secreted frizzle-related protein 5 (292) may be useful in early identification of women
1232 in whom GDM will be identified later in pregnancy. There is an ongoing search to identify the
1233 optimum method to detect GDM in early pregnancy.

1234 B. Towards a consensus on detection and diagnosis

1235 Based on analysis of OGTT results from the Danish Odense Cohort Study (293,294),
1236 McIntyre et al (293) have questioned the universal use of the value ≥ 92 mg/dl (5.1 mmol/l) as
1237 the FPG threshold for a diagnosis of GDM by the IADPSG (262) and WHO (263) 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 (295,296).

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 (297). This approach also includes recommendations for low resources 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 hours later. In circumstance of very limited resources or in remote locations far from laboratories, the only way of estimating glycemia is by point of care finger stick.

The controversy surrounding the optimal way to diagnose GDM continues, despite calls for global agreement on a common approach. In 2021 a group of obstetricians reviewed the strengths and weaknesses of the 1-step and 2-step approaches to diagnose GDM (298). The authors favored the one-step procedure, but concluded that diagnostic thresholds should be confirmed by a large multi-institutional RCT. However, there is no assurance that such a RCT would end the GDM controversy. Definitive prospective clinical trials are needed to unequivocally establish a universal and pragmatic strategy to diagnose and follow-up GDM.

URINE GLUCOSE

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

1. **Description/introduction/terminology**

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

2. **Use/rationale**

Measurement of glucose in the urine, once the hallmark of diabetes care in the home setting, has now been replaced by SMBG (see above). Semiquantitative urine glucose monitoring should be considered only for patients who are unable to or refuse to perform SMBG, since urine glucose concentration does not accurately reflect plasma glucose concentration (299). Notwithstanding these limitations, urine glucose monitoring is supported by the IDF in those situations where blood glucose monitoring is not accessible or affordable, particularly in resource poor settings (300). 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 (301).

Although urine glucose is detectable in patients with grossly increased blood glucose concentrations, it provides no information about blood glucose concentrations below the variable renal glucose threshold [~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.

3. **Analytical Considerations**

Qualitative, semiquantitative and quantitative methods are available to measure glucose in urine (92). Semiquantitative test-strip methods that utilize specific reactions for glucose are recommended. Commercially available strips use the glucose oxidase reaction (92). The strip is moistened with freshly voided urine and after 10 seconds the color is compared to a color chart. Test methods that detect reducing substances are not recommended as they are subject to numerous interferences, including numerous drugs, and non-glucose sugars. When used, single voided urine samples are recommended (299).

4. **Interpretation**

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

KETONE TESTING

1. **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 patients with diabetes mellitus as adjuncts for both diagnosis and ongoing monitoring of diabetic ketoacidosis (DKA). Measurements of ketone bodies are performed both in an office/hospital setting and by patients 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.

2. Use/Rationale

Recommendation: Patients who are prone to ketosis (those with type 1 diabetes, history of diabetic ketoacidosis (DKA), or treated with SGLT-2 inhibitors) should measure ketones in urine or blood if they have unexplained hyperglycemia or symptoms of ketosis (abdominal pain, nausea), and implement sick day rules and/or seek medical advice if urine or blood ketones are 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 patients with known diabetes mellitus or in previously undiagnosed patients presenting with hyperglycemia suggest impending or established DKA, a medical emergency. The two major mechanisms responsible for the high ketone concentrations in patients with diabetes are increased production from triglycerides and decreased utilization in the liver, both a result of absolute or relative insulin deficiency and increased counter-regulatory hormones including cortisol, epinephrine, glucagon, and growth hormone (302).

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 towards formation of βOHB in any condition that alters the redox state of hepatic mitochondria to increase concentrations of NADH such as hypoxia, fasting, metabolic disorders (including DKA) and alcoholic ketoacidosis. Thus, assay methods for ketones that do not include measurement of βOHB may provide misleading clinical information by underestimating total ketone body concentration (299,303).

The presence of urine ketones is highly sensitive for DKA or significant ketosis, with high negative predictive value suggesting utility in ruling out DKA (304,305). Some blood glucose meters also have the capacity to measure blood ketones. Compared to testing urine ketones, children with type 1 diabetes (and caregivers) were more likely to measure blood ketones during periods of illness, and those randomized to blood ketone testing had almost half the number of emergency department visits or hospitalizations (306). The ADA recommends that ketosis-prone people with diabetes mellitus check urine or blood ketones in situations characterized by symptoms of illness and deterioration in glycemic control, in order to detect and pre-empt DKA ketoacidosis (307). 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 (307).

3. Analytical Considerations

Urine ketones

A. Preanalytical

Normally, the concentrations of ketones in the urine are below the detection limits of commercially available testing materials. False-positive results have been reported with highly colored urine and in the presence of several sulfhydryl containing drugs, including angiotensin-converting enzyme inhibitors (305). 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 patients in the home setting, control materials (giving both negative and positive readings) are not commercially available but would be desirable to assure accuracy of test results.

B. 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 (305). 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 patient 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 (299,308).

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)

A. 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 one week at 4 °C and for at least several weeks at –20 °C (long-term stability data are not available for most assay methods).

B. 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 (305). The principle of the enzymatic methods is that βOHB in the presence of NAD is converted to AcAc and NADH by β-hydroxybutyrate dehydrogenase (308). Under alkaline conditions (pH 8.5-9.5), the reaction favors formation of AcAc from βOHB. The NADH produced can be quantified spectrophotometrically (usually kinetically) with use of a peroxidase reagent. Most methods permit use of whole blood, plasma, or serum specimens (required volumes are generally 200 uL or less). Some methods permit analysis of multiple analytes and are designed for point-of-care testing. Several methods are available as hand-held meters, which are FDA-approved in the US for both laboratory use or for home use by patients. 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 (305,309).

4. **Interpretation**

A. Urine ketone determinations

In a patient with known diabetes mellitus or in a patient not previously diagnosed with diabetes, but 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 associated with type 1 diabetes, it may rarely occur in type 2 patients (310). The introduction of SGLT-inhibitors has resulted in an increase in cases of DKA in patients with type 2 diabetes and an even greater increase in patients with type 1 diabetes treated off-label. Since the SGLT inhibitors decrease the hyperglycemia that otherwise attends DKA, patients are often instructed to check urine ketone concentrations (or blood ketones or βOHB) at any sign of illness (310). Patients 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 (299).

B. 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 (299,302). Blood ketone determinations that measure βOHB specifically are useful for both diagnosis (303,305) and ongoing monitoring of DKA (302,303). 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 (311). 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 (312).

Reference intervals for βOHB differ among assay methods, but concentrations in healthy individuals fasted overnight are generally <0.5 mmol/L. Patients 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.

5. **Emerging considerations & knowledge gaps**

Since hospitalization rates for DKA are increasing (313), 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) (303).

HEMOGLOBIN A1c

1. Description/introduction/terminology

Glycation refers to the nonenzymatic attachment of glucose to available amino groups on proteins. The extent of glycation reflects the exposure of the protein to mean glycemia integrated over time as a function of the lifespan and turnover of the protein. Hemoglobin in the erythrocyte has an average circulating lifespan of approximately 120 days and glycated hemoglobin therefore usually indicates the average glucose concentration over the preceding ~60-90 days. The terms glycated hemoglobin, glycohemoglobin, glycosylated and glucosylated hemoglobin, HbA1, HbA1c, 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). HbA1c is the specific glycated species that is modified by glucose on the N-terminal valine of the hemoglobin beta chain. Assay methods that measure total glycated hemoglobins (e.g., boronate affinity methods) should be calibrated to report results equivalent to HbA1c to harmonize results. HbA1 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 HbA1c to facilitate communication with patients. 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 (52)) and UKPDS (51,54)) used assay methods that quantified HbA1c. In order to harmonize results, most clinical studies of glucose control recommend the use of HbA1c assays that are traceable to the DCCT assay, as was done in the UKPDS. In this paper, we use the abbreviation GHb to include all forms of glycated hemoglobin and HbA1c 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 are available. Concentrations of these glycated proteins also reflect mean glycemia, but over a much shorter time (15-30 days, reflecting the turnover of albumin) than GHb (60-90 days) (299,314–319). However, the clinical utility of glycated proteins other than hemoglobin has not been clearly established. Only one published study has convincingly demonstrated a relationship between glycated protein levels and the chronic complications of diabetes (320).

2. Use/rationale

A. Screening/Diagnosis

Recommendation: Laboratory-based HbA1c testing can be used to diagnose

a) diabetes, with a value ≥ 6.5% (≥48 mmol/mol) diagnostic of diabetes, and

b) prediabetes (or high risk for diabetes) with a HbA1c level of 5.7% to 6.4% (39-46 mmol/mol)

A (moderate)

The role of HbA1c in the diagnosis of diabetes was first proposed and implemented in 2009 (22), made possible by improved assay standardization through the NGSP and IFCC, and new data demonstrating the association between HbA1c concentrations and risk for retinopathy (22) Guidelines have been updated over time (27). Several technical advantages of HbA1c testing compared with glucose testing, such as its pre-analytic stability and decreased biological variability (321), also played a role. Finally, the clinical convenience of the HbA1c assay, which requires no patient fasting or stress (glucose tolerance) tests, compared with glucose-based diagnosis, has led to increasing use of HbA1c testing for diagnosis. A HbA1c value of 6.5% (48 mmol/mol) or greater is considered diagnostic. Confirmation with a repeated HbA1c test on a different sample or a glucose-based test is recommended (27,322). The frequency of HbA1c testing for diagnosis has not been established, but guidelines similar to those for glucose-based testing seem appropriate (27). HbA1c assays are not recommended for screening for or diagnosis of gestational diabetes (see GDM section). Screening for diabetes will also identify populations with HbA1c that are increased but not high enough to qualify as diabetes (≥6.5%). Although the risk for developing diabetes follows HbA1c levels as a continuum, i.e., higher values are associated with higher risk for future development of diabetes (323–325) , an International Expert Committee (22) recommended HbA1c levels from 6.0 to 6.4% and the ADA has recommended HbA1c levels from 5.7 to 6.4% (27) 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: POC HbA1C 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 HbA1c methods that are NGSP-certified should be used to diagnose (or screen for) diabetes. The ADA has cautioned that POCT devices for HbA1c should not be used for diagnosis (307). Although several point-of-care HbA1c assays are NGSP-certified, the test is CLIA-waived in the US and proficiency testing is not necessary. Therefore, minimal objective information is available concerning their performance in the hands of non-laboratory personnel who often measure HbA1c with POCT devices. Several published evaluations revealed that few POCT devices for HbA1c met acceptable analytical performance criteria (326). A meta-analysis published in 2017 revealed continuing problems with the accuracy of POCT devices (327). 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 one device, including when it was used by non-laboratory clinical staff (328). Laboratories or sites that perform these tests need to have a CLIA certificate, be inspected, and must meet the CLIA quality standards (329). These standards include specified personnel requirements (including documented annual competency assessments) and participation three times per year in an approved proficiency testing program). It is not intended for sites that only do waived testing. 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 HbA1c devices should not be used for diagnosis of or screening for diabetes.

B. Monitoring

Recommendation: HbA1c should be measured routinely (usually every 3 months until acceptable, individualized targets are achieved and then no less than every 6 months) in most patients with diabetes mellitus to document their degree of glycemic control. A (moderate)

Measurement of HbA1c is widely used for routine monitoring of long-term glycemic status in patients with diabetes mellitus. HbA1c 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 all patients with diabetes (299,330). The ADA, virtually all other endocrinology specialty organizations, and non-specialty organizations have recommended measurement of HbA1c in all patients with diabetes to document the degree of glycemic control and assess response to therapy (61,331). The recommended specific treatment goals for HbA1c are based on the results of prospective randomized clinical trials, most notably the DCCT in type 1 diabetes (52) and the UKPDS in type 2 diabetes (54). These trials have documented an association between glycemic control, as quantified by longitudinal determinations of HbA1c, and risks for the development and progression of chronic complications of diabetes (50,51). More importantly, they have established a salutary role of "intensive" glycemic control aimed at achieving near normal glycemia, as measured by HbA1c levels, on long-term diabetic complications (52,54).

a. Testing frequency

There is no consensus on the optimal frequency of HbA1c testing. The ADA recommends (61): "The frequency of HbA1c 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 HbA1c 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" (61). These testing recommendations are for non-pregnant patients with either type 1 or type 2 diabetes. In addition, all patients with diabetes who are admitted to hospital should have HbA1c measured if the result of testing in the previous 3 months is not available (61). Studies have established that serial (quarterly for one year) measurements of HbA1c are associated with large improvements in HbA1c values in patients with type 1 diabetes (332).

b. Target Levels/Treatment Goals

Recommendation: Treatment goals should be based on ADA recommendations which include maintaining HbA1c concentrations <7% (53 mmol/mol) for many nonpregnant patients with diabetes and more stringent goals in selected individual patients if this can be achieved without significant hypoglycemia or other adverse effects of treatment.

Somewhat higher ranges are recommended for children and adolescents and are appropriate for patients with limited life expectancy, extensive co-morbid illnesses, a history of severe hypoglycemia and advanced complications. *(Note that these values are applicable only if the assay method is certified by the NGSP as traceable to the DCCT reference.)* ***A (high)***

The ADA recommends that in general a HbA1c target less than 7% (53 mmol/mol) is desirable for many nonpregnant adults, with higher values recommended for children and adolescents (27), balancing the acute risks of hypoglycemia against the long-term benefits on complications. HbA1c measurements are a routine component of the clinical management of patients with diabetes mellitus. Based principally on the results of the DCCT in type 1 diabetes and the UKPDS in type 2 diabetes, the ADA has recommended that a primary goal of therapy is a HbA1c value < 7% (53 mmol/mol) for many patients (61). Other endocrine specialty clinical organizations recommend HbA1c targets similar to the ADA, ranging from 6.5% to 7% (48 to 53 mmol/mol), although higher levels have been suggested by non-specialty organizations (333,334). These HbA1c values apply only to assay methods that are certified as traceable to the DCCT reference, with non-diabetic reference interval approximately 4-6% HbA1c (20-42 mmol/mol). In the DCCT, each 10% reduction in HbA1c (e.g., 12 vs. 10.8% or 8 vs. 7.2%) was associated with a 44% lower risk for the progression of diabetic retinopathy (51). Comparable risk reductions were found in the UKPDS (54). It should also be noted that in the DCCT and UKPDS decreased HbA1c was associated with increased risk for severe hypoglycemia.

HbA1c 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 individual patients, more stringent targets than 7% (53 mmol/mol) can be pursued, provided that this goal can be achieved without substantial hypoglycemia or other adverse effects of treatment. Such patients might include those with short duration of diabetes, diet-treated type 2 diabetes, and long life expectancy (61). Moreover, the introduction of CGM devices that alarm with low blood glucose concentrations and semi-

automated pumps that suspend insulin infusion as glucose concentrations decrease have facilitated achieving target HbA1c levels with less risk for hypoglycemia (335). Conversely, in patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications or extensive comorbid conditions, higher HbA1c goals should be chosen.

Recommendation: During pregnancy and in preparation for pregnancy, women with diabetes should try to achieve HbA1c goals that are more stringent than in the non-pregnant state, aiming ideally for <6.0% 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, HbA1c testing and maintenance of specified concentrations in patients 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 HbA1c 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 (336). ADA recommendations include a HbA1c <6% (42 mmol/mol) during pregnancy in patients with preexisting diabetes, recognizing that changes in red blood cell turnover during pregnancy in non-diabetic women lowers usual HbA1c concentrations, if it can be achieved without "significant" hypoglycemia (255).

3. Analytical Considerations

A. Preanalytical

a. Patient variables- age and race

HbA1c 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 HbA1c. Published data show age-related increases in mean HbA1c in non-diabetic populations of approximately 0.1% per decade after age 30 years (337,338). Careful phenotyping of subjects with OGTT supports an increase in HbA1c with age, even after removing patients with otherwise undiagnosed diabetes and persons with impaired glucose tolerance from the study population (339). The increase in HbA1c levels with age generally parallel other measures of glycemia. The clinical implications of the small, but statistically significant, progressive increase of "normal" HbA1c levels with aging remains to be determined (340).

The effects of race on HbA1c values remain controversial. Several studies have suggested a relatively higher HbA1c in African-American and Hispanic populations than in Caucasian 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 (338,341,342). An analysis of 11,092 adults showed that blacks had mean HbA1c values 0.4% higher than whites (339). However, race did not modify the association between the HbA1c concentration and adverse cardiovascular outcomes or death (339). In addition, a study among races showed that all measures of glycemia, including HbA1c, fructosamine, and glycated albumin, were higher, in parallel among African-Americans compared with Caucasians, and that the measures were similarly associated with risk of nephropathy, retinopathy and CVD in the different races (343). The consistency of

glycemic measurements within races and the similar relationship of each glycemic measurement with complications in African Americans suggests that higher HbA1c measurements in African Americans reflects, at least in part, higher glycemic exposure and not just a difference in the relationship between mean glycemia and HbA1c levels. The HbA1c-derived average glucose (ADAG) study, which included frequent measures of glucose, did not show a significantly different relationship between calculated mean glucose during three months and HbA1c at the end of the three months between Africans/African-Americans and Caucasians; however, the size of the African/African-American population was relatively small, limiting the interpretation of this finding (344). A study in type 1 diabetes demonstrated a difference in the relationship between mean average glucose measured with CGM and HbA1c in African Americans compared with Caucasians (345). At the same average glucose values, HbA1c was approximately 0.4% higher in African American patients than Caucasians.

b. Other patient-related factors and interfering factors

Recommendation: Laboratories should be aware of potential interferences, including hemoglobin variants that may affect HbA1c 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: HbA1c results in patients with disorders that affect erythrocyte 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 cell turnover, hemoglobin variants or other interfering factors compromise interpretation of HbA1c test results, although they reflect a shorter period of average glycemia than HbA1c. GPP

Recommendation: HbA1c cannot be measured 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 erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers HbA1c test results, compared with mean glycemia, regardless of the assay method (299). One study has suggested that differences in mean red cell half-life that may range from approximately 48 to 68 (mean 58 and 1 SD of 4.5 to 6.5) days may explain some of the inter-individual variability in the relationship between measured average glucose and HbA1c levels (346).

Vitamins C and E are reported to lower test results falsely, possibly by inhibiting glycation of hemoglobin (347,348). Iron-deficiency anemia is reported to increase test results (349). Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction are reported to interfere with some assay methods, falsely increasing results (315,350). 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 erythrocyte survival) (351–353) for a review, see (350). 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, HbA1c can be measured accurately in most individuals heterozygous for hemoglobin variants (see http://www.ngsp.org/factors.asp for a summary of published studies). It is important to emphasize that HbA1c cannot be measured in individuals with homozygous hemoglobin variants (e.g., HbSS, HbCC, HbEE) or two variant hemoglobins, like HbSC; they have no HbA therefore do not have HbA1c. In this situation, or if altered erythrocyte turnover interferes with the relationship between mean blood glucose values and HbA1c, 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 HbA1c assay method. A list of interfering factors for specific assays is maintained on the NGSP website (www.ngsp.org). In selecting an assay method, the laboratory should take into consideration characteristics of the patient population served, e.g., high prevalence of hemoglobin variants.

c. Sample collection, handling, and storage

Blood can be obtained by venipuncture or by finger-stick capillary sampling. Blood tubes should contain anticoagulant as specified by the manufacturer of the HbA1c assay method (EDTA can be used unless otherwise specified by the manufacturer). Sample stability is assay method specific (354,355). In general, whole blood samples are stable for up to 1 week at 4° C (355). For most methods, whole blood samples stored at –70° C or colder are stable long-term (at least one 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 (356–358) 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 (357,358). In addition, the sample collection kits should be approved for clinical use by appropriate authorities.

B. Analytical

a. Traceability of HbA1c methods

Recommendation: Laboratories should use only HbA1c assay methods that are certified by the NGSP as traceable to the DCCT reference. The manufacturers of HbA1c assays should also show traceability to the IFCC reference method. GPP

There are >300 HbA1c assay methods in current clinical use. Many of these use high throughput automated systems dedicated to HbA1c determinations. Most methods can be classified into groups based on assay principle (69,299,315). The first group includes methods that quantify GHb based on charge differences between glycated and non-glycated components. Examples include cation-exchange chromatography and capillary electrophoresis. The second group includes methods that separate components based on structural differences between glycated and non-glycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify HbA1c, defined as hemoglobin A with glucose attached to the NH2-terminus valine of one or both beta chains. Other methods quantify "total glycated hemoglobin," which includes both HbA1c and other hemoglobin-glucose adducts (i.e., internal glucose-lysine adducts, and terminal glucose-alpha chain NH2-terminus valine adducts). Enzymatic methods to specificall measure HbA1c are also commercially available. Generally, results of methods using different assay principles show excellent inter-assay correlation, and there are no convincing data to show that any one method type or analyte is clinically superior to any other. The ADA recommends that laboratories use only assay methods that are certified as traceable to the DCCT GHb reference (61); these results are reported as HbA1c (299,315,333,359).

Recommendation: Laboratories that measure HbA1c 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 National Glycohemoglobin Standardization Program (NGSP), initiated under the auspices of the AACC and endorsed by the ADA, has standardized GHb test results among laboratories to DCCT-equivalent HbA1c values (360–362) and focused on improving world-wide 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 HbA1c and is a CLSI designated comparison method (363). 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 Pathologist proficiency testing survey has documented a steady improvement in comparability of GHb values among laboratories, both within-method and between-method (360,361,364). The NGSP website provides detailed information on the certification process and maintains a listing of certified assay methods (updated monthly) and factors that are known to interfere with specific methods (NGSP website: http://www.ngsp.org).

The IFCC has developed a higher order reference method and reference materials for HbA1c analysis that was approved in 2001 (365,366). Analysis is performed by cleaving hemoglobin with endoproteinase Glu-C and separating the resulting glycated and non-glycated N-terminal β chain hexapeptides by HPLC (366). Quantification of the hexapeptides is performed with electrospray

ionization mass spectrometry or capillary electrophoresis. The 2 methods use the same primary reference materials and the results are essentially identical. HbA1c is measured as the ratio of glycated to non-glycated N-terminal peptide and is reported as mmol beta N1-deoxyfructosyl-hemoglobin per mol hemoglobin. Of note, the preparation and measurement of samples using this method is laborious, expensive and time-consuming and was never envisioned as a practical means of assaying clinical samples. It is only used for manufacturers to standardize the assays. Like the NGSP, the IFCC has established a network of reference laboratories (367). The IFCC offers manufacturers calibrators and controls and a monitoring program (367).

b. Analytical performance goals and quality control

Recommendation: The goals for imprecision for HbA_{1c} measurement are intra-laboratory CV <1.5% and inter-laboratory CV <2.5% (using at least two 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, intra-laboratory CVs <3% (368)or <2% (14)and inter-laboratory CV <5% (368) have been proposed. The prior version of these guidelines recommended intra-laboratory CV <2% and inter-laboratory CV <3.5% (14,15). Intraindividual CVs in healthy persons are very small (<2%) and many current assay methods can achieve intra-laboratory CVs <1.5% and inter-laboratory CVs <2.0% among different laboratories using the same method (369). 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% HbA1c between successive patient samples is due to a significant change in glycemic control (when HbA1c is 7% (53 mmol/mol)) (364). In addition,

if a method has no bias, a CV of 3.5% is necessary to have 95% confidence that the HbA1c result for a patient with a "true" HbA1c of 7% (53 mmol/mol) will be between 6.5 and 7.5% (48 and 58 mmol/mol) (364). Based on the currently available technologies and the clinical need for low CVs, we recommend intra-laboratory CV <1.5% and inter-laboratory CV <2.5%.

Bias is the deviation of a result from the true value. Criteria based on biological variation have been suggested to establish analytic performance targets. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation database, which uses a systematic review that is regularly updated, recommends a desirable bias no more than 1.2% for HbA1c (370). To minimize differences among laboratories in the diagnosis of diabetes in individuals whose HbA1c 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 ^{0}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.

c. Removal of labile GHb

Formation of HbA1c includes an intermediate Schiff base which is called "pre-A1c" or labile A1c (371). This material is formed rapidly with hyperglycemia and could interfere with some HbA1c assay methods if not completely removed or separated. Currently available automated assays either remove the labile pre-HbA1c during the assay process or they do not measure the labile product.

4. Interpretation

A. Laboratory-physician interactions

The laboratory should work closely with physicians who order HbA1c 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 (independent of any shortened erythrocyte survival), the physician 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 HbA1c test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS (52,54). This information is available on the NGSP website. For example, each 1% (~11 mmol/mol) change in HbA1c is related to a change in mean plasma glucose of approximately 1.6 mmol/L (29 mg/dL). Reporting HbA1c results with a calculated estimated average glucose (eAG) will eliminate the need for health care providers or patients to perform these calculations themselves. The equation generated by the ADAG study is generally considered the most reliable one to date (344).

There is some evidence to suggest that immediate feedback to patients at the time of the clinic visit with HbA1c test results improves their long-term glycemic control (372,373). However, not all publications support this observation (374) and additional studies are needed to resolve this question before the strategy can be generally recommended. It is possible to have HbA1c test results available at the time of the clinic visit by either having the patient go to the laboratory shortly before the scheduled clinic visit or by having a rapid assay system convenient to the clinic.

B. Clinical application

a. Reporting

HbA1c values in patients with diabetes are a continuum; they range from within the non-diabetic reference interval in a small percentage of patients whose mean plasma glucose concentrations are close to those of non-diabetic individuals, to markedly increased values, e.g., two- to threefold higher levels than the non-diabetic mean of approximately 5%, in some patients, reflecting an extreme degree of hyperglycemia. Proper interpretation of HbA1c test results requires that physicians understand the relationship between HbA1c values and mean plasma glucose, the kinetics of HbA1c, and specific assay limitations/interferences (299). Small changes in HbA1c (e.g., +/- 0.3% HbA1c) over time may reflect assay variability rather than a true change in glycemic status (364).

Recommendation: Hemoglobin A1c (HbA1c) should be reported as a percentage of total hemoglobin or as mmol/mol of total hemoglobin . GPP

HbA1c 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 (375). Comparison of pooled blood samples between the IFCC and the NGSP (DCCT-aligned) networks has revealed a linear relationship (termed the master equation): (NGSP% = (0.915 x IFCC%) + 2.152) (366). Clinical results reported in IFCC units (mmol/mol) correlate tightly with NGSP results reported in percent.

Recommendation: HbA1c may also be reported as estimated average glucose (eAG) to facilitate comparison with the self-monitoring results obtained by patients and make the interpretation of the HbA1c more accessible to patients. GPP

Several studies have demonstrated a close mathematical relationship between the HbA1c concentration and mean glycemia that should allow expression of HbA1c as an estimated average glucose concentration (eAG) (344,376,377). The eAG is helpful in translating the HbA1c results into the same glucose levels as SMBG and CGM for the purposes of clinical management and therapeutic adjustments.

An international agreement recommended that both NGSP and IFCC units be reported (378,379), with reporting of eAG left to the discretion of individual countries; however, universal reporting of HbA1c has not been adopted, with some countries, like the US, usually reporting HbA1c as a % of total hemoglobin and eAG, while others, such as the UK, report results in IFCC mmol/mol units with or without eAG.

b. 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, non-diabetic test subjects should be nonobese and have FPG <5.6 mmol/L (100 mg/dL) and, ideally, a 2-hour glucose <11.1 mmol/L (200 mg/dL) during an OGTT. For many years, HbA1c reference intervals were 4-6% (20-42 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., 4.5-5.5% (26-37 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 in patients.

c. Out-of-range specimens

Recommendation: Laboratories should verify by repeat testing specimens with HbA1c results below the lower limit of the reference interval or greater than 15% HbA1c. B (low)

The laboratory should repeat testing for all sample results below the lower limit of the reference interval and, if confirmed, the physician should be informed to see if the patient has a variant hemoglobin or evidence of red cell destruction. If possible, the repeat measurement of HbA1c should be performed using 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% HbA1c (140

mmol/mol) should be repeated and, if confirmed, the possibility of a hemoglobin variant should be considered (350). Any result that does not correlate with the clinical impression should also be investigated. Comparison of suspicious HbA1c results with other glycated protein assays (e.g., fructosamine, glycated albumin) may be informative.

5. Emerging Considerations & knowledge gaps/research needs

A. Capillary kits for measurement of HbA1c

Capillary blood sample kits have been used in research studies and shown to perform well compared with whole venous samples when assayed with a high-performance chromatography method (356,357). 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.

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

Further studies are needed to determine if other glycated proteins such as fructosamine or glycated serum albumin are clinically useful for routine monitoring of patients' 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 HbA1c. Further studies are also needed to determine if measurements of advanced glycation end-products (AGEs) are clinically useful as predictors of risk for chronic diabetes complications (380). Only one study in a subset of DCCT patients 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 HbA1c values over time (381). The clinical role of such measurements remains undefined. Similarly, the role of noninvasive methods using light to measure tissue glycation transdermally is undefined.

C. *Global harmonization of HbA1c 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 (378,379) needs to be carried out with education of health care providers and patients. Some believe that reporting eAG should complement the current reporting of HbA1c in NGSP-DCCT aligned units (%) and the newer IFCC results (mmol/mol), since the eAG results will be in the same units (mmol/L or mg/dL) as patients' self-monitoring. Educational campaigns will be necessary to ensure clear understanding of this assay (and the reported units) that is central to diabetes management.

GENETIC MARKERS

1. **Description/introduction/terminology**

Type 1 diabetes results from a selective autoimmune destruction of the pancreatic beta cell functional mass, eventually leading to an absolute lack of insulin and consequent hyperglycemia. The mode of inheritance is complex, and around 80% to 85% of newly diagnosed patients occur sporadically without familial aggregation. Among identical twins or

HLA-identical siblings of type 1 diabetes patients, about 20-30% 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 type 1 diabetes patients diagnosed before age 30 years have the HLA haplotypes *DRB1*04-DQAI*03:01-BI*03:02(DR4-DQ8), DRBI*03-DQA1*05:01-BI*02:01 (DR3-DQ2.5),* or both (382). These haplotypes are common in the general population and are necessary, but not sufficient, for type 1 diabetes.

2. **Use/rationale**

A. Diagnosis/Screening

a. 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 patients with type 1 diabetes. Typing for genetic markers and the use of genetic risk scores is recommended for patients 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, valuable information including treatment options can be obtained with definition of diabetes-associated mutations. A (moderate)

Genetic markers are in general of limited clinical value in the diagnosis, classification and management of pediatric patients with diabetes. However, an exception is the mutational analyses established for classification of diabetes in the neonate (383–386) as well as in young patients with

2012 a dominant family history of diabetes, often referred to as maturity onset diabetes of the young
2013 (MODY) (386,387) (Table 8). Type 1 or autoimmune diabetes is strongly associated with HLA
2014 DR and DQ genes. Typing of the class II major histocompatibility antigens or HLA DRB1, DQA1
2015 and DQB1 is not diagnostic for type 1 diabetes. HLA-DQ A1 and B1 genotyping can be useful to
2016 signal absolute risk of diabetes. The HLA-DQA1*03:01-B1*03:02 (DQ8) and HLA-
2017 DQA1*05:01-B1*02:01 (DQ2) haplotypes, alone or in combination, may account for up to 90%
2018 of children and young adults with type 1 diabetes (382). Both haplotypes may be present in 30-
2019 40% of a Caucasian population and HLA is therefore necessary, but not sufficient, for disease. The
2020 HLA DQ and DR genes are by far the most important determinants for the risk of developing a
2021 first beta cell autoantibody such as either insulin autoantibodies (IAA) or glutamic acid
2022 decarboxylase autoantibodies (GADA) following an environmental exposure by e.g. enterovirus
2023 (388). Once beta cell autoimmunity has developed, HLA genes do not seem to contribute to the
2024 risk of progression to clinical onset of type 1 diabetes (389).

2025 Thus, HLA-DR-DQ typing can be used only to increase or decrease the probability of type
2026 1 diabetes and cannot be recommended for routine clinical diagnosis or classification (390).
2027 Precision in the genetic characterization of type 1 diabetes may be extended by typing for
2028 polymorphisms in several genetic loci identified in genome wide association studies (388,391).
2029 Non-HLA genetic factors include the genes for insulin (INS), PTPN22, CTLA-4 and several others
2030 (388,389). These additional genetic factors may assist in assigning a probability of the diagnosis
2031 of type 1 diabetes of uncertain etiology, and genetic risk scores for type 1 diabetes have been
2032 developed (392).

2033 It is possible to screen newborn children to identify those at increased risk of developing
2034 type 1 diabetes (393). 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 (390,392,394). Nevertheless, this strategy cannot be recommended until there is a proven intervention available to delay or prevent the disease (395). There is some evidence that early diagnosis may prevent hospitalization with ketoacidosis and preserve residual beta cells (395). The rationale for the approach is thus placed below under emerging considerations.

b. Type 2 diabetes and MODY

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

Type 2 diabetes: Fewer than 5% of patients with type 2 diabetes have been resolved on a molecular genetic basis and, not surprisingly, most of these have an autosomal dominant form of the disease or very high degrees of insulin resistance. Type 2 diabetes is a heterogenous polygenic disease with both resistance to the action of insulin and defective insulin secretion (3,4). 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 (396,397). 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 (398,399).

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 patients and their relatives is technically feasible. The reduced cost of sequencing and emerging new technologies makes it possible to identify mutations and properly classify MODY patients 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.

B. 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 sulphonylurea rather than with insulin (383,385,400).

3. **Rationale**

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 (401). 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.

The rationale for genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself (27). Such diabetes may be secondary to the obesity associated with Prader-Willi syndrome, which maps to chromosome 15 q, or to the absence of adipose tissue inherent to recessive Seip-Berardinelli syndrome of generalized lipodystrophy mapping to chromosome 9q34 (18,402). 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 (396,397). 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] (27).

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.

A. 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 microgram DNA from one 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.

B. 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.

4. **Interpretation**

The risk of type 1 diabetes etiology and pathogenesis in the general population may be determined by HLA-DQ typing, which contribute as much as 50% of familial susceptibility (403). 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 alpha and beta chain heterodimers. Persons 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. By contrast, individuals with the DRB1*15-DQA1*02:01-DQB1*06:02 haplotype are protected from type 1 diabetes at a young age (404). 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 (389,405). 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 1:10 in the general population. The risk of type 1 diabetes in HLA-identical siblings of a proband with type 1 diabetes is 1:4, while siblings who have HLA-haplotype identity have a 1:12 risk and those with no shared haplotype a 1:100 risk (406). Genome wide association studies have confirmed a number of non-HLA genetic factors that increase the risk of a first appearing beta-cell autoantibody or type 1 diabetes, both in first degree relatives of type 1 diabetes patients and in the general population (388,389,407,408). Combining HLA and non-HLA polymorphisms in genetic risk scores has improved the selection subjects at risk of type 1 diabetes into prevention clinical trials.

5. Emerging considerations & 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 (406,409,410). The prospect of genotyping is to identify pathophysiological variants and provide personalized medicine.

AUTOIMMUNE MARKERS

1. **Description/introduction/terminology**

The pathogenesis of type 1 diabetes is strongly associated with several immune abnormalities most prominently islet autoantibodies, but also co-occurrence of other organ-specific autoimmune diseases such as autoimmune thyroid disease and celiac disease. The islet autoantibodies are directed against insulin (IAA), GAD65 (GADA), IA-2 (IA-2A) or ZnT8 (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 (411,412). The islet autoantibody biomarkers are useful to predict and classify type 1 diabetes.

2. **Use/rationale**

Recommendation: Standardized islet autoantibody tests are recommended for classification of diabetes in adults who phenotypically overlap with type 1 diabetes (such as thin and onset at age <40) or in adults with questionable diagnostic criteria for type 2 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 and symptoms. GPP

Recommendation: Standardized islet autoantibody tests are recommended in prospective studies of children at increased genetic risk of type 1 diabetes following HLA typing at birth or in first degree relatives of type 1 diabetes patients. B (low)

A therapeutic intervention that will prevent diabetes has yet to be identified (413). Therefore, although several islet autoantibodies have been detected in individuals with type 1 diabetes, measurement of these has limited use outside of clinical studies. Currently islet autoantibodies are not used in routine management of patients with diabetes. This section will focus on the pragmatic aspects of clinical laboratory testing for islet autoantibodies.

A. Diagnosis

The clinical onset of type 1 diabetes is related to the loss of the functional beta-cell mass. In most of these patients, the loss of function is associated with an autoimmune attack (414). This is termed type 1A or immune mediated diabetes. Islet autoantibodies comprise autoantibodies to 1) islet cell cytoplasm (ICA), 2) native insulin, termed insulin autoantibodies (IAA) (415), 3) glutamic acid decarboxylase (GADA) (416–418), 4) islet antigen-2, IA-2A (417) and IA-2betaA

(also known as phogrin) (419) and 5) three variants of the ZnT8 transporter (ZnT8A) (420,421). Autoantibody markers are usually present in 85-90% of individuals with type 1 diabetes when fasting hyperglycemia is initially detected (27). Autoimmune destruction of the islet beta 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). 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. Patients with type 1A diabetes have a significantly increased risk of other autoimmune disorders, including celiac disease, Graves' disease, thyroiditis, Addison's disease, and atrophic gastritis along with pernicious anemia (422). As many as 1:4 females with type 1 diabetes have autoimmune thyroid disease while 1:280 patients develop adrenal autoantibodies and adrenal insufficiency. A few patients with type 1 diabetes (type 1B, idiopathic) have no known etiology and no evidence of autoimmunity. Many of these patients are of African or Asian origin.

B. Screening

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

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

Only about 15% of newly diagnosed type 1 diabetes patients have a first degree relative with the disease (423). The risk of developing type 1 diabetes in relatives of patients with the disease is ~ 5%, which is 15-fold higher than the risk in the general population (1:250-300 lifetime risk). Screening relatives of type 1 diabetes patients for islet autoantibodies can identify those at high risk of the disease. However, as many as 1-2% of healthy individuals may have either IAA, GADA, IA-2A or ZnT8A alone and are at low risk of type 1 diabetes (424). Children with only one autoantibody may revert to negativity, but their risk of type 1 diabetes remains between not having an islet autoantibody to being persistent single autoantibody positive. Because of the low prevalence of type 1 diabetes (~0.3% in the general population), the positive predictive value of a single islet autoantibody is low (411). The presence of multiple islet autoantibodies (IAA, GADA, IA-2A/IA-2betaA or ZnT8A) is associated with a risk of type 1 diabetes of > 90% (411,425,426). 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 (427) nor from type 1 diabetes later in life. Because islet autoantibodies in these individuals have substantially reduced predictive significance, these subjects are often excluded from prevention trials.

Approximately 5-10% of Caucasian adult patients who present with type 2 diabetes phenotype have islet autoantibodies (428), particularly GADA, which predict insulin dependency. This has been termed latent autoimmune diabetes of adult (LADA) (429), type 1,5 diabetes (430)

or slowly progressive insulin-dependent diabetes (SPIDDM) (431). Although GADA-positive diabetes patients progress to absolute insulinopenia faster than do autoantibody-negative patients, some autoantibody-negative adults with type 2 diabetes also progress (albeit more slowly) to insulin dependence with time. Some of these patients may show T cell reactivity to islet cell components (430). There is limited utility for islet autoantibody testing in patients with type 2 diabetes because the institution of insulin therapy is based on glucose control. At diagnosis of pediatric diabetes, absence of all four islet autoantibodies and modest hyperglycemia (HbA1c <7.5% [58 mmol/mol]) proved useful for the detection of MODY (386). Routine testing for GADA in adults with newly diagnosed diabetes could better define autoimmune diabetes.

C. Monitoring/Prognosis

Recommendation: There is currently no role for measurement of islet autoantibodies in the monitoring of patients 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 (432). However, 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 (433). 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 (434). 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 (435), 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 (436); c) to screen non-diabetic family members 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 (437,438). 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 patients 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 are determined by indirect immunofluorescence on frozen sections of human pancreas (439). ICA measure the degree of binding of immunoglobulin to islet sections and are compared to a WHO standard serum available from the National Institute of Biological Standards and Control

(440). 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 radio isotopic method that calculates the displaceable insulin radioligand binding after the addition of excess non-radiolabeled insulin (441) is recommended. Results are reported as positive when the specific antibody binding exceeds the 99th percentile or possibly the mean + 2 (or 3) SD for healthy persons. IAA binding is not normally distributed. Each laboratory needs to assay at least 100-200 healthy individuals to determine the distribution of binding. An important caveat concerning IAA determination is that insulin antibodies develop following insulin therapy even in those persons who use human insulin. Data from the Diabetes Autoantibody Standardization Program (DASP) (442) and the NIDDK workshop (443) demonstrate that the interlaboratory variability for IAA is inappropriately large.

GADA, IA-2A and ZnT8A are determined in standardized radio binding assays using coupled *in vitro* transcription translation to label the autoantigens (444) or with commercially available non-radiolabelled 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 (443,445).

3. **Interpretation**

GADA may be present in 60-80% of newly diagnosed patients with type 1 diabetes, but the frequency varies with gender and age. GADA in both patients and healthy subjects is associated with HLA DR3-DQA1*05:01-B1*02:01. IA-2A may be present in about 40-80% of newly diagnosed type 1 diabetes patients, but the frequency is highest in the young and decreases with increasing age. 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. IAA are positive in more than 70-80% of children who develop type 1 diabetes before age 5 years, but in fewer than 40% of individuals developing diabetes after age 12. IAA are associated with HLA DR4-DQA1*03:01-B1*03:02 and with INS VNTR (382). ICA is found in about 75-85% of new onset type 1 diabetes patients.

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 (446).

The presence of islet autoantibodies suggests that insulin is the most appropriate therapeutic option, especially in a young person. 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 subsets of patients, including Hispanic and African American children with a potential diagnosis of non-autoimmune diabetes, adults with islet autoantibodies but clinically classified with type 2 diabetes, and children with transient hyperglycemia. Most non-diabetic individuals who have only one autoantibody may never develop diabetes as the 10 year risk is about 15% (411). Although expression of multiple islet autoantibodies is associated with greatly increased risk of diabetes (424,447), approximately 10% of individuals presenting with new onset diabetes express only a single autoantibody (448). 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 (449).

The following suggestions have been proposed (405) 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. These strategies will reduce both false positive and negative results.

4. Emerging Considerations & knowledge gaps/research needs

Since immunoassays for IAA, GADA IA-2A/IA-2betaA and ZnT8A are available, a panel of these autoantibodies can be used in screening studies (450).

It is likely that other islet autoantigens will be discovered, which could lead to additional diagnostic and predictive tests for type 1 diabetes. Autoantibody screening on finger-stick blood samples as dried blood spots appears feasible. In those individuals who are islet autoantibody positive, HLA-DR-DQ genotyping or an analysis of Genetic Risk Score (390,394) will help define the risk of type 1 diabetes.

Many relatives of type 1 diabetes patients have been screened for IAA, GADA, IA-2A and ZnT8A to enroll double autoantibody positive subjects in prevention trials (451). After many years of negative studies of various immune interventions, there is some evidence that the anti-CD3 monoclonal antibody teplizumab delays progression to type 1 diabetes in high-risk individuals (432).

Several clinical trials to prevent or intervene in type 1 diabetes are being actively pursued in relatives of patients with type 1 diabetes or in the general population based on islet autoantibodies and HLA-DR-DQ genotypes or genetic risk scores. Research subjects with two or more islet autoantibodies undergo an OGTT, allowing randomization 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. The staging of presymptomatic autoimmune type 1 diabetes should prove useful for future secondary prevention trials. For example, the TrialNet oral insulin prevention trial was a mixture of stage 1 and 2 subjects, while only stage 2 subjects were enrolled in the anti-CD3 teplizumab trial. Additional trials of other antigen-based immunotherapies, adjuvants, cytokines and T cell accessory molecule blocking agents are likely in the future (452). Decreased islet autoimmunity will be an important outcome measure of these therapies.

URINE ALBUMIN

1. **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 (453), and these definitions have been adopted by the

ADA. There are now three categories of albuminuria (Figure 1, Table 10) which have been renamed. These are:

- **A1 - Normal to Mildly Increased Albuminuria:** urine albumin/creatinine ratio (uACR) <30 mg/g (<3 mg/mmol). This is equivalent to 24-hour albumin excretion rate (AER) <30 mg/d and urine protein:creatinine ratio (uPCR) <150 mg/g (<15 mg/mmol).
- **A2 - Moderately Increased Albuminuria:** uACR 30–299 mg/g (3–29 mg/mmol). This is equivalent to AER 30–299 mg/d and uPCR 150–499 mg/g (15–49 mg/mmol).
- **A3 - Severely Increased Albuminuria** uACR ≥300 mg/g (≥30 mg/mmol). This is equivalent to AER ≧300 mg/d, protein excretion rate (PER ≧500 mg/d) and uPCR ≧500 mg/g (>50 mg/mmol).

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

2. **Use/Rationale**

A. Diagnosis/Screening

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

Diabetes is associated with a high rate of cardiovascular events and is also the leading cause of end-stage renal disease in the Western world (454). 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 levels of albumin are used (455–457).

Moderately increased albuminuria (stage A2, Figure 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 (458,459).

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 patients (age > 75 years) or with life expectancy < 20 years may not be at increased risk of kidney failure requiring replacement therapy during their lifetimes, they will be at moderately increased risk of cardiovascular mortality, with severity of chronic kidney disease (CKD)

acting as a risk multiplier (460,461). In such patients, the predictive role of reducing moderately increased albuminuria in the context of cardiovascular outcomes has become clearer over the last five years. The FIGARO outcome trial (462) 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 patients with diabetes and/or kidney disease for albuminuria (463,464). Moreover, cardiovascular risk may extend below the lower limit of 30 mg/d (465–467), reinforcing the notion that albuminuria is a continuous variable for cardiovascular risk (468–470).

An eGFR of <60 mL/min, regardless of the presence of moderately increased albuminuria, is an independent cardiovascular risk marker (453). 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 (458).

B. Monitoring

Although the urine albumin: creatinine ratio appears entirely acceptable for screening, limited data are available for its use in monitoring the response to therapy. Post hoc analyses of clinical trials indicate that the albumin: creatinine ratio is a reasonable method to assess change over time (471). The KDIGO and ADA guidelines recommend annual quantitative testing for urine albumin in adults with diabetes, using morning spot albumin to creatinine ratio measurement (458,472,473).

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/1.73m^2 (61). SGLT2 inhibitors and finerenone, a nonsteroidal mineralocorticoid receptor antagonists, also reduce albuminuria in clinical trials of advanced diabetic kidney disease (474–476). 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 GFR.

i. Evolving Changes in eGFR Measurement

At the time of publication of this guideline, a strong consensus was developing to use an equation for estimating GFR that, unlike the CKD-EPI equation, eliminates racial status and improves specificity by adding cystatin C when possible. The rationale is the inequities noted in the race-based equation for eGFR. A special panel was convened and a more equitable equation was proposed involving cystatin C (477,478). Using cystatin C with serum creatinine improves the accuracy of the CKD-EPI equation. 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 (479). Cystatin C may also detect kidney dysfunction at an earlier stage than creatinine in people with diabetes (480).

C. Prognosis

Albuminuria above 30 mg/g creatinine and eGFR <60 ml/min (Figure 1) have prognostic significance. In multiple epidemiological studies moderately increased albuminuria is an independent risk marker for cardiovascular death (481–483). In 80% of patients with type 1

diabetes and moderately increased albuminuria, urine albumin excretion can increase by as much as 10 – 20% per year, with more than half the patients developing severely increased albuminuria (> 300 mg albumin/day) in 10 –15 years. Once this occurs, most patients 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 – 40% of patients with Stage A2 albuminuria (Figure 1) progress to an eGFR <60 ml/min/1.73m^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 patient does not die from a cardiovascular event) kidney disease usually progresses to stage 4 and even stage 5. Approximately 20% develop end-stage kidney disease and almost all will have severely increased albuminuria despite achievement of blood pressure goals (484). Moderately increased 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, ~20% of people with diabetes progress to end stage kidney disease without an increase in moderately increased albuminuria (488).

3. Analytical Considerations

A. Preanalytical

Recommendation: First morning void urine sample should be used for measurement of albumin: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 patient should be well hydrated and should not have ingested food within the preceding 2 hours 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 even moderately increased in patients with diabetes (489). The albumin to creatinine ratio is the best method to predict renal events in patients 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-hour albumin excretion, the albumin concentration and the albumin: creatinine ratio over 3-4 weeks. The last two were measured in the 24-hour urine sample, the first morning void and random untimed urine. In healthy volunteers, the lowest CVi was observed for the albumin concentration in the first morning void (36%) and for the albumin: creatinine ratio in that sample (31%) (491). Others have validated the reliability of a first-morning void sample (464,492,493). To minimize variability, all collections should be at the same time of day and patients should preferably not have ingested food for at least 2 hours (494).

Transient increases of urine albumin excretion are reported with short-term hyperglycemia, exercise, urine tract infections, sustained blood pressure elevation, heart failure, fever, and hyperlipidemia (473).

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).

B. Analytical

a. Quantitative

Recommendation: The analytical performance goals for urine albumin measurement should be between-day precision ≤6%, bias ≤7%-13% and total allowable error of ≤24%-30%. B (moderate)

Analytical goals can be based on biological variation, expert opinion, opinion of clinicians or state of the art (94). A 2014 study compared 17 commercially available urine albumin measurement procedures to an isotope dilution mass spectrometry reference measurement procedure (498). Mean biases were large and ranged from 35% to 34% at 15 mg/L. The authors concluded that calibration bias was the main source of error for differences among methods and precision was adequate for most assays. Based on the performance of measurement procedures, the National Kidney Disease Education Program (NKDEP) Laboratory Working Group in 2017 recommended the following analytical performance goals for measurement of urine albumin:

between-day precision ≤6%, bias ≤7%-13% and total allowable error of ≤24%-30% (499). The analytical measurement range for urine albumin should be 2 mg/L to 400 mg/L (499).

b. Semi-quantitative 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 semi-quantitative 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, ie, 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 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 patients) 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% to 86%) and 93% (CI, 84% to 97%), respectively, for the semiquantitative test (501). Sensitivity and specificity estimates for the quantitative test were 96% (CI, 78% to 99%) and 98% (CI, 93% to 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, five of which were in the 2014 review and evaluated performance of ACR. The pooled sensitivity and specificity at each cutoff point were as follows: ACR >30 mg/g, 0.82 (95% confidence interval:0.76–0.87) and 0.88 (0.83–0.91); ACR 30–300 mg/g, 0.72 (0.68–0.77) and 0.82 (0.76–0.89); and ACR >300 mg/g, 0.84 (0.71–0.90) and 0.97 (0.95–0.99), respectively. An important limitation of all these data is that the dipstick methods were compared to local laboratory methods, which, as indicated above, exhibit large biases (498).

A cost effectiveness analysis of 1881 patients with diabetes published in 2020 evaluated medical costs of CKD and concluded that semi-quantitative uACR dipstick method could be an

appropriate screening tool for albuminuria in diabetic patients. 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 seconds) between dipping and scanning, which can result in an incomplete reaction. It is therefore critical that 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 semi-quantitative uACR dipsticks from proteinuria dipsticks. Chemical strip methods for total protein are not sensitive when the urine albumin concentration is 20 – 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: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.

4. **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 patients 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 2 of 3 tests repeated at intervals over a period of a 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. StageA2 albuminuria (30-299 mg/g) on one occasion is indicative of persistent albuminuria 50-75% of the time, while stage A3 albuminuria (≥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 semi-quantitative uACR dipstick testing as a useful approach when quantitative analysis is not possible. Advantages of semi-quantitative testing include relatively high specificity and use as point-of-care testing which, if appropriately

implemented, can improve access (particularly in resource-limited settings) and eliminate the need for shipping samples and delays in getting a test result.

Frequency of measurement

Recommendation: If eGFR is <60 ml/min/1.73m^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 2 of 3 tests performed within a period of 3 – 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 (507). 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 such testing in individual patients (508).

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 40-50% 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 30-50%

and ideally try to achieve uACR of <30 mg/g. This is difficult in many cases, but annual measurement of albuminuria is useful to assess risk and treatment.

MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES

I. INSULIN AND PRECURSORS

1. **Use**

A. Diagnosis

Recommendation: In most patients 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 patients 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 (509). Increased insulin concentration is a surrogate marker for insulin resistance, although accurate measurement of insulin sensitivity requires the use of complex methods, such as the hyperinsulinemic euglycemic clamp technique, which are generally confined to research laboratories. Due to the critical role of insulin resistance in the pathogenesis of type 2 diabetes, hyperinsulinemia would also appear to be a logical risk predictor for incident type 2 diabetes.

Earlier studies may not have controlled well for undiagnosed diabetes, glycemic measures, body mass index, or other confounders (509). Subsequent analyses suggest that insulin values do not add significantly to diabetes risk prediction carried out using more traditional clinical and laboratory measurements (510), and that measures of insulin resistance (which include insulin measurements) predicted risk of diabetes or CAD only moderately, with no threshold effects (511). Consequently, it seems of greater clinical importance to quantify the consequences of the insulin resistance and hyperinsulinemia (or hyperproinsulinemia) rather than the hormone values themselves, i.e., by measuring blood pressure, body mass index, degree of glucose tolerance, and plasma lipid/lipoprotein concentrations. It is these variables that are the focus of clinical interventions, not plasma insulin or proinsulin concentrations (510,511).

The clinical utility of measuring insulin, C-peptide or proinsulin concentrations to help select the best antihyperglycemic agent for initial therapy in patients with type 2 diabetes is a question that arises from consideration of the pathophysiology of type 2 diabetes. In theory, the lower the pre-treatment insulin concentration, the more appropriate might be insulin, or an insulin secretagogue, as the drug of choice to initiate treatment. While this line of reasoning may have some intellectual appeal, there is no evidence that measurement of plasma insulin or proinsulin concentrations will lead to more efficacious treatment of patients 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 (512). 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 patients 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 (5,513). 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 non-diabetic hypoglycemia to rule out hypoglycemia due to surreptitious insulin administration (512).

In the past, some advocated insulin or C-peptide assays in the evaluation and management of patients with the 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 (body mass index, presence of acanthosis nigricans) and routine measurements of C-peptide or insulin are not recommended by ACOG (514).

2. **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 (515). In 2009, an insulin standardization workgroup of the ADA, in conjunction with NIDDK, CDC, and EASD, called for harmonization of insulin assay results through traceability to an isotope dilution liquid chromatography/tandom mass spectrometry reference (516). 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 (517), 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 world-wide harmonization of C-peptide (518).

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 United States Centers for Medicare and Medicaid Services (CMS) that Medicare patients must have C-peptide measured in order to be eligible for coverage of insulin pumps. Initially, the requirement was that the C-peptide be ≤ 0.5 ng/mL; however because of non-comparability of results from different assays resulting in denial of payment for some patients with values above 0.5 ng/mL, the requirement now states that the C-peptide should be ≤110% of the lower limit of the reference interval of the laboratory's measurement method (519).

II. INSULIN ANTIBODIES

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

Given sufficiently sensitive techniques, insulin antibodies can be detected in any patient being treated with exogenous insulin (520,521). In most patients, 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, usually occurs in insulin-treated

patients with type 2 diabetes, and the cause and effect relationships between the magnitude of the increase in insulin antibodies and the degree of insulin resistance is unclear (521). There are several therapeutic approaches for treating these patients and a quantitative estimate of the concentration of circulating insulin antibodies does not appear to be of significant benefit.

Table 1

Classification of diabetes mellitus[a]

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

[a]From the ADA (27).

Table 2: Rating scale for the quality of the evidence

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

Table 3: Grading the Strength of Recommendations

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

- There is very low quality evidence but very strong agreement and very high levels of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.

C. THERE IS INSUFFICIENT INFORMATION TO MAKE A RECOMMENDATION

Grade C is applied in the following circumstances:

- Evidence is lacking or scarce or of very low quality, and the balance of benefits and harms cannot be determined, and there is no or very low level of agreement of experts for or against adoption of the recommendation.
- At any level of evidence – particularly if the evidence is heterogeneous or inconsistent, indirect, or inconclusive – if there is no agreement of experts for or against adoption of the recommendation.

GPP. GOOD PRACTICE POINT

Good Practice Points (GPPs) are recommendations mostly driven by expert consensus and professional agreement, and are based on the below listed information and/or professional experience, or widely accepted standards of best practice. This category mostly applies to technical (e.g. pre-analytical, analytical, post-analytical), organizational, economic or quality management aspects of laboratory practice. In these cases evidence often comes from observational studies, audit reports, case series or case studies, non-systematic 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.

2791
2792

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]

[a]In the absence of unequivocal hyperglycemia, diagnosis requires abnormal results on two different tests (glucose and HbA1c) on the same day or two abnormal results from samples obtained on different days.

Adapted from the ADA (27).

[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 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.

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.

Table 6. Comparison of Selected Accuracy Standards for Glucose Meters

	Required meter results	At glucose concentrations
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

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.

Table 7 Screening for and diagnosis of GDM [a]

One-step strategy
Perform a 75-g OGTT, with plasma glucose measurement when patient is fasting and at 1 and 2 h, at 24–28 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 24–28 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), 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 two* of the following four plasma glucose levels (measured fasting and at 1, 2, and 3 h during OGTT) are met or exceeded (Carpenter-Coustan criteria [244]):
• 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)

GDM, gestational diabetes mellitus; GLT, glucose load test; OGTT, oral glucose tolerance test.

[a] From the ADA (27).

* American College of Obstetricians and Gynecologists notes that one elevated value can be used for diagnosis (254).

Table 8. Causes of 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 (171)
	FOXP3	X-linked	Permanent: immunodysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome: autoimmune diabetes, autoimmune thyroid disease, exfoliative dermatitis; insulin requiring

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.

From the ADA (27)

Table 9 Staging of type 1 diabetes [a]

	Stage 1	Stage 2
Characteristics	• Autoimmunity	• Autoimmunity
	• Normoglycemia	• Dysglycemia
	• Presymptomatic	• Presymptomatic
Diagnostic criteria	• Multiple islet autoantibodies	• Islet autoantibodies (usually multiple)
	• No IGT or IFG	• Dysglycemia: IFG and/or IGT
		• FPG 100–125 mg/dL (5.6–6.9 mmol/L)
		• 2-h PG 140–199 mg/dL (7.8–11.0 mmol/L)
		• A1C 5.7–6.4% (39–47 mmol/mol) or ≥10% increase in A1C

FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; 2-h PG, 2-h plasma glucose.

[a]Adapted from the ADA (27).

Table 10 Definitions of albuminuria [a]

	Unit of measure		
	mg/24 h	**µg/min**	**mg/g creatinine**
Normal	<30	<20	<30
Moderately increased albuminuria (formerly microalbuminuria)	30–299	20–199	30–299
Severely increased albuminuria[b]	≥300	≥200	≥300

[a]Adapted from the ADA (473).

[b]Also called "overt nephropathy."

FIGURE 1. The KDIGO HeatMap of staging and CKD/CV risk [a]

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 Legend

Fig. 1: 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.

[a]From the ADA (473)

References

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014 Jan 1;37(Supplement_1):S81–90.
2. Castaño L, Eisenbarth GS. Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat. Annu Rev Immunol. 1990;8:647–79.
3. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988 Dec;37(12):1595–607.
4. Sacks DB, McDonald JM. The pathogenesis of type II diabetes mellitus. A polygenic disease. Am J Clin Pathol. 1996 Feb;105(2):149–56.
5. Balasubramanyam A, Garza G, Rodriguez L, Hampe CS, Gaur L, Lernmark A, et al. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. Diabetes Care. 2006 Dec;29(12):2575–9.
6. IDF Diabetes Atlas [Internet]. 10th ed. International Diabetes Federation; 2021 [cited 2022 May 23]. Available from: https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf
7. National Diabetes Statistics Report | Diabetes | CDC [Internet]. 2022 [cited 2022 Aug 9]. Available from: https://www.cdc.gov/diabetes/data/statistics-report/index.html
8. Chan JCN, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA. 2009 May 27;301(20):2129–40.
9. Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, et al. Prevalence of diabetes among men and women in China. N Engl J Med. 2010 Mar 25;362(12):1090–101.
10. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2017. Diabetes Care. 2018;41(5):917–28.
11. American Diabetes Association. Economic costs of diabetes in the U.S. In 2007. Diabetes Care. 2008 Mar;31(3):596–615.
12. Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, et al. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes Care. 2005 Sep;28(9):2130–5.
13. Sacks D, 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(6):750–86.
14. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care. 2011 Jun;34(6):e61-99.
15. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem. 2011 Jun;57(6):e1–47.
16. Group NDD. Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. Diabetes. 1979 Dec 1;28(12):1039–57.
17. WHO Expert Committee on Diabetes Mellitus: second report. World Health Organ Tech Rep Ser. 1980;646:1–80.
18. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997 Jul;20(7):1183–97.

19. Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, et al. Comparison of fasting and 2-hour glucose and HbA1c levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diabetes Care. 1997 May;20(5):785–91.
20. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ. 1994 May 21;308(6940):1323–8.
21. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia [Internet]. WHO and International Diabetes Federation; 2006 Apr [cited 2022 May 23]. Available from: https://www.who.int/publications/i/item/definition-and-diagnosis-of-diabetes-mellitus-and-intermediate-hyperglycaemia
22. International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care. 2009 Jul;32(7):1327–34.
23. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation [Internet]. Geneva: World Health Organization; 2011 [cited 2022 May 23]. 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 HbA1c for diabetes diagnosis. Diabetes Res Clin Pract. 2011 Sep;93(3):310–1.
25. American Diabetes Association. Tests of glycemia in diabetes. Diabetes Care. 2000 Jan;23 Suppl 1:S80-82.
26. Harris MI. Undiagnosed NIDDM: clinical and public health issues. Diabetes Care. 1993 Apr;16(4):642–52.
27. American Diabetes Association Professional Practice Committee. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S17–38.
28. Nathan DM, Bennett PH, Crandall JP, Edelstein SL, Goldberg RB, Kahn SE, et al. Does diabetes prevention translate into reduced long-term vascular complications of diabetes? Diabetologia. 2019 Aug;62(8):1319–28.
29. American Diabetes Association. Type 2 diabetes in children and adolescents. Diabetes Care. 2000 Mar;23(3):381–9.
30. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002 Feb 7;346(6):393–403.
31. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001 May 3;344(18):1343–50.
32. 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 Jun 2;162(11):765–76.
33. Icks A, Rathmann W, Haastert B, John J, Löwel H, Holle R, et al. Cost-effectiveness of type 2 diabetes screening: results from recently published studies. Gesundheitswesen. 2005 Aug;67 Suppl 1:S167-171.
34. Perry RC, Shankar RR, Fineberg N, McGill J, Baron AD, Early Diabetes Intervention Program (EDIP). HbA1c 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 Mar;24(3):465–71.

35. 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 Feb;26(2):485–90.
36. Dallo FJ, Weller SC. Effectiveness of diabetes mellitus screening recommendations. Proc Natl Acad Sci USA. 2003 Sep 2;100(18):10574–9.
37. The cost-effectiveness of screening for type 2 diabetes. CDC Diabetes Cost-Effectiveness Study Group, Centers for Disease Control and Prevention. JAMA. 1998 Nov 25;280(20):1757–63.
38. 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 May 4;140(9):689–99.
39. Glümer C, Yuyun M, Griffin S, Farewell D, Spiegelhalter D, Kinmonth AL, et al. What determines the cost-effectiveness of diabetes screening? Diabetologia. 2006 Jul;49(7):1536–44.
40. Kahn R, Alperin P, Eddy D, Borch-Johnsen K, Buse J, Feigelman J, et al. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. Lancet. 2010 Apr 17;375(9723):1365–74.
41. Zhou X, Siegel KR, Ng BP, Jawanda S, Proia KK, Zhang X, et al. Cost-effectiveness of Diabetes Prevention Interventions Targeting High-risk Individuals and Whole Populations: A Systematic Review. Diabetes Care. 2020 Jul 1;43(7):1593–616.
42. Greenberg RA, Sacks DB. Screening for diabetes: is it warranted? Clin Chim Acta. 2002 Jan;315(1–2):61–9.
43. Herman WH, Hoerger TJ, Brandle M, Hicks K, Sorensen S, Zhang P, et al. The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. Ann Intern Med. 2005 Mar 1;142(5):323–32.
44. Genuth S, Alberti KGMM, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care. 2003 Nov;26(11):3160–7.
45. Forouhi NG, Balkau B, Borch-Johnsen K, Dekker J, Glumer C, Qiao Q, et al. The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. Diabetologia. 2006 May;49(5):822–7.
46. Tai ES, Goh SY, Lee JJM, Wong MS, Heng D, Hughes K, 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 Jul;27(7):1728–34.
47. Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, 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 Aug;23(8):1108–12.
48. Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, Shochat T, et al. Normal fasting plasma glucose levels and type 2 diabetes in young men. N Engl J Med. 2005 Oct 6;353(14):1454–62.
49. 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 Apr;43(4):894–902.
50. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes. 1995 Aug;44(8):968–83.

51. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000 Aug 12;321(7258):405–12.
52. The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. New England Journal of Medicine. 1993;329(14):977–86.
53. Nathan DM, Cleary PA, Backlund JYC, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005 Dec 22;353(25):2643–53.
54. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998 Sep 12;352(9131):837–53.
55. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med. 2004 Sep 21;141(6):421–31.
56. Ray KK, Seshasai SRK, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D, 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 May 23;373(9677):1765–72.
57. 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 Oct 9;359(15):1577–89.
58. Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, Goff DC, Bigger JT, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med. 2008 Jun 12;358(24):2545–59.
59. ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med. 2008 Jun 12;358(24):2560–72.
60. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med. 2009 Jan 8;360(2):129–39.
61. American Diabetes Association Professional Practice Committee. 6. Glycemic Targets: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S83–96.
62. Howe-Davies S, Simpson RW, Turner RC. Control of maturity-onset diabetes by monitoring fasting blood glucose and body weight. Diabetes Care. 1980 Oct;3(5):607–10.
63. Muir A, Howe-Davies SA, Turner RC. General practice care of non-insulin-dependent diabetes with fasting blood glucose measurements. Am J Med. 1982 Nov;73(5):637–40.
64. 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 Dec 27;284(24):3157–9.
65. Bruns DE, Metzger BE, Sacks DB. Diagnosis of Gestational Diabetes Mellitus Will Be Flawed until We Can Measure Glucose. Clin Chem. 2020 Feb 1;66(2):265–7.
66. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. Clin Chem. 2009 May;55(5):850–2.
67. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem. 1989 Feb;35(2):315–7.

68. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In: Clinical laboratory methods and disgnosis. St. Louis: C.V. Mosby Co.; p. 149–92.
69. Rifai N. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2017.
70. Sacks DB. Tietz textbook of clinical chemistry and molecular diagnostics - 4th edition. 4th ed. St. Louis: Elsevier Saunders; 2018. 518–538 p.
71. Wilson SS, Guillan RA, Hocker EV. Studies of the stability of 18 chemical constituents of human serum. Clin Chem. 1972 Dec;18(12):1498–503.
72. 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 Dec;22(12):2031–3.
73. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem. 2009 May;55(5):1019–21.
74. Uchida K, Matuse R, Toyoda E, Okuda S, Tomita S. A new method of inhibiting glycolysis in blood samples. Clin Chim Acta. 1988 Feb 29;172(1):101–8.
75. Fobker M. Stability of glucose in plasma with different anticoagulants. Clin Chem Lab Med. 2014 Jul;52(7):1057–60.
76. 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 Jun;62(6):886–7.
77. van den Berg S a. A, Thelen MHM, Salden LPW, van Thiel SW, Boonen KJM. It takes acid, rather than ice, to freeze glucose. Sci Rep. 2015 Mar 9;5:8875.
78. 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 Dec;49(18):1406–11.
79. 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 Jul 6;67(7):1032–4.
80. 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 May;47(7–8):625–8.
81. 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 Oct;62(4):545–52.
82. 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 May;61(3):169–79.
83. Carstensen B, Lindström J, Sundvall J, Borch-Johnsen K, Tuomilehto J, DPS Study Group. Measurement of blood glucose: comparison between different types of specimens. Ann Clin Biochem. 2008 Mar;45(Pt 2):140–8.
84. Miles RR, Roberts RF, Putnam AR, Roberts WL. Comparison of serum and heparinized plasma samples for measurement of chemistry analytes. Clin Chem. 2004 Sep;50(9):1704–6.

85. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem. 2002 Dec;48(12):2242–7.
86. Larsson-Cohn U. Differences between capillary and venous blood glucose during oral glucose tolerance tests. Scand J Clin Lab Invest. 1976 Dec;36(8):805–8.
87. Lind T, de Groot HAVC, Brown G, Cheyne GA. Observations on Blood Glucose and Insulin Determinations. Br Med J. 1972 Aug 5;3(5822):320–3.
88. Tchobroutsky G. Blood glucose levels in diabetic and non-diabetic subjects. Diabetologia. 1991 Feb 1;34(2):67–73.
89. 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 Aug;6(8):647–58.
90. 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 Nov;14(11):989–93.
91. Chia CW, Egan JM, Ferrucci L. Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. Circ Res. 2018 Sep 14;123(7):886–904.
92. Sacks DB. Carbohydrates. In: In Rifai, Chiu R, Young I, Burnham C, Wittwer C, editors. Tietz Textbook of Laboratory Medicine. 7th ed. St. Louis: Elsevier; 2023. p. 353.e1-353.e23.
93. Miller WG, Myers GL, Ashwood ER, Killeen AA, Wang E, Ehlers GW, et al. State of the art in trueness and interlaboratory harmonization for 10 analytes in general clinical chemistry. Arch Pathol Lab Med. 2008 May;132(5):838–46.
94. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. Clin Chem. 1999 Mar;45(3):321–3.
95. 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 Mar;33(3):157–69.
96. Fraser CG. The necessity of achieving good laboratory performance. Diabet Med. 1990 Jul;7(6):490–3.
97. 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 Apr;45(4):561–6.
98. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, 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 Mar;39(3):298–305.
99. Sebastián-Gámbaro MA, Lirón-Hernández FJ, Fuentes-Arderiu X. Intra- and inter-individual biological variability data bank. Eur J Clin Chem Clin Biochem. 1997 Nov;35(11):845–52.
100. 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 Jul 23;167(14):1545–51.
101. 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 Feb;51(2):450–2.

102. Aarsand AK, Díaz-Garzón J, Fernandez-Calle P, Guerra E, Locatelli M, Bartlett WA, 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 Sep;64(9):1380–93.
103. González-Lao E, Corte Z, Simón M, Ricós C, Coskun A, Braga F, et al. Systematic review of the biological variation data for diabetes related analytes. Clin Chim Acta. 2019 Jan;488:61–7.
104. Howanitz PJ, Cembrowski GS, Steindel SJ, Long TA. Physician goals and laboratory test turnaround times. A College of American Pathologists Q-Probes study of 2763 clinicians and 722 institutions. Arch Pathol Lab Med. 1993 Jan;117(1):22–8.
105. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in critically ill patients. N Engl J Med. 2001 Nov 8;345(19):1359–67.
106. 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 Dec;25(12):2903–13.
107. Harris MI, Cowie CC, Howie LJ. Self-monitoring of blood glucose by adults with diabetes in the United States population. Diabetes Care. 1993 Aug;16(8):1116–23.
108. American Diabetes Association Professional Practice Committee. 7. Diabetes Technology: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S97–112.
109. 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 Dec 1;163(11):861–8.
110. Siu AL. Screening for Abnormal Blood Glucose and Type 2 Diabetes Mellitus: U.S. Preventive Services Task Force Recommendation Statement. Annals of Internal Medicine. 2015 Dec 1;163(11):861–9.
111. Sell-Monitoring of Blood Glucose. Diabetes Care. 1996 Jan 1;19(Supplement_1):S62–6.
112. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. Endocr Rev. 1991 Nov;12(4):356–71.
113. American Diabetes Association. Standards of Medical Care in Diabetes--2011. Diabetes Care. 2011 Jan 1;34(Supplement_1):S11–61.
114. Simon J, Gray A, Clarke P, Wade A, Neil A, Farmer A. 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 May 22;336(7654):1177–80.
115. 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 Jan 18;1:CD005060.
116. Farmer AJ, Perera R, Ward A, Heneghan C, Oke J, Barnett AH, 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 Feb 27;344:e486.
117. Machry RV, Rados DV, Gregório GR de, 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 Aug;142:173–87.
118. 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. Journal of Diabetes Science and Technology. 2018 Jan;12(1):183–9.
119. Young LA, Buse JB, Weaver MA, Vu MB, Mitchell CM, Blakeney T, 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 Jul 1;177(7):920–9.
120. 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 Oct;17(10):1110–23.
121. Ellison JM, Stegmann JM, Colner SL, Michael RH, Sharma MK, Ervin KR, et al. Rapid changes in postprandial blood glucose produce concentration differences at finger, forearm, and thigh sampling sites. Diabetes Care. 2002 Jun;25(6):961–4.
122. Burnett RW, D'Orazio P, Fogh-Andersen N, Kuwa K, Külpmann WR, Larsson L, et al. IFCC recommendation on reporting results for blood glucose. Clin Chim Acta. 2001 May;307(1–2):205–9.
123. D'Orazio P, Burnett RW, Fogh-Andersen N, Jacobs E, Kuwa K, Külpmann WR, et al. Approved IFCC Recommendation on Reporting Results for Blood Glucose (Abbreviated). Clinical Chemistry. 2005 Sep 1;51(9):1573–6.
124. Steffes MW, Sacks DB. Measurement of circulating glucose concentrations: the time is now for consistency among methods and types of samples. Clin Chem. 2005 Sep;51(9):1569–70.
125. Self-Monitoring Blood Glucose Test Systems for Over-the-Counter Use [Internet]. U.S. Food & Drug Administration Center for Devices and Radiological Health; 2020 [cited 2022 May 23]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/self-monitoring-blood-glucose-test-systems-over-counter-use
126. Blood Glucose Monitoring Test Systems for Prescription Point-of-Care Use [Internet]. U.S. Food & Drug Administration Center for Devices and Radiological Health; 2020 [cited 2022 May 23]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/blood-glucose-monitoring-test-systems-prescription-point-care-use
127. In vitro diagnostic test systems — Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus [Internet]. International Standards Organization; 2013 [cited 2022 May 23]. Available from: http://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/49/54976.html
128. POCT12A3E | Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities, 3rd Edition [Internet]. Clinical & Laboratory Standards Institute. [cited 2022 Mar 1]. Available from: https://clsi.org/standards/products/point-of-care-testing/documents/poct12/
129. Ekhlaspour L, Mondesir D, Lautsch N, Balliro C, Hillard M, Magyar K, et al. Comparative Accuracy of 17 Point-of-Care Glucose Meters. J Diabetes Sci Technol. 2017 May;11(3):558–66.
130. Boyd JC, Bruns DE. Quality Specifications for Glucose Meters: Assessment by Simulation Modeling of Errors in Insulin Dose. Clinical Chemistry. 2001 Feb 1;47(2):209–14.
131. Campos-Náñez E, Fortwaengler K, Breton MD. Clinical Impact of Blood Glucose Monitoring Accuracy: An In-Silico Study. J Diabetes Sci Technol. 2017 Nov;11(6):1187–95.
132. Fortwaengler K, Campos-Náñez E, Parkin CG, Breton MD. The Financial Impact of Inaccurate Blood Glucose Monitoring Systems. J Diabetes Sci Technol. 2018 Mar;12(2):318–24.
133. Nichols JH, Brandler ES, Fantz CR, Fisher K, Goodman MD, Headden G, et al. A Multicenter Evaluation of a Point-of-Care Blood Glucose Meter System in Critically Ill Patients. J Appl Lab Med. 2021 Jul 7;6(4):820–33.

134. 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 Sep 1;7(5):1282–7.
135. 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 Aug 25;10(2):336–42.
136. 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 Jan;13(1):55–62.
137. Ceriotti F, Kaczmarek E, Guerra E, Mastrantonio F, Lucarelli F, Valgimigli F, 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 Mar;9(2):268–77.
138. Inman M, Kyle BD, Lyon ME. Contribution of Glucose Meter Error to Misclassification of Neonatal Glycemic Status. JAMA Pediatr. 2021 May 1;175(5):453–5.
139. 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 Sep;10(5):1161–8.
140. Tirimacco R, Koumantakis G, Erasmus R, Mosca A, Sandberg S, Watson ID, et al. Glucose meters - fit for clinical purpose. Clin Chem Lab Med. 2013 May;51(5):943–52.
141. 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 Jul;13(4):734–43.
142. Vanavanan S, Santanirand P, Chaichanajarernkul U, Chittamma A, Dubois JA, Shirey T, et al. Performance of a new interference-resistant glucose meter. Clin Biochem. 2010 Jan;43(1–2):186–92.
143. 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 Oct;28(10):1272–6.
144. Lv H, Zhang G jun, Kang X xiong, Yuan H, Lv Y wei, Wang W wen, et al. Factors interfering with the accuracy of five blood glucose meters used in Chinese hospitals. J Clin Lab Anal. 2013 Sep;27(5):354–66.
145. 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 May;11(3):577–83.
146. 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 Mar 1;7(2):389–98.
147. Wada Y, Nakamura T, Kaneshige M, Takahashi S, Fujinaga H, Tsukamoto K, et al. Evaluation of two glucose meters and interference corrections for screening neonatal hypoglycemia. Pediatr Int. 2015 Aug;57(4):603–7.
148. 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 Mar;9(2):262–7.
149. Teodorczyk M, Cardosi M, Setford S. Hematocrit compensation in electrochemical blood glucose monitoring systems. J Diabetes Sci Technol. 2012 May 1;6(3):648–55.

150. Tendl KA, Christoph J, Bohn A, Herkner KR, Pollak A, Prusa AR. 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 Sep;51(9):1747–54.
151. 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 Nov;58(11):1067–70.
152. Lyon ME, Lyon AW. N-Acetylcysteine Interference with a Glucose Dehydrogenase Linked Glucose Meter. J Diabetes Sci Technol. 2021 Mar 14;1932296821999416.
153. Kelly BN, Haverstick DM, Bruns DE. Interference in a glucose dehydrogenase-based glucose meter revisited. Clin Chim Acta. 2012 Apr 11;413(7–8):829–30.
154. Miller KM, Beck RW, Bergenstal RM, Goland RS, Haller MJ, McGill JB, et al. Evidence of a strong association between frequency of self-monitoring of blood glucose and hemoglobin A1c levels in T1D exchange clinic registry participants. Diabetes Care. 2013 Jul;36(7):2009–14.
155. Ziegler R, Heidtmann B, Hilgard D, Hofer S, Rosenbauer J, Holl R, et al. Frequency of SMBG correlates with HbA1c and acute complications in children and adolescents with type 1 diabetes. Pediatr Diabetes. 2011 Feb;12(1):11–7.
156. Schütt M, Kern W, Krause U, Busch P, Dapp A, Grziwotz R, et al. 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 Jul;114(7):384–8.
157. Yu-Fei W, Wei-Ping J, Ming-Hsun W, Miao-O C, Ming-Chang H, Chi-Pin 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 Sep;11(5):953–65.
158. 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 Sep;12(5):992–1001.
159. 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 Aug 14;10(2):366–77.
160. Beck RW, Riddlesworth T, Ruedy K, Ahmann A, Bergenstal R, Haller S, 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 24;317(4):371–8.
161. 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 Aug;8(4):947–51.
162. Lind M, Polonsky W, Hirsch IB, Heise T, Bolinder J, Dahlqvist S, 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 24;317(4):379–87.
163. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group, Tamborlane WV, Beck RW, Bode BW, Buckingham B, Chase HP, et al. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med. 2008 Oct 2;359(14):1464–76.
164. 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 May;8(3):516–22.
165. van Beers CAJ, DeVries JH, Kleijer SJ, Smits MM, Geelhoed-Duijvestijn PH, Kramer MHH, 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(11):893–902.
166. Pratley RE, Kanapka LG, Rickels MR, Ahmann A, Aleppo G, Beck R, et al. Effect of Continuous Glucose Monitoring on Hypoglycemia in Older Adults With Type 1 Diabetes: A Randomized Clinical Trial. JAMA. 2020 16;323(23):2397–406.
167. 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 05;388(10057):2254–63.
168. 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 Jul;1(3):e00023.
169. Charleer S, De Block C, Van Huffel L, Broos B, Fieuws S, Nobels F, 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(2):389–97.
170. 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 May;22(5):337–45.
171. Castellana M, Parisi C, Di Molfetta S, Di Gioia L, Natalicchio A, Perrini 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 Jun;8(1):e001092.
172. Evans M, Welsh Z, Ells S, Seibold A. The Impact of Flash Glucose Monitoring on Glycaemic Control as Measured by HbA1c: A Meta-analysis of Clinical Trials and Real-World Observational Studies. Diabetes Ther. 2020 Jan;11(1):83–95.
173. Yoo HJ, An HG, Park SY, Ryu OH, Kim HY, Seo JA, 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 Oct;82(1):73–9.
174. 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 May 1;5(3):668–75.
175. Beck RW, Riddlesworth TD, Ruedy K, Ahmann A, Haller S, Kruger D, 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 Sep 19;167(6):365–74.
176. Martens T, Beck RW, Bailey R, Ruedy KJ, Calhoun P, Peters AL, 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 Jun 8;325(22):2262–72.
177. 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 Feb 1;8(1):55–73.

178. Yaron M, Roitman E, Aharon-Hananel G, Landau Z, Ganz T, Yanuv I, et al. Effect of Flash Glucose Monitoring Technology on Glycemic Control and Treatment Satisfaction in Patients With Type 2 Diabetes. Diabetes Care. 2019 Jul;42(7):1178–84.
179. Laffel LM, Kanapka LG, Beck RW, Bergamo K, Clements MA, Criego A, 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 16;323(23):2388–96.
180. Wong JC, Foster NC, Maahs DM, Raghinaru D, Bergenstal RM, Ahmann AJ, et al. Real-Time Continuous Glucose Monitoring Among Participants in the T1D Exchange Clinic Registry. Diabetes Care. 2014 Oct;37(10):2702–9.
181. 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 Jun;39(6):e81-82.
182. Mauras N, Beck R, Xing D, Ruedy K, Buckingham B, Tansey M, et al. 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 Feb;35(2):204–10.
183. Tsalikian E, Fox L, Weinzimer S, Buckingham B, White NH, Beck R, et al. Feasibility of prolonged continuous glucose monitoring in toddlers with type 1 diabetes. Pediatr Diabetes. 2012 Jun;13(4):301–7.
184. 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 Jul 30;13(5):2923–6.
185. Vergier J, Samper M, Dalla-Vale F, Ventura V, Baucher F, Joubert F, et al. Evaluation of flash glucose monitoring after long-term use: A pediatric survey. Prim Care Diabetes. 2019;13(1):63–70.
186. Landau Z, Abiri S, Gruber N, Levy-Shraga Y, Brener A, Lebenthal Y, 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 Dec;55(12):1303–10.
187. Deja G, Kłeczek M, Chumięcki M, Strzała-Kłeczek A, Deja R, Jarosz-Chobot P. The usefulness of the FlashStyle Libre system in glycemic control in children with type 1 diabetes during summer camp. Pediatr Endocrinol Diabetes Metab. 2018;24(1):11–9.
188. Feig DS, Donovan LE, Corcoy R, Murphy KE, Amiel SA, Hunt KF, et al. Continuous glucose monitoring in pregnant women with type 1 diabetes (CONCEPTT): a multicentre international randomised controlled trial. Lancet. 2017 Nov 25;390(10110):2347–59.
189. 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 [Internet]. 2016 Jan 27 [cited 2022 May 23];6. Available from: https://www.nature.com/articles/srep19920
190. Prepared for the March 29, 2018 meeting of the Clinical Chemistry and Clinical Toxicology Devices Panel P160048 Eversense Continuous Glucose Monitoring System Senseonics, Inc. [Internet]. U.S. Food & Drug Administration; 2018 Mar [cited 2020 Sep 5]. Available from: https://www.fda.gov/media/112110/download
191. PMA P160030/S017: FDA Summary of Safety and Effectiveness Data. FreeStyle Libre 14 Day Flash Glucose Monitoring System. [Internet]. Abbott Diabetes Care Inc.; 2018 [cited

2020 May 12]. Available from:
https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160030S017B.pdf
192. PMA P160007: FDA Summary of Safety and Effectiveness Data [Internet]. 2018 [cited 2020 May 12]. Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160007b.pdf
193. EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR Dexcom G6 Continuous Glucose Monitoring System [Internet]. U.S. Food & Drug Administration; [cited 2020 May 12]. Available from:
https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170088.pdf
194. Facchinetti A. Continuous Glucose Monitoring Sensors: Past, Present and Future Algorithmic Challenges. Sensors (Basel). 2016 Dec 9;16(12):E2093.
195. Aleppo G, Ruedy KJ, Riddlesworth TD, Kruger DF, Peters AL, Hirsch I, 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 Apr;40(4):538–45.
196. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International Consensus on Use of Continuous Glucose Monitoring. Diabetes Care. 2017 Dec 1;40(12):1631–40.
197. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. Diabetes Care. 2019 Aug 1;42(8):1593–603.
198. Enforcement Policy for Non-Invasive Remote Monitoring Devices Used to Support Patient Monitoring During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency (Revised) [Internet]. 2020 [cited 2020 Sep 2]. Available from:
https://www.fda.gov/media/136290/download
199. Zhang R, Liu S, Jin H, Luo Y, Zheng Z, Gao F, et al. Noninvasive Electromagnetic Wave Sensing of Glucose. Sensors (Basel). 2019 Mar 7;19(5):E1151.
200. 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;6:51119–29.
201. 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 Physics Letters. 2017 Mar 1;14:035603.
202. Chen TL, Lo YL, Liao CC, Phan QH. Noninvasive measurement of glucose concentration on human fingertip by optical coherence tomography. J Biomed Opt. 2018 Apr;23(4):1–9.
203. Yamakoshi Y, Matsumura K, Yamakoshi T, Lee J, Rolfe P, Kato Y, et al. Side-scattered finger-photoplethysmography: experimental investigations toward practical noninvasive measurement of blood glucose. J Biomed Opt. 2017 Jun 1;22(6):67001.
204. Mesch M, Zhang C, Braun PV, Giessen H. Functionalized hydrogel on plasmonic nanoantennas for noninvasive glucose sensing. ACS Photonics. 2015 Apr 15;2(4):475–80.
205. 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 Sensors Journal. 2019;
206. Omkar, Yu W, Huang S. T-Shaped Patterned Microstrip Line for Noninvasive Continuous Glucose Sensing. IEEE Microwave and Wireless Components Letters. 2018;

207. 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. p. 1649–52.
208. 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 Oct 4;7(1):12650.
209. Yadav J, Singh V, Murari BM. Investigations on Multisensor-Based Noninvasive Blood Glucose Measurement System. Journal of Medical Devices [Internet]. 2017 Jul 31 [cited 2022 Mar 1];11(3). Available from: https://research.vit.ac.in/publication/investigations-on-multisensor-based-noninvasive
210. Chowdhury MK, Srivastava A, Sharma N, Sharma S. Noninvasive blood glucose measurement utilizing a newly designed system based on modulated ultrasound and infrared light. International Journal of Diabetes in Developing Countries. 2015;
211. Kitazaki T, Kawashima N, Yamamoto N, Nomura H, Kang H, Nishiyama A, 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 Mar;24(3):1–7.
212. Zhai Q, Gong S, Wang Y, Lyu Q, Liu Y, Ling Y, et al. Enokitake Mushroom-like Standing Gold Nanowires toward Wearable Noninvasive Bimodal Glucose and Strain Sensing. ACS Appl Mater Interfaces. 2019 Mar 13;11(10):9724–9.
213. 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 Mar 19;91(6):3778–83.
214. Liu J, Liu R, Xu K. Accuracy of Noninvasive Glucose Sensing Based on Near-Infrared Spectroscopy. Appl Spectrosc. 2015 Nov;69(11):1313–8.
215. 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 May 15;179:250–4.
216. Haxha S, Jhoja J. Optical Based Noninvasive Glucose Monitoring Sensor Prototype. IEEE Photonics Journal [Internet]. 2016 Dec 15 [cited 2022 Mar 2];8(6). Available from: https://ieeexplore.ieee.org/document/7782291
217. 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 Jan;39(1):166.
218. Hu Y xiang, Liu R, Zhang W, Xu K xin. Application of Two-Dimensional Near-Infrared Correlation Spectroscopy in the Specificity Analysis of Noninvasive Blood Glucose Sensing. 2017 Feb;
219. 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 Sep;71(9):2177–86.
220. Lü X feng, Zhang T lin, Xiao F, Li G, Wang Y. Noninvasive Blood Glucose Analysis Based on Near-Infrared Reflectance Spectroscopy. Guang Pu Xue Yu Guang Pu Fen Xi. 2016 Jul;36(7):2312–7.

221. Kasahara R, Kino S, Soyama S, Matsuura Y. Noninvasive glucose monitoring using mid-infrared absorption spectroscopy based on a few wavenumbers. Biomed Opt Express. 2018 Jan 1;9(1):289–302.
222. 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. Applied Physics B: Lasers and Optics. 2018 May 1;124:75.
223. Pandey R, Paidi SK, Valdez TA, Zhang C, Spegazzini N, Dasari RR, et al. Noninvasive Monitoring of Blood Glucose with Raman Spectroscopy. Acc Chem Res. 2017 Feb 21;50(2):264–72.
224. Shih WC, Bechtel KL, Rebec MV. Noninvasive glucose sensing by transcutaneous Raman spectroscopy. J Biomed Opt. 2015 May;20(5):051036.
225. Zheng Y, Zheng Y, Zhu X, Wang Z, Hou Z, Gao F, et al. Noninvasive blood glucose detection using a miniature wearable Raman spectroscopy system. Chin Opt Lett, COL. 2017 Aug 10;15(8):083001.
226. Shih WC. Constrained regularization for noninvasive glucose sensing using Raman spectroscopy. Journal of Innovative Optical Health Sciences. 2014 Nov 25;8:1550022.
227. Li N, Zang H, Sun H, Jiao X, Wang K, Liu TCY, et al. A Noninvasive Accurate Measurement of Blood Glucose Levels with Raman Spectroscopy of Blood in Microvessels. Molecules. 2019 Apr 17;24(8):E1500.
228. 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 Oct 1;63(10 Pt 1):3016–25.
229. Vrba J, Karch J, Vrba D. Phantoms for Development of Microwave Sensors for Noninvasive Blood Glucose Monitoring. International Journal of Antennas and Propagation. 2015 Mar 1;2015:1–5.
230. Xiao X, Li Q. A Noninvasive Measurement of Blood Glucose Concentration by UWB Microwave Spectrum. IEEE Antennas and Wireless Propagation Letters. 2017;
231. Turgul V, Kale I. Characterization of the complex permittivity of glucose/water solutions for noninvasive RF/Microwave blood glucose sensing. 2016 IEEE International Instrumentation and Measurement Technology Conference Proceedings. 2016;
232. Choi H, Luzio S, Beutler J, Porch A. Microwave noninvasive blood glucose monitoring sensor: Human clinical trial results. 2017 IEEE MTT-S International Microwave Symposium (IMS). 2017;
233. Zhang R, Gao F, Feng X, Liu S, Kishor R, Luo Y, et al. Noninvasive photoacoustic measurement of glucose by data fusion. Analyst. 2017 Aug 7;142(16):2892–6.
234. Pai PP, De A, Banerjee S. Accuracy Enhancement for Noninvasive Glucose Estimation Using Dual-Wavelength Photoacoustic Measurements and Kernel-Based Calibration. IEEE Transactions on Instrumentation and Measurement. 2018;
235. Zhang R, Gao F, Feng X, Jin H, Zhang S, Liu S, et al. “Guide Star” Assisted Noninvasive Photoacoustic Measurement of Glucose. ACS Sens. 2018 Dec 28;3(12):2550–7.
236. 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. 2016 IEEE SENSORS. 2016;
237. 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: 2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2016. p. 6477–80.
238. 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: 2015 IEEE SENSORS. 2015. p. 1–4.
239. Small GW. Chemometrics and near-infrared spectroscopy: Avoiding the pitfalls. TrAC Trends in Analytical Chemistry. 2006 Dec 1;25(11):1057–66.
240. 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 Jun;18(6):366–76.
241. Segman YJ. Device and Method for Noninvasive Glucose Assessment. J Diabetes Sci Technol. 2018 Nov;12(6):1159–68.
242. 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 Nov;12(6):1178–83.
243. Segman Y. Combination non-invasive and invasive bioparameter measuring device [Internet]. US8948833B2, 2015 [cited 2022 Mar 3]. Available from: https://patents.google.com/patent/US8948833B2/fi
244. 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 Nov;12(6):1169–77.
245. Ascaso FJ, Huerva V. Noninvasive Continuous Monitoring of Tear Glucose Using Glucose-Sensing Contact Lenses. Optom Vis Sci. 2016 Apr;93(4):426–34.
246. Ruan JL, Chen C, Shen JH, Zhao XL, Qian SH, Zhu ZG. A Gelated Colloidal Crystal Attached Lens for Noninvasive Continuous Monitoring of Tear Glucose. Polymers (Basel). 2017 Mar 28;9(4):E125.
247. Baca JT, Finegold DN, Asher SA. Tear glucose analysis for the noninvasive detection and monitoring of diabetes mellitus. Ocul Surf. 2007 Oct;5(4):280–93.
248. 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 Jul;53(7):1370–2.
249. 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 Feb 4;86(3):1902–8.
250. 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 Nov 15;49:204–9.
251. 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 Nov 1;83(21):8341–6.
252. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008 May 8;358(19):1991–2002.

253. 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 Oct 24;16:E146.
254. Committee on Practice Bulletins—Obstetrics. ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus. Obstet Gynecol. 2018;131(2):e49–64.
255. American Diabetes Association Professional Practice Committee. 15. Management of Diabetes in Pregnancy: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S232–43.
256. O'sullivan JB, Mahan CM. CRITERIA FOR THE ORAL GLUCOSE TOLERANCE TEST IN PREGNANCY. Diabetes. 1964 Jun;13:278–85.
257. Vandorsten JP, Dodson WC, Espeland MA, Grobman WA, Guise JM, Mercer BM, et al. NIH consensus development conference: diagnosing gestational diabetes mellitus. NIH Consens State Sci Statements. 2013 Mar 4;29(1):1–31.
258. National Institutes of Health consensus development conference statement: diagnosing gestational diabetes mellitus, March 4-6, 2013. Obstet Gynecol. 2013 Aug;122(2 Pt 1):358–69.
259. Sacks DB. Diagnosis of gestational diabetes mellitus: it is time for international consensus. Clin Chem. 2014 Jan;60(1):141–3.
260. Metzger BE, Persson B, Lowe LP, Dyer AR, Cruickshank JK, Deerochanawong C, et al. Hyperglycemia and adverse pregnancy outcome study: neonatal glycemia. Pediatrics. 2010 Dec;126(6):e1545-1552.
261. Yogev null, Chen null, Hod null, Coustan null, Oats null, McIntyre null, et al. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: preeclampsia. Am J Obstet Gynecol. 2010 Mar;202(3):255.e1-7.
262. 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. Dia Care. 2010 Mar 1;33(3):676–82.
263. Colagiuri S, Falavigna M, Agarwal MM, Boulvain M, Coetzee E, Hod M, et al. Strategies for implementing the WHO diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Diabetes Res Clin Pract. 2014 Mar;103(3):364–72.
264. American Diabetes Association. Standards of Medical Care in Diabetes--2014. Diabetes Care. 2014 Jan 1;37(Supplement_1):S14–80.
265. 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 Mar 11;384(10):895–904.
266. Coustan DR, Dyer AR, Metzger BE. One-step or 2-step testing for gestational diabetes: which is better? Am J Obstet Gynecol. 2021 Dec;225(6):634–44.
267. McIntyre HD, Oats JJN, Kihara AB, Divakar H, Kapur A, Poon LC, 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 Aug;154(2):189–94.
268. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS, et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005 Jun 16;352(24):2477–86.

269. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med. 2009 Oct 1;361(14):1339–48.
270. 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 May;33(5):964–8.
271. Landon MB, Rice MM, Varner MW, Casey BM, Reddy UM, Wapner RJ, et al. Mild gestational diabetes mellitus and long-term child health. Diabetes Care. 2015 Mar;38(3):445–52.
272. Lowe WL, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, et al. Association of Gestational Diabetes With Maternal Disorders of Glucose Metabolism and Childhood Adiposity. JAMA. 2018 Sep 11;320(10):1005–16.
273. Lowe WL, Scholtens DM, Kuang A, Linder B, Lawrence JM, Lebenthal Y, et al. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal Gestational Diabetes Mellitus and Childhood Glucose Metabolism. Diabetes Care. 2019;42(3):372–80.
274. 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 Jun;113(6):1307–12.
275. 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 Sep;26(13):1268–72.
276. Mosca A, Paleari R, Dalfrà MG, Di Cianni G, Cuccuru I, Pellegrini G, et al. Reference intervals for hemoglobin A1c in pregnant women: data from an Italian multicenter study. Clin Chem. 2006 Jun;52(6):1138–43.
277. 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 May 13;369:m1361.
278. Ratner RE, Christophi CA, Metzger BE, Dabelea D, Bennett PH, Pi-Sunyer X, et al. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. J Clin Endocrinol Metab. 2008 Dec;93(12):4774–9.
279. Li Z, Cheng Y, Wang D, Chen H, Chen H, Ming WK, 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.
280. Standardization of the oral glucose tolerance test. Report of the Committee on Statistics of the American Diabetes Association June 14, 1968. Diabetes. 1969 May;18(5):299–307.
281. Daly N, Flynn I, Carroll C, Farren M, McKeating A, Turner MJ. Impact of Implementing Preanalytical Laboratory Standards on the Diagnosis of Gestational Diabetes Mellitus: A Prospective Observational Study. Clin Chem. 2016 Feb;62(2):387–91.
282. Diabetes in America, 3rd Edition | NIDDK [Internet]. National Institute of Diabetes and Digestive and Kidney Diseases. [cited 2019 Aug 27]. Available from: https://www.niddk.nih.gov/about-niddk/strategic-plans-reports/diabetes-in-america-3rd-edition
283. Zhu WW, Yang HX, Wei YM, Yan J, Wang ZL, Li XL, 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 Mar;36(3):586–90.

284. Wexler DJ, Powe CE, Barbour LA, Buchanan T, Coustan DR, Corcoy R, 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 Aug;132(2):496–505.
285. 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 Jan;39(1):75–81.
286. Wei YM, Liu XY, Shou C, Liu XH, Meng WY, Wang ZL, 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 Apr 20;132(8):883–8.
287. Mills JL, Jovanovic-Peterson L, Knopp R, Aarons J, Conley M, Park E, et al. Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study. Metabolism. 1998 Sep;47(9):1140–4.
288. Powe CE. Early Pregnancy Biochemical Predictors of Gestational Diabetes Mellitus. Curr Diab Rep. 2017 Feb;17(2):12.
289. Mañé L, Flores-Le Roux JA, Benaiges D, Rodríguez M, Marcelo I, Chillarón JJ, et al. Role of First-Trimester HbA1c as a Predictor of Adverse Obstetric Outcomes in a Multiethnic Cohort. J Clin Endocrinol Metab. 2017 Feb 1;102(2):390–7.
290. Fong A, Serra AE, Gabby L, Wing DA, Berkowitz KM. Use of hemoglobin A1c as an early predictor of gestational diabetes mellitus. Am J Obstet Gynecol. 2014 Dec;211(6):641.e1-7.
291. Ghosh P, Luque-Fernandez MA, Vaidya A, Ma D, Sahoo R, Chorev M, et al. Plasma Glycated CD59, a Novel Biomarker for Detection of Pregnancy-Induced Glucose Intolerance. Diabetes Care. 2017 Jul;40(7):981–4.
292. Oztas E, Ozler S, Ersoy E, Ersoy AO, Tokmak A, Ergin M, et al. Prediction of gestational diabetes mellitus by first trimester serum secreted frizzle-related protein-5 levels. J Matern Fetal Neonatal Med. 2016;29(9):1515–9.
293. 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 Jul;41(7):1339–42.
294. Kyhl HB, Jensen TK, Barington T, Buhl S, Norberg LA, Jørgensen JS, et al. The Odense Child Cohort: aims, design, and cohort profile. Paediatr Perinat Epidemiol. 2015 May;29(3):250–8.
295. 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 Sep;33(9):2018–20.
296. McIntyre HD, Gibbons KS, Ma RCW, Tam WH, Sacks DA, Lowe J, 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 Sep;167:108353.
297. Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, 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 Oct;131 Suppl 3:S173–211.
298. 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 Apr;44(4):858–64.

299. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, et al. Tests of glycemia in diabetes. Diabetes Care. 2004 Jul;27(7):1761–73.
300. Clinical Guidelines Task Force. Global Guideline for Type 2 Diabetes [Internet]. International Diabetes Federation; 2012 [cited 2022 Mar 31]. Available from: file:///C:/Users/mhill/Downloads/IDF-Guideline-for-Type-2-Diabetes.pdf
301. International Diabetes Federation Guideline Development Group. Global guideline for type 2 diabetes. Diabetes Res Clin Pract. 2014 Apr;104(1):1–52.
302. Dhatariya KK, Glaser NS, Codner E, Umpierrez GE. Diabetic ketoacidosis. Nat Rev Dis Primers. 2020 May 14;6(1):40.
303. 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 Feb 1;45(2):267–72.
304. Schwab TM, Hendey GW, Soliz TC. Screening for ketonemia in patients with diabetes. Ann Emerg Med. 1999 Sep;34(3):342–6.
305. 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 Aug 23;13(9):E837.
306. 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 Mar;23(3):278–84.
307. American Diabetes Association. Standards of Medical Care in Diabetes--2022. Diabetes Care. 2022 Jan;45(Supplement 1):S1-SXXX.
308. Sacks DB. Diabetes Mellitus. In: Tietz Textbook of Laboratory Medicine. 7th ed. St. Louis: Elsevier; 2023. p. 502–43.
309. Yu HYE, Agus M, Kellogg MD. Clinical utility of Abbott Precision Xceed Pro® ketone meter in diabetic patients. Pediatr Diabetes. 2011 Nov;12(7):649–55.
310. Danne T, Garg S, Peters AL, Buse JB, Mathieu C, Pettus JH, 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 Jun;42(6):1147–54.
311. Noyes KJ, Crofton P, Bath LE, Holmes A, Stark L, Oxley CD, 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 Jun;8(3):150–6.
312. 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.
313. 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 May;43(5):1057–64.
314. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med. 1981 Feb;70(2):325–30.
315. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Chem. 1986 Oct;32(10 Suppl):B64-70.

316. 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 Nov;23(5):403–5.
317. 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 Jul;40(7 Pt 1):1317–21.
318. 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 May 19;288(6429):1484–6.
319. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care. 1995 Apr;18(4):440–7.
320. Nathan DM, McGee P, Steffes MW, Lachin JM, DCCT/EDIC Research Group. Relationship of glycated albumin to blood glucose and HbA1c values and to retinopathy, nephropathy, and cardiovascular outcomes in the DCCT/EDIC study. Diabetes. 2014 Jan;63(1):282–90.
321. Sacks DB. A1C versus glucose testing: a comparison. Diabetes Care. 2011 Feb;34(2):518–23.
322. 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 07;169(3):156–64.
323. Droumaguet C, Balkau B, Simon D, Caces E, Tichet J, Charles MA, et al. Use of HbA1c in predicting progression to diabetes in French men and women: data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care. 2006 Jul;29(7):1619–25.
324. Edelman D, Olsen MK, Dudley TK, Harris AC, Oddone EZ. Utility of hemoglobin A1c in predicting diabetes risk. J Gen Intern Med. 2004 Dec;19(12):1175–80.
325. Little RR, England JD, Wiedmeyer HM, Madsen RW, Pettitt DJ, Knowler WC, et al. Glycated haemoglobin predicts progression to diabetes mellitus in Pima Indians with impaired glucose tolerance. Diabetologia. 1994 Mar;37(3):252–6.
326. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A1c point-of-care instruments do not meet the general accepted analytical performance criteria. Clin Chem. 2010 Jan;56(1):44–52.
327. Hirst JA, McLellan JH, Price CP, English E, Feakins BG, Stevens RJ, et al. Performance of point-of-care HbA1c test devices: implications for use in clinical practice - a systematic review and meta-analysis. Clin Chem Lab Med. 2017 Feb 1;55(2):167–80.
328. Nathan DM, Griffin A, Perez FM, Basque E, Do L, Steiner B. Accuracy of a Point-of-Care Hemoglobin A1c Assay. J Diabetes Sci Technol. 2019 Nov;13(6):1149–53.
329. CLIA Brochures | CMS [Internet]. [cited 2022 Aug 26]. Available from: https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CLIA_Brochures
330. American Diabetes Association. Implications of the Diabetes Control and Complications Trial. Diabetes Care. 2000 Jan;23 Suppl 1:S24-26.
331. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, et al. 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 Aug;49(8):1711–21.

332. 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 Oct 11;323(15):1021–5.
333. Berg AH, Sacks DB. Haemoglobin A1c analysis in the management of patients with diabetes: from chaos to harmony. J Clin Pathol. 2008 Sep;61(9):983–7.
334. Qaseem A, Vijan S, Snow V, Cross JT, Weiss KB, Owens DK, et al. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A1c targets. A guidance statement from the American College of Physicians. Ann Intern Med. 2007 Sep 18;147(6):417–22.
335. Brown SA, Kovatchev BP, Raghinaru D, Lum JW, Buckingham BA, Kudva YC, et al. Six-Month Randomized, Multicenter Trial of Closed-Loop Control in Type 1 Diabetes. N Engl J Med. 2019 31;381(18):1707–17.
336. Kitzmiller JL, Block JM, Brown FM, Catalano PM, Conway DL, Coustan DR, et al. Managing Preexisting Diabetes for Pregnancy. Diabetes Care. 2008 May;31(5):1060–79.
337. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care. 2008 Oct;31(10):1991–6.
338. Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. Ann Intern Med. 2010 Jun 15;152(12):770–7.
339. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010 Mar 4;362(9):800–11.
340. Little RR, Sacks DB. HbA1c: how do we measure it and what does it mean? Curr Opin Endocrinol Diabetes Obes. 2009 Apr;16(2):113–8.
341. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, et al. Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care. 2007 Oct;30(10):2453–7.
342. Saaddine JB, Fagot-Campagna A, Rolka D, Narayan KMV, Geiss L, Eberhardt M, 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 Aug;25(8):1326–30.
343. Parrinello CM, Sharrett AR, Maruthur NM, Bergenstal RM, Grams ME, Coresh J, et al. Racial Differences in and Prognostic Value of Biomarkers of Hyperglycemia. Diabetes Care. 2016 Apr;39(4):589–95.
344. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, et al. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008 Aug;31(8):1473–8.
345. Bergenstal RM, Gal RL, Connor CG, Gubitosi-Klug R, Kruger D, Olson BA, et al. Racial Differences in the Relationship of Glucose Concentrations and Hemoglobin A1c Levels. Ann Intern Med. 2017 Jul 18;167(2):95–102.
346. Malka R, Nathan DM, Higgins JM. Mechanistic modeling of hemoglobin glycation and red blood cell kinetics enables personalized diabetes monitoring. Sci Transl Med. 2016 Oct 5;8(359):359ra130.
347. Davie SJ, Gould BJ, Yudkin JS. Effect of vitamin C on glycosylation of proteins. Diabetes. 1992 Feb;41(2):167–73.

348. 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 Jan;14(1):68–72.
349. Tarim O, Küçükerdoğan A, Günay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int. 1999 Aug;41(4):357–62.
350. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem. 2001 Feb;47(2):153–63.
351. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW, Krejs GJ. Evaluation of HbA1c determination methods in patients with hemoglobinopathies. Diabetes Care. 2000 Mar;23(3):339–44.
352. 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 A1c by fifteen methods. Clin Chim Acta. 2021 Nov;522:31–5.
353. Little RR, La'ulu SL, Hanson SE, Rohlfing CL, Schmidt RL. Effects of 49 Different Rare Hb Variants on HbA1c Measurement in Eight Methods. J Diabetes Sci Technol. 2015 Jul;9(4):849–56.
354. Rohlfing CL, Hanson S, Tennill AL, Little RR. Effects of whole blood storage on hemoglobin a1c measurements with five current assay methods. Diabetes Technol Ther. 2012 Mar;14(3):271–5.
355. 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 Feb;9(1):36–42.
356. Beck RW, Bocchino LE, Lum JW, Kollman C, Barnes-Lomen V, Sulik M, et al. An Evaluation of Two Capillary Sample Collection Kits for Laboratory Measurement of HbA1c. Diabetes Technol Ther. 2021 Aug;23(8):537–45.
357. Nathan DM, Krause-Steinrauf H, Braffett BH, Arends VL, Younes N, McGee P, et al. Comparison of central laboratory HbA1c measurements obtained from a capillary collection versus a standard venous whole blood collection in the GRADE and EDIC studies. PLOS ONE. 2021 Nov 15;16(11):e0257154.
358. 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.
359. 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 Jan;40(1):138–44.
360. Little RR, Rohlfing C, Sacks DB. The National Glycohemoglobin Standardization Program: Over 20 Years of Improving Hemoglobin A1c Measurement. Clin Chem. 2019 Jul;65(7):839–48.
361. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE, et al. The national glycohemoglobin standardization program: a five-year progress report. Clin Chem. 2001 Nov;47(11):1985–92.
362. Goldstein DE, Little RR. Bringing order to chaos: the National Glycohemoglobin Standardization Program. Contemp Int Med. 1997;9:27–32.
363. Steffes M, Cleary P, Goldstein D, Little R, Wiedmeyer HM, Rohlfing C, et al. Hemoglobin A1c 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 Apr;51(4):753–8.
364. Little RR, Rohlfing CL, Sacks DB, National Glycohemoglobin Standardization Program (NGSP) Steering Committee. Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care. Clin Chem. 2011 Feb;57(2):205–14.
365. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med. 2002 Jan;40(1):78–89.
366. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, et al. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem. 2004 Jan;50(1):166–74.
367. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem. 2008 Feb;54(2):240–8.
368. Marshall SM, Barth JH. Standardization of HbA1c measurements: a consensus statement. Ann Clin Biochem. 2000 Jan;37 (Pt 1):45–6.
369. Sacks DB. Participant Summary for Hemoglobin A1C Survey 2020 Set GH5-C. College of American Pathologists; 2020.
370. European Federation of Clinical Chemistry and Laboratory Medicine. EFLM Biological Variation [Internet]. Biological Variation Database. [cited 2022 Mar 3]. Available from: https://biologicalvariation.eu/
371. Goldstein DE, Peth SB, England JD, Hess RL, Da Costa J. Effects of acute changes in blood glucose on HbA1c. Diabetes. 1980 Aug;29(8):623–8.
372. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA1c levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. Diabetes Care. 1999 Nov;22(11):1785–9.
373. 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 HbA1c on glycemic control in patients with type 2 diabetes: the Glycemic Optimization with Algorithms and Labs at Point of Care (GOAL A1C) trial. Diabetes Care. 2006 Jan;29(1):1–8.
374. Khunti K, Stone MA, Burden AC, Turner D, Raymond NT, Burden M, et al. Randomised controlled trial of near-patient testing for glycated haemoglobin in people with type 2 diabetes mellitus. Br J Gen Pract. 2006 Jul;56(528):511–7.
375. Geistanger A, Arends S, Berding C, Hoshino T, Jeppsson JO, Little R, et al. Statistical methods for monitoring the relationship between the IFCC reference measurement procedure for hemoglobin A1c and the designated comparison methods in the United States, Japan, and Sweden. Clin Chem. 2008 Aug;54(8):1379–85.
376. Murata GH, Hoffman RM, Duckworth WC, Wendel CS, Shah JH, Diabetes Outcomes in Veterans Study. Contributions of weekly mean blood glucose values to hemoglobin A1c in insulin-treated type 2 diabetes: the Diabetes Outcomes in Veterans Study (DOVES). Am J Med Sci. 2004 Jun;327(6):319–23.
377. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007 Nov;50(11):2239–44.

378. Hanas R, John G, International HbA(1c) Consensus Committee. 2010 consensus statement on the worldwide standardization of the hemoglobin A1c measurement. Clin Chem. 2010 Aug;56(8):1362–4.
379. Sacks DB. 2011 consensus meeting on the worldwide standardization of hemoglobin A(1c) measurement. Clin Chem. 2013 May;59(5):857–8.
380. Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, et al. Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med. 1991 Sep 19;325(12):836–42.
381. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, 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 HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes. 1999 Apr;48(4):870–80.
382. Graham J, Hagopian WA, Kockum I, Li LS, Sanjeevi CB, Lowe RM, et al. Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. Diabetes. 2002 May;51(5):1346–55.
383. Edghill EL, Flanagan SE, Patch AM, Boustred C, Parrish A, Shields B, et al. 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 Apr;57(4):1034–42.
384. Støy J, Greeley SAW, Paz VP, Ye H, Pastore AN, Skowron KB, et al. Diagnosis and treatment of neonatal diabetes: a United States experience. Pediatr Diabetes. 2008 Oct;9(5):450–9.
385. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab. 2008 Apr;4(4):200–13.
386. Carlsson A, Shepherd M, Ellard S, Weedon M, Lernmark Å, Forsander G, 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 Jan;43(1):82–9.
387. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med. 2001 Sep 27;345(13):971–80.
388. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009 Jun;41(6):703–7.
389. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med. 2009 Apr 16;360(16):1646–54.
390. Sharp SA, Rich SS, Wood AR, Jones SE, Beaumont RN, Harrison JW, 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 Feb;42(2):200–7.
391. Robertson CC, Inshaw JRJ, Onengut-Gumuscu S, Chen WM, Santa Cruz DF, Yang H, et al. Fine-mapping, trans-ancestral and genomic analyses identify causal variants, cells, genes and drug targets for type 1 diabetes. Nat Genet. 2021 Jul;53(7):962–71.
392. Ferrat LA, Vehik K, Sharp SA, Lernmark Å, Rewers MJ, She JX, et al. A combined risk score enhances prediction of type 1 diabetes among susceptible children. Nat Med. 2020 Aug;26(8):1247–55.

393. Hagopian WA, Lernmark A, Rewers MJ, Simell OG, She JX, Ziegler AG, et al. TEDDY--The Environmental Determinants of Diabetes in the Young: an observational clinical trial. Ann N Y Acad Sci. 2006 Oct;1079:320–6.
394. Bonifacio E, Beyerlein A, Hippich M, Winkler C, Vehik K, Weedon MN, et al. Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: A prospective study in children. PLoS Med. 2018 Apr;15(4):e1002548.
395. Barker JM, Goehrig SH, Barriga K, Hoffman M, Slover R, Eisenbarth GS, et al. Clinical characteristics of children diagnosed with type 1 diabetes through intensive screening and follow-up. Diabetes Care. 2004 Jun;27(6):1399–404.
396. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat Genet. 2018 Nov;50(11):1505–13.
397. Mansour Aly D, Dwivedi OP, Prasad RB, Käräjämäki A, Hjort R, Thangam M, et al. Genome-wide association analyses highlight etiological differences underlying newly defined subtypes of diabetes. Nat Genet. 2021 Nov;53(11):1534–42.
398. Viñuela A, Varshney A, van de Bunt M, Prasad RB, Asplund O, Bennett A, et al. Genetic variant effects on gene expression in human pancreatic islets and their implications for T2D. Nat Commun. 2020 Sep 30;11(1):4912.
399. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008 Nov 20;359(21):2208–19.
400. Pipatpolkai T, Usher S, Stansfeld PJ, Ashcroft FM. New insights into KATP channel gene mutations and neonatal diabetes mellitus. Nat Rev Endocrinol. 2020 Jul;16(7):378–93.
401. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med. 2000 Sep 7;343(10):702–9.
402. Taylor SI, Arioglu E. Genetically defined forms of diabetes in children. J Clin Endocrinol Metab. 1999 Dec;84(12):4390–6.
403. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. Pediatr Diabetes. 2018 May;19(3):346–53.
404. Redondo MJ, Kawasaki E, Mulgrew CL, Noble JA, Erlich HA, Freed BM, 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 Oct;85(10):3793–7.
405. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet. 2001 Jul 21;358(9277):221–9.
406. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet. 2016 Jun 4;387(10035):2331–9.
407. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007 Jun 7;447(7145):661–78.
408. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet. 2007 Jul;39(7):857–64.
409. Pucci M, Benati M, Lo Cascio C, Montagnana M, Lippi G. The challenges of diagnosing diabetes in childhood. Diagnosis (Berl). 2020 Jul 20;dx-2020-0036.

410. 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 Mar 21;dc202872.
411. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013 Jun 19;309(23):2473–9.
412. Vehik K, Bonifacio E, Lernmark Å, Yu L, Williams A, Schatz D, et al. Hierarchical Order of Distinct Autoantibody Spreading and Progression to Type 1 Diabetes in the TEDDY Study. Diabetes Care. 2020 Sep;43(9):2066–73.
413. Warshauer JT, Bluestone JA, Anderson MS. New Frontiers in the Treatment of Type 1 Diabetes. Cell Metab. 2020 Jan 7;31(1):46–61.
414. 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 Jul;15(7):539–53.
415. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, et al. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science. 1983 Dec 23;222(4630):1337–9.
416. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature. 1990 Sep 13;347(6289):151–6.
417. 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 Jan;89(1):283–92.
418. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest. 1993 Oct;92(4):1608–16.
419. Lu J, Li Q, Xie H, Chen ZJ, Borovitskaya AE, Maclaren NK, 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 USA. 1996 Mar 19;93(6):2307–11.
420. Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA. 2007 Oct 23;104(43):17040–5.
421. Wenzlau JM, Liu Y, Yu L, Moua O, Fowler KT, Rangasamy S, et al. A common nonsynonymous single nucleotide polymorphism in the SLC30A8 gene determines ZnT8 autoantibody specificity in type 1 diabetes. Diabetes. 2008 Oct;57(10):2693–7.
422. 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 Feb 1;180(2):135–44.
423. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G, 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 Jun 13;373(9680):2027–33.
424. Maclaren N, Lan M, Coutant R, Schatz D, Silverstein J, Muir A, 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 Jun;12(4):279–87.

425. Jacobsen LM, Bocchino L, Evans-Molina C, DiMeglio L, Goland R, Wilson DM, et al. The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. Diabetologia. 2020 Mar;63(3):588–96.
426. 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 Jun 23;dc201836.
427. Schott M, Schatz D, Atkinson M, Krischer J, Mehta H, Vold B, 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 Dec;7(6):865–72.
428. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, et al. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. Lancet. 1997 Nov 1;350(9087):1288–93.
429. 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 Aug;24(8):1460–7.
430. 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 Dec;54 Suppl 2:S62-67.
431. Kobayashi T, Tanaka S, Harii N, Aida K, Shimura H, Ohmori M, 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 Oct;1079:60–6.
432. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, et al. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. New England Journal of Medicine. 2019 Aug 15;381(7):603–13.
433. Steck AK, Larsson HE, Liu X, Veijola R, Toppari J, Hagopian WA, et al. Residual beta-cell function in diabetes children followed and diagnosed in the TEDDY study compared to community controls. Pediatr Diabetes. 2017 Dec;18(8):794–802.
434. 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 Feb;49(2):218–24.
435. Ziegler AG, Kick K, Bonifacio E, Haupt F, Hippich M, Dunstheimer D, et al. Yield of a Public Health Screening of Children for Islet Autoantibodies in Bavaria, Germany. JAMA. 2020 Jan 28;323(4):339–51.
436. 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 Mar;22 Suppl 2:B59-64.
437. 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 Nov;39(11):1329–33.
438. Füchtenbusch M, Ferber K, Standl E, Ziegler AG. Prediction of type 1 diabetes postpartum in patients with gestational diabetes mellitus by combined islet cell autoantibody screening: a prospective multicenter study. Diabetes. 1997 Sep;46(9):1459–67.
439. Gleichmann H, Bottazzo GF. Progress toward standardization of cytoplasmic islet cell-antibody assay. Diabetes. 1987 May;36(5):578–84.

440. Mire-Sluis AR, Gaines Das R, Lernmark A. The World Health Organization International Collaborative Study for islet cell antibodies. Diabetologia. 2000 Oct;43(10):1282–92.
441. Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA. A novel micro-assay for insulin autoantibodies. J Autoimmun. 1997 Oct;10(5):473–8.
442. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes. 2003 May;52(5):1128–36.
443. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, 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 Jul;95(7):3360–7.
444. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, et al. A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia. 1994 Apr;37(4):344–50.
445. Lampasona V, Pittman DL, Williams AJ, Achenbach P, Schlosser M, Akolkar B, et al. Islet Autoantibody Standardization Program 2018 Workshop: Interlaboratory Comparison of Glutamic Acid Decarboxylase Autoantibody Assay Performance. Clin Chem. 2019 Sep;65(9):1141–52.
446. 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 May 30;346(22):1685–91.
447. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, et al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes. 1996 Jul;45(7):926–33.
448. Andersson C, Kolmodin M, Ivarsson SA, Carlsson A, Forsander G, Lindbald B, et al. Islet cell antibodies (ICA) identify autoimmunity in children with new onset diabetes mellitus negative for other islet cell antibodies. Pediatric Diabetes. 2014 Aug;15(5):336–44.
449. Siljander H, Härkönen T, Hermann R, Simell S, Hekkala A, Salonsaari RT, 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 Oct;25(7):615–22.
450. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. Diabetes Care. 2005 May;28(5):1068–76.
451. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care. 2015 Oct;38(10):1964–74.
452. Primavera M, Giannini C, Chiarelli F. Prediction and Prevention of Type 1 Diabetes. Front Endocrinol (Lausanne). 2020;11:248.
453. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. Kidney Int. 2020 Oct;98(4S):S1–115.
454. Thurlow JS, Joshi M, Yan G, Norris KC, Agodoa LY, Yuan CM, et al. Global Epidemiology of End-Stage Kidney Disease and Disparities in Kidney Replacement Therapy. Am J Nephrol. 2021;52(2):98–107.
455. Davidson MB, Bazargan M, Bakris G, Peters Harmel A, Campese V, Basta E. ImmunoDip: an improved screening method for microalbuminuria. Am J Nephrol. 2004 Jun;24(3):284–8.

456. Khosla N, Sarafidis PA, Bakris GL. Microalbuminuria. Clin Lab Med. 2006 Sep;26(3):635–53, vi–vii.
457. 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(2):324–9.
458. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. American Journal of Kidney Diseases. 2007 Feb;49(2):S12–154.
459. Vassalotti JA, Stevens LA, Levey AS. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. Am J Kidney Dis. 2007 Aug;50(2):169–80.
460. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C yuan. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004 Sep 23;351(13):1296–305.
461. Beddhu S, Boucher RE, Sun J, Balu N, Chonchol M, Navaneethan S, et al. Chronic kidney disease, atherosclerotic plaque characteristics on carotid magnetic resonance imaging, and cardiovascular outcomes. BMC Nephrol. 2021 Feb 24;22(1):69.
462. Pitt B, Filippatos G, Agarwal R, Anker SD, Bakris GL, Rossing P, et al. Cardiovascular Events with Finerenone in Kidney Disease and Type 2 Diabetes. N Engl J Med. 2021 Dec 9;385(24):2252–63.
463. 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 Nov;25(11):2103–4; author reply 2104.
464. 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 Nov;20(11):2402–7.
465. 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 Sep;260(3):231–7.
466. 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 Jul;46(1):33–7.
467. 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 Jul;19(7):728–34; discussion 735-736.
468. 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 May;30(5):1248–54.
469. 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 Aug;49(2–3):187–94.
470. Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med. 2003 Dec 2;139(11):901–6.

471. Ibsen H, Olsen MH, Wachtell K, Borch-Johnsen K, Lindholm LH, Mogensen CE, et al. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for endpoint reduction in hypertension study. Hypertension. 2005 Feb;45(2):198–202.
472. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003 Dec;42(6):1206–52.
473. American Diabetes Association Professional Practice Committee. 11. Chronic Kidney Disease and Risk Management: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S175–84.
474. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. The New England Journal of Medicine. 2019 Jun 13;380:2295–306.
475. Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, et al. Dapagliflozin in Patients with Chronic Kidney Disease. N Engl J Med. 2020 Oct 8;383(15):1436–46.
476. Bakris GL, Agarwal R, Anker SD, Pitt B, Ruilope LM, Rossing P, et al. Effect of Finerenone on Chronic Kidney Disease Outcomes in Type 2 Diabetes. New England Journal of Medicine. 2020 Dec 3;383(23):2219–29.
477. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR without Race. N Engl J Med. 2021 Nov 4;385(19):1737–49.
478. Miller WG, Kaufman HW, Levey AS, Straseski JA, Wilhelms KW, Yu HY (Elsie), et al. 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. Clinical Chemistry. 2021 Dec 16;hvab278.
479. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012 Jul 5;367(1):20–9.
480. Asmamaw T, Genet S, Menon M, Tarekegn G, Chekol E, Geto Z, 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;13:4727–35.
481. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011 Jul;80(1):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 Apr 6;293(13):1609–16.
483. Yuyun MF, Khaw KT, Luben R, Welch A, Bingham S, Day NE, et al. 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 Feb;33(1):189–98.
484. Duka I, Bakris G. Influence of microalbuminuria in achieving blood pressure goals. Curr Opin Nephrol Hypertens. 2008 Sep;17(5):457–63.

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(5):469–70.
486. Mok Y, Ballew SH, Stacey RB, Rossi J, Koton S, Kucharska-Newton A, et al. Albuminuria and Prognosis Among Individuals With Atherosclerotic Cardiovascular Disease: The ARIC Study. J Am Coll Cardiol. 2021 Jul 6;78(1):87–9.
487. Mok Y, Ballew SH, Sang Y, Grams ME, Coresh J, Evans M, et al. Albuminuria as a Predictor of Cardiovascular Outcomes in Patients With Acute Myocardial Infarction. J Am Heart Assoc. 2019 Apr 16;8(8):e010546.
488. Steinke JM, Sinaiko AR, Kramer MS, Suissa S, Chavers BM, Mauer M, et al. 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 Jul;54(7):2164–71.
489. Miller WG, Bruns DE, Hortin GL, Sandberg S, Aakre KM, McQueen MJ, et al. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem. 2009 Jan;55(1):24–38.
490. Lambers Heerspink HJ, Gansevoort RT, Brenner BM, Cooper ME, Parving HH, Shahinfar S, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol. 2010 Aug;21(8):1355–60.
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 Jan;13(1):35–7.
492. Gansevoort RT, Verhave JC, Hillege HL, Burgerhof JGM, Bakker SJL, de Zeeuw D, et al. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int Suppl. 2005 Apr;(94):S28-35.
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 Oct;17(5):254–7.
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 Feb;20(2):436–43.
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 Oct;36(10):993–7.
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 Dec;37(12):2120–3.
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 Dec;16(12 Pt 2):2101–8.
498. Bachmann LM, Nilsson G, Bruns DE, McQueen MJ, Lieske JC, Zakowski JJ, 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 Mar;60(3):471–80.
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 Nov 1;2(3):423–9.

500. McTaggart MP, Newall RG, Hirst JA, Bankhead CR, Lamb EJ, Roberts NW, et al. Diagnostic accuracy of point-of-care tests for detecting albuminuria: a systematic review and meta-analysis. Ann Intern Med. 2014 Apr 15;160(8):550–7.
501. Shin JI, Chang AR, Grams ME, Coresh J, Ballew SH, Surapaneni A, et al. Albuminuria Testing in Hypertension and Diabetes: An Individual-Participant Data Meta-Analysis in a Global Consortium. Hypertension. 2021 Sep;78(4):1042–52.
502. Mejia JR, Fernandez-Chinguel JE, Dolores-Maldonado G, Becerra-Chauca N, Goicochea-Lugo S, Herrera-Añazco P, et al. Diagnostic accuracy of urine dipstick testing for albumin-to-creatinine ratio and albuminuria: A systematic review and meta-analysis. Heliyon. 2021 Nov;7(11):e08253.
503. Kim Y, Park S, Kim MH, Song SH, Lee WM, Kim HS, 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(1):e0227694.
504. Chronic kidney disease: assessment and management [Internet]. National Institute for Health and Care Excellence; 2021 [cited 2022 Mar 7]. Available from: https://www.nice.org.uk/guidance/ng203
505. Shaikh A, Seegmiller JC, Borland TM, Burns BE, Ladwig PM, Singh RJ, et al. Comparison between immunoturbidimetry, size-exclusion chromatography, and LC-MS to quantify urinary albumin. Clin Chem. 2008 Sep;54(9):1504–10.
506. American Diabetes Association Professional Practice Committee. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Medical Care in Diabetes—2022. Diabetes Care. 2021 Dec 16;45(Supplement_1):S185–94.
507. Tangri N, Grams ME, Levey AS, Coresh J, Appel LJ, Astor BC, et al. Multinational Assessment of Accuracy of Equations for Predicting Risk of Kidney Failure: A Meta-analysis. JAMA. 2016 Jan 12;315(2):164–74.
508. 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 Sep;22(9):1555–60.
509. Després JP, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med. 1996 Apr 11;334(15):952–7.
510. Wilson PWF, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med. 2007 May 28;167(10):1068–74.
511. Rutter MK, Wilson PWF, Sullivan LM, Fox CS, D'Agostino RB, Meigs JB. Use of alternative thresholds defining insulin resistance to predict incident type 2 diabetes mellitus and cardiovascular disease. Circulation. 2008 Feb 26;117(8):1003–9.
512. 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 Jul 26;379(4):376–85.
513. Leighton E, Sainsbury CA, Jones GC. A Practical Review of C-Peptide Testing in Diabetes. Diabetes Ther. 2017 Jun;8(3):475–87.
514. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology. ACOG Practice Bulletin No. 194: Polycystic Ovary Syndrome. Obstet Gynecol. 2018 Jun;131(6):e157–71.

515. Marcovina S, Bowsher RR, Miller WG, Staten M, Myers G, Caudill SP, et al. Standardization of insulin immunoassays: report of the American Diabetes Association Workgroup. Clin Chem. 2007 Apr;53(4):711–6.
516. Miller WG, Thienpont LM, Van Uytfanghe K, Clark PM, Lindstedt P, Nilsson G, et al. Toward standardization of insulin immunoassays. Clin Chem. 2009 May;55(5):1011–8.
517. Staten MA, Stern MP, Miller WG, Steffes MW, Campbell SE, Insulin Standardization Workgroup. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diabetes Care. 2010 Jan;33(1):205–6.
518. Little RR, Wielgosz RI, Josephs R, Kinumi T, Takatsu A, Li H, et al. Implementing a Reference Measurement System for C-Peptide: Successes and Lessons Learned. Clin Chem. 2017 Sep;63(9):1447–56.
519. Infusion Pumps: C-Peptide Levels as a Criterion for Use | Guidance Portal [Internet]. Centers for Medicare & Medicaid Services; 2005 [cited 2022 Mar 7]. Available from: https://www.hhs.gov/guidance/document/infusion-pumps-c-peptide-levels-criterion-use
520. Censi S, Mian C, Betterle C. Insulin autoimmune syndrome: from diagnosis to clinical management. Ann Transl Med. 2018 Sep;6(17):335.
521. 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 Jan;7(1):R47–55.