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

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ABSTRACT

Background: Multiple laboratory tests are used in the diagnosis and management of patients with diabetes mellitus. The quality of the scientific evidence supporting the use of these assays varies substantially.

Approach: An expert committee compiled evidence-based recommendations for the use of laboratory analysis in patients with diabetes. A new system was developed to grade the overall quality or strength of the evidence and the strength of the recommendations. A draft of the guidelines was posted on the Internet and was presented at the Arnold O. Beckman Conference in November, 2007. The document was modified in response to oral and written comments. The guidelines were reviewed by the Professional Practice Committee of the American Diabetes Association.

Content: In addition to the longstanding criteria based on measurement of venous plasma glucose, diabetes can be diagnosed by demonstrating increased hemoglobin (Hb) A_{1c} concentrations in the blood. Monitoring of glycemic control is performed by the patients measuring their own plasma or blood glucose with meters and by laboratory analysis of HbA_{1c}. The potential roles of non-invasive glucose monitoring, genetic testing, and measurement of autoantibodies, microalbumin, proinsulin, C-peptide and other analytes are addressed.

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

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Nonstandard abbreviations: OGTT, oral glucose tolerance test; FPG, fasting plasma glucose; IMD, immune-mediated diabetes; SMBG, self-monitoring of blood glucose; GHb, glycated hemoglobin; DCCT, Diabetes Control and Complications Trial; UKPDS, United Kingdom Prospective Diabetes Study; ADA, American Diabetes Association; NGSP, National Glycohemoglobin Standardization Program; CI, confidence intervals; GDM, gestational diabetes mellitus; WHO, World Health Organization; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; DKA, diabetic ketoacidosis; AcAc, acetoacetate; βHBA, β hydroxybutyrate; CAP, College of American Pathologists; MODY, maturity onset diabetes of the young; ICA, islet-cell cytoplasm antibodies; GAD_{65}, 65-kDa isoform of glutamic acid decarboxylase; IAA, insulin autoantibodies; JDF, Juvenile Diabetes Foundation; FDA, Food and Drug Administration; HDL, high density lipoprotein; LDL, low density lipoprotein; CAD, coronary artery disease; CDC, Centers for Disease Control; HbA_{1c}, hemoglobin A_{1c}; NHANES, National Health and Nutrition Examination Survey; FPG, fasting plasma glucose; IDF, International Diabetes Federation; CLSI, Clinical and Laboratory Standards Institute

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EXECUTIVE SUMMARY

Key recommendations of the guideline are summarized below. Capital letters denote the grade of recommendations and categories in brackets refer to the strength of the underlying body of evidence supporting each recommendation. Detailed description of the grading system used is given in Table 1-2.

1. Glucose

Recommendation	Grade
Glucose measured in venous plasma should be used to establish the diagnosis of diabetes.	A (high)
Glucose should be measured in venous plasma when used for screening of high-risk individuals.	B (moderate)
Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes.	GPP
Outcome studies are needed to determine the effectiveness of screening.	C (moderate)
Routine measurement of plasma glucose concentrations in an accredited laboratory is not recommended as the primary means of monitoring or evaluating therapy in individuals with diabetes.	B (low)

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Blood for fasting plasma glucose analysis should be drawn in the morning after the subject has fasted overnight (at least 8 h).	B (low)
To minimize glycolysis, ideally the sample tube should immediately be placed in an ice slurry and plasma should be separated from the cells as soon as possible. If this cannot be achieved within 30 min, a tube containing a glycolytic inhibitor such as sodium fluoride or citrate buffer should be used for collecting the sample.	B (moderate)
FPG values of 6.1 – 6.9 mmol/L (110-125 mg/dL) should be repeated and individuals with FPG of 5.3 – 5.7 mmol/L (96-104 mg/dL) should be considered for follow-up at 1 year intervals.	B (moderate)
Based on biological variation, glucose measurement should have analytical imprecision ≤2.9%, bias ≤2.2% and total error ≤6.9%. To avoid misclassification of patients, the goal for glucose analysis should be to minimize total analytical error and methods should be without measureable bias.	GPP

2. Meters

There are insufficient published data to support a role for portable meters and skin-prick (finger-stick) blood samples in the diagnosis of diabetes or for population screening.	C (moderate)

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Recommendation	Grade
The imprecision of the results, coupled with the substantial differences among meters, precludes their use in the diagnosis of diabetes and limits their usefulness in screening for diabetes.	A (moderate)
Self-monitoring of blood glucose (SMBG) is recommended for all insulin-treated patients with diabetes. For type 1 patients, SMBG is recommended three or more times a day.	A (high)
In patients with type 2 diabetes treated with diet and oral agents, SMBG may help achieve better control, particularly when therapy is initiated or changed. Data are insufficient, however, to claim an associated improvement of health outcomes. The role of SMBG in patients with stable type 2 diabetes controlled by diet alone is not known.	C (high)
Patients should be instructed in the correct use of glucose meters, including quality control. Comparison between SMBG and concurrent laboratory glucose analysis should be performed at regular intervals to evaluate the performance of the meters in the patient's hands.	B (moderate)

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Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. No published study has reported results that meet the goals proposed by the ADA of less than 5% total error. Manufacturers should work to improve the imprecision of current meters, with an intermediate goal of limiting total error, for 95% of samples, to ≤15% at glucose concentrations ≥5.6 mmol/L (100 mg/dL) and to <0.8 mmol/L (15 mg/dL) at glucose concentrations <5.6 mmol/L (100 mg/dL). Lower total error would be desirable and may prove necessary in tight glucose control protocols and for avoiding hypoglycemia in all settings.

C (low)

We recommend meters that measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories.

GPP

Studies are needed to determine the analytical goals (quality specifications) for glucose meters in self monitoring of blood glucose and in intensive care units.

C (moderate)

Recommendations for future research: Important end-points in studies of SMBG should include, at a minimum, HbA_{1c} and frequency of hypoglycemic episodes to ascertain whether improved meters enable patients to achieve better glucose control. For studies of meter use in intensive or critical care, important end-points include mean blood glucose, frequency of hypoglycemia and variability of glucose control. Ideally, outcomes (e.g., long-term complications) should also be examined.

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3. Continuous Minimally-Invasive Glucose Analyses

Real-time continuous glucose monitoring (CGM) in conjunction with intensive insulin regimens can be a useful tool to lower HbA_{1c} in selected adults (age > 25 years) with type 1 diabetes.	A (high)
Although the evidence for HbA_{1c} lowering is less strong in children, teens, and younger adults, real-time CGM may be helpful in these groups. Success correlates with adherence to ongoing use of the device.	B (moderate)
Real-time CGM may be a supplemental tool to SMBG in those with hypoglycemia unawareness and/or frequent episodes of hypoglycemia.	B (low)
Patients require extensive training in using the device. Available devices must be calibrated with SMBG readings and the latter are recommended for making treatment changes.	GPP

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4. Noninvasive Glucose Analysis

No noninvasive sensing technology is currently approved for clinical glucose measurements of any kind. Major technological hurdles must be overcome before noninvasive sensing technology will be sufficiently reliable to replace existing portable meters, implantable biosensors, or minimally invasive technologies.	C (very low)

5. Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test is not recommended for the routine diagnosis of type 1 or type 2 diabetes mellitus. It is recommended for establishing the diagnosis of gestational diabetes mellitus and for evaluation postpartum after gestational diabetes.	A (moderate)

6. Gestational Diabetes Mellitus (GDM)

All women of average or high risk of gestational diabetes mellitus (GDM) should undergo GDM testing at 24-28 weeks of gestation.	A (high)
GDM should be diagnosed by a 75 g OGTT using the IADPSG criteria derived from the HAPO study.	A (moderate)

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7. Urinary Glucose

Semi-quantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus.	B (low)

8. Ketone Testing

Recommendation	Grade
Ketones should be measured in urine or blood by patients with diabetes in the home setting and in the clinic/hospital setting as an adjunct to the diagnosis of diabetic ketoacidosis.	GPP
Urine ketone determinations should not be used to diagnose or monitor the course of diabetic ketoacidosis (DKA).	GPP
Blood ketone determinations that rely on the nitroprusside reaction should be used only as an adjunct to diagnose DKA and should not be used to monitor treatment of DKA. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA.	B (moderate)

9. Hemoglobin A_{1c}

Recommendation	Grade
Hemoglobin A_{1c} (HbA_{1c}) should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control.	A (moderate)
Laboratories should use only HbA_{1c} assay methods that are certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference. The manufacturers of assays for HbA_{1c} should also show traceability to the IFCC reference method.	GPP

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Laboratories that measure HbA_{1c} should participate in a proficiency-testing program, such as the CAP Glycohemoglobin Survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network.	GPP
Laboratories should be aware of potential interferences, including hemoglobinopathies that may affect HbA_{1c} test results depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population. In addition, disorders that affect erythrocyte turnover may cause spurious results regardless of the method used.	GPP
Desirable specifications for HbA_{1c} measurement are intra-laboratory CV <2% and inter-laboratory CV <3.5%. At least two control materials with different mean values should be analyzed as an independent measure of assay performance.	B (low)
Laboratories should verify by repeat testing specimens with HbA_{1c} results below the lower limit of the reference interval or greater than 15% HbA_{1c}.	B (low)

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Treatment goals should be based on ADA recommendations which include generally maintaining HbA_{1c} concentrations <7% and in individual patients as close to the non-diabetic range as safely possible. Somewhat higher ranges are recommended for children and adolescents and should be considered in patients with projected limited lifespans, owing to advanced age or co-morbid illnesses. (Note that these values are applicable only if the assay method is certified by the NGSP as traceable to the DCCT reference.)	A (high)
HbA_{1c} testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or are not meeting treatment goals.	GPP
The HbA_{1c} assay may be used for the diagnosis of diabetes, with values $\geq$ 6.5% being diagnostic. Similar to its use in the management of diabetes, factors that interfere with or adversely affect the HbA_{1c} assay will preclude its use in diagnosis. When a HbA_{1c} assay is not available, or cannot be interpreted in a patient, glucose-based testing should be used for diagnosis.	A (moderate)

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10. Genetic Markers

Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, including neonatal diabetes, valuable information can be obtained with definition of diabetes-associated mutations.	A (moderate)

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

11. Autoimmune Markers

Islet cell autoantibodies are recommended for screening of non-diabetic family members who wish to donate part of their pancreas for transplantation to a relative with end stage type 1 diabetes. B (low)

Islet cell autoantibodies are not recommended for routine diagnosis of diabetes but standardized islet cell autoantibody tests may be used for classification of diabetes in adults and in prospective studies of children at genetic risk for type 1 diabetes following HLA typing at birth. B (low)

Screening of patients with type 2 diabetes is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies of type 2 diabetes patients to identify possible mechanisms of secondary failures to type 2 diabetes treatment. B (low)

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Screening relatives of patients with type 1 diabetes or persons in the general population for islet cell autoantibodies is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies of children selected at birth following HLA testing for type 1 diabetes high risk HLA genotypes.	B (low)
There is currently no role for measurement of islet cell autoantibodies in the monitoring of patients in clinical practice. Islet cell autoantibodies are measured in research protocols and some clinical trials as surrogate end-points.	B (low)
It is important that islet cell autoantibodies be measured only in an accredited laboratory with an established quality control program and participation in a proficiency testing program.	GPP

12. Microalbuminuria

Annual microalbuminuria testing of patients without macroalbuminuria or clinical proteinuria should begin in pubertal or postpubertal individuals five years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment.	B (moderate)
Microalbuminuria is a continuous risk marker for cardiovascular events which appear to start at concentrations of 20 ug/min.	B (moderate)

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The analytical CV of methods to measure microalbuminuria should be <15%. — B (moderate)

Semiquantitative or qualitative screening tests for microalbuminuria should be positive in >95% of patients with microalbuminuria to be useful for screening. Positive results must be confirmed by analysis in an accredited laboratory. — GPP

Currently available dipstick tests are not sensitive enough to be used reliably to make a diagnosis of microalbuminuria. — C (low)

Acceptable samples to test for increased urinary albumin excretion are timed (e.g., 12 or 24 hour) collections for measurement of albumin concentration and timed or untimed samples for measurement of the albumin:creatinine ratio. For screening, an untimed sample for albumin measurement (without creatinine) may be considered if a concentration cutoff is used that allows high sensitivity for detection of an increased albumin excretion rate. — B (moderate)

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Optimal time for spot urine collection is the early morning and fasting. To minimize variability all collections should be at the same time of day and preferably fasting for at least 2 hours. — GPP

Recommendation	Grade
A urine albumin of <30 µg/mg creatinine, while considered “normal”, should be reassessed annually. If the value is ≥30 µg/mg, changes should be reassessed after six to 12 months if antihypertensive therapy is required, or annually in those who are normotensive.	B (moderate)

13. Miscellaneous Potentially Important Analytes

Recommendation	Grade
There is no role for routine testing for insulin, C-peptide or proinsulin in most patients with diabetes. Differentiation between type 1 and type 2 diabetes may in most cases be made based on the clinical presentation and subsequent course. These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 and type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis.	B (moderate)
There is no role for measurement of insulin concentration in the assessment of cardiometabolic risk, as knowledge of this value does not alter the management of these patients.	B (moderate)
Since current measures of insulin are poorly harmonized, a standardized insulin assay should be developed to encourage the development of measures of insulin sensitivity that will be practical for clinical care.	GPP

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

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized and over-produced, resulting in hyperglycemia. The disease is classified into several categories. The revised classification, published in 1997 (1) is indicated in Table 3. Type 1 diabetes mellitus, formerly known as insulin-dependent diabetes mellitus (IDDM) or juvenile onset 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, formerly known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes, 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 ~7% (ranging from 5 to 15%) of pregnancies, usually remits after delivery and is a major risk factor for the development of type 2 diabetes later in life. Other types of diabetes are rare. Type 2 is the most common form, accounting for 85-95% of diabetes in developed countries. Some patients cannot be clearly classified as type 1 or type 2 diabetes (5).

Diabetes is a common disease. The current worldwide prevalence is estimated to be ~250 million and it is expected to reach 380 million by 2025 (6). The prevalence of diabetes (based on FPG) in adults in the U.S. in 1999-2002 was 9.3%, of which 30% was undiagnosed (7). The most recent data, derived from the 2005-2006 National Health and Nutrition Examination Survey (NHANES) using both FPG and 2-h oral glucose tolerance test (OGTT), show a prevalence of diabetes in the U.S. in persons aged ≥20 years of 12.9% (equivalent to ~40 million) (8). Of these, 40% (~16 million) are undiagnosed. The prevalence of diabetes has also increased in other parts of the world. For example, recent estimates suggest 110 million diabetic individuals in Asia in 2007 (9), but the true number is

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likely to be substantially greater as China alone was thought to have 92.4 million adults with diabetes in 2008 (10).

The worldwide costs of diabetes in 2007 were approximately $232 billion and are likely to be $302 billion by 2025 (6). In 2007 the costs of diabetes in the U.S. were estimated to be $174 billion (11). 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 2008 to £9 billion per year). The high costs of diabetes are attributable to care for both acute conditions (such as hypoglycemia and ketoacidosis) and debilitating complications (12). The latter include both 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 (13). About 3.8 million people worldwide were estimated to have died from diabetes-related causes in 2007 (6).

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The NACB issued its "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus" in 2002 (14). These recommendations were reviewed and updated using an evidence-based approach, especially in key areas where new evidence has emerged since the 2002 publication. The process of updating guideline recommendations followed the "Standard Operating Procedures for Preparing, Publishing, and Editing National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines" and the key steps as well as the grading system used to define the strength of evidence and recommendations are detailed in the Preamble (see Supplement). A new system was developed to grade both the overall quality or strength of the evidence (Table 2) and the strength of recommendations (Table 3).

This guideline primarily focuses 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

American Diabetes Association (ADA) guidelines. The ADA publishes in January each year a supplement, titled Clinical Practice Recommendations, in Diabetes Care. This is a compilation of all ADA position statements related to clinical practice and is an important resource for health care professionals who care for people with diabetes. The NACB 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. Testing lipids and related cardiovascular risk factors is addressed in a separate NACB guideline.

To facilitate comprehension and assist the reader, each analyte is divided into several headings and subheadings (listed in parentheses). These are use (diagnosis, screening, monitoring and prognosis), rationale (diagnosis and screening), analytical considerations (preanalytical [including reference values] 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.

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GLUCOSE

1. **Use**

A. Diagnosis/Screening

Recommendation: Glucose measured in venous plasma should be used to establish the diagnosis of diabetes.

A (high)

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

Recommendation: Outcome studies are needed to determine the effectiveness of screening.

C (moderate)

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The diagnosis of diabetes is established by identifying the presence of hyperglycemia. For many years the only method recommended for diagnosis was a direct demonstration of hyperglycemia by measuring increased glucose concentrations in the plasma (15, 16). In 1979, a set of criteria based on the distribution of glucose concentrations in high risk populations was established to standardize the diagnosis (15). These recommendations were endorsed by the World Health Organization (WHO) (16). In 1997, the diagnostic criteria were modified (1) to better identify subjects at risk of retinopathy and

nephropathy (17, 18). The revised criteria comprised: (a) fasting plasma glucose (FPG) ≥ 7.0 mmol/L (126 mg/dL), (b) 2-h postload glucose ≥ 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT) or (c) symptoms of diabetes and a casual (i.e., regardless of the time of the preceding meal) plasma glucose ≥11.1 mmol/L (200 mg/dL) (Table 4) (1). If any one of these three criteria is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis. (Note that repeat testing is not required in patients who have unequivocal hyperglycemia i.e., >11.1 mmol/L (200 mg/dL) with symptoms consistent with hyperglycemia.) The WHO and IDF recommend either FPG or 2-h postload glucose using the same cutoffs as the ADA (19). Although included as a criterion, the OGTT was not recommended by the Expert Committee in 1997 for routine clinical use in non-pregnant individuals, although the WHO continues to advocate its use (see OGTT section below). In 2009 an International Expert Committee (20), with members appointed by the ADA, EASD and IDF, recommended that diabetes be diagnosed by measurement of hemoglobin A_{1c} (HbA_{1c}), which reflects long-term blood glucose concentrations (see HbA_{1c} section below). The ADA has endorsed the use of HbA_{1c} for diagnosis of diabetes (21).

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Testing to detect type 2 diabetes in asymptomatic people, previously controversial, is now recommended for those at risk of developing the disease (21, 22). The ADA proposes that all asymptomatic people aged 45 years or more, particularly those who are overweight (BMI ≥ 25 kg/m^2) or obese, should be screened in a health care setting. If HbA_{1c} is not used, either FPG or 2-h OGTT or both are appropriate for screening (21). The FPG is more convenient, more reproducible, less costly and easier to administer than the 2-h OGTT. Thus, the ADA recommends the FPG test over OGTT as the initial glucose-based screening test (21). The IDF recommends that the health service in each country should decide whether to implement screening for diabetes (23). FPG is the suggested test. In contrast, the International Expert Committee and ADA have recommended that HbA_{1c} be used for screening for diabetes (20, 21, 24) (see section on HbA_{1c} below). If 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. Screening should be considered at a younger age or be carried out more frequently in individuals at increased risk of diabetes (see Ref (21) for conditions associated with increased risk). Because of the increasing prevalence of type 2 diabetes in children, screening of children is now advocated (25). Starting at age 10 years, testing should be performed every 2 years in overweight individuals who have two other risk factors, namely family history, race/ethnicity recognized to increase risk and signs of insulin resistance (25). Despite these recommendations and the demonstration that interventions can delay, and sometimes prevent, the onset of type 2 diabetes in individuals with impaired glucose tolerance (IGT) (26, 27), there is as yet no published evidence that treatment based on screening has an effect on long-term complications. In addition, there is a lack of consensus in the published literature as to which screening procedure, FPG, OGTT and/or HbA_{1c}, is the most appropriate (20, 28-30). Based on evaluation of NHANES III data, a strategy to screen whites who are ≥40 years and other propulations ≥30 years of age with FPG has been proposed (31). 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 (32). Interestingly, screening was more cost-effective at ages younger than the 45 years currently recommended. In contrast, screening targeted to individuals with hypertension reduces the QALY from $360,966 to $34,375, with ages 55 to 75 years being most cost-effective (33). Modelling run on one million individuals suggests there is considerable uncertainty as to whether screening for diabetes would be cost effective (34). Long-term outcome studies are necessary to provide evidence to resolve the question of the efficacy of screening for diabetes (35).

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) (36). This change is contentious and has not been accepted by all organizations (19, 37). The rationale is based on data that individuals with FPG values between 5.6

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mmol/L (100 mg/dL) and 6.05 mmol/L (109 mg/dL) are at increased risk for the development of type 2 diabetes (38, 39). More recent evidence indicates that FPG concentrations even lower than 5.6 mmol/L (100 mg/dL) are associated with a graded risk for type 2 diabetes (40). 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.

B. Monitoring/Prognosis

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

B (low)

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There is a direct relationship between the degree of chronic plasma glucose control and the risk of late renal, retinal and neurological complications. This correlation has been documented in epidemiologic studies and in clinical trials for both type 1 (41) and type 2 (42) diabetes. The important causal role of hyperglycemia in the development and progression of complications has been documented in clinical trials. Persons with type 1 diabetes who maintain lower average plasma glucose concentrations exhibit a significantly lower incidence of microvascular complications, namely diabetic

retinopathy, nephropathy and neuropathy (43). Although intensive insulin therapy reduced hypercholesterolemia by 34%, the risk of macrovascular disease was not significantly decreased in the original analysis (43). Longer follow up documented a significant reduction in cardiovascular disease in patients with type 1 diabetes treated with intensive glycemic control (44). The effects of tight glycemic control on microvascular complications in patients with type 2 diabetes (45) are similar to those with type 1 diabetes, taking into account the differences in glycemia achieved between the active intervention and control groups in the various trials. Intensive plasma glucose control in patients with type 2 diabetes significantly reduced microvascular complications. While meta-analyses suggest that intensive glycemic control in individuals with type 2 diabetes reduces cardiovascular disease (46, 47), 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 United Kingdom Prospective Diabetes Study (UKPDS) population supported a benefit of intensive therapy on macrovascular disease (48), but three other recent trials failed to demonstrate a significant difference in macrovascular disease outcomes between very intensive treatment strategies achieving HbA_{1c} concentrations of approximately 6.5% compared with the control groups who had HbA_{1c} conentrations 0.8 to 1.1% higher (49-51). One study even observed higher cardiovascular mortality in the intensive treatment arm (49). In both the Diabetes Control and Complications Trial (DCCT) and UKPDS, patients in the intensive group maintained lower median plasma 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 (21, 52).

Neither random nor fasting glucose concentrations should be measured in an accredited laboratory as the primary means of routine outpatient monitoring of patients with diabetes. Laboratory

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plasma glucose testing can be used to supplement information from other testing, to test the accuracy of self-monitoring (see below) or when adjusting the dose of oral hypoglycemic agents (22, 53). 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 (53, 54). Finally, plasma glucose measured in an accredited laboratory should be used to aid in the management of hospitalized patients to achieve recommended targets of glucose control (21).

2. **Rationale**

A. Diagnosis

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

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B. Screening

Screening is recommended for several reasons. The onset of type 2 diabetes is estimated to occur ~4-7 (or more) years before clinical diagnosis (55) and epidemiological evidence indicates that complications may begin several years before clinical diagnosis. Furthermore, it is estimated that 40% of people in the U.S. with type 2 diabetes are undiagnosed (8). Notwithstanding this recommendation,

there is no published evidence that population screening for hyperglycemia provides any long-term benefit. Outcome studies that examine the potential long-term benefits of screening are ongoing.

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)

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Recommendation: To minimize glycolysis, ideally the sample tube should immediately be placed in an ice slurry and plasma should be separated from the cells as soon as possible. If this cannot be achieved within 30 min, a tube containing a glycolytic inhibitor such as sodium fluoride or citrate buffer should be used for collecting the sample.

B (moderate)

Blood should be drawn in the morning after an overnight fast (no caloric intake for at least 8 h) during which time the subject may consume water ad lib (1). 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 (56). Glucose concentrations decrease *ex vivo* with time in whole blood due to glycolysis. The rate of glycolysis—reported to

average 5-7% (~0.6 mmol/L; 10 mg/dL) per hour (57)—varies with the glucose concentration, temperature, white blood cell count and other factors (58). 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. 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 fluoride (57). After 4 h, the glucose concentration is stable in whole blood for 72 h at room temperature in the presence of fluoride (57). (Note that leukocytosis will increase glycolysis even in the presence of fluoride if the white cell count is very high.) In separated, nonhemolyzed, sterile serum without fluoride the glucose concentration is stable for 8 h at 25 °C and 72 h at 4 °C (59). A recent study showed that acidification of blood using citrate buffer inhibits *in vitro* glycolysis more effectively than fluoride (60). The mean glucose concentration in samples at 37 °C decreased by 0.3% at 2 h and 1.2% at 24 h when blood was drawn into tubes containing citrate buffer, sodium fluoride and EDTA. To minimize glycolysis, the cells should be separated from plasma within minutes. Because this is usually impractical, blood should immediately be placed in ice-water and cells should be removed within 30 min (19, 60).

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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 blood (19, 21).] The molality of glucose (i.e., amount of glucose per unit water mass) in whole blood and plasma is identical. 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 (61). (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, more recent studies found that glucose concentrations in plasma are slightly higher than serum. The differences observed were ~0.2 mmol/L (3.6 mg/dL) (62), ~2% (63) or 0.9% (60). Other studies indicate that glucose values measured in serum and plasma are essentially the same (64, 65). 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. Nevertheless, carefully controlled studies are necessary to unequivocally resolve this question. Measurement of glucose in serum (rather than plasma) is not recommended by clinical organizations for the diagnosis of diabetes (19, 21). The glucose concentrations during an OGTT in capillary blood are significantly higher than those in venous blood (mean of 1.7 mmol/L (30 mg/dL), equivalent to 20-25% (66)), but the mean difference in fasting samples is only 0.1 mmol/L (2 mg/dL) (66, 67).

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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 – 6.1 mmol/L (74-110 mg/dL) (68). Note that the ADA and WHO criteria (19, 21), not the reference values, are used for the diagnosis of diabetes. Moreover, the threshold for diagnosis of hypoglycemia is variable. The reference values are not useful to diagnose these conditions. In adults, mean FPG increases with increasing age from the third to the sixth decade (69), but does not increase significantly after age 60 (70, 71). By contrast, glucose concentrations after a glucose challenge are substantially higher in older individuals (70, 71). Evidence of an association of increasing insulin resistance with age is inconsistent (72). Aging appears to influence glucose homeostasis and visceral obesity seems to be responsible for the reported decrease in glucose tolerance that is continuous beginning in middle-age (73).

B. Analytical

Recommendation: FPG values of 6.1 – 6.9 mmol/L (110-125 mg/dL) should be repeated and individuals with FPG of 5.3 – 5.7 mmol/L (96-104 mg/dL) should be considered for follow-up at 1 year intervals.

B (moderate)

Recommendation: Based on biological variation, glucose measurement should have analytical imprecision ≤2.9%, bias ≤2.2% and total error ≤6.9%. To avoid misclassification of patients, the goal for glucose analysis should be to minimize total analytical error and methods should be without measureable bias.

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Glucose is measured almost exclusively by enzymatic methods. Analysis of proficiency surveys conducted 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. (68). A very few laboratories (<1%) use glucose dehydrogenase. Enzymatic methods for glucose analysis are relatively well standardized. At a plasma glucose concentration of ~7.5 mmol/L (135 mg/dL), imprecision among laboratories using the same method had a CV ≤2.6% (68). 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 the hexokinase and glucose oxidase methods are essentially the same (74). However, significant biases were observed among different instruments (74). If similar biases occur with plasma, patients near the diagnostic threshold could be misclassified.

No consensus has been achieved on the goals for glucose analysis. Numerous criteria have been proposed to establish analytic goals. These include expert opinion (consensus conferences), opinion of clinicians, regulation, state of the art and biological variation (75). A rational and realistic recommendation that has received some support is to use biological criteria as the basis for analytic goals. It has been suggested that imprecision should not exceed one half of the within-subject biological CV (76, 77). For plasma glucose, a CV $\leq$ 2.2% has been suggested as a target for imprecision, with 0% bias (77). Although this recommendation was proposed for within-laboratory error, it would be desirable to achieve this goal for inter-laboratory imprecision to minimize differences among laboratories in the diagnosis of diabetes in individuals whose glucose concentrations are close to the threshold value. Therefore, the goal for glucose analysis should be to minimize total analytical error and methods should be without measureable bias. A national or international program using commutable samples (e.g., fresh frozen plasma) that eliminate matrix effects, with accuracy based grading using values derived with a reference method, should be developed to assist in the achievement of this objective.

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4. **Interpretation**

Despite the low analytical imprecision at the diagnostic decision limits of 7.0 mmol/L (126 mg/dL) and 11.1 mmol/L (200 mg/dL), classification errors may occur. Knowledge of intraindividual (within-person) variability of FPG concentrations is essential for meaningful interpretation of patient values. An early study, which repeated the OGTT in 31 nondiabetic adults at 48 h intervals, revealed that FPG

in 22 subjects (77%) varied by <10% and in 30 subjects (97%) varied by <20% between the two values (78). 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 (79-81). Larger studies have revealed intraindividual CVs of 4.8-7.1% for FPG in 246 normal and 80 previously undiagnosed individuals with diabetes, respectively (81). 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%) (82). 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) (83). If a CV (within-person biological) of 5.7% is applied to a true glucose concentration of 7.0 mmol/L (126 mg/dL), the 95% CI would encompass glucose concentrations of 6.2-7.8 mmol/L (112-140 mg/dL). If the 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 is substantially greater than analytic variability. Using biological variation as the basis for deriving analytical performance characteristics (75), the following desirable specifications for glucose have been proposed (84): analytical imprecision ≤2.9%, bias ≤2.2% and total error ≤6.9%.

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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 or treatment of diabetic ketoacidosis (DKA), rapid analysis is desirable. A turnaround time of 30 min has been proposed (85). However, this value is based on suggestions of clinicians and no outcome data have been published that validate this figure. Inpatient management of diabetic patients may on occasion require a rapid turnaround time (minutes, not hours). Similarly, for protocols with intensive glucose control in critically ill patients (86), rapid glucose measurements are required to calculate the dose of insulin. Bedside monitoring with glucose meters (see below) has been adopted by many as a practical solution.

Frequency of measurement: The frequency of measurement of plasma glucose is dictated by the clinical situation. The ADA, WHO and IDF recommend that an increased FPG or abnormal OGTT must be confirmed to establish the diagnosis of diabetes (19, 87). Screening by FPG is recommended every 3 years beginning at age 45, 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 and by assessment of GHb 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**

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

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METERS

Portable meters for measurement of blood glucose concentrations are used in three major settings: i) in acute and chronic care facilities (including intensive care units); ii) in physicians' offices and iii) by patients at home, work and school. The last, self-monitoring of blood glucose (SMBG), was performed at least once a day by 40% and 26% of individuals with type 1 and 2 diabetes, respectively, in the United States in 1993 (88). The overall rate of daily use of SMBG increased to 40.6% in 1997 and 63.4% in 2006 among adults with diabetes in the US (89). The ADA listed the following indications for SMBG: i) achievement and maintenance of glycemic control; ii) prevention and detection of hypoglycemia; iii) avoidance of severe hyperglycemia; iv) adjusting to changes in life-style and v) determining the need for initiating insulin therapy in GDM (90). It is recommended that most individuals with diabetes attempt to achieve and maintain blood glucose concentrations as close to those found in non-diabetic individuals as is safely possible.

1. **Use**

A. Diagnosis/Screening

Recommendation: There are insufficient published data to support a role for portable meters and skin-prick (finger-stick) blood samples in the diagnosis of diabetes or for population screening.

C (moderate)

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Recommendation: The imprecision of the results, coupled with the substantial differences among meters, precludes their use in the diagnosis of diabetes and limits their usefulness in screening for diabetes.

A (moderate)

The glucose-based criteria for the diagnosis of diabetes are based upon outcome data (the risk of micro- and macrovascular disease) correlated with plasma glucose concentrations—both fasting and 2 h after a glucose load—assayed in an accredited laboratory (1). Whole blood is used in portable meters. Although most portable meters have been programmed to report a plasma glucose concentration, the imprecision of the current meters (see below) precludes their use in the diagnosis of diabetes. Similarly, screening by portable meters, although attractive because of convenience, ease and accessibility, would generate many false positives and false negatives.

B. Monitoring/Prognosis

Recommendation: SMBG is recommended for all insulin-treated patients with diabetes. For type 1 patients, SMBG is recommended three or more times a day.

A (high)

Recommendation: In patients with type 2 diabetes treated with diet and oral agents, SMBG may help achieve better control, particularly when therapy is initiated or changed. Data are insufficient, however,

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to claim an associated improvement of health outcomes. The role of SMBG in patients with stable type 2 diabetes controlled by diet alone is not known.

C (high-moderate)

SMBG is recommended for all insulin-treated patients with diabetes. Tight glycemic control can decrease microvascular complications in individuals with type 1 (43) or type 2 (45) diabetes. Intensive glycemic control in patients with type 1 diabetes was achieved in the DCCT by participants performing SMBG at least four times per day (43). Therapy in patients with type 2 diabetes in the UKPDS (45) was adjusted according to FPG concentrations – SMBG was not evaluated.

The role of SMBG in individuals with type 2 diabetes has generated considerable controversy (91, 92). Faas *et al.* (93) reviewed eleven studies, published between 1976 and 1996, that evaluated SMBG in patients with type 2 diabetes. Only one of the published studies reported that SMBG produced a significant improvement in GHb. The authors of the review concluded that the efficacy of SMBG in type 2 diabetes is questionable (93). Similar conclusions were drawn in a meta-analysis (94), in a sample of patients with type 2 diabetes in the NHANES (95) and in the Freemantle Diabetes Study (96). Two randomized trials have assessed the use of glucose meters in individuals with type 2 diabetes (97, 98). One (97) had statistical power to detect a 0.5% reduction in HbA_{1c}, but reported only a modest decrease (0.3%) of HbA_{1c} in poorly controlled patients treated with oral agents. The second study (98) failed to demonstrate a significant difference in HbA_{1c} in patients who were assigned to use meters compared to those who weren't.

For individuals with type 2 diabetes, cross-sectional and longitudinal observational studies in several countries have failed to demonstrate an improvement of glycemic control, as measured by

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mean HbA_{1c} concentrations, associated with use of SMBG (99-101). This lack of effect was seen in individuals treated with insulin, oral agents or both. Frequency of meter use did not predict HbA_{1c}.

A 2005 Cochrane review (102, 103) of self monitoring in individuals with type 2 diabetes not using insulin concluded that SMBG might be effective in improving glucose control. There was insufficient evidence to study if it was beneficial in improving quality of life, well-being or patient satisfaction, or in decreasing the number of hypoglycaemic episodes.

The randomized controlled DiGEM Trial (104) studied people with type 2 diabetes, a third of whom were treated with diet alone. The investigators concluded that "evidence is not convincing of an effect of self monitoring blood glucose in improving glycaemic control [as assessed by HbA_{1c}] compared with usual care in reasonably well controlled non-insulin treated patients with type 2 diabetes." A cost-effectiveness analysis of data from the DiGEM trial concluded that "self monitoring of blood glucose with or without additional training in incorporating the results into self care was associated with higher costs and lower quality of life in patients with non-insulin treated type 2 diabetes. In light of this, and no clinically significant differences in other outcomes, self monitoring of blood glucose is unlikely to be cost effective in addition to standardised usual care" (105)

The later ESMON study (106), a randomized controlled trial of SMBG in newly diagnosed people with diabetes not treated with insulin, found no benefit of SMBG on glycemic control, but did find higher scores on a depression subscale.

Finally, a 2009 review (107) addressed recent large randomized trials of tight glycemic control in patients with type 2 diabetes, a major rationale for use of SMBG in these patients. It concluded that "tight glycemic control burdens patients with complex treatment programs, hypoglycemia, weight gain, and costs and offers uncertain benefits in return".

2. **Rationale**

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Knowledge of ambient plasma or blood glucose concentrations is used by insulin-requiring patients, particularly those with type 1 diabetes, as an aid in determining appropriate insulin doses at different times of the day (90). Patients adjust the amount of insulin according to their plasma or blood glucose concentration. Frequent SMBG is particularly important for tight glycemic control in type 1 diabetes.

Hypoglycemia is a major, potentially life-threatening complication of the treatment of diabetes. The risk of hypoglycemia increases significantly with pharmacologic therapy directed towards maintaining the glycemic range as close to those found in non-diabetic individuals as is safely possible (43, 45). 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 (43, 45). Furthermore, many patients with diabetes, particularly those with type 1, lose the autonomic warning symptoms that normally precede neuroglycopenia ("hypoglycemic unawareness") (108), increasing the risk of hypoglycemia. SMBG can be useful for detecting asymptomatic hypoglycemia and allowing patients to avoid major hypoglycemic episodes.

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3. **Analytical Considerations**

A. Preanalytical

Recommendation: Patients should be instructed in the correct use of glucose meters, including quality control. Comparison between SMBG and concurrent laboratory glucose analysis should be performed at regular intervals to evaluate the performance of the meters in the patient's hands.

B (moderate)

Numerous factors can interfere with glucose analysis with portable meters. Several of these, such as improper application, timing and removal of excess blood (59), have been eliminated by advances in technology. Important variables that may influence the results of bedside glucose monitoring include changes in hematocrit (109), altitude, environmental temperature or humidity, hypotension, hypoxia and high triglyceride concentrations (110). Furthermore, most meters are inaccurate at very high or very low glucose concentrations. Another important factor is variability of results among different glucose meters. Different assay methods and architectures result in lack of correlation among meters, even from a single manufacturer. In fact, two meters of the same brand have been observed to differ substantially in accuracy (111, 112). Patient factors are also important, particularly adequate training. Recurrent education at clinic visits and comparison of SMBG with concurrent laboratory glucose analysis improved the accuracy of patients' blood glucose readings (113). In addition, it is important to evaluate the patient's technique at regular intervals (21).

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B. Analytical

Recommendation: Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. No published study has reported results that meet the goals proposed by the ADA of less than 5% total error. Manufacturers should work to improve the imprecision of current meters, with an intermediate goal of limiting total error, for 95% of samples, to ≤15% at glucose concentrations ≥5.6 mmol/L (100 mg/dL) and to <0.8 mmol/L (15 mg/dL) at glucose

concentrations <5.6 mmol/L (100 mg/dL). Lower total error would be desirable and may prove necessary in tight glucose control protocols and for avoiding hypoglycemia in all settings.

C (low)

Recommendation: We recommend meters that measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories.

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Virtually all glucose meters use strips that contain enzymes such as glucose oxidase and glucose dehydrogenase. A drop of whole blood is applied to a strip that contains all the reagents necessary for the assay. Some meters have a porous membrane that separates erythrocytes and analysis is performed on the resultant plasma. Meters can be calibrated to report plasma glucose values, even when the sample is whole blood. An IFCC working group recommended that glucose meters report concentrations of glucose in plasma, irrespective of the sample type or technology (114, 115); this approach can improve harmonization and allow comparision with laboratory-generated results (116). The meters use reflectance photometry or electrochemistry to measure the rate of the reaction or the final concentration of the products. The meters provide digital readouts of glucose concentration. Manufacturers claim reportable concentration ranges as large as 33.3 mmol/L (600 mg/dL), e.g., 0-33.3 mmol/L (0-600 mg/dL).

Several important technological advances decrease operator error. These include automatic commencement of timing when both the sample and the strip are in the meter, smaller sample volume requirements, an error signal if sample volume is inadequate, “lock out” if controls are not assayed,

and bar code readers to identify the lot of strips. Moreover, meters store up to several hundred results that can subsequently be downloaded for analysis. Together these improvements have improved performance of new meters (117, 118). Nonetheless, meter performance in the hands of patients does not equal potential performance as judged by performance in the hands of skilled medical technologists (119) .

Numerous analytical goals have been proposed for the performance of glucose meters. The rationale for these is not always clear. In 1987 the ADA recommended a goal of total error (user plus analytical) of < 10% at glucose concentrations of 1.7-22.2 mmol/L (30-400 mg/dL) 100% of the time (120). In addition, it was proposed that values should differ by ≤ 15% from those obtained by a laboratory reference method. The recommendation was modified in response to the significant reduction in complications by tight glucose control in the DCCT. The revised performance goal, published in 1996 (90), is for total analytical error < 5%. To our knowledge, there are no published studies of patients with diabetes achieving the ADA goal of analytic error of <5% with any glucose meters.

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The less stringent CLSI (formerly NCCLS) recommendations are that, for 95% of the samples, the difference between meter and laboratory measurements of glucose be (a) <20% when laboratory glucose is >5.5 mmol/L (100 mg/dL) and (b) <0.83 mmol/L (15 mg/dL) of laboratory glucose when the glucose concentration is ≤5.5 mmol/L (100 mg/dL)(121). The 2003 International Organization for Standardization (ISO) recommendations (122) propose that for test readings >4.2 mmol/L (75 mg/dL), the discrepancy between meters and accredited laboratory should be <20%; for glucose ≤ 4.2 mmol/L (75 mg/dL), the discrepancy should not exceed 0.83 mmol/L (15 mg/dL) in 95% of samples. At the time of writing, both the CLSI and ISO recommendations were undergoing revision.

These criteria serve as *de facto* minimal quality requirements for manufacturers wishing to sell meters. With these criteria, a concentration of 2.5 mmol/L (45 mg/dL) may be read as 1.7 mmol/L (30

mg/dL) or 3.3 mmol/L (60 mg/dL) and be considered acceptable. Such errors do not appear to be acceptable for reliable detection of hypoglycemia. Similarly, errors of 20% can lead to errors in insulin dosing which, when combined with other factors, can lead to hypoglycemia.

Different approaches to establishing quality requirements have been proposed by others. Clarke (123) developed an Error Grid that attempts to define clinically important errors by identifying fairly broad target ranges. In another approach, 201 patients with longstanding type 1 diabetes were questioned to estimate quality expectations for glucose meters (124). Based on patients' perceptions of their needs and of their reported actions in response to changes in measured glucose concentrations, a goal for analytical quality at hypoglycemic concentrations was a CV of 3.1%. Excluding hypoglycemia, the analytical CV to meet the expectations of 75% of the patients was 6.4%-9.7%. The authors recommended an analytical CV of 5%, with a bias ≤5% (124). A third approach used simulation modeling of errors in insulin dose (125). The results revealed that meters that achieve both a CV and a bias <5% rarely lead to major errors in insulin dose. However, to provide the intended insulin dosage 95% of the time, the bias and CV needed to be <1%-2%, depending upon the dosing schedule for insulin and the ranges of glucose concentrations for the individual patient (125). No meters have been shown to achieve CVs of 1-2% in routine use in the hands of patients.

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The lack of consensus on quality goals for glucose meters reflects the absence of agreed objective criteria. Using the same biological variation criteria described above for glucose analysis in accredited laboratories (Section 4, Interpretation), a biological goal would be total error ≤6.9%, with imprecision (as CV of measurements over several days or weeks) ≤ 2.9% and bias ≤2.2% (84). However, additional studies are necessary to define a goal that is related to medical needs.

Current meters exhibit performance superior to prior generations of meters (117, 118). A variety of studies of newer analyzers have documented imprecision (CV) of about 2% in the hands of trained workers. Nonetheless, there is room for improvement. In a study conducted under carefully

controlled conditions in which all assays were performed by a single medical technologist, only about 50% of analyses met the ADA criterion of <5% deviation from reference values (117). Another study that evaluated meter performance in 226 hospitals by split-samples analyzed simultaneously on meters and laboratory glucose analyzers revealed that 45.6%, 25% and 14% differed from each other by > 10%, > 15% and > 20%, respectively (126). In another study, none of the meters met the ADA criterion (127). In an evaluation in which “all testing was performed by trained study staff in an inpatient Clinical Research Center setting”, only 81% of results of meter results were within 10% of results from an accredited laboratory using a hexokinase method (128). We are aware of no studies that document patient-generated results that meet the ADA criteria. Moreover, an analysis of published studies of glucose meters demonstrated that the studies suffered from deficiencies in study design, methodology and reporting (129), raising the possibility that reported total error underestimates the true total error of the meters. A standardized method for evaluation of meters has been developed in Norway (129), and the Norwegian Health Authorities have decided that all SMBG instruments marketed in Norway should be examined by a similar procedure (130). Results of evaluations of 9 brands of meters according to this method showed that 3 of 9 meters did not meet the ISO criteria and none met the ADA criteria in the hands of patients (130).

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Glucose meters are also used to support tight control of glucose in patients in intensive care units settings. A seminal randomized controlled trial of Van den Berghe reported a 34% reduction of mortality in surgical ICU patients managed according to a tight glucose control protocol (86). A meta-analysis seven years later of multiple randomized controlled trials of tight glucose control failed to identify any improved outcomes, but did find an increased incidence of hypoglycemia (131). A Perspective article (132) pointed out that the seminal study of Van den Berghe used a precise and accurate glucose analyzer and collected central blood samples, whereas subsequent studies often used glucose meters and finger-stick samples. The integrity of results of finger-stick samples can be

compromised by factors such as shock, hypoxia and low hematocrit that are common in these settings (133). Moreover, the error of glucose meters may compound the problem and compromise the ability to control blood glucose and avoid hypoglycemia. Simulation modeling studies have demonstrated that errors in glucose measurement lead to marked degradation of glycemic control in tight glucose control protocols (134). In a 2005 study of ICU patients (135), the agreement of meter results with accredited laboratory results was found to be poor: among 767 paired results, the 95% limits of agreement were +2.4 to -1.5 mmol/L (+43.1 to -27.2 mg/dL); the performance did not meet the CLSI/ISO criteria for glucose meters. The authors concluded that "for the individual patient, bedside glucose meter measurement gives an unreliable estimate of plasma glucose" (135). Hoedemakers et al (136), in a study of 197 arterial blood samples from ICU patients, reported that the evaluated meter did not meet the ISO total-error criteria. They also demonstrated that total error was greater when meters were used in ICU patients than in non-ICU patients. A later paper, also studying arterial blood from ICU patients, measured glucose in 239 samples by a portable meter and by a laboratory method and found that the meter results did not meet the CLSI/ISO criteria (137). Similarly, a 2005 study of arterial, venous and capillary samples from a mixed medical-surgical ICU of a tertiary care hospital in Canada had found that meters did not meet proposed CLSI goals, but that a blood gas analyzer did (138).

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Recommendation: Studies are needed to determine the analytical goals (quality specifications) for glucose meters in self monitoring of blood glucose and in intensive care units.

C (moderate)

Recommendations for future research: Important end-points in studies of SMBG should include, at a minimum, HbA_{1c} and frequency of hypoglycemic episodes to ascertain whether improved meters

enable patients to achieve better glucose control. For studies of meter use in intensive or critical care, important end-points include mean blood glucose, frequency of hypoglycemia and variability of glucose control. Ideally, outcomes (e.g., long-term complications) should also be examined.

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Frequency of measurement

SMBG should be performed at least 3 times per day in patients with type 1 diabetes. Monitoring less frequently than 3 times a day results in a deterioration of glycemic control (90, 139, 140). Self-monitoring is performed by patients much less frequently than recommended. Data from NHANES III collected between 1988 and 1994 reveal that SMBG was performed at least once a day by 39% of patients taking insulin and 5-6% of those treated with oral agents or diet alone (95). Moreover, 29% and 65% of patients treated with insulin and oral agents, respectively, monitored their blood glucose less than once per month. However, no evaluation has been performed to verify that 3 times a day is ideal or whether some other frequency would improve glycemic control. For example, adjustment of insulin therapy in women with GDM according to the results of post-prandial, rather than pre-prandial, plasma glucose concentrations improved glycemic control and reduced the risk of neonatal complications (141). The optimal frequency of SMBG for patients with type 2 diabetes is unknown.

Current ADA recommendations suggest that SMBG be performed 3 or more times per day by patients treated with multiple daily injections of insulin (21), and that “SMBG is useful in achieving glycemic goals” in other patients. The last statement is based on expert opinion.

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CONTINUOUS MINIMALLY-INVASIVE GLUCOSE ANALYSES

1. **Use**

Recommendation: Real-time continuous glucose monitoring (CGM) in conjunction with intensive insulin regimens can be a useful tool to lower HbA_{1c} in selected adults (age > 25 years) with type 1 diabetes.

A (high)

Recommendation: Although the evidence for HbA_{1c} lowering is less strong in children, teens, and younger adults, real-time CGM may be helpful in these groups. Success correlates with adherence to ongoing use of the device.

B (moderate)

Recommendation: Real-time CGM may be a supplemental tool to SMBG in those with hypoglycemia unawareness and/or frequent episodes of hypoglycemia.

B (low)

Recommendation: Patients require extensive training in using the device. Available devices must be calibrated with SMBG readings and the latter are recommended for making treatment changes.

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GPP

The development of a device for "continuous" *in vivo* monitoring of glucose concentrations in blood is a very high priority as patients are required to control their plasma glucose more closely (21, 43, 142). The first device approved by the FDA for minimally-invasive interstitial fluid glucose sensing, the transcutaneous "GlucoWatch Biographer", is no longer on the market. Subsequently, several implanted-catheter systems were approved (Table 5). The initial device in the latter category is the "Continuous Glucose Monitoring System" (CGMS) (Medtronic), a system that does not provide real-time data to the patient; but rather is worn for three days and then returned to the provider's office for its data to be downloaded for trend analyses. More recently, a number of real-time devices, with which patients can see both current glucose concentrations and trends, have become commercially available. These include, in the U.S., the Guardian Real-Time (Medtronic Diabetes), the Seven Plus System (DexCom), and the Freestyle Navigator (Abbott). Continuous glucose monitoring devices require calibration and confirmation of accuracy with conventional SMBG, and the FDA advises using the latter for treatment decisions, such as calculating pre-meal insulin doses.

Clinical studies of these devices, generally in highly selected populations, had primarily been limited to assessments of their accuracy, or short-term trials demonstrating reductions in the time patients spend in hypo- and hyperglycemic ranges (143). A systematic review of trials of the non- real-time CGMS device suggests that it does not significantly lower HbA_{1c} compared to SMBG (144). In 2008, a large 26-week randomized trial of 322 type 1 diabetes patients showed that adults age 25 and older using intensive insulin therapy and real-time CGM experienced a 0.5% reduction in HbA_{1c} (from ~ 7.6% to 7.1%) compared to usual intensive insulin therapy with SMBG (145). Sensor use in children, teens and adults to age 24 did not lower HbA_{1c} significantly, and there was no significant difference in hypoglycemia in any group. The greatest predictor of HbA_{1c} reduction in this study for all age groups

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was frequency of sensor use, which was lower in younger age groups. Although CGM is an evolving technology, emerging data suggest that, in appropriately selected patients who are motivated to wear it most of the time, it may offer benefit. CGM may be particularly useful in those patients with hypoglycemia unawareness and/or frequent episodes of hypoglycemia, and studies in this area are ongoing.

2. Rationale

The first goal for developing a reliable *in vivo* continuous glucose sensor is to detect unsuspected hypoglycemia. The importance of this goal has been increasingly appreciated with the recognition that strict glucose control is accompanied by a marked increase in the risk of hypoglycemia (43, 142). Therefore, a sensor designed to detect severe hypoglycemia alone would be of value. In contrast, a full-range, reliable *in vivo* continuous glucose monitor is a prerequisite for the development of a closed-loop pump or "artificial pancreas" that would measure blood glucose concentrations and automatically adjust insulin administration.

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3. Analytical Considerations

Methods to sample biological fluids in a continuous and minimally invasive way vary among test systems (Table 5). The underlying fundamental concept is that the concentration of glucose in the interstitial fluid correlates with blood glucose. The implanted sensors employ multiple detection systems including enzyme- (usually glucose oxidase), electrode- and fluorescence-based techniques. Alternatives to enzymes as glucose recognition molecules are being developed, including artificial glucose "receptors" (146, 147). Fluorescence technologies include use of engineered molecules, which

exhibit altered fluorescence intensity or spectral characteristics upon binding glucose, or use of competitive binding assays that employ two fluorescent molecules in the fluorescent resonance energy transfer (FRET) technique (148-152).

4. **Interpretation**

The subcutaneous sensors are generally worn for a number of days, and require calibration several times per day with SMBG readings. A few small studies have examined their accuracy compared to SMBG and/or to plasma glucose assays. For the Medtronic CGMS Gold device, the mean absolute difference between sensor readings and blood glucose readings was 15.0% +/- 12.2% for 735 paired samples, while the GlucoDay microdialysis device had a mean absolute difference of 13.6 +/- 10.2% for 1156 paired samples (153). For both devices, accuracy was lowest in the hypoglycemic ranges. Approximately 97% of values for both fell within zones A and B of a Clarke error grid analysis, with none falling in zone E (153). A study of 91 insulin-treated patients using the DexCom showed that 95% of 6767 paired glucose values fell within Clark error grid zones A and B, with mean absolute difference of 21.2% (143).

Currently, there are no analytical goals for non- and minimally-invasive glucose analyses. Such standards will clearly need to be different for different proposed uses. For example, the reliability, precision and accuracy requirements for a glucose sensor that is linked to a system that automatically adjusts insulin doses will be much more stringent than those for a sensor designed to trigger an alarm in cases of apparent extreme hyper- or hypoglycemia. It seems intuitively obvious that a larger imprecision can be tolerated in instruments that make frequent readings during each hour than in an instrument used only 2 or 3 times per day to adjust a major portion of a person's daily insulin dose.

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5. **Emerging considerations**

With the approvals of several self-monitoring continuous glucose sensors by the FDA, it is anticipated that there will be renewed efforts to bring other technologies forward into clinical studies. Ultimately, we shall see improved methods for non-invasive or minimally-invasive glucose measurements that will complement current self glucose monitoring techniques.

NONINVASIVE GLUCOSE ANALYSIS

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Recommendation: No noninvasive sensing technology is currently approved for clinical glucose measurements of any kind. Major technological hurdles must be overcome before noninvasive sensing technology will be sufficiently reliable to replace existing portable meters, implantable biosensors, or minimally invasive technologies.

C (very low)

1. **Use**

Noninvasive glucose sensing technologies represent a group of potential analytical methods for measuring blood glucose concentrations without implanting a probe or collecting a sample of any type.

The most commonly explored methods involve passing a selected band of non-ionizing electromagnetic radiation (light) through a vascular region of the body and then determining the *in vivo* glucose concentration from an analysis of the resulting light or spectrum (Table 5). The distinguishing feature of this approach is a lack of physical contact between the sample matrix and a measurement probe. The only functional interaction is light passing through the sample.

A truly noninvasive method will be painless in operation and capable of continuous readings over time. In addition, noninvasive sensing technology may be less expensive to implement compared to existing technologies that demand either a fresh test-strip for each measurement or a new implantable probe that requires multiple daily calibration measurements with fresh test-strips. Furthermore, most noninvasive strategies offer the potential for measuring multiple analytes from a single noninvasive measurement. The development of this technology is driven by the features of low cost, painless and continuous operation with no reagents or waste for disposal.

Reports in the peer-reviewed literature describe noninvasive measurements based on a variety of techniques, such as absorption spectroscopy, photoacoustic spectroscopy, Raman scattering, static light scattering, polarimetry, and optical coherent tomography (154-157). Potential applications include discrete home glucose testing, continuous home glucose monitoring, nocturnal hypoglycemia alarm, physician's office measurements, point-of-care monitoring, screening for diabetes, and control of hyperglycemia in critically ill patients. To date, none of these potential applications has been realized.

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2. **Rationale**

Indirect and direct methods are being developed for noninvasive glucose sensing. Indirect methods rely on the impact of *in vivo* glucose concentrations on a measurable parameter. The classic

example of this approach is the effect of blood glucose concentrations on the scattering properties of skin (158). Changes in blood glucose substantially affect the refractive index difference between skin cells and the surrounding interstitial fluid, thereby altering the scattering coefficient of skin. This parameter can be measured in a number of ways, including ocular coherent tomography. Impedance of skin and the aggregation properties of red blood cells are other indirect approaches.

Direct methods measure a property of the glucose molecule itself. Vibrational spectroscopy is the primary direct method and generally involves either mid-infrared, near infrared, photoacoustic or Raman scattering spectroscopy. The basis of these measurements is the unique spectral signature of glucose relative to the background tissue matrix.

Selectivity is the primary factor that must be addressed for either indirect or direct approaches. The lack of an isolated sample precludes the use of physical separations or chemical reactions to enhance measurement selectivity. All the analytical information must originate from the noninvasive signal. Ultimately, success of any approach demands a full understanding of the fundamental basis of selectivity. To this end, basic research efforts are paramount to establish such a level of understanding.

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3. **Analytical Considerations**

It should no longer be acceptable to publish results that simply demonstrate the ability to follow glucose transients during simple glucose tolerance tests (159). This ability is well established in the literature for numerous approaches, both indirect and direct. In fact, it is rather easy to monitor optical changes that correlate with *in vivo* glucose concentrations during glucose tolerance tests. It is considerably more difficult, however, to demonstrate that such measurements are reliable and selective. Reliability and selectivity must be the focus of the next generation of research. Indeed, the FDA

considers all noninvasive sensing technologies to be high-risk medical devices and premarket approval documentation will be required for commercialization in the U.S. (160).

Many reports of attempts to measure glucose noninvasively lack sufficient information to judge the likelihood that glucose is truly being measured. Interpretation of such clinical data is complicated by the common use of multivariate statistically methods, such as partial least squares regression and artificial neural networks. These multivariate methods are prone to spurious correlations that can generate apparently functional glucose measurements in the complete absence of glucose-specific analytical information (161, 162). Given this known limitation of these multivariate methods, care must be used in their implementation. Tests for spurious correlations (163-165) must be developed and implemented with all future clinical data to avoid reports of false success.

Despite the limitations noted above, real progress is being made to further the development of noninvasive glucose sensing technologies (166, 167). Rigorous testing of noninvasive technologies must be continued in concert with efforts to understand the underlying chemical basis of selectivity. Issues of calibration stability must also be investigated. Overall progress demands advances in both instrumentation and methods of data analysis. For each, meaningful benchmarks must be established to allow rigorous inter- and intra-laboratory comparisons.

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ORAL GLUCOSE TOLERANCE TEST (OGTT)

Recommendation: The oral glucose tolerance test is not recommended for the routine diagnosis of type 1 or type 2 diabetes mellitus. It is recommended for establishing the diagnosis of gestational diabetes mellitus and for evaluation postpartum after gestational diabetes.

A (moderate)

1. **Use**

The OGTT, once the gold standard for diagnosing diabetes mellitus, is now not recommended by the ADA for routinely diagnosing either type 1 or type 2 diabetes, but continues to be recommended in a limited fashion by the WHO) (19, 168, 169). Did you mean throughout the document or just here? The oral glucose challenge (or glucose tolerance test) continues to be recommended by both the ADA and the WHO for establishing the diagnosis of GDM (see below). Neither group recommends use of the extended 3-5 h glucose tolerance test in routine practice.

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2. **Rationale**

Inability to respond appropriately to a glucose challenge, i.e., glucose intolerance, represents the fundamental pathological defect in diabetes mellitus. The rationale for the ADA not recommending that the glucose tolerance test be used routinely to diagnose type 1 and 2 diabetes is that appropriate use of FPG could identify approximately the same prevalence of abnormal glucose metabolism in the population as the OGTT. Furthermore, the OGTT is impractical in ordinary practice. The consensus was that a 2 h plasma glucose cutoff of $\geq$11.1 mmol/L (200 mg/dL) should be used as it was predictive

of the occurrence of microangiopathy. However, approximately only one-fourth of the individuals with 2 h plasma glucose ≥11.1 mmol/L (200 mg/dL) have a FPG ≥7.8 mmol/L (140 mg/dL), which was the FPG previously recommended to diagnose diabetes mellitus. The currently recommended FPG value of 7.0 mmol/L (126 mg/dL) corresponds better to a 2-h value in the OGTT of ≥11.1 mmol/L (200 mg/dL), and thus with development of complications.

Use of the OGTT to classify individuals with impaired glucose tolerance (IGT) and diabetes remains controversial. Several studies (170-173) indicate that individuals classified with IGT by the OGTT (WHO criteria) have increased risk of cardiovascular disease, but many of these individuals do not have impaired fasting glucose (IFG) by the ADA criteria. Furthermore, the OGTT (WHO criteria) identifies diabetes in approximately 2% more individuals than the FPG (ADA criteria) (174) [see Emerging Considerations below]. Finally, diabetic patients with both abnormal FPG and 2 h OGTT have a higher risk of premature death than those with only an increased FPG concentration (175). Use of the OGTT remains controversial with some studies suggesting that its value is limited (176, 177), while in 2006 the WHO re-iterated its support of the OGTT (19).

3. Analytical considerations

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The reproducibility of the OGTT has received considerable attention. In numerous studies, the reproducibility of the OGTT in classifying patients ranges from 50-66% (178, 179). Possible factors contributing to the lack of reproducibility include biologic variation of plasma glucose concentrations, the variable effects of administration of a hyperosmolar glucose solution on gastric emptying and the extensive patient preparation that is required (178, 180, 181).

4. Interpretation

a) Diagnosis of type 1 and 2 diabetes. The ADA and WHO have different recommendations

1) ADA: Not recommended for routine clinical use except in pregnant women (1).

2) WHO: When the FPG concentration is in the IFG range [6.1 mmol/L (110 mg/dL)-7.0 mmol/L (126 mg/dL)] an OGTT is recommended (169). After three days of unrestricted diet and an overnight fast (8-14 h), FPG is measured, followed by the oral ingestion of 75 g anhydrous glucose (or partial hydrolysates of starch of the equivalent carbohydrate content) in 250-300 ml of water over 5 min. For children, the dose is 1.75 g glucose/kg up to 75 g glucose. Blood samples are collected 2 h after the load, and plasma glucose analyzed. Results are interpreted as detailed in Table 6.

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5. **Emerging considerations**

The main issues of controversy are: 1) the lower sensitivity of FPG compared to the OGTT in diagnosing diabetes mellitus, 2) the value of classifying individuals as having IGT (recommended by WHO, but not the ADA) and 3) the appropriate use in GDM (see GDM section).

The lower sensitivity of FPG compared to the OGTT in diagnosing diabetes mellitus is closely linked to epidemiological evidence that the OGTT better identifies patients at risk for developing complications of diabetes. This includes assessment of the risk of developing cardiovascular disease (182) and of predicting increased risk of death (183). The continuing use of the OGTT to diagnose diabetes mellitus has been supported by the WHO (19).

GESTATIONAL DIABETES MELLITUS (GDM)

1. **Use**

Recommendation: All women of average or high risk of gestational diabetes mellitus (GDM) should undergo GDM testing at 24-28 weeks of gestation.

A (high)

The OGTT is used universally to identify states of increased glucose concentrations in pregnancy, commonly called gestational diabetes mellitus. The oral glucose challenge (or glucose tolerance test) continues to be recommended by both the ADA and the WHO for establishing the diagnosis of GDM. Neither group recommends use of the extended 3-5 h glucose tolerance test in routine practice. Controversy about the diagnostic criteria for GDM has continued because the strategies for detection and diagnosis are in the case of ADA criteria, based on a paradigm for identifying mothers at risk for development of diabetes mellitus in the future, or in the case of criteria recommended by WHO and others, simply represent the same values that are used to classify concentrations of plasma glucose in non-pregnant individuals (Table 6). However, two randomized clinical trials have now demonstrated benefit from the treatment of "mild" GDM. Both studies found that treatment of GDM can reduce both serious adverse outcomes and the frequency of large babies (macrosomia) (184, 185).

2. **Rationale**

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GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy (1). Following recent discussions, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended that high-risk women who have diabetes at their initial prenatal visit using standard criteria (Table 4) receive a diagnosis of overt, not gestational, diabetes (21). The IADPSG recommendations are not identical to the criteria for non-pregnant individuals in that an OGTT result with FPG <7.0 and 2-hr value >11.1 is not called “overt diabetes”.

The ADA states that because of the risks of GDM to the mother and the neonate, screening and diagnosis are warranted (21). The 4th International Workshop Conference on GDM (186) and subsequently the ADA (187) recommended that at the time patients enroll for prenatal care, screening for GDM be initiated by carrying out a “risk assessment” as follows:

a. Low risk patients require no testing.

Low risk status is limited to women meeting ALL of the following:

- Age < 25 years
- Weight normal before pregnancy
- Weight normal at birth (added by 5th Workshop Conference (188)
- Member of an ethnic group with a low prevalence of GDM
- No known diabetes in first-degree relatives
- No history of abnormal glucose tolerance
- No history of poor obstetric outcome

b. Average risk patients (all patients who fall between low and high risk). They should be tested at 24-28 weeks of gestation (see below for testing strategy).

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c. High risk patients. They are defined as having any of the following and should undergo immediate testing:

- Marked obesity
- Personal history of GDM
- Glycosuria
- Strong family history of diabetes

Reports based on the retrospective application of this strategy in the U.S. (189, 190)and Spain (191) indicate that few (<10%) pregnant women qualify as “low risk” in these populations.

3. **Analytical Considerations**

These have been addressed earlier in the glucose and OGTT sections.

4. **Interpretation**

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Recommendation: GDM should be diagnosed by a 75 g OGTT using the IADPSG criteria derived from the HAPO study.

A (moderate)

The ADA recommends that average and high risk patients receive a glucose challenge test following one of two methods:

a. One-step: Perform either a 100 g or 75 g OGTT. This one-step approach may be cost-effective in high-risk patients or populations (e.g., some Native-American groups).

- The 100 g OGTT is the most commonly used, standard test supported by outcome data. Two or more of the venous plasma glucose concentrations indicated in Table 7 must be met or exceeded for a positive diagnosis.

- Alternatively, a 75 g OGTT can be performed, but it is not as well validated as the 100 g test. In the 75 g test, diagnostic criteria for plasma glucose values are the same as for the 100 g test, except that there is no 3 h measurement. Two or more of the venous plasma glucose values must equal or exceed the cut-offs to diagnose GDM.

b. Two-step: The first step is a 50 g oral glucose load (the patient does not need to be fasting), followed by a plasma glucose determination at 1-hr. A plasma glucose value ≥7.8 mmol/L (140 mg/dL) indicates the need for definitive testing. A value of > 7.2 mmol/L (130 mg/dL) has been used as a cutoff value by some as it will detect approximately 10% more diabetic patients, but many more individuals will require definitive testing. The second and definitive test is one of the two OGTTs described above.

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5. **Emerging Considerations**

a. Developments between 2002 and 2010

The 5th International Workshop Conference on GDM was held in November 2005 and the Proceedings published in July 2007 (188). Strategies that had been recommended for screening and diagnosis by the 4th Conference were endorsed with minimal modifications. Considerable emphasis

was placed on postpartum evaluation and classification. The literature on postpartum testing with OGTT and/or FPG was reviewed and additional data were presented confirming the recommendations of the 4th GDM Conference (186) that performing FPG measurements alone to classify glucose tolerance status postpartum fails to identify a substantial proportion of women who have IGT or diabetes when tested by the OGTT. With the support of additional new data, the 5th Workshop conferees (188) strongly endorsed the use of a 75 g OGTT for early (2-3 months) and one postpartum evaluation after GDM in order to provide more informed counseling regarding future pregnancies, potential risks with use of hormonal contraception and future risk of progression to diabetes. The ADA now endorses the use of an OGTT for postpartum evaluation (21).

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b. Future expectations

The screening and diagnostic criteria for GDM will almost certainly be modified extensively in the near future. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study was a large (~25,000 pregnant women) prospective multinational epidemiologic study to assess adverse outcomes as a function of maternal glycemia (192). The study revealed strong, graded, predominantly linear associations between maternal glycemia and primary study outcomes, namely frequency of birthweight >90 percentile, delivery by Cesarean section, clinical neonatal hypoglycemia and cord serum insulin [C-peptide] concentrations >90th percentile of values in HAPO study population. Associations remain strong with adjustments made for multiple, potentially confounding factors. Strong associations were also found with infant adiposity (193) and some secondary outcomes including risks of shoulder dystocia and/or birth injury and with preeclampsia (192). On the strength of these results, an expert Consensus Panel appointed by IADPSG has recently recommended "outcome based" criteria for the classification of glucose concentrations in pregnancy (194). These recommendations (Table 8) will be

adopted by the ADA in 2011 and are currently under consideration by the American College of Obstetrics and Gynecology in the US and by corresponding groups in other countries. If adopted, the incidence of GDM will be substantially higher than in the past (only one increased value is required to diagnose GDM) and the treatment will require additional resources. However, the two trials that focused on treatment of "mild GDM" achieved improvement in outcomes with only 10-20% of the patients requiring pharmacological treatment in addition to medical nutritional therapy (184, 185).

URINARY GLUCOSE

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

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1. **Use**

Semiquantitative urine glucose testing, 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 (142, 195). 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 (23).

2. **Rationale**

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 concentration of the urine affects urine glucose concentrations and only average glucose values between voidings are reflected, further minimizing the value of urine glucose determinations.

3. **Analytical Considerations**

Semiquantitative test-strip methods utilizing specific reactions for glucose are recommended. Commercially available strips utilize the glucose oxidase reaction (196). 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 (142).

4. **Interpretation**

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

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KETONE TESTING

1. Use

Recommendation: Ketones should be measured in urine or blood by patients with diabetes in the home setting and in the clinic/hospital setting as an adjunct to the diagnosis of diabetic ketoacidosis.

GPP

The ketone bodies, acetoacetate (AcAc), acetone, and β-hydroxybutyric acid (βHBA), 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 routinely performed both in an office/hospital setting and by patients at home. The ADA recommends that ketosis-prone patients with diabetes mellitus check urine or blood ketones in situations characterized by deterioration in glycemic control, in order to detect and pre-empt diabetic ketoacidosis (21, 197).

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2. Rationale

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 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 (198).

The principal ketone bodies βHBA and AcAc are normally present in approximately equimolar amounts. Acetone, usually present in only small quantities, is derived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βHBA is shifted towards formation of βHBA 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 (199-201). Thus, assay methods for ketones that do not include measurement of βHBA may provide misleading clinical information by underestimating total ketone body concentration (202).

3. **Analytical Considerations**

A. Urine ketones

1. Pre-analytical: 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 sulfhydyl containing drugs, including angiotensin-converting enzyme inhibitors (203). 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 (204). 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

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positive readings) are not commercially available but would be desirable to assure accuracy of test results.

2. Analytical: Several assay principles have been described. Most commonly used is the colorimetric reaction that occurs between ketones and nitroprusside (sodium nitroferricyanide), resulting in a purple color (196). 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 βHBA (196).

B. Blood ketones

1. 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, βHBA, the predominant ketone body in DKA, is not detected.

For specific determinations of βHBA, 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

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

2. 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 βHBA, enzymatic methods for quantification of βHBA appear to be the most widely used for routine clinical management (205-207). The principle of the enzymatic methods is that βHBA in the presence of NAD is converted to AcAc and NADH by β-hydroxybutyrate dehydrogenase. Under alkaline conditions (pH 8.5-9.5), the reaction favors formation of AcAc from βHBA. 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 µL 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 for both laboratory use (e.g., BioScanner Ketone, PolymerTechnology Systems, Indianapolis, IN) or for home use by patients (MediSense Precision Xtra, Abbott Laboratories, Abbott Park, IL and Stanbio Laboratory, Boeme, TX). 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.

4. **Interpretation**

A. Urine ketone determinations

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Recommendation: Urine ketone determinations should not be used to diagnose or monitor the course of DKA.

GPP

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. Although DKA is most commonly associated with type 1 diabetes mellitus, it may rarely occur in type 2 patients (208). 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 (202).

B. Blood ketone determinations

Recommendation: Blood ketone determinations that rely on the nitroprusside reaction should be used only as an adjunct to diagnose DKA and should not be used to monitor treatment of DKA. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA.

B (moderate)

Blood ketone determinations that rely on the nitroprusside reaction should be used with caution for diagnosis of DKA as results do not quantify βHBA, the predominant ketone in DKA. The test should not be used to monitor the course of therapy since AcAc and acetone may increase as βHBA

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falls during successful therapy (142, 198-202). Blood ketone determinations that measure βHBA specifically are useful for both diagnosis and ongoing monitoring of DKA (209-211). Reference intervals for βHBA differ among assay methods, but concentrations in healthy individuals fasted overnight are generally <0.5 mmol/L. Patients with well-documented diabetic ketoacidosis [serum CO_2 <17 mmol/L, arterial pH <7.3, plasma glucose >14.9 mmol/L (250 mg/dL)] generally have βHBA concentrations >2 mmol/L.

5. **Emerging considerations**

Further studies are needed to determine

- whether blood ketone determinations by patients with diabetes mellitus are preferable (e.g., better accepted by patients than urine testing, more prompt diagnosis of DKA) to urine ketone determinations;
- if the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH).

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HEMOGLOBIN A_{1c}

1. **Use**

Recommendation: Hemoglobin A_{1c} (HbA_{1c}) should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control. A (moderate)

Measurement of glycated proteins, primarily HbA_{1c}, is widely used for routine monitoring of long-term glycemic status in patients with diabetes mellitus.[1] HbA_{1c} is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications (142, 212). HbA_{1c} testing and maintenance of specified concentrations during pregnancy 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 HbA_{1c} values during pregnancy decreases congenital malformations, large-for-date infants, and the complications of pregnancy and delivery that can otherwise occur when glycemic control is not carefully managed (213). A recent consensus statement (213) recommends an HbA_{1c} <6% in these patients, if it can be achieved without excessive hypoglycemia. HbA_{1c} is also being used increasingly by quality assurance programs to assess the quality of diabetes care (e.g., requiring that health-care providers document the frequency of HbA_{1c} testing in patients with diabetes and the proportion of patients with HbA_{1c} values below a specified value) (214, 215).

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[1] The terms glycated hemoglobin, glycohemoglobin, "glycosylated" (which should not be used) hemoglobin, HbA_1 and HbA_{1c} have all been used to refer to hemoglobin that has been modified by the nonenzymatic addition of glucose residues. However, these terms are not interchangeable. Glycated hemoglobins comprise HbA1 and other hemoglobin-glucose adducts, while HbA1 is made up of HbA_{1a}, HbA_{1b} and HbA_{1c}. HbA_{1c} is the major component of HbA1, accounting for ~80% of HbA1. In order to eliminate this confusing nomenclature, the term "A1c test" has been suggested to facilitate communication. As described in the text, most of the clinical outcome data that are available for the effects of metabolic control on complications (at least for the DCCT and UKPDS) used assay methods that quantified HbA_{1c}. In this paper, we use the abbreviation GHb to include all forms of glycated hemoglobin.

The ADA and other organizations that have addressed this issue recommend measurement of HbA_{1c} in patients with both type 1 and type 2 diabetes, to document the degree of glycemic control and assess response to therapy (21, 216). The ADA has recommended specific treatment goals for HbA_{1c} based on the results of prospective randomized clinical trials, most notably the DCCT in type 1 diabetes (43, 212) and the UKPDS in type 2 diabetes (45). These trials have documented the relationship between glycemic control, as quantified by longitudinal determinations of HbA_{1c}, and risks for the development and progression of chronic complications of diabetes. Since different GHb assays can give different GHb values, the ADA recommends that laboratories use only assay methods that are certified as traceable to the DCCT GHb reference (21, 202); these results are reported as HbA_{1c}. The ADA recommends that in general a HbA_{1c} target less than 7% is desirable for nonpregnant adults, with higher values recommended for children and adolescents (21). HbA_{1c} goals should be individualized based on the potential for benefit with regard to long-term complications balanced against the increased risk for hypoglycemia that attends intensive therapy. For specific patients, HbA_{1c} concentrations as close to the non-diabetic range (<6.1%) as safely possible can be pursued. In older patients with limited lifespans. the risk of hypoglycemia may outweigh any possible benefits and higher HbA_{1c} goals should be chosen. Other clinical organizations recommend similar HbA_{1c} targets, ranging from 6.5% to 7% (52).

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2. **Rationale**

Glycated proteins are formed post-translationally from the slow, non-enzymatic reaction between glucose and free amino groups on proteins (217). For hemoglobin, the rate of synthesis of GHb is principally a function of the concentration of glucose to which the erythrocytes are exposed integrated over the time of exposure. GHb is a clinically useful index of mean glycemia during the

preceding 120 days, the average lifespan of erythrocytes (142, 217-220). Several studies have demonstrated a close mathematical relationship between the HbA_{1c} concentration and mean glycemia that should allow expression of HbA_{1c} as an estimated average glucose concentration (219, 221-223). Concentrations of other blood-based glycated proteins (e.g., glycated serum/plasma proteins, "fructosamine") also reflect mean glycemia, but over a much shorter time (15-30 days) than GHb (60-120 days) (142, 217-220, 224, 225). However, clinical utility of glycated proteins other than hemoglobin has not been clearly established and there is no convincing evidence that relates their concentration to the chronic complications of diabetes (142, 202).

3. **Analytical Considerations**

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

GPP

Recommendation: Laboratories that measure HbA_{1c} should participate in a proficiency-testing program, such as the CAP Glycohemoglobin Survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network.

GPP

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There are ~100 different GHb assay methods in current use. These range from low throughput research laboratory component systems and manual minicolumn methods to high throughput automated systems dedicated to HbA_{1c} determinations. Most methods can be classified into one of two groups based on assay principle (142, 196, 218). The first group includes methods that quantify GHb based on charge differences between glycated and nonglycated components. Examples include cation-exchange chromatography and agar gel electrophoresis. The second group includes methods that separate components based on structural differences between glycated and nonglycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify HbA_{1c}, defined as hemoglobin A with glucose attached to the NH_2-terminus valine of one or both beta chains. Other methods quantify "total glycated hemoglobin," which includes both hemoglobin A_{1c} and other hemoglobin-glucose adducts (e.g., glucose-lysine adducts and glucose-alpha chain NH_2-terminus valine adducts). Generally, results of methods using different assay principles show excellent correlation, and there are no convincing data to show that any one method or analyte is clinically superior to any other. However, the reported GHb results from the same blood sample could differ considerably among methods unless they are standardized to a common reference (e.g., without standardization, the same blood sample could be read as 7% in one laboratory and 9% in another) (52, 142, 218, 226-229).

In 1996, the National Glycohemoglobin Standardization Program (NGSP) was initiated to standardize GHb test results among laboratories to DCCT-equivalent values (229). The rationale for standardizing GHb test results to DCCT values was that the DCCT had determined the relationship between the results of a specific GHb test (HbA_{1c}) and long-term complications in patients with type 1 diabetes (43, 142, 202). The NGSP was developed under the auspices of the AACC and is endorsed by the ADA, which recommends that laboratories use only GHb methods that have passed certification testing by the NGSP (21, 142). In addition, the ADA recommends that all laboratories performing GHb

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testing participate in the CAP proficiency testing survey for HbA_{1c} which uses fresh whole-blood specimens (230).

The NGSP laboratory network includes a variety of certified assay methods, each calibrated to the DCCT reference. The DCCT reference is a high-performance liquid chromatographic cation-exchange method that quantifies HbA_{1c} and is a CLSI designated comparison method (231). The assay method has been used since 1978 and has demonstrated good long-term precision (between-run CVs consistently <3%) (230). 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. Certification is valid for one year. An important adjunct to the program is the GHb proficiency testing survey administered by CAP. Since 1996 (starting with a pilot project including 500 laboratories and expanded to all laboratories in 1998), the survey has utilized fresh whole blood samples with NGSP-assigned target values. Since initiation of the NGSP in 1996, the survey has documented a steady improvement in comparability of GHb values among laboratories, both within-method and between-method (232). In 2007, CAP initiated "accuracy-based" grading with the value of each sample assigned by the NGSP Network. The objective is to reduce bias and imprecision among assays. The NGSP website provides detailed information on the certification process and maintains a listing of certified assay methods (updated monthly) and factors that are know to interfere with specific methods (NGSP website: http://www.ngsp.org).

In 1997 the IFCC formed a committee to develop a reference method for HbA_{1c} analysis based on peptide mapping and the method was accepted in 2001 (233, 234). Analysis is performed by cleaving hemoglobin with endoproteinase Glu-C and separating the resulting glycated and non-glycated N-terminal hexapeptides by HPLC. Quantification of the hexapeptides is performed with electrospray ionization mass spectrometry or capillary electrophoresis. HbA_{1c} is measured as the ratio of glycated to non-glycated N-terminal peptide and is reported as mmol deoxy fructosyl hemoglobin

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per mol hemoglobin. Of note, the preparation and measurement of samples using this method is laborious, very expensive and time-consuming and was never envisoned as a practical means of assaying clinical samples. It will only be used by manufacturers to standardize the assays. Like the NGSP, the IFCC has a network of laboratories (235). Comparison of pooled blood samples between the IFCC and the NGSP (DCCT-aligned) networks has revealed a linear relationship:

(NGSP HbA_{1c} = 0.915 (IFCC-HbA_{1c}) + 2.15%, $r^2 = 0.998$) (234).

Although the clinical values using assays standardized with the new IFCC method correlate tightly with NGSP values, the absolute values of HbA_{1c} reported differ by 1.5-2.0% HbA_{1c}. Concern regarding the clinical impact of reporting HbA_{1c} results in new units (mmol/mol) and the differences in HbA_{1c} reported led to an agreement among IFCC and the major diabetes organizations to report HbA_{1c} results as the IFCC result, as the equivalent NGSP DCCT-aligned result (percent based on the master regression equation shown above), and as a calculated estimate of average glucose (eAG), based on the A_{1c}-Derived Average Glucose (ADAG) study (223, 236). Notwithstanding the agreement, it appears unlikely that universal reporting of HbA_{1c} will be adopted.

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A. Preanalytical

a. Patient variables

Recommendation: Laboratories should be aware of potential interferences, including hemoglobinopathies, that may affect HbA_{1c} test results depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population. In addition, disorders that affect erythrocyte turnover may cause spurious results regardless of the method used.

GPP

HbA_{1c} results are not significantly affected by acute fluctuations in blood glucose concentrations, such as those that occur with illness or after meals. The effects of age on HbA_{1c} are controversial (237-239). Some studies show age-related increases in HbA_{1c}, approximately 0.1% per decade after age 30 years. Other reports show little or no increase. Differences in results among the studies may be due to differences in the selection of study subjects; when studies are restricted to participants with normal glucose tolerance (i.e., normal fasting and postprandial plasma glucose concentrations), little or no age-related increase in HbA_{1c} has been found. However, a recent study with careful phenotyping of subjects with OGTT supports an increase in HbA_{1c} with age, even after removing patients with otherwise undiagnosed diabetes, and persons with impaired glucose tolerance from the study population (240, 241). The clinical implications of the small, but statistically significant, progressive increase of "normal" HbA_{1c} levels with aging remains to be determined (242). The effects of race on HbA_{1c} values are similarly controversial. Several studies have suggested a relatively higher HbA_{1c} in African-American and Hispanic populations than in Caucasian populations at the same level of glycemia (243). However, the measurement of chronic glucose levels in these studies has not been frequent enough to capture adequately the actual mean glycemia. The ADAG study, which included frequent measures of glucose, did not show a significantly different relationship between calculated mean glucose during three months and HbA_{1c} 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 (223).

Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers HbA_{1c} test results regardless of the

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assay method (142). Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin (244, 245), but vitamin C may increase values with some assays (244). Iron-deficiency anemia is reported to increase test results (246). There is no significant effect of food intake on test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction are reported to interfere with some assay methods, falsely increasing results (218, 247)

Several hemoglobinopathies (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) (248-250); for a review, see (247). Depending on the particular hemoglobinopathy and assay method, results can be either falsely increased or decreased. Some methods may give a value in the reference range for a nondiabetic patient with a hemoglobin variant, but this is not an assurance that no interference is present; the interference may be subtle in the reference range, but may increase steadily with increasing HbA_{1c}. Boronate affinity chromatographic assay methods and immunoassays are generally considered to be less affected by hemoglobinopathies than methods that separate glycated and nonglycated components based on charge differences. In some instances, such as with most 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 altered erythrocyte turnover interferes with the relationship between mean blood glucose values and HbA_{1c}, or if a suitable assay method is not available for interfering hemoglobinopathies, alternative non-hemoglobin-based methods for assessing long-term glycemic control may be useful (247).

Since interferences are method specific, product instructions from the manufacturer should be reviewed before use of the HbA_{1c} assay method. A list of interfering factors for specific assays is maintained on the NGSP website (www.ngsp.org). In selecting an assay method, the laboratory should

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take into consideration characteristics of the patient population served, e.g., high prevalence of hemoglobinopathies.

b. Sample collection, handling, and storage

Blood can be obtained by venipuncture or by fingerprick capillary sampling (251, 252). Blood tubes should contain anticoagulant as specified by the manufacturer of the HbA_{1c} assay method (EDTA can be used unless otherwise specified by the manufacturer). Sample stability is assay method specific (253, 254). In general, whole blood samples are stable for up to 1 week at 4 °C (254)**.** 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.

A number of convenient blood collection systems have been introduced, including filter paper and small vials containing stabilizing/lysing reagent (255-257) These systems are designed for field collection of specimens with routine mailing to the laboratory and are generally matched to specific assay methods. They 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.

B. Analytical

a. Performance goals and quality control

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Recommendation: Desirable specifications for HbA_{1c} measurement are intra-laboratory CV <2% and inter-laboratory CV <3.5%. At least two control materials with different mean values should be analyzed as an independent measure of assay performance.

B (low)

Several expert groups have presented recommendations for assay performance. Early reports recommended that interassay CV be < 5% at normal and diabetic GHb concentrations (258). More recent reports suggest lower CVs (e.g., intralaboratory <3% (259) or <2% (260) and interlaboratory <5% (259)). Intraindividual CVs in healthy persons are very small (<2%) and many current assay methods can achieve intralaboratory CVs <2% and interlaboratory CVs <3% (261). We recommend intra-laboratory CV <2% and interlaboratory CV <3.5%. For a single method, the goal should be interlaboratory CV <3%.

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

Reference intervals: The laboratory should determine its own reference interval according to CLSI guidelines (CLSI Document C28A) even if the manufacturer has provided one. Non-diabetic test subjects should be nonobese and have FPG <5.6 mmol/L (100 mg/dL) and, ideally, a 2 hour post-oral glucose tolerance test PG <11.1 mmol/L. For NGSP-certified assay methods, the SD for the reference

interval is generally 0.5% GHb or less, resulting in a 95% CI of 2 % HbA_{1c} or lower (e.g., mean hemoglobin A_{1c} +/- 2 SD = 5.0 +/- 1.0%). For assay methods that are NGSP-certified, reference intervals should not deviate significantly (e.g. > 0.5%) from the 4-6 % range. Note that ADA treatment target values derived from the DCCT and UKPDS (22), not the reference intervals, are used to evaluate metabolic control in patients.

b. Out-of-range specimens

Recommendation: Laboratories should verify by repeat testing specimens with HbA_{1c} results below the lower limit of the reference interval or greater than 15% HbA_{1c}.

B (low)

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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. In addition, sample results greater than 15% HbA_{1c} should be repeated and, if confirmed, the possibility of a hemoglobin variant should be considered (247). Any result that does not correlate with the clinical impression should also be investigated.

c. Removal of labile GHb

Formation of GHb includes an intermediate Schiff base which is called “pre-A_{1c}” or labile A_{1c} (262). This material is formed rapidly with hyperglycemia and interferes with some HbA_{1c} assay

methods, primarily those that are charge-based. Most currently available automated assays remove the labile pre- HbA_{1c} 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 HbA_{1c} testing. Proper interpretation of test results requires an understanding of the assay method, including its known interferences. For example, if the assay method is affected by hemoglobinopathies (independent of any shortened erythrocyte survival) or uremia, 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 HbA_{1c} test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS (43, 142, 202). This information is available on the NGSP website. For example, each 1% change in HbA_{1c} is related to a change in mean plasma glucose of approximately 1.6 mmol/L (29 mg/dL). Reporting HbA_{1c} results with a calculated estimated average glucose (eAG) will eliminate the need for health care providers or patients to perform these calculations themselves. The equation generated by the ADAG study is the most reliable one to date (223)**.**

There is some evidence to suggest that immediate feedback to patients at the time of the clinic visit with HbA_{1c} test results improves their long-term glycemic control (263, 264). However, not all publications support this observation (265) and additional studies are needed to confirm these findings before this strategy can be generally recommended. It is possible to achieve the goal of having HbA_{1c}

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test results available at the time of the clinic visit by either having the patient send in a blood sample shortly before the scheduled clinic visit or by having a rapid assay system convenient to the clinic.

B. Clinical application

Recommendation: Treatment goals should be based on ADA recommendations which include generally maintaining HbA_{1c} concentrations <7% and in individual patients as close to the non-diabetic range as safely possible. Somewhat higher ranges are recommended for children and adolescents and should be considered in patients with projected limited lifespans, owing to advanced age or co-morbid illnesses. (Note that these values are applicable only if the assay method is certified by the NGSP as traceable to the DCCT reference.)

A (high)

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Treatment goals: HbA_{1c} measurements are now a routine component of the clinical management of patients with diabetes mellitus. Based principally on the results of the DCCT, the ADA has recommended that a primary goal of therapy is a HbA_{1c} value < 7% (21). Targets less than 7% may be considered for individual patients, for example in diet-treated type 2 diabetes. Similar targets are recommended by other major clinical organizations (52). However, recent studies using multiple medications to treat type 2 diabetes and aiming for HbA_{1c} concentrations <6.5% have not demonstrated consistent benefits, and no benefit with regard to macrovascular disease compared with interventions that achieved HbA_{1c} values 0.8 to 1.1% higher (49-51). The ACCORD study demonstrated increased mortality with very intensive diabetes therapy (HbA_{1c} 6.4% vs 7.5%). These HbA_{1c} values apply only to assay methods that are certified as traceable to the DCCT reference, with reference interval

approximately 4-6% HbA_{1c}. In the DCCT, each 10% reduction in HbA_{1c} (e.g., 12 vs. 10.8% or 8 vs. 7.2%) was associated with approximately 45% lower risk for the progression of diabetic retinopathy (41). Comparable risk reductions were found in the UKPDS (212). It should also be noted that in the DCCT and UKPDS decreased HbA_{1c} was associated with increased risk for severe hypoglycemia.

Recommendation: HbA_{1c} testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or are not meeting treatment goals.

GPP

Testing frequency: There is no consensus on the optimal frequency of HbA_{1c} testing. The ADA recommends (21): “For any individual patient, the frequency of HbA_{1c} testing should be dependent on the clinical situation, the treatment regimen used and the judgment of the clinician.” In the absence of well-controlled studies that suggest a definite testing protocol, expert opinion recommends HbA_{1c} testing “at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients whose therapy has changed or who are not meeting glycemic goal” (21). These testing recommendations are for non-pregnant patients with either type 1 or type 2 diabetes. Diabetes quality assurance programs (e.g., ADA Provider Recognition Program and HEDIS 2000 (214, 215)) have generally required documentation of the percentage of patients with diabetes who have had at least one HbA_{1c} determination during the preceding year. Studies have established that serial (quarterly for one year) measurements of HbA_{1c} result in large improvements in HbA_{1c} values in patients with type 1 diabetes (266).

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Interpretation: HbA_{1c} values in patients with diabetes are a continuum; they range from within the 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 increases in some patients, reflecting an extreme degree of hyperglycemia. Proper interpretation of HbA_{1c} test results requires that physicians understand the relationship between HbA_{1c} values and mean plasma glucose, the kinetics of HbA_{1c}, and specific assay limitations/interferences (142). Small changes in HbA_{1c} (e.g., +/- 0.3% HbA_{1c}) over time may reflect assay variability rather than a true change in glycemic status (232).

5. **Emerging Considerations**

A. Use of HbA_{1c} for diabetes screening/diagnosis

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Recommendation: The HbA_{1c} assay may be used for the diagnosis of diabetes, with values $\geq$ 6.5% being diagnostic. Similar to its use in the management of diabetes, factors that interfere with or adversely affect the HbA_{1c} assay will preclude its use in diagnosis. When a HbA_{1c} assay is not available, or cannot be interpreted in a patient, glucose-based testing should be used for diagnosis.

A (moderate)

The role of HbA_{1c} in the diagnosis of diabetes has been considered for several years (19, 24, 36, 186). In the past, the lack of standardization has been a major barrier. With improved standardization through the NGSP, and new data demonstrating the association between HbA_{1c} concentrations and risk for retinopathy, an International Expert Committee recommended the use of HbA_{1c} in the diagnosis of

diabetes (20). In making its recommendation, the Committee also considered several technical advantages of HbA_{1c} testing compared with glucose testing, such as its pre-analytic stability and decreased biological variability. Finally, the clinical convenience of the HbA_{1c} assay, which requires no patient preparation or timing, compared with glucose-based diagnosis convinced the Committee to recommend HbA_{1c} testing for diagnosis. A value of 6.5% or greater was considered diagnostic based on the observed relationship with retinopathy in more than 28,000 persons. When a HbA_{1c} assay is not available, or cannot be interpreted in a patient owing, for example, to a chronic hemolytic anemia, glucose-based testing should be used for diagnosis. For diagnosis, the International Expert Committee recommended that a positive test ($\geq$ 6.5%) be confirmed with a repeat assay. The frequency of testing for diagnosis has not been established, but guidelines similar to those for glucose-based testing seem appropriate. The ADA has endorsed the use of HbA_{1c} for the diagnosis of diabetes (Table 4) (21), as has the Endocrine Society (267). The ADA and other national and international organizations are considering the implications and other practical considerations of the recommendations of the International Expert Committee, especially as they affect screening and diagnosis programs.

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

Further studies are needed to determine if other glycated proteins such as fructosamine are clinically useful for routine monitoring of patients' glycemic status. 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 (268). 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

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than the mean HbA_{1c} values (269). The clinical role of such measurements remains undefined. Similarly, the role of noninvasive methods using light to measure glycation transdermally (Glucoscout and others) is undefined.

C. Global harmonization of HbA_{1c} testing and uniform reporting of results.

As noted above, the NGSP has largely succeeded in standardizing the GHb assay across methods and laboratories. Furthermore, the IFCC standardization, providing a more stable and chemically discrete standard, is being implemented worldwide. Implementation of the reporting recommendations (270) needs to be carried out with education of health care providers and patients. Some believe that reporting eAG should compliment the current reporting in NGSP-DCCT aligned units (%) and the new IFCC results (mmol/mol), since the eAG results will be in the same units (mmol/L or mg/dL) as patients' self-monitoring; however, educational campaigns will be necessary to ensure clear understanding of this assay that is central to diabetes management.

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GENETIC MARKERS

1. **Use**

A. Diagnosis/Screening

a. Type 1 diabetes

Recommendation: Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, including neonatal diabetes, valuable information can be obtained with definition of diabetes-associated mutations.

A (moderate)

Genetic markers are currently of limited clinical value in the evaluation and management of patients with diabetes. However, mutational analysis is rapidly emerging for classification of diabetes in the neonate (271-273) as well as in young patients with a dominant family history of diabetes often refered to a Maturity Onset Diabetes of the Young (MODY) (274). Type 1 or autoimmune diabetes is strongly associated with the HLA DR and DQ genes. HLA-DQ A1 and B1 genotyping can be useful to indicate absolute risk of diabetes (see Table 9). The HLA-DQ A1*0301-B1*0302 or A1*0501-B1*0201 haplotypes, alone or in combination, may account for up to 90% of children and young adults with type 1 diabetes (275). These two haplotypes may be present in 30-40% of a Caucasian population and HLA is therefore necessary but not sufficient for disease. The HLA DQ and DR genetic factors are by far the most important determinants for type 1 diabetes risk (276). HLA typing may be used in combination with islet autoantibody analyses to exclude type 1 diabetes to assist in the diagnosis of genetic forms of diabetes.

As indicated below, HLA-DR/DQ typing can be useful to indicate modified risk of type 1 diabetes in persons with positive islet cell autoantibodies, since protective alleles do not prevent the appearance of islet cell autoantibodies (most often as single autoantibodies), but may delay the onset of clinical diabetes. Typing of the class II major histocompatibility antigens or HLA DRB1, DQA1 and DQB1 is not diagnostic for type 1 diabetes. However, some haplotypes form susceptibility, while

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others provide significant delay or even protection. Thus, HLA-DR/DQ typing can be used only to increase or decrease the probability of type 1 diabetes presentation and cannot be recommended for routine clinical diagnosis or classification (277).

The precision in the genetic characterization of type 1 diabetes may be extended by typing for polymorphisms in several genetic factors identified in genome wide association studies (278). Non-HLA genetic factors include the genes for insulin (INS), PTPN22, CTLA-4 and several others (276, 278). These additional genetic factors may assist in assigning a probability of the diagnosis of type 1 diabetes of uncertain etiology (279).

It is possible to screen newborn children to identify those at increased risk for developing type 1 diabetes (280-282). This strategy cannot be recommended until there is a proven intervention available to delay or prevent the disease (283). There is some evidence that early diagnosis may prevent hospitalization with ketoacidosis and preserve residual beta cells (284). The rationale for the approach is thus placed below under emerging considerations.

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b. Type 2 diabetes and MODY

Recommendation: There is no role for routine genetic testing in patients 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 affected genes is therefore highly complex. Recent genome wide association studies have identified more than 30 genetic factors, which increase the risk for type 2 diabetes (285, 286). However, the risk alleles in these loci all have relatively small effects (odds ratios 1.1 to 1.3) and do not significantly enhance our ability to predict risk of type 2 diabetes (287).

MODY: Mutation detection for MODY patients and their relatives is technically feasible. The reduced cost of sequencing and emerging new technologies make it possible to identify mutations and properly classify MODY patients based on specific mutations. As direct automated sequencing of genes becomes standard, it is likely that detection of specific diabetes mutations will become routine.

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B. Monitoring/Prognosis

Although genetic screening may provide information about prognosis and could be useful for genetic counseling, genotype may not correlate with the phenotype. In addition to environmental factors, interactions among multiple quantitative trait loci expressions may be involved. Genetic identification of a defined MODY will have value for anticipating the prognosis. Infants with neonatal diabetes due to a mutation in the KCNj11 (KIR6.2) gene may be treated with sulphonylurea rather than with insulin (271, 272).

2. **Rationale**

The HLA system, which has a fundamental role in the adaptive immune response, exhibits considerable genetic complexity. The HLA complex on chromosome 6 contains class I and II genes that code for several polypeptide chains (288). The major (classic) class I genes are HLA-A, B and C. The loci of class II genes are designated by three letters: the first (D) indicates the class, the second (M, O, P, Q or R) the family and the third (A or B) the chain. Both classes of molecules are heterodimers; class I consists of an α chain and β2-microglobulin, while class II has α and β chains. The function of the HLA molecules is to present short peptides, derived from pathogens or autoantigens, to T cells to initiate the adaptive immune response (288). Genetic studies have revealed an association between certain HLA alleles and autoimmune diseases. These diseases include, but are not confined to, ankylosing spondylitis, celiac disease, Addison's disease and type 1 diabetes (288). Not only the disease but also autoantibodies, which are markers of the disease pathogenesis, are often associated with HLA DRB1, DQA1 and DQB1, indicating that self-peptides may also be presented to T cells (275).

Genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself (1). 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 (1, 289). There are over 60 distinct genetic disorders associated with glucose intolerance or frank diabetes. Many forms of type 2 diabetes (which are usually strongly familial) will probably be understood in defined genetic terms. The complexity of genetic factors which contribute to type 2 diabetes risk is substantial (285, 286). Several genetic factors for MODY have been identified, and there are large numbers of individual mutants. Persons 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

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associated with long term complications of diabetes or as severe as typical type 1 diabetes [e.g., hepatocyte nuclear factor (HNF) mutations] (290).

Five different MODYs have been identified. MODY-1, 3, 4, and 5 all result from mutations in the genes encoding transcription factors that regulate the expression of genes in pancreatic β cells. These genes are hepatocyte nuclear factor-4α (HNF-4α) in MODY-1, HNF-1α in MODY-3, HNF-1β in MODY-5, and insulin promoter factor-1 (IPF-1) in MODY-4. It has been shown that homozygous mutations of the IPF-1 gene leads to pancreatic agenesis and that heterozygous mutations of IPF-1 genes results in MODY-4 (289). The modes of action of the HNF lesions in MODY is still not clear. It is likely that mutation in HNF-1α, 1β, and 4α cause diabetes because they impair insulin secretion. MODY-2 is caused by mutations in the glucokinase gene. The product of the gene is an essential enzyme in the glucose-sensing mechanism of the β cells, and mutations in this gene lead to partial deficiencies of insulin secretion.

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3. **Analytical Considerations**

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. Serological HLA typing should be replaced by molecular methods, since antibodies with a mixture of specificities and cross reactivities have been estimated to give inaccurate results in approximately 15% of typings.

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 100 to 200 µg DNA from 10 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. The MODYs have substitution, deletion or insertion of nucleotides in the coding region of the genes. These are detected by PCR. The detailed protocols for the detection of specific mutations are beyond the scope of this review.

4. **Interpretation**

To screen for the propensity for type 1 diabetes in general populations, HLA-D genes are the most important, contributing as much as 50% of familial susceptibility (291). HLA-DQ genes appear to be central to the HLA associated risk of type 1 diabetes, albeit DR genes may be independently involved (for reviews, see (292, 293). The heterodimeric proteins that are expressed on antigen presenting cells, B lymphocytes, platelets and activated T cells, but not other somatic cells, are composed of cis and trans complementated α and β chain heterodimers. Thus, in any individual four possible DQ dimers are encoded. Persons at the highest genetic risk for type 1 diabetes are those in whom all four DQ combinations meet this criterion. Thus, persons heterozygous for HLA-DRB1*04 -DQA1*0301-DQB1*0302 and DRB1*03- DQA1*0501-DQB1*0201 are the most susceptible, with an

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absolute life-time risk of type 1 diabetes in the general population of about 1:12. Persons who are protected from developing type 1 diabetes at a young age are those with DRB1*15-DQA1*0201-DQB1*0602 (haplotypes in particular (294). Individuals with DRB1*11 or 04 who also have DQB1*0301 are not likely to develop type 1 diabetes at a young age. HLA-DR is also involved in susceptibility to type 1 diabetes in that the B1*0401 and 0405 subtypes of DRB1*04 are susceptible, while the 0403 and 0406 subtypes are negatively associated with the disease, even when found in HLA genotypes with the susceptible DQA1*0301-DQB1*0302. DR molecules are heterodimers also, however the DRα chain is invariant in all persons. Additional DRβ chains (B3, B4 and B5) are not important.

Class II MHC is involved in antigen presentation to CD4 helper cells, and the above associations are likely to be explained by defective affinities to islet cell antigenic peptides, leading to persistence of T helper cells which escape thymic ablation. Class I HLA is also implicated in type 1 diabetes. Multiple non-HLA loci also contribute to susceptibility to type 1 diabetes (292). For example, the variable nucleotide tandem repeat (VNTR) upstream from the insulin (INS) gene on chromosome 11q is also useful for predicting the development of type 1 diabetes, with alleles with the longest VNTR having protective effects. Typing newborn infants for both 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 (293). Genome wide association studies have confirmed the following non-HLA genetic factors to increase the risk for type 1 diabetes, both in first degree relatives of type 1 diabetes patients and in the general population: INS VNTR, CTLA-4, PTPN22 and others (276, 278, 295, 296).

5. **Emerging considerations**

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The sequencing of the human genome and the formation of consortia demonstrate advances in the identification of the genetic bases for both type 1 and type 2 diabetes. This progress should ultimately result in family counseling, prognostic information and the selection of optimal treatment (289, 297) .

AUTOIMMUNE MARKERS

1. **Use**

Recommendation: Islet cell autoantibodies are recommended for screening of non-diabetic family members who wish to donate part of their pancreas for transplantation to a relative with end stage type 1 diabetes.

B (low)

Recommendation: Islet cell autoantibodies are not recommended for routine diagnosis of diabetes but standardized islet cell autoantibody tests may be used for classification of diabetes in adults and in prospective studies of children at genetic risk for type 1 diabetes following HLA typing at birth.

B (low)

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No therapeutic intervention has been identified that will prevent diabetes (292, 293). Therefore, although several islet cell autoantibodies have been detected in individuals with type 1 diabetes, measurement of these has limited use outside of clinical studies. Currently islet cell 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 cell autoantibodies.

A. Diagnosis/Screening

a. Diagnosis

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In type 1 diabetes the pancreatic islet β cells are destroyed and lost. In the vast majority of these patients, the destruction is mediated by an autoimmune attack (169). This is termed type 1A or immune mediated diabetes (Table 3). Islet cell autoantibodies comprise autoantibodies to islet cell-cytoplasm (ICA), to native insulin, referred to as insulin autoantibodies or IAA (298), to glutamic acid decarboxylase (GAD65A) (299-301), to two insulinoma antigen-2, IA-2A (302) and IA-2βA (also know as phogrin) (303) and to three variants of the ZnT8 transporter (ZnT8A) (304, 305). Autoantibody markers of the immune destruction are usually present in 85-90% of individuals with type 1 diabetes when fasting hyperglycemia is initially detected (1). Autoimmune destruction of the β cells has multiple genetic predispositions and is modulated by undefined environmental influences. The autoimmunity may be present for months or years prior to the onset of hyperglycemia and subsequent symptoms of diabetes. Patients with type 1A diabetes have a significantly increased risk of other autoimmune disorders including celiac disease, Graves' disease, thyroiditis, Addison's disease, and pernicious anemia (123). 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 minority of

patients with type 1 diabetes (type 1B, idiopathic) have no known aetiology and no evidence of autoimmunity. Many of these patients are of African or Asian origin.

b. Screening

Recommendation: Screening relatives of patients with type 1 diabetes or persons in the general population for islet cell autoantibodies is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies of children selected at birth following HLA testing for type 1 diabetes high risk HLA genotypes. B (low)

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Only about 15% of newly diagnosed type 1 diabetes patients have a first degree relative with the disease (306). 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 cell autoantibodies can identify those at high risk for the disease. However, as many as 1-2% of healthy individuals have a single autoantibody and are at low risk of developing type 1 diabetes (307). Because of the low prevalence of type 1 diabetes (~0.3% in the general population), the positive predictive value of a single islet cell autoantibody will be low (293). The presence of multiple islet cell autoantibodies (IAA, GAD65A, IA-2A/IA-2βA or ZnT8A) is associated with a risk of type 1 diabetes of > 90% (304, 307, 308). However, until cost effective screening strategies can be developed for young children and effective intervention therapy to prevent the clinical onset of the disease become available, such testing cannot be recommended outside of a research setting.

Children with certain HLA-DR and/or DQB1 chains (*0602/*0603/*0301) are mostly protected from type 1 diabetes, but not from developing islet cell autoantibodies (309). Because islet cell autoantibodies in these individuals have substantially reduced predictive significance, these subjects are often excluded from prevention trials.

Recommendation: Screening of patients with type 2 diabetes is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies of type 2 diabetes patients to identify possible mechanisms of secondary failures to type 2 diabetes treatment.

B (low)

Approximately 5-10 % of Caucasian adult patients who present with type 2 diabetes phenotype also have islet cell autoantibodies (310), particularly GAD65A, which predict insulin dependency. This has been termed latent autoimmune diabetes of adulthood (LADA) (311), type 1,5 diabetes (312) or slowly progressive insulin-dependent diabetes (SPIDDM) (313). Although GAD65A-positive diabetic patients progress faster to absolute insulinopenia than do antibody-negative patients, many antibody-negative (type 2) diabetic adults also progress (albeit more slowly) to insulin dependency with time. Some of these patients may show T cell reactivity to islet cell components (312). There is limited utility for islet cell autoantibody testing in patients with type 2 diabetes because the institution of insulin therapy is based on glucose control.

B. Monitoring/Prognosis

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Recommendation: There is currently no role for measurement of islet cell autoantibodies in the monitoring of patients in clinical practice. Islet cell autoantibodies are measured in research protocols and some clinical trials as surrogate end-points.

B (low)

No acceptable therapy has been demonstrated to prolong survival of islet cells once diabetes has been diagnosed or to prevent the clinical onset of diabetes in islet-cell-autoantibody-positive subjects (292). Thus, repeated testing for islet cell autoantibodies to monitor islet cell autoimmunity is not clinically useful at present. In islet cell or pancreas transplantation, the presence or absence of islet cell autoantibodies may clarify whether subsequent failure of the transplanted islets is due to recurrent autoimmune disease or to rejection (314). When a partial pancreas has been transplanted from an identical twin or HLA-identical sibling, appearance of islet cell autoantibodies may raise consideration of 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 cell autoantibodies may be useful in the following situations: a) to identify a subset of adults initially thought to have type 2 diabetes, but have islet cell autoantibody markers of type 1 diabetes and progress to insulin dependency (315); b) to screen non-diabetic family members who wish to donate a kidney or part of their pancreas for transplantation; c) to screen women with GDM to identify those at high risk of progression to type 1 diabetes and d) to distinguish type 1 from type 2 diabetes in children to institute insulin therapy at the time of diagnosis (316, 317). For example, some pediatric diabetologists are now treating children thought to have type 2 diabetes with oral medications, but treat autoantibody positive children immediately with insulin. However, it is possible to follow patients who are islet cell autoantibody

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positive to the point of metabolic decompensation and then institute insulin therapy. The DPT-1 study failed to show a protective effect of parenteral insulin (318).

2. **Rationale**

The presence of islet cell autoantibodies suggests that insulin therapy is the most appropriate therapeutic option especially in a young person. Conversely, in children or young people without islet cell autoantibodies, consideration may be given to a trial of oral agents and life style changes other than insulin therapy. There is not unanimity of opinion, but the presence of islet cell 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 cell autoantibodies but clinically classified as having type 2 diabetes, and children with transient hyperglycemia. The majority of non-diabetic individuals who have only one autoantibody may never develop diabetes. Although expression of multiple islet cell autoantibodies is associated with greatly increased diabetes risk (307, 308), approximately 20% of individuals presenting with new onset diabetes express only a single autoantibody. Prospective studies of children indicate that islet cell autoantibodies may be transient indicating that an islet autoantibody may have disappeared prior to the onset of hyperglycemia or diabetes symptoms (319).

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3. **Analytical Considerations**

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

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For IAA, a radioisotopic method that calculates the displaceable insulin radioligand binding after the addition of excess non-radiolabelled insulin (320) 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. It is noted that insulin autoantibody 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) demonstrate that the interlaboratory variability for IAA is inappropriately large (321).

GAD65A and IA-2A are determined in standardized radiobinding assays performed with ^{35}S-labeled recombinant human GAD65 or IA-2 generated by coupled *in vitro* transcription translation using [^{35}S]methionine or other ^{35}S- or ^{3}H-labeled amino acids (322). Commercially available methods for GAD65A and IA-2A are available either as a radioimmunoassay with ^{125}I-labeled GAD65 (truncated at the N-terminal end to promote solubility) or IA-2, respectively. In addition, immunoassays for both GAD65A and IA-2A are commercially available. Major efforts have been made to standardize GAD65A and IA-2A measurements (321, 323). A WHO-standard for both GAD65A and IA-2A is established and amounts of GAD65A and IA-2A are expressed in international units (324). The binding of labeled autoantigen to autoantibodies is normally distributed. Cut-off values should be determined from 100-200 sera obtained from healthy individuals. GAD65A and IA-2A should be reported as positive when the signal exceeds the 99th percentile. Comparison of multiple

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laboratories worldwide is carried out in the DASP, a proficiency testing program organized by the Centers for Disease Control (CDC) under the auspicies of the Immunology of Diabetes Society (http://www.idsoc.org/). Commercially available GAD65A and IA-2A methods are also participating in the DASP program demonstrating that it should be possible not only to harmonize participating laboratories but also eventually to standardized GAD65A and IA-2A (323).

ICA are determined by indirect immunofluorescence on frozen sections of human pancreas (325). ICA measure the degree of binding of imunoglobulin to the islets and are compared to a WHO standard serum available from the National Institute of Biological Standards and Control (324). 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 method is cumbersome and has proven difficult to standardize. The number of laboratories which still carry out the ICA test has decreased markedly and the test is no longer included in the DASP program.

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4. **Interpretation**

GAD65A may be present in ~ 60-80% of newly diagnosed patients with type 1 diabetes, but the frequency varies with gender and age. GAD65A in both patients and healthy subjects are associated with HLA DR3-DQA1*0501-B1*0201. IA-2A may be present in about 40-50% of newly diagnosed type 1 diabetes patients but the frequency is highest in the young and decreases with increasing age. IA-2A are associated with HLA DR4-DQA1*0301-B1*0302. 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*0301-B1*0302 and with INS VNTR (275). ICA is found in about 75-85% of new onset patients.

The ICA assay is labor-intensive and difficult to standardize, and marked inter-laboratory variability in sensitivity and specificity has been demonstrated in workshops (297, 326). Few clinical laboratories are likely to implement this test. The immunoassays are more reproducible and amendable to standardization (321). Measurement of T cell reactivity in peripheral blood is theoretically appealing but the variability of such assays precludes their use in a clinical setting (327, 328).

Autoantibody-positivity is reported (by definition) in healthy individuals despite an absence of family history of autoimmune diseases. Islet cell autoantibodies are no exception. If one autoantibody is found, the others should be assayed because the risk of type 1 diabetes increases if two or more autoantibodies are positive (318).

The following suggestions have been proposed (292) as a rational approach to the use of autoantibodies in diabetes: a) antibody 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 false positive and negative results.

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5. **Emerging Considerations**

Since immunoassays for IAA, GAD65A IA-2A/IA-2βAand ZnT8A are now available, a panel of these autoantibodies is currently used in screening studies (329). As ICA assays are difficult to standardize, their use has declined substantially.

It is likely that other islet cell antigens 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 quite feasible in future. In those individuals who are islet cell autoantibody positive, HLA-DR/DQ genotyping will help define absolute risk of type 1 diabetes.

Several clinical trials to prevent or intervene with type 1 diabetes are being actively pursued (329). Such trials can now be done in relatives of patients with type 1 diabetes or in the general population on the basis of the islet cell autoantibody and HLA-DR/DQ genotype status. Risk can be assessed by islet cell autoantibodies alone, without the need for evaluation of endogenous insulin reserves as was done for the US DPT-1 trial (318). Islet cell 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. Potential interventional therapies (for type 1 diabetes) undergoing clinical trials include oral (329) or nasal insulin (330) given to non-diabetic - but islet cell autoantibody positive - relatives of individuals with type 1 diabetes or HLA high-risk children with islet cell autoantibodies. Phase II clinical trials with alum-formulated GAD65 report no adverse events and some preservation of endogenous insulin production in GAD65A-positive diabetes patients (331, 332). Additional trials of other antigen-based immunotherapies, adjuvants, cytokines and T cell accessory molecule blocking agents are likely in the future (283). Decreased islet cell autoimmunity will be one important outcome measure of these therapies.

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MICROALBUMINURIA

Microalbuminuria is a well established cardiovascular risk marker, where increases over time to macroalbuminuria (>300 mg/day) are associated with increased risk for the development of end-stage renal disease. Annual testing for microalbuminuria is recommended by all major guidelines for patients with diabetes and/or kidney disease. To be useful, semi-quantitative or qualitative screening tests must

be shown to be positive in >95% of patients with microalbuminuria. Positive results of such tests must be confirmed by quantitative testing in an accredited laboratory.

1. **Use**

Recommendation: Annual microalbuminuria testing of patients without macroalbuminuria or clinical proteinuria should begin in pubertal or postpubertal individuals five years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment. B (moderate)

Recommendation: Microalbuminuria is a continuous risk marker for cardiovascular events which appear to start at concentrations of 20 ug/min. B (moderate)

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A. Diagnosis/Screening

Diabetes is associated with a very high cardiovascular event rate and is the leading cause of end-stage renal disease in the Western world (333). Early detection of risk markers such as small amounts of albumin in the urine (termed "microalbuminuria") relies upon tests for urinary excretion of albumin. Conventional qualitative tests (chemical strips or "dipsticks") for albuminuria do not detect the small increases of urinary albumin excretion. For this purpose, tests for "microalbuminuria" are used (Table 10) (334-336). Microalbuminuria has been defined by the Joint National Committee (JNC) 7, ADA and National Kidney Foundation (NKF) (21, 337, 338) as excretion of 30 – 300 mg of albumin /24 h, or 20

– 200 μg/min or 30 – 300 μg/mg creatinine (Table 11) on two of three urine collections. Recent data, however, suggest that risk extends below the lower limit of 20 μg/min (339-341), reinforcing the notion that this is a continuous variable in regard to cardiovascular risk (342-344).

The JNC 7, NKF and ADA all recommend annual quantitative testing for urine albumin in adults with diabetes using morning spot albumin:creatinine measurement (21, 337, 338). Subjects should be fasting. Optimal time for spot urine collection is the early morning, but to minimize variability all collections should be at the same time of day and preferably fasting for at least 2 hours (345).

Positive tests represent “clinical albuminuria or macroalbuminuria” in these guidelines, corresponding to protein excretion > 300 mg/24 hours, > 200 μg/min or > 300 μg/mg creatinine (Table 11). In these patients, quantitative measurement of urine albumin excretion is used in the assessment of the severity of albuminuria and its progression, in planning treatment, and in determining the impact of therapy. Measurement of estimated glomerular filtration rate (eGFR) to properly assess stage of kidney disease can be calculated by knowing the serum creatinine, age, sex and race of the patient (346). An eGFR of <60 mL/min, regardless of the presence of microalbuminuria, is an independent cardiovascular risk factor (338) A urine albumin of <30 μg/mg creatinine, while considered “normal”, should be reassessed annually. If the value is ≥ 30 μg/mg, changes should be reassessed after 6 to 12 months if antihypertensve therapy is required or annually in those who are normotensive (337). For children with type 1 diabetes, testing for microalbuminuria is recommended to begin after puberty and after 5 years duration of diabetes. It should be noted that most longitudinal cohort studies report significant increases in prevalence of microalbuminuria only after diabetes has been present for 5 years (337, 347).

In the algorithms of both the NKF and ADA for urine protein testing (333), the diagnosis of microalbuminuria requires the demonstration of increased albumin excretion (as defined above) on 2

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of 3 tests repeated at intervals over a period of a 3 to 6 months, and exclusion of conditions that "invalidate" the test (Fig. 1).

A. Prognosis

Microalbuminuria has prognostic significance. In multiple epidemiological studies it is an independent risk marker for cardiovascular death (348, 349). In 80% of people with type 1 diabetes and microalbuminuria, urinary albumin excretion can increase by as much as 10 – 20 % per year, with development of clinical proteinuria (> 300 mg albumin/day) in 10 –15 years in a little more than half the people. After the development of clinical grade proteinuria, most (> 90 %) patients go on to develop decreased glomerular filtration rate and, given enough time, end-stage renal disease. In type 2 diabetes, 20 – 40 % of patients with microalbuminuria progress to overt nephropathy, but by 20 years after overt nephropathy only ~ 20 % develop end-stage renal disease. In addition, patients with diabetes (type 1 or type 2) and microalbuminuria are at increased risk for cardiovascular disease. It should be noted that microalbuminuria alone does NOT indicate increased risk for progression to end stage kidney disease nor kidney disease per se; hypertension needs to be present for the risk of progression (350, 351). Moreover, about 20% of people progress to end stage kidney disease without an increase in microalbuminuria (352). Another factor that indicates progression is an increase in microalbuminuria to macroalbuminuria over time despite achievement of blood pressure goals (353).

B. Monitoring

The roles of routine urinalysis and albumin measurements are less clear in patients with a diagnosis of microalbuminuria. Some have advocated urine protein testing to monitor treatment, which

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may include improved glycemic control, more assiduous control of hypertension, dietary protein restriction and therapy with blockers of the renin angiotensin system (333). Several factors are known to slow the rate of urinary albumin excretion or to prevent its development. These include reducing blood pressure (with a blocker of the renin angiotensin system as part of the regimen), glycemic control and lipid lowering therapy (44, 354-356).

2. Rationale

Early detection of microalbuminuria allows early intervention with a goal of reducing cardiovascular risk and delaying the onset of overt diabetic nephropathy. Thus, it is a signal for more intensive efforts to reduce cardiovascular risk factors.

Microalbuminuria rarely occurs with short duration of type 1 diabetes or before puberty. Thus, testing is less urgent in these situations. Nevertheless, the difficulty in precisely dating the onset of type 2 diabetes warrants initiation of annual testing at the time diagnosis of diabetes. While older patients (age > 75 years or life expectancy < 20 years) may not be at risk for clinically significant nephropathy due to a short projected life-span, they will be at higher cardiovascular risk. In such patients, the role of treating microalbuminuria is far from clear. Published studies have demonstrated that it is cost effective to screen all patients with diabetes and/or kidney disease for microalbuminuria (357, 358).

3. Analytical Considerations

A. Analytical

Recommendation: The analytical CV of methods to measure microalbuminuria should be <15%.

B (moderate)

Analytical goals can be related to the degree of biological variation, with less precision required for analytes that vary widely in subjects to be tested. Detection limit and imprecision data are summarized in Table 10. Commercially available quantitative methods for microalbuminuria have documented detection limits of ~ 20 ug/L or less. Within-run imprecision and day-to-day (total) imprecision are well within the analytical goal of ~ 15 %, and often much less. Most methods, but not all, agree well with each other and support a reference interval of 2 – 20 µg albumin/mg creatinine (359) .

The within-person variation of albumin excretion is large in people without diabetes and even higher in patients with diabetes. Howey *et al.* (360) studied day-to-day variation, over 3-4 weeks, of the 24-hour albumin excretion, the concentration of albumin and the albumin:creatinine ratio. The last two were measured in the 24-hour urine sample and as well as the first morning void and random untimed urine. In healthy volunteers, the lowest within-person CVs were found for the concentration of albumin in the first morning void (36 %) and for the albumin:creatinine ratio in that sample (31%) (360). Multiple studies have evaluated the best procedure to assess microalbuminuria. Most studies have found that the spot urine albumin:creatinine concentration in the first morning void, rather than 24-hour urinary excretion of albumin or timed collection, is the most practical and reliable way to assess microalbuminuria (357, 361, 362).

To keep analytical CV less than half the biological CV, an analytical goal of 18% CV has been proposed (360). Alternatively, if the albumin:creatinine ratio is to be used, one may calculate the need for somewhat lower imprecision (that is, a better precision) to accommodate the lower biological CV

for the ratio and the imprecision contributed by the creatinine measurement. Assuming a CV of 5 % for the measurement of creatinine, we calculate a goal of 14.7% for the analytical CV for albumin when it is used to estimate the albumin:creatinine ratio. A goal of 15% appears reasonable to accommodate use of the measured albumin concentration for calculation of either timed excretion rate or the albumin:creatinine ratio.

Recommendation: Semiquantitative or qualitative screening tests for microalbuminuria should be positive in >95% of patients with microalbuminuria to be useful for screening. Positive results must be confirmed by analysis in an accredited laboratory.

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Qualitative (or semiquantitative) assays have been proposed as screening tests for microalbuminuria. To be useful, screening tests must have high detection rates for abnormal samples, i.e., a high clinical sensitivity. Although many studies have assessed the ability of reagent strips ("dipstick" methods) to detect increased albumin concentrations in urine, the important question is whether the method can detect microalbuminuria, that is, increased albumin excretion rate or its surrogate, increased albumin:creatinine ratio. We can find no documentation of any test in which the sensitivity for detection of an increased albumin excretion rate consistently reached 95% in more than one study.

In a large study (363), the sensitivity for detection of an albumin excretion rate > 30 mg/24 hours was 91 % when the test was performed by a single laboratory technician, 86 % when performed by nurses, and 66 % when performed by general practitioners. In two subsequent studies (364, 365), the sensitivities were 67 % - 86 %. False-positive results also appear to be common, with rates as high

as 15 % (363). Thus it appears that at least some of the tests, especially as used in practice, have the wrong characteristics for screening because of low sensitivity (high false negative rates), and positive results must be confirmed by a laboratory method.

Recommendation: Currently available dipstick tests are not sensitive enough to be used reliably to make a diagnosis of microalbuminuria.

C (low)

Chemical strip methods are not sensitive when the albumin concentration in the urine is in the range of 20 – 50 mg/L. Thus, no recommendation can be made for the use of any specific screening test. "Dipstick" tests for microalbuminuria cannot be recommended as replacement for the quantitative tests.

The available "dipstick" methods for microalbuminuria do not appear to lend themselves to viable screening strategies either in the physician's office or for home testing. Usual screening tests (e.g., for phenylketonuria) have low false negative rates, and, thus, only positive results require confirmation by a quantitative method. If a screening test has low sensitivity, negative results, too, must be confirmed, a completely untenable approach. With semiquantitative tests, it may be possible (or, indeed, necessary) to use a cutoff below 20 mg/L to ensure detection of samples with albumin > 20 mg/L as measured by laboratory methods.

Recent studies have compared selected dipstick methods to laboratory assays and one dipstick was found to have >95% sensitivity (334, 336). One such study evaluated an office screening test that uses a monoclonal antibody against human serum albumin (ImmunoDip) (334). Screening 182 patient samples with this method using an albumin:creatinine ratio of ≥30 μg/mg as positive yielded a

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sensitivity of 96%, a specificity of 80%, a positive predictive value of 66% and a negative predictive value of 98%. In a separate study, 165 patients had the HemoCue albumin concentration point of care system compared with Clinitek Microalbumin and Chemstrip Micral test, as well as an HPLC assay for spot albumin:creatinine measurement (336). Further studies are needed before the "dipstick" tests for microalbuminuria can be recommended as replacement for the quantitative tests. The use of the qualitative tests at the point of care is reasonable only when it can be shown that it eliminates quantitative testing in a sizeable proportion of patients and detects those patients who have early renal disease.

B. Premeasurement

Recommendation: Acceptable samples to test for increased urinary albumin excretion are timed (e.g., 12 or 24 hour) collections for measurement of albumin concentration and timed or untimed samples for measurement of the albumin:creatinine ratio. For screening, an untimed sample for albumin measurement (without creatinine) may be considered if a concentration cutoff is used that allows high sensitivity for detection of an increased albumin excretion rate.

B (moderate)

Recommendation: Optimal time for spot urine collection is the early morning and fasting. To minimize variability all collections should be at the same time of day and preferably fasting for at least 2 hours.

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Collection of 24-hour samples has disadvantages, specifically because many samples are collected inadequately and total creatinine is not routinely checked to evaluate adequacy of collection. The albumin:creatinine ratio appears to be an acceptable alternative. The ratio has a within-person, biological variation similar to that of the excretion rate, and correlates well with timed excretion as well as with albumin concentration in a first morning void of urine (360). For the ratio, a first-morning void sample is preferable as this sample has a lower within-person variation than the ratio in a random sample of urine during the day (360). Although the ratio appears entirely acceptable for screening, limited data are available for its use in monitoring the response to therapy. Recent post hoc analyses of clinical trials, however, have found that the albumin:creatinine ratio is a reasonable method to assess change over time (366).

Albumin is stable in untreated urine stored at 4 °C or 20°C for at least a week (367). Neither centrifugation nor filtration appears necessary before storage at – 20 °C or – 80 °C (368). 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 (368). Urinary albumin excretion rate reportedly has no marked diurnal variation in diabetes, but does in essential hypertension (369).

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4. **Interpretation**

A. Nonanalytical sources of variation

Transient increases of urinary albumin excretion have been reported with short-term hyperglycemia, exercise, urinary tract infections, marked hypertension, heart failure, acute febrile illness and hyperlipidemia(333).

B. Frequency of measurement

Recommendation: A urine albumin of <30 μg/mg creatinine, while considered “normal”, should be reassessed annually. If the value is ≥30 μg/mg, changes should be reassessed after six to 12 months if antihypertensive therapy is required, or annually in those who are normotensive.

B (moderate)

The NKF, ADA and JNC 7 recommend annual measurement in patients with albumin:creatinine ratios of <30 μg/mg. After the documentation of a diagnosis of microalbuminuria (i.e., with results as defined above on 2 of 3 tests performed within a period of 3 – 6 months), repeated testing is reasonable to determine whether a chosen therapy is effective. It may also be useful in determining the rate of progression of disease and thus support planning for care of end-stage renal disease. Although the ADA recommendations suggest that such testing is not generally needed before puberty, testing may be considered on an individual basis if it appears appropriate because of early onset of diabetes, poor control or family history of diabetic nephropathy. The duration of diabetes prior to puberty was reported to be is an important risk factor in this age group and thus can be used to support such testing in individual patients (370).

MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES

I. INSULIN AND PRECURSORS

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1. **Use**

A. Diagnosis

Recommendation: There is no role for routine testing for insulin, C-peptide or proinsulin in most patients with diabetes. Differentiation between type 1 and type 2 diabetes may in most cases be made based on the clinical presentation and subsequent course. These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 and type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis. B (moderate)

Recommendation: There is no role for measurement of insulin concentration in the assessment of cardiometabolic risk, as knowledge of this value does not alter the management of these patients. B (moderate)

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In the last several years, interest has increased in the possibility that measurements of the concentration of plasma insulin and its precursors might be of clinical benefit. In particular, evidence has been published that increased concentrations of insulin and/or proinsulin in nondiabetic individuals predict the development of coronary artery disease (CAD) (371). Although this possibility may be scientifically valid, its clinical value is questionable. Increased insulin concentration is a surrogate

marker which can be used to estimate resistance to insulin-mediated glucose disposal, and can identify individuals at risk for developing Syndrome X, also known as the insulin resistance syndrome (372). 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 (373, 374). 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 glycemic status and other confounders. More recent analyses suggest that insulin values do not add significantly to diabetes risk prediction carried out using more traditional clinical and laboratory measurements (375), and that measures of insulin resistance (that include insulin measurements) predicted risk of diabetes or CAD only moderately, with no threshold effects (376). 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, 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 (375, 376).

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 fasting hypoglycemia (377). The diagnosis

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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 in 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 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). 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 possible factitious hypoglycemia due to surreptitious insulin administration (378).

In the past, some advocated use of insulin assays in the evaluation and management of patients with the polycystic ovary syndrome. Women with this syndrome manifest insulin resistance by androgen excess as well as abnormalities of carbohydrate metabolism; both abnormalities may respond to treatment with metformin or thiazolidinediones. While clinical trials have generally evaluated insulin resistance using the hyperinsulinemic euglycemic clamp, fasting glucose to insulin ratios, and other modalities, the optimal laboratory evaluation of these patients in routine clinical care is not clearly defined. It is unclear whether assessing insulin resistance through insulin measurement has any advantage over assessment of physical signs of insulin resistance (body mass index, presence of acanthosis nigricans) and routine measurements of insulin are not recommended by the American College of Obstetrics and Gynecology (379).

2. **Analytical Considerations**

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Recommendation: Since current measures of insulin are poorly harmonized, a standardized insulin assay should be developed to encourage the development of measures of insulin sensitivity that will be practical for clinical care.

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Although assayed for over 40 years, there is no standardized method available to measure serum insulin (380). Attempts to harmonize insulin assays using commercial insulin reagent sets result in greatly discordant results (381). Recently, 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 (382). 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 (383). Considerable imprecision among laboratories is also observed for measurement of C-peptide. Comparison of 15 laboratories which used 9 different routine C-peptide assay methods, found within- and between-run CVs as high as >10% and 18%, respectively (384). A committee has been established under the auspices of the CDC to harmonize C-peptide analysis.

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; previously called Health Care Financing Administration (HCFA)) 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 (385).

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 (380). In the vast majority of patients, the titer of insulin antibodies is low, and their presence is of no clinical significance. Very low values are seen in patients treated exclusively with human recombinant insulin (386). However, on occasion the titer of insulin antibodies in the circulation can be quite high and 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. 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.

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The prior version of these guidelines (14) contained short sections on amylin and leptin, both of which were the focus of active clinical studies. The evidence accumulated in the last seven to eight years has failed to identify any clinical value in measuring these analytes in patients with diabetes. Similarly, although cardiovascular disease is the major cause of mortality for persons with diabetes, no evidence supports the measurement of non-traditional cardiovascular risk factors for routine assessment of risk in patients with diabetes. These sections have, therefore, been removed.

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Table 1: Grading the Quality/Strength of Evidence

The overall quality/strength of the *body* of evidence is scored on a 4-point scale:

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.

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Table 2: Grading the Strength of Recommendations

A. The NACB strongly recommends adoption

Strong recommendations *for* adoption at Level A are made 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, or

Strong recommendations *against* adoption at Level A are made 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.

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Table 2: Grading the Strength of Recommendations

B. The NACB recommends adoption

Recommendations *for* adoption at Level B are made 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.

Recommendations *against* adoption at Level B are made 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.
- 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.

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Table 2: Grading the Strength of Recommendations

C. The NACB concludes that the evidence or the level of agreement between experts is insufficient to make recommendations

Level C is applied in the following circumstances:

- Evidence is lacking, 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. The NACB recommends it as 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 where the question does not directly address health-related outcome aspects of care. 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.

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Table 3: Classification of Diabetes Mellitus*

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. Gestational diabetes mellitus

*From ADA (387)

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Table 4: Criteria for the Diagnosis of Diabetes[*]

Any one of the following is diagnostic

1. $HbA_{1c} \geq 6.5\%$[1]

OR

2. FPG ≥ 7.0 mmol/L (126 mg/dL)[2]

OR

3. 2-h plasma glucose ≥ 11.1 mmol/L (200 mg/dL) during an OGTT[3]

OR

4. Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/L (200 mg/dL)[4]

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeating the same test on a different day. Mixing different methods to diagnose diabetes should be avoided.

[*] From ADA (387)

[1] The test should be performed in a laboratory that is NGSP certified and standardized to the DCCT assay.

[2] Fasting is defined as no caloric intake for at least 8 h.

[3] The OGTT should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g of anhydrous glucose dissolved in water.

[4] Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia and unexplained weight loss.

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Table 5: Minimally- and Non-invasive Methodology for *in Vivo* Glucose Monitoring*

1. Transcutaneous needle-type enzyme electrodes
2. Totally implanted sensors
   - Enzyme electrodes
   - Near infrared fluorescence-based
3. Sampling technologies
   - Microdialysis
   - Reverse iontophoresis
4. Non-invasive technologies
   - Light scattering
   - Raman spectroscopy
   - Near - or mid-infrared spectroscopy
   - Photoacoustic spectroscopy

*Adapted from Pickup et al. (388)

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Table 6: WHO Criteria for Interpreting 2 h OGTT*

	0 h	**2 h**
Impaired Fasting Glucose[a]	≥ 6.1 mmol/L (110 mg/dL) < 7.0 (126)	< 7.8 (140)
Impaired Glucose Tolerance[b]	< 7.0 (126)	≥ 7.8 (140) - < 11.1 (200)
Diabetes[c]	≥ 7.0 (126)	≥ 11.1 (200)

Values are for venous plasma glucose using a 75 g oral glucose load.

*From WHO (19)

[a] If 2-h glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded.

[b]Both fasting and 2-h value need to meet criteria

[c]Either fasting or 2-h measurement can be used. Any single positive result should be repeated on a separate day.

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Table 7: 100 g Oral Glucose Tolerance Test

	mmol/L	mg/dL
Fasting	5.3	95
1 h	10.0	180
2 h	8.6	155
3 h	7.8	140

The test should be done in the morning after an overnight fast of between 8 and 14 h and after a unrestricted diet (≥ 150 g carbohydrate per day) and unlimited physical activity. The subject should be seated and should not smoke throughout the test.

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Table 8: Screening for and Diagnosis of GDM

Glucose Measure	Glucose Concentration Threshold+		Percent ≥ Threshold†
	mmol/L	mg/dL	Cumulative
FPG	5.1	92	8.3
1-hr PG	10.0	180	14.0
2-hr PG	8.5	153	16.1*

+One or more of these values from a 75 g OGTT must be equaled or exceeded for the diagnosis of GDM.

†Cumulative proportion of HAPO cohort equaling or exceeding those thresholds

*In addition, 1.7% of participants in the initial cohort were unblinded because of a FPG >5.8 mmol/L (105 mg/dL) or 2-hr OGTT values >11.1 mmol/L (200 mg/dL) bringing the total to 17.8%.

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Table 9: Lifetime Risk of Type 1 Diabetes in First-Degree Relatives*

(proband diagnosed before age 20)

Relative	Risk (%)
Parents	2.2 ± 0.6%
Children	5.6 ± 2.8%
Siblings	6.9 ± 1.3%
HLA non-identical sib	1.2%
HLA haploidentical sib	4.9%
HLA identical sib	15.9%
Identical twin	30-40%
General population	0.3%

*From Harrison (293)

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Table 10: Review of Assays to Assess Albuminuria

Method	Inter-assay CV	Detection Limit
Immunonephelometry (Beckman Array Analyzer)	4.2% at 12.1 mg/L 5.3% at 45 mg/L	2 mg/L
Immunoturbimetry (Dade-Behring Turbimeter)	4.1% at 10.6 mg/L 2.2% at 77.9 mg/L	6 mg/L
Hemocue (Point of Care)	2.2% at 77.9 mg/L 4,3% at 82 mg/L	5 mg/L
Radioimmunoassay	9.2% at 12.2 mg/dL 4.8% at 33 mg/L	16 µg/L

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Table 11: Definitions of Microalbuminuria and Clinical Albuminuria*

	mg/24 h	ug/min	ug/mg creatinine
Normal	<30	<20	<30
Microalbuminuria	30-300	20-200	30-300
Clinical albuminuria[†]	>300	>200	>300

[†]Also called “overt nephropathy”

*From ADA (21)

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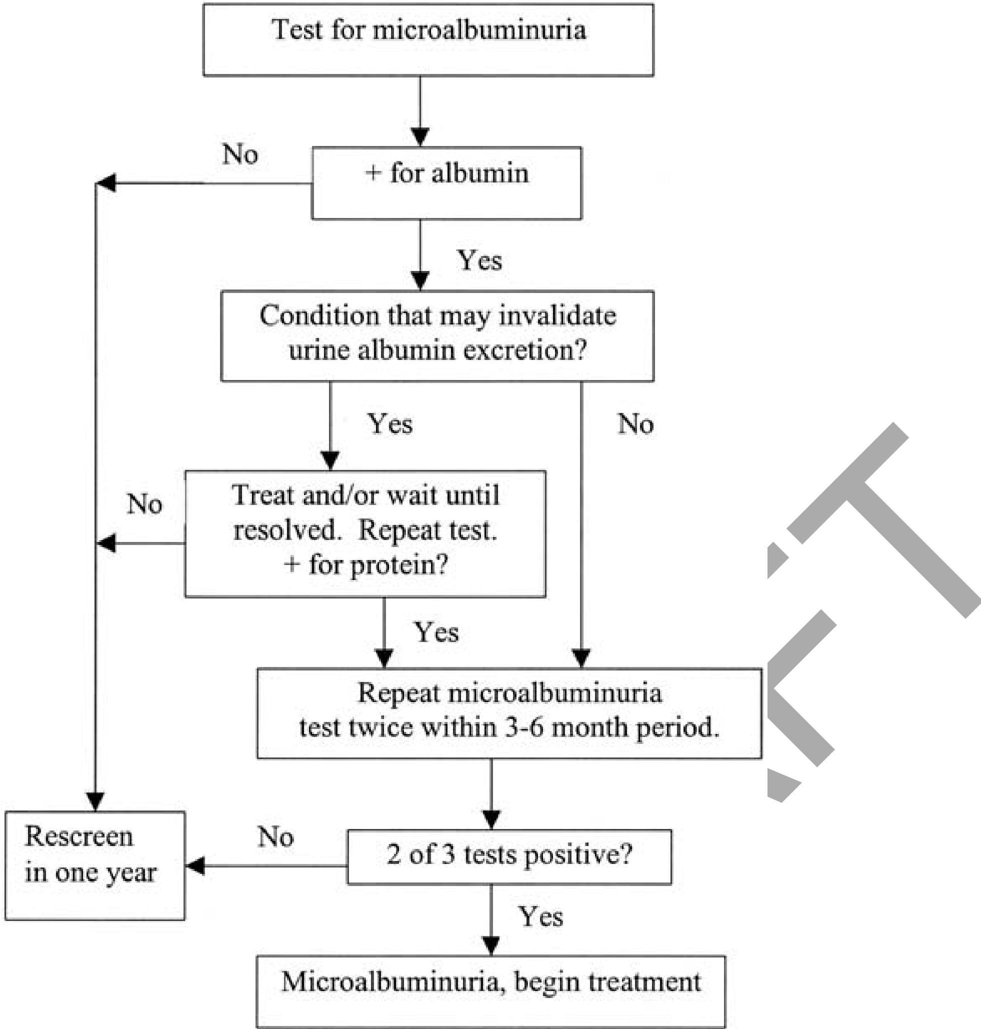

Fig. 1. Algorithm for urine protein testing

References

1. American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.
2. Castano 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;37:1595-607.
4. Sacks DB, McDonald JM. The pathogenesis of type II diabetes mellitus. A polygenic disease. Am J Clin Pathol 1996;105:149-56.
5. Balasubramanyam A, Garza G, Rodriguez L, Hampe CS, Gaur L, Lernmark A, Maldonado MR. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. Diabetes Care 2006;29:2575-9.
6. International Diabetes Federation. Diabetes Atlas, 3rd Edition. Brussels, Belgium: International Diabetes Federation, 2008.
7. Cowie CC, Rust KF, Byrd-Holt DD, Eberhardt MS, Flegal KM, Engelgau MM, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. Diabetes Care 2006;29:1263-8.
8. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care 2009;32:287-94.
9. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, Hu FB. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 2009;301:2129-40.
10. 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;362:1090-101.
11. American Diabetes Association. Economic costs of diabetes in the U.S. in 2007. Diabetes Care 2008;31:596-615.
12. Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med 1993;328:1676-85.
13. 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;28:2130-5.
14. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002;48:436-72.
15. NDDG. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. Diabetes 1979;28:1039-57.
16. World Health Organization. WHO Expert Committee on Diabetes Mellitus: second report. World Health Organ Tech Rep Ser 1980;646:1-80.
17. 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;20:785-91.
18. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, Knowler WC. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ 1994;308:1323-8.
19. World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycermia: Report of a WHO/IDF Consultation. Geneva: World Health Org, 2006.
20. The International Expert Committee. International Expert Committee Report on the Role of the A1c Assay in the Diagnosis of Diabetes. Diabetes Care 2009;32:1327-34.

DRAFT

21. American Diabetes Association. Standards of medical care in diabetes--2010. Diabetes Care 2010;33 Suppl 1:S11-61.
22. American Diabetes Association. Tests of glycemia in diabetes. Diabetes Care 2001;24 (Suppl 1):S80-2.
23. International Diabetes Federation Task Force. Global guideline for Type 2 diabetes. Brussels: International Diabetes Federation, 2005:1-11pp.
24. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab 2008;93:2447-53.
25. American Diabetes Association. Type 2 diabetes in children and adolescents. Diabetes Care 2000;23:381-9.
26. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.
27. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen 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;344:1343-50.
28. Icks A, Rathmann W, Haastert B, John J, Lowel H, Holle R, Giani G. Cost-effectiveness of type 2 diabetes screening: results from recently published studies. Gesundheitswesen 2005;67 Suppl 1:S167-71.
29. Perry RC, Shankar RR, Fineberg N, McGill J, Baron AD. 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;24:465-71.
30. Jesudason DR, Dunstan K, Leong D, Wittert GA. Macrovascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and HbA(1c) for cost-effective screening. Diabetes Care 2003;26:485-90.
31. Dallo FJ, Weller SC. Effectiveness of diabetes mellitus screening recommendations. Proc Natl Acad Sci U S A 2003;100:10574-9.
32. CDC Diabetes Cost-effectiveness Study Group. The cost-effectiveness of screening for type 2 diabetes. JAMA 1998;280:1757-63.
33. Hoerger TJ, Harris R, Hicks KA, Donahue K, Sorensen S, Engelgau M. Screening for type 2 diabetes mellitus: a cost-effectiveness analysis. Ann Intern Med 2004;140:689-99.
34. Glumer C, Yuyun M, Griffin S, Farewell D, Spiegelhalter D, Kinmonth AL, Wareham NJ. What determines the cost-effectiveness of diabetes screening? Diabetologia 2006;49:1536-44.
35. Greenberg RA, Sacks DB. Screening for diabetes: is it warranted? Clin Chim Acta 2002;315:61-9.
36. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160-7.
37. 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;49:822-7.
38. Tai ES, Goh SY, Lee JJ, 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;27:1728-34.
39. Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, Knowler WC. The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 2000;23:1108-12.
40. 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;353:1454-62.

DRAFT

41. DCCT. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968-83.
42. Stratton IM, Adler AI, Neil HA, 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;321:405-12.
43. DCCT. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
44. Nathan DM, Cleary PA, Backlund JY, 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;353:2643-53.
45. U.K. 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). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-53.
46. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, Golden SH. Meta-analysis: glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141:421-31.
47. Ray KK, Seshasai SR, 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;373:1765-72.
48. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577-89.
49. Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, Buse JB, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59.
50. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-72.
51. 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;360:129-39.
52. Berg AH, Sacks DB. Haemoglobin A1c analysis in the management of patients with diabetes: from chaos to harmony. J Clin Pathol 2008;61:983-7.
53. Howe-Davies S, Simpson RW, Turner RC. Control of maturity-onset diabetes by monitoring fasting blood glucose and body weight. Diabetes Care 1980;3:607-10.
54. Muir A, Howe-Davies SA, Turner RC. General practice care of non-insulin-dependent diabetes with fasting blood glucose measurements. Am J Med 1982;73:637-40.
55. Harris MI. Undiagnosed NIDDM: clinical and public health issues. Diabetes Care 1993;16:642-52.
56. Troisi RJ, Cowie CC, Harris MI. Diurnal variation in fasting plasma glucose: implications for diagnosis of diabetes in patients examined in the afternoon. JAMA 2000;284:3157-9.
57. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315-7.
58. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In: Sonnenwirth A, Jarett L, eds. Clinical Laboratory Methods and Diagnosis. St. Louis, MO: C.V. Mosby Co., 1980:149-92.
59. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. St. Louis: Elsevier Saunders, 2006:837-902.

DRAFT

60. Gambino R, Piscitelli J, Ackattupathil TA, Theriault JL, Andrin RD, Sanfilippo ML, Etienne M. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem 2009;55:1019-21.
61. Ladenson JH, Tsai LM, Michael JM, Kessler G, Joist JH. Serum versus heparinized plasma for eighteen common chemistry tests: is serum the appropriate specimen? Am J Clin Pathol 1974;62:545-52.
62. Stahl M, Jorgensen LG, Hyltoft Petersen P, Brandslund I, de Fine Olivarius N, Borch-Johnsen K. Optimization of preanalytical conditions and analysis of plasma glucose. 1. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. Scand J Clin Lab Invest 2001;61:169-79.
63. Carstensen B, Lindstrom J, Sundvall J, Borch-Johnsen K, Tuomilehto J. Measurement of blood glucose: comparison between different types of specimens. Ann Clin Biochem 2008;45:140-8.
64. Boyanton BL, Jr., Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242-7.
65. Miles RR, Roberts RF, Putnam AR, Roberts WL. Comparison of serum and heparinized plasma samples for measurement of chemistry analytes. Clin Chem 2004;50:1704-5.
66. Larsson-Cohn U. Differences between capillary and venous blood glucose during oral glucose tolerance tests. Scand J Clin Lab Invest 1976;36:805-8.
67. Lind T, de Groot HA, Brown G, Cheyne GA. Observations on blood glucose and insulin determinations. Br Med J 1972;3:320-3.
68. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th ed. St. Louis: Elsevier Saunders, 2010:in press.
69. Tchobroutsky G. Blood glucose levels in diabetic and non-diabetic subjects. Diabetologia 1991;34:67-73.
70. Blunt BA, Barrett-Connor E, Wingard DL. Evaluation of fasting plasma glucose as screening test for NIDDM in older adults. Rancho Bernardo Study. Diabetes Care 1991;14:989-93.
71. DECODE. Consequences of the new diagnostic criteria for diabetes in older men and women. DECODE Study (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe). Diabetes Care 1999;22:1667-71.
72. Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U. Insulin action and age. European Group for the Study of Insulin Resistance (EGIR). Diabetes 1996;45:947-53.
73. Imbeault P, Prins JB, Stolic M, Russell AW, O'Moore-Sullivan T, Despres JP, et al. Aging per se does not influence glucose homeostasis: in vivo and in vitro evidence. Diabetes Care 2003;26:480-4.
74. 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;132:838-46.
75. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. Clin Chem 1999;45:321-3.
76. Stockl D, Baadenhuijsen H, Fraser CG, Libeer JC, Petersen PH, Ricos C. Desirable routine analytical goals for quantities assayed in serum. Discussion paper from the members of the external quality assessment (EQA) Working Group A on analytical goals in laboratory medicine. Eur J Clin Chem Clin Biochem 1995;33:157-69.
77. Fraser CG. The necessity of achieving good laboratory performance. Diabet Med 1990;7:490-3.
78. Olefsky JM, Reaven GM. Insulin and glucose responses to identical oral glucose tolerance tests performed forty-eight hours apart. Diabetes 1974;23:449-53.

DRAFT

79. Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE, Turner RC. Within- and between-subject variation in commonly measured anthropometric and biochemical variables. Clin Chem 1999;45:561-6.
80. Sebastian-Gambaro MA, Liron-Hernandez FJ, Fuentes-Arderiu X. Intra- and inter-individual biological variability data bank. Eur J Clin Chem Clin Biochem 1997;35:845-52.
81. Mooy JM, Grootenhuis PA, de Vries H, Kostense PJ, Popp-Snijders C, Bouter LM, Heine RJ. Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. Diabetologia 1996;39:298-305.
82. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. Arch Intern Med 2007;167:1545-51.
83. Lacher DA, Hughes JP, Carroll MD. Estimate of biological variation of laboratory analytes based on the third national health and nutrition examination survey. Clin Chem 2005;51:450-2.
84. Westgard QC. Desirable specifications for total error, imprecision, and bias, derived from biologic variation. http://www.westgard.com/biodatabase1.htm (11 July 2009).
85. 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;117:22-8.
86. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001;345:1359-67.
87. American Diabetes Association. Standards of medical care in diabetes --2009. Diabetes Care 2009;32 (Suppl 1):S13-61.
88. Harris MI, Cowie CC, Howie LJ. Self-monitoring of blood glucose by adults with diabetes in the United States population. Diabetes Care 1993;16:1116-23.
89. Self-monitoring of blood glucose among adults with diabetes - United States, 1997-2006. JAMA 2007;298:2861-63.
90. American Diabetes Association. Self-monitoring of blood glucose. Diabetes Care 1996;19 (Supp 1):S62-6.
91. Ipp E, Aquino RL, Christenson P. Point: Self-monitoring of blood glucose in type 2 diabetic patients not receiving insulin: the sanguine approach. Diabetes Care 2005;28:1528-30.
92. Davidson MB. Counterpoint: Self-monitoring of blood glucose in type 2 diabetic patients not receiving insulin: a waste of money. Diabetes Care 2005;28:1531-3.
93. Faas A, Schellevis FG, Van Eijk JT. The efficacy of self-monitoring of blood glucose in NIDDM subjects. A criteria-based literature review. Diabetes Care 1997;20:1482-6.
94. Coster S, Gulliford MC, Seed PT, Powrie JK, Swaminathan R. Self-monitoring in Type 2 diabetes mellitus: a meta-analysis. Diabet Med 2000;17:755-61.
95. Harris MI. Frequency of blood glucose monitoring in relation to glycemic control in patients with type 2 diabetes. Diabetes Care 2001;24:979-82.
96. Davis WA, Bruce DG, Davis TM. Is self-monitoring of blood glucose appropriate for all type 2 diabetic patients? The Fremantle Diabetes Study. Diabetes Care 2006;29:1764-70.
97. Guerci B, Drouin P, Grange V, Bougneres P, Fontaine P, Kerlan V, et al. Self-monitoring of blood glucose significantly improves metabolic control in patients with type 2 diabetes mellitus: the Auto-Surveillance Intervention Active (ASIA) study. Diabetes Metab 2003;29:587-94.
98. Davidson MB, Castellanos M, Kain D, Duran P. The effect of self monitoring of blood glucose concentrations on glycated hemoglobin levels in diabetic patients not taking insulin: a blinded, randomized trial. Am J Med 2005;118:422-5.

DRAFT

99. Franciosi M, Pellegrini F, De Berardis G, Belfiglio M, Di Nardo B, Greenfield S, et al. Self-monitoring of blood glucose in non-insulin-treated diabetic patients: a longitudinal evaluation of its impact on metabolic control. Diabet Med 2005;22:900-6.
100. Karter AJ, Parker MM, Moffet HH, Spence MM, Chan J, Ettner SL, Selby JV. Longitudinal study of new and prevalent use of self-monitoring of blood glucose. Diabetes Care 2006;29:1757-63.
101. Martin S, Schneider B, Heinemann L, Lodwig V, Kurth HJ, Kolb H, Scherbaum WA. Self-monitoring of blood glucose in type 2 diabetes and long-term outcome: an epidemiological cohort study. Diabetologia 2006;49:271-8.
102. Welschen LM, Bloemendal E, Nijpels G, Dekker JM, Heine RJ, Stalman WA, Bouter LM. Self-monitoring of blood glucose in patients with type 2 diabetes who are not using insulin. Cochrane Database Syst Rev 2005:CD005060.
103. Welschen LM, Bloemendal E, Nijpels G, Dekker JM, Heine RJ, Stalman WA, Bouter LM. Self-monitoring of blood glucose in patients with type 2 diabetes who are not using insulin: a systematic review. Diabetes Care 2005;28:1510-7.
104. Farmer A, Wade A, Goyder E, Yudkin P, French D, Craven A, et al. Impact of self monitoring of blood glucose in the management of patients with non-insulin treated diabetes: open parallel group randomised trial. BMJ 2007;335:132.
105. 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;336:1177-80.
106. O'Kane MJ, Bunting B, Copeland M, Coates VE. Efficacy of self monitoring of blood glucose in patients with newly diagnosed type 2 diabetes (ESMON study): randomised controlled trial. BMJ 2008;336:1174-7.
107. Montori VM, Fernandez-Balsells M. Glycemic control in type 2 diabetes: time for an evidence-based about-face? Ann Intern Med 2009;150:803-8.
108. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. Endocr Rev 1991;12:356-71.
109. Tang Z, Lee JH, Louie RF, Kost GJ. Effects of different hematocrit levels on glucose measurements with handheld meters for point-of-care testing. Arch Pathol Lab Med 2000;124:1135-40.
110. American Diabetes Association. Consensus statement on self-monitoring of blood glucose. Diabetes Care 1994;17:81-6.
111. Tate PF, Clements CA, Walters JE. Accuracy of home blood glucose monitors. Diabetes Care 1992;15:536-8.
112. Chan JC, Wong RY, Cheung CK, Lam P, Chow CC, Yeung VT, et al. Accuracy, precision and user-acceptability of self blood glucose monitoring machines. Diabetes Res Clin Pract 1997;36:91-104.
113. Kabadi UM, O'Connell KM, Johnson J, Kabadi M. The effect of recurrent practice at home on the acceptability of capillary blood glucose readings. Accuracy of self blood glucose testing. Diabetes Care 1994;17:1110-23.
114. Burnett RW, D'Orazio P, Fogh-Andersen N, Kuwa K, Kulpmann WR, Larsson L, et al. IFCC recommendation on reporting results for blood glucose. Clin Chim Acta 2001;307:205-9.
115. D'Orazio P, Burnett RW, Fogh-Andersen N, Jacobs E, Kuwa K, Kulpmann WR, et al. Approved IFCC recommendation on reporting results for blood glucose (abbreviated). Clin Chem 2005;51:1573-6.
116. Steffes MW, Sacks DB. Measurement of circulating glucose concentrations: the time is now for consistency among methods and types of samples. Clin Chem 2005;51:1569-70.

DRAFT

117. Weitgasser R, Gappmayer B, Pichler M. Newer portable glucose meters--analytical improvement compared with previous generation devices? Clin Chem 1999;45:1821-5.
118. Bohme P, Floriot M, Sirveaux MA, Durain D, Ziegler O, Drouin P, Guerci B. Evolution of analytical performance in portable glucose meters in the last decade. Diabetes Care 2003;26:1170-5.
119. Skeie S, Thue G, Nerhus K, Sandberg S. Instruments for self-monitoring of blood glucose: comparisons of testing quality achieved by patients and a technician. Clin Chem 2002;48:994-1003.
120. American Diabetes Association. Consensus statement on self-monitoring of blood glucose. Diabetes Care 1987;10:93-9.
121. Barr JT, Betschart J, Bracey A. Ancillary (bedside) blood glucose testing in acute and chronic care facilities. NCCLS 1994;14:1-14.
122. ISO. ISO 15197, First edition 2003-05-01, In vitro diagnostic test systems — Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus. Switzerland: ISO, 2003.
123. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl SL. Evaluating clinical accuracy of systems for self-monitoring of blood glucose. Diabetes Care 1987;10:622-8.
124. Skeie S, Thue G, Sandberg S. Patient-derived quality specifications for instruments used in self-monitoring of blood glucose. Clin Chem 2001;47:67-73.
125. Boyd JC, Bruns DE. Quality specifications for glucose meters: assessment by simulation modeling of errors in insulin dose. Clin Chem 2001;47:209-14.
126. Novis DA, Jones BA. Interinstitutional comparison of bedside blood glucose monitoring program characteristics, accuracy performance, and quality control documentation: a College of American Pathologists Q-Probes study of bedside blood glucose monitoring performed in 226 small hospitals. Arch Pathol Lab Med 1998;122:495-502.
127. Brunner GA, Ellmerer M, Sendlhofer G, Wutte A, Trajanoski Z, Schaupp L, et al. Validation of home blood glucose meters with respect to clinical and analytical approaches. Diabetes Care 1998;21:585-90.
128. Weinzimer SA, Beck RW, Chase HP, Fox LA, Buckingham BA, Tamborlane WV, et al. Accuracy of newer-generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) inpatient exercise study. Diabetes Technol Ther 2005;7:675-80; discussion 81-3.
129. Mahoney J, Ellison J. Assessing the quality of glucose monitor studies: a critical evaluation of published reports. Clin Chem 2007;53:1122-8.
130. Kristensen GB, Nerhus K, Thue G, Sandberg S. Standardized evaluation of instruments for self-monitoring of blood glucose by patients and a technologist. Clin Chem 2004;50:1068-71.
131. Wiener RS, Wiener DC, Larson RJ. Benefits and risks of tight glucose control in critically ill adults: a meta-analysis. JAMA 2008;300:933-44.
132. Scott MG, Bruns DE, Boyd JC, Sacks DB. Tight glucose control in the intensive care unit: are glucose meters up to the task? Clin Chem 2009;55:18-20.
133. Dungan K, Chapman J, Braithwaite SS, Buse J. Glucose measurement: confounding issues in setting targets for inpatient management. Diabetes Care 2007;30:403-9.
134. Boyd JC, Bruns DE. Monte Carlo simulation in establishing analytical quality requirements for clinical laboratory tests meeting clinical needs. Methods Enzymol 2009;467:411-33.
135. Finkielman JD, Oyen LJ, Afessa B. Agreement between bedside blood and plasma glucose measurement in the ICU setting. Chest 2005;127:1749-51.
136. Hoedemaekers CW, Klein Gunnewiek JM, Prinsen MA, Willems JL, Van der Hoeven JG. Accuracy of bedside glucose measurement from three glucometers in critically ill patients. Crit Care Med 2008;36:3062-6.

DRAFT

137. Meynaar IA, van Spreuwel M, Tangkau PL, Dawson L, Sleeswijk Visser S, Rijks L, Vlieland TV. Accuracy of AccuChek glucose measurement in intensive care patients. Crit Care Med 2009;37:2691-6.
138. Kanji S, Buffie J, Hutton B, Bunting PS, Singh A, McDonald K, et al. Reliability of point-of-care testing for glucose measurement in critically ill adults. Crit Care Med 2005;33:2778-85.
139. Schiffrin A, Belmonte M. Multiple daily self-glucose monitoring: its essential role in long-term glucose control in insulin-dependent diabetic patients treated with pump and multiple subcutaneous injections. Diabetes Care 1982;5:479-84.
140. Nathan D. The importance of intensive supervision in determining the efficacy of insulin pump therapy. Diabetes Care 1983;6:295-7.
141. de Veciana M, Major CA, Morgan MA, Asrat T, Toohey JS, Lien JM, Evans AT. Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. N Engl J Med 1995;333:1237-41.
142. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM, Sacks DB. Tests of glycemia in diabetes. Diabetes Care 2004;27:1761-73.
143. Garg S, Zisser H, Schwartz S, Bailey T, Kaplan R, Ellis S, Jovanovic L. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor: a randomized controlled trial. Diabetes Care 2006;29:44-50.
144. Chetty VT, Almulla A, Odueyungbo A, Thabane L. The effect of continuous subcutaneous glucose monitoring (CGMS) versus intermittent whole blood finger-stick glucose monitoring (SBGM) on hemoglobin A1c (HBA1c) levels in Type I diabetic patients: a systematic review. Diabetes Res Clin Pract 2008;81:79-87.
145. Tamborlane WV, Beck RW, Bode BW, Buckingham B, Chase HP, Clemons R, et al. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 2008;359:1464-76.
146. Haupt K, Mosbach K. Plastic antibodies: developments and applications. Trends Biotechnol 1998;16:468-75.
147. Chen G, Guan Z, Chen CT, Fu L, Sundaresan V, Arnold FH. A glucose-sensing polymer. Nat Biotechnol 1997;15:354-7.
148. James TC, Sananayake DRAS, Shinkai S. A glucose-selective molecular fluorescence sensor. Angewandte Chemie International Edition in English 1994;33:2207-09.
149. Birch DJS, Imhof RE. Time-domain fluorescence spectroscopy using time-correlated single-photon counting. Topics in Fluorescence Spectroscopy 1994;1:1-95.
150. Tolosa L, Szmacinski H, Rao G, Lakowicz JR. Lifetime-based sensing of glucose using energy transfer with a long lifetime donor. Anal Biochem 1997;250:102-8.
151. Rolinski OJ, Birch DJS, McCartney LJ, Pickup JC. Near-infrared assay for glucose determination. Soc Photo-optical Instrumentation Engineers Proc 1999;3602:6-14.
152. Marvin JS, Hellinga HW. Engineering biosensors by introducing fluorescent allosteric signal transducers: construction of a novel glucose sensor. J Am Chem Soc 1998;120:7-11.
153. Wentholt IM, Vollebregt MA, Hart AA, Hoekstra JB, DeVries JH. Comparison of a needle-type and a microdialysis continuous glucose monitor in type 1 diabetic patients. Diabetes Care 2005;28:2871-6.
154. Khalil OS. Non-invasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium. Diabetes Technol Ther 2004;6:660-97.
155. Arnold MA, Small GW. Noninvasive glucose sensing. Anal Chem 2005;77:5429-39.
156. Tura A, Maran A, Pacini G. Non-invasive glucose monitoring: assessment of technologies and devices according to quantitative criteria. Diabetes Res Clin Pract 2007;77:16-40.
157. Sieg A, Guy RH, Delgado-Charro MB. Noninvasive and minimally invasive methods for transdermal glucose monitoring. Diabetes Technol Ther 2005;7:174-97.

DRAFT

158. Kohl M, Essenpreis M, Cope M. The influence of glucose concentration upon the transport of light in tissue-simulating phantoms. Phys Med Biol 1995;40:1267-87.
159. Heise HM, Lampen P. Transcutaneous glucose measurements using near-infrared spectroscopy: validation of statistical calibration models. Diabetes Care 2000;23:1208-10.
160. Gutman S, Bernhardt P, Pinkos A, Moxey-Mims M, Knott T, Cooper J. Regulatory aspects of noninvasive glucose measurements. Diabetes Technol Ther 2002;4:779-81.
161. Rhiel MH, Amrhein MI, Marison IW, von Stockar U. The influence of correlated calibration samples on the prediction performance of multivariate models based on mid-infrared spectra of animal cell cultures. Anal Chem 2002;74:5227-36.
162. Arnold MA, Liu L, Olesberg JT. Selectivity assessment of noninvasive glucose measurements based on analysis of multivariate calibration vectors. J Diabetes Sci Technol 2007;1:454-62.
163. Small GW. Chemometrics and near-infrared spectroscopy: Avoiding the pitfalls. Trends in Anal Chem 2006;25:1057-66.
164. Gabrielsson J, Trygg J. Recent developments in multivariate calibration. Crit Rev Anal Chem 2006;36:243-55.
165. Arnold MA, Small GW, Xiang D, Qui J, Murhammer DW. Pure component selectivity analysis of multivariate calibration models from near-infrared spectra. Anal Chem 2004;76:2583-90.
166. Arnold MA, Olesberg JT, Small GW. Near-infrared spectroscopy for noninvasive glucose sensing. In: Cunningham D, Stenken JA, eds. Analytical Chemistry of *In Vivo* Glucose Measurements: Wiley Chemical Analysis Series, 2009:357-90.
167. Shih W-C, Bechtel KB, Feld MS. Noninvasive glucose sensing with Raman spectroscopy. In: Cunningham D, Stenken JA, eds. Analytical Chemistry of *In Vivo* Glucose Measurements: Wiley Chemical Analysis Series, 2009:391-419.
168. The Expert Committee. The expert committee on the diagnosis and classification of diabetes mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1999;22:S5-S19.
169. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.
170. Gimeno S, Ferreira S, Franco L, Iunes M. Comparison of glucose tolerance categories according to World Health Organization and American Diabetes Association diagnostic criteria in a population-based study in Brazil. The Japanese-Brazilian Diabetes Study Group. Diabetes Care 1998;21:1889-92.
171. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study [see comments]. Diabetes Care 1999;22:920-4.
172. Perry RC, Baron AD. Impaired glucose tolerance. Why is it not a disease? Diabetes Care 1999;22:883-5.
173. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. Diabetes Res Clin Pract 1999;44:21-6.
174. Harris MI, Eastman RC, Cowie CC, Flegal KM, Eberhardt MS. Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria [see comments]. Diabetes Care 1997;20:1859-62.
175. Balkau B. The DECODE study. Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe. Diabetes Metab 2000;26:282-6.

DRAFT

176. Balkau B, Forhan A, Eschwege E. Two hour plasma glucose is not unequivocally predictive for early death in men with impaired fasting glucose: more results from the Paris Prospective Study. Diabetologia 2002;45:1224-30.
177. Norberg M, Eriksson JW, Lindahl B, Andersson C, Rolandsson O, Stenlund H, Weinehall L. A combination of HbA1c, fasting glucose and BMI is effective in screening for individuals at risk of future type 2 diabetes: OGTT is not needed. J Intern Med 2006;260:263-71.
178. Ko GT, Chan JC, Woo J, Lau E, Yeung VT, Chow CC, Cockram CS. The reproducibility and usefulness of the oral glucose tolerance test in screening for diabetes and other cardiovascular risk factors. Ann Clin Biochem 1998;35:62-7.
179. Libman IM, Barinas-Mitchell E, Bartucci A, Robertson R, Arslanian S. Reproducibility of the oral glucose tolerance test in overweight children. J Clin Endocrinol Metab 2008;93:4231-7.
180. Moses RG, Patterson MJ, Regan JM, Chaunchaiyakul R, Taylor NA, Jenkins AB. A non-linear effect of ambient temperature on apparent glucose tolerance. Diabetes Res Clin Pract 1997;36:35-40.
181. Ganda OP, Day JL, Soeldner JS, Connon JJ, Gleason RE. Reproducibility and comparative analysis of repeated intravenous and oral glucose tolerance tests. Diabetes 1978;27:715-25.
182. Barzilay JI, Spiekerman CF, Wahl PW, Kuller LH, Cushman M, Furberg CD, et al. Cardiovascular disease in older adults with glucose disorders: comparison of American Diabetes Association criteria for diabetes mellitus with WHO criteria [see comments]. Lancet 1999;354:622-5.
183. DECODE Study Group. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe [see comments]. Lancet 1999;354:617-21.
184. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477-86.
185. 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;361:1339-48.
186. Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. Diabetes Care 1998;21:B161-7.
187. American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2000;23:S77-9.
188. Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care 2007;30 Suppl 2:S251-60.
189. Williams CB, Iqbal S, Zawacki CM, Yu D, Brown MB, Herman WH. Effect of selective screening for gestational diabetes. Diabetes Care 1999;22:418-21.
190. Danilenko-Dixon DR, Van Winter JT, Nelson RL, Ogburn PL, Jr. Universal versus selective gestational diabetes screening: application of 1997 American Diabetes Association recommendations. Am J Obstet Gynecol 1999;181:798-802.
191. Corcoy R, Garcia-Patterson A, Pau E, Pascual E, Altirriba O, Adelantado JM, de Leiva A. Is selective screening for gestational diabetes mellitus worthwhile everywhere? Acta Diabetol 2004;41:154-7.
192. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991-2002.
193. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes 2009;58:453-9.

DRAFT

194. International Association of Diabetes and Pregnancy Study Groups. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676-82.
195. American Diabetes Association. Tests of glycemia in diabetes. Diabetes Care 1999;22:S77-9.
196. Sacks DB. Diabetes mellitus. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics, 5th ed. St. Louis: Elsevier Saunders, 2010:in press.
197. Kitabchi AE, Umpierrez GE, Murphy MB, Barrett EJ, Kreisberg RA, Malone JI, Wall BM. Hyperglycemic crises in diabetes. Diabetes Care 2004;27 Suppl 1:S94-102.
198. Kreisberg RA. Diabetic ketoacidosis: new concepts and trends in pathogenesis and treatment. Ann Intern Med 1978;88:681-95.
199. Owen OE, Trapp VE, Skutches CL, Mozzoli MA, Hoeldtke RD, Boden G, Reichard GA, Jr. Acetone metabolism during diabetic ketoacidosis. Diabetes 1982;31:242-8.
200. Stephens JM, Sulway MJ, Watkins PJ. Relationship of blood acetoacetate and 3-hydroxybutyrate in diabetes. Diabetes 1971;20:485-9.
201. Porter WH, Yao HH, Karounos DG. Laboratory and clinical evaluation of assays for beta-hydroxybutyrate. Am J Clin Pathol 1997;107:353-8.
202. American Diabetes Association. Tests of glycemia (Position Statement). Diabetes Care 2000;23 (Suppl 1):S80-2.
203. Csako G. Causes, consequences, and recognition of false-positive reactions for ketones. Clin Chem 1990;36:1388-9.
204. Rosenbloom AL, Malone JI. Recognition of impending ketoacidosis delayed by ketone reagent strip failure. JAMA 1978;240:2462-4.
205. McMurray CH, Blanchflower WJ, Rice DA. Automated kinetic method for D-3-hydroxybutyrate in plasma or serum. Clin Chem 1984;30:421-5.
206. Koch DD, Feldbruegge DH. Optimized kinetic method for automated determination of beta-hydroxybutyrate. Clin Chem 1987;33:1761-6.
207. D'arrigo T. Beyond blood glucose. Diabetes Forecast 1999;52:37-8.
208. Westphal SA. The occurrence of diabetic ketoacidosis in non-insulin-dependent diabetes and newly diagnosed diabetic adults. Am J Med 1996;101:19-24.
209. Wiggam MI, O'Kane MJ, Harper R, Atkinson AB, Hadden DR, Trimble ER, Bell PM. Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxybutyrate concentration as the endpoint of emergency management. A randomized controlled study. Diabetes Care 1997;20:1347-52.
210. Umpierrez GE, Watts NB, Phillips LS. Clinical utility of beta-hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diabetes Care 1995;18:137-8.
211. Noyes KJ, Crofton P, Bath LE, Holmes A, Stark L, Oxley CD, Kelnar CJ. Hydroxybutyrate near-patient testing to evaluate a new end-point for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. Pediatr Diabetes 2007;8:150-6.
212. American Diabetes Association. Implications of the Diabetes Control and Complications Trial (position statement). Diabetes Care 2000;23 (Suppl 1):S24-6.
213. Kitzmiller JL, Block JM, Brown FM, Catalano PM, Conway DL, Coustan DR, et al. Managing preexisting diabetes for pregnancy: summary of evidence and consensus recommendations for care. Diabetes Care 2008;31:1060-79.
214. Davidson MB. Diabetes research and diabetes care. Where do we stand? Diabetes Care 1998;21:2152-60.
215. American Diabetes Association. Provider Notes: the newsletter of the ADA/NCQA Provider Recognition Program. 2000;1:1-4.

DRAFT

216. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, Zinman B. Management of hyperglycaemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia 2006;49:1711-21.
217. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med 1981;70:325-30.
218. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Chem 1986;32:B64-70.
219. Svendsen PA, Lauritzen T, Soegaard U, Nerup J. Glycosylated haemoglobin and steady-state mean blood glucose concentration in Type 1 (insulin-dependent) diabetes. Diabetologia 1982;23:403-5.
220. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin measured by automated affinity HPLC correlates with both short-term and long-term antecedent glycemia. Clin Chem 1994;40:1317-21.
221. Murata GH, Hoffman RM, Duckworth WC, Wendel CS, Shah JH. 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;327:319-23.
222. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50:2239-44.
223. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the hemoglobin A1c assay into estimated average glucose values. Diabetes Care 2008;31:1473-8.
224. Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. Br Med J (Clin Res Ed) 1984;288:1484-6.
225. 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;18:440-7.
226. Little RR, Wiedmeyer HM, England JD, Wilke AL, Rohlfing CL, Wians FH, Jr., et al. Interlaboratory standardization of measurements of glycohemoglobins. Clin Chem 1992;38:2472-8.
227. Bodor GS, Little RR, Garrett N, Brown W, Goldstein DE, Nahm MH. Standardization of glycohemoglobin determinations in the clinical laboratory: three years of experience. Clin Chem 1992;38:2414-8.
228. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Effect of calibration on dispersion of glycohemoglobin values determined by 111 laboratories using 21 methods. Clin Chem 1994;40:138-44.
229. Goldstein DE, Little RR. Bringing order to chaos: the National Glycohemoglobin Standardization Program. Contemp Int Med 1997;9:27-32.
230. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE. The national glycohemoglobin standardization program: a five-year progress report. Clin Chem 2001;47:1985-92.
231. DCCT. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. Clin Chem 1987;33:2267-71.
232. Little RR, Rohlfing CL, Sacks DB. Status of HbA1c measurement and goals for improvement: From chaos to order for improving diabetes care. Clin Chem 2011;in press.
233. 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;40:78-89.
234. 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

DRAFT

schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166-74.

235. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson J, et al. The IFCC reference measurement system for HbA1c: a 6-year progress report. Clin Chem 2008;54:240-8.
236. Sacks DB. Translating hemoglobin A1c into average blood glucose: Implications for clinical chemistry. Clin Chem 2008;54:1756-58.
237. Wiener K, Roberts NB. Age does not influence levels of HbA1c in normal subject. QJ Med 1999;92:169-73.
238. Kilpatrick ES, Dominiczak MH, Small M. The effects of ageing on glycation and the interpretation of glycaemic control in Type 2 diabetes. QJ Med 1996;89:307-12.
239. Nuttall FQ. Effect of age on the percentage of hemoglobin A1c and the percentage of total glycohemoglobin in non-diabetic persons. J Lab Clin Med 1999;134:451-3.
240. 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;31:1991-6.
241. 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;152:770-7.
242. Little RR, Sacks DB. HbA1c: how do we measure it and what does it mean? Curr Opin Endocrinol Diabetes Obes 2009;16:113-8.
243. 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;30:2453-7.
244. Davie SJ, Gould BJ, Yudkin JS. Effects of vitamin C on glycosylation of proteins. Diabetes Care 2000;23:339-44.
245. Ceriello A, Giugliano D, Quatraro A, Donzella C, Dipalo G, Lefebvre PJ. Vitamin E reduction of protein glycosylation in diabetes. New prospect for prevention of diabetic complications? Diabetes Care 1991;14:68-72.
246. Tarim O, Kucukerdogan A, Gunay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999;41:357-62.
247. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin [Review]. Clin Chem 2001;47:153-63.
248. Roberts WL, Chiasera JM, Ward-Cook KM. Glycohemoglobin results in samples with hemoglobin C or S trait: a comparison of four test systems. Clin Chem 1999;45:906-9.
249. Weykamp CW, Penders TJ, Muskiet FA, van der Slik W. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 methods. Clin Chem 1993;39:1717-23.
250. 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;23:339-44.
251. Rendell M, Brannan C, Nierenberg J, Rasbold K, Hestorff T. Fingerstick glycosylated hemoglobin, plasma protein, and albumin. Diabetes Care 1987;10:629-32.
252. Ferrell RE, Hanis CL, Aguilar L, Tulloch B, Garcia C, Schull WJ. Glycosylated hemoglobin determination from capillary blood samples. Utility in an epidemiologic survey of diabetes. Am J Epidemiol 1984;119:159-66.
253. Little RR, England JD, Wiedmeyer HM, Goldstein DE. Effects of whole blood storage on results for glycosylated hemoglobin as measured by ion-exchange chromatography, affinity chromatography, and colorimetry. Clin Chem 1983;29:1113-5.

DRAFT

254. Little RR, Rohlfing CL, Tennill AL, Connolly S, Hanson S. Effects of sample storage conditions on glycated hemoglobin measurement: evaluation of five different high performance liquid chromatography methods. Diabetes Technol Ther 2007;9:36-42.
255. Baglin SK, Brown AS. Two capillary blood-collection techniques for estimating glycohemoglobin compared. Clin Chem 1995;41:330-2.
256. Voss EM, Cembrowski GS, Clasen BL, Spencer ML, Ainslie MB, Haig B. Evaluation of capillary collection system for HbA1c specimens. Diabetes Care 1992;15:700-1.
257. Little RR, Wiedmeyer HM, D. H. A simple blood collection device for analysis of glycohemoglobin (GHB). Clin Chem 1998;44:A139.
258. Baynes JW, Bunn HF, Goldstein D, Harris M, Martin DB, Peterson C, Winterhalter K. National Diabetes Data Group: report of the expert committee on glucosylated hemoglobin. Diabetes Care 1984;7:602-6.
259. Marshall SM, Barth JH. Standardization of HbA1c measurements: a consensus statement. Ann Clin Biochem 2000;37:45-6.
260. Goodall I, Colman PG, Schneider HG, McLean M, Barker G. Desirable performance standards for HbA(1c) analysis - precision, accuracy and standardisation: consensus statement of the Australasian Association of Clinical Biochemists (AACB), the Australian Diabetes Society (ADS), the Royal College of Pathologists of Australasia (RCPA), Endocrine Society of Australia (ESA), and the Australian Diabetes Educators Association (ADEA). Clin Chem Lab Med 2007;45:1083-97.
261. Sacks DB. CAP Surveys: Participant Summary for Glycohemoglobin Survey 2010 Set GH2-A. Northfield, IL: College of American Pathologists, 2010.
262. Goldstein DE, Peth SB, England JD, Hess RL, Da Costa J. Effects of acute changes in blood glucose on HbA1c. Diabetes 1980;29:623-8.
263. 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;22:1785-9.
264. Kennedy L, Herman WH, Strange P, Harris A. 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;29:1-8.
265. Khunti K, Stone MA, Burden AC, Turner D, Raymond NT, Burden M, Baker R. Randomised controlled trial of near-patient testing for glycated haemoglobin in people with type 2 diabetes mellitus. Br J Gen Pract 2006;56:511-7.
266. Larsen ML, Horder M, Mogensen EF. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. N Engl J Med 1990;323:1021-5.
267. American Association of Clinical Endocrinologists Board of Directors/American College of Endocrinologists Board of Trustees. American Association of Clinical Endocrinologists/American College of Endocrinology statement on the use of hemoglobin A1c for the diagnosis of diabetes. Endocr Pract 2010;16:155-6.
268. 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;325:836-42.
269. 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;48:870-80.
270. American Diabetes Association. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the

DRAFT

Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care 2007;30:2399-400.
271. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab 2008;4:200-13.
272. 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;57:1034-42.
273. Stoy J, Greeley SA, Paz VP, Ye H, Pastore AN, Skowron KB, et al. Diagnosis and treatment of neonatal diabetes: a United States experience. Pediatr Diabetes 2008;9:450-9.
274. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345:971-80.
275. 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;51:1346-55.
276. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009;360:1646-54.
277. Nagi DK, Hendra TJ, Ryle AJ, Cooper TM, Temple RC, Clark PM, et al. The relationships of concentrations of insulin, intact proinsulin and 32-33 split proinsulin with cardiovascular risk factors in type 2 (non-insulin-dependent) diabetic subjects. Diabetologia 1990;33:532-7.
278. 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.
279. Nadler ST, Stoehr JP, Schueler KL, Tanimoto G, Yandell BS, Attie AD. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. Proc Natl Acad Sci USA 2000;97:11371-6.
280. Ziegler AG, Bachmann W, Rabl W. Prophylactic insulin treatment in relatives at high risk for type 1 diabetes. Diabetes Metab Rev 1993;9:289-93.
281. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, et al. Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia 1996;39:807-12.
282. 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;1079:320-6.
283. Kukreja A, Maclaren NK. Autoimmunity and diabetes. J Clin Endocrinol Metab 1999;84:4371-8.
284. 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;27:1399-404.
285. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331-6.
286. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341-5.
287. 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;359:2208-19.
288. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med 2000;343:702-9.

DRAFT

289. Taylor SI, Arioglu E. Genetically defined forms of diabetes in children. J Clin Endocrinol Metab 1999;84:4390-6.
290. Fajans SS, Bell GI, Bowden DW, Halter JB, Polonsky KS. Maturity onset diabetes of the young (MODY). Diabet Med 1996;13:S90-5.
291. Todd JA. Genetics of type 1 diabetes. Pathol Biol (Paris) 1997;45:219-27.
292. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221-9.
293. Harrison LC. Risk assessment, prediction and prevention of type 1 diabetes. Pediatric Diabetes 2001;2:71-82.
294. 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;85:3793-7.
295. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.
296. 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;39:857-64.
297. Maclaren NK, Kukreja A. Type 1 diabetes. In: Sly WS, ed. The metabolic and molecular bases of inherited disease, 8th ed. St. Louis: McGraw-Hill, 2001:1471-88.
298. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL. Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 1983;222:1337-9.
299. 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 [published erratum appears in Nature 1990 Oct 25;347(6295):782]. Nature 1990;347:151-6.
300. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 1992;89:283-92.
301. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 1993;92:1608-16.
302. Lan MS, Wasserfall C, Maclaren NK, Notkins AL. IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 1996;93:6367-70.
303. 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;93:2307-11.
304. 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 U S A 2007;104:17040-5.
305. 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;57:2693-7.
306. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009;373:2027-33.

DRAFT

307. 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;12:279-87.
308. 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;45:926-33.
309. Schott M, Schatz D, Atkinson M, Krischer J, Mehta H, Vold B, Maclaren N. GAD65 autoantibodies increase the predictability but not the sensitivity of islet cell and insulin autoantibodies for developing insulin dependent diabetes mellitus. J Autoimmun 1994;7:865-72.
310. 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 [published erratum appears in Lancet 1998 Jan 31;351(9099):376]. Lancet 1997;350:1288-93.
311. Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (Latent Autoimmune Diabetes of the Adult): Definition, characterization, and potential prevention. Diabetes Care 2001;24:1460-7.
312. Palmer JP, Hampe CS, Chiu H, Goel A, Brooks-Worrell BM. Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age? Diabetes 2005;54 Suppl 2:S62-7.
313. 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;1079:60-6.
314. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. Diabetes 2000;49:218-24.
315. Zimmet P, Turner R, McCarty D, Rowley M, Mackay I. Crucial points at diagnosis. Type 2 diabetes or slow type 1 diabetes. Diabetes Care 1999;22:B59-64.
316. Petersen JS, Dyrberg T, Damm P, Kuhl C, Molsted-Pedersen L, Buschard K. GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. Diabetologia 1996;39:1329-33.
317. Fuchtenbusch 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;46:1459-67.
318. Diabetes Prevention Trial - Type 1 Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 2002;346:1685-91.
319. Siljander H, Harkonen 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;25:615-22.
320. Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA. A novel micro-assay for insulin autoantibodies. J Autoimmun 1997;10:473-8.
321. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes 2003;52:1128-36.
322. 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;37:344-50.
323. Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008;51:846-52.

DRAFT

324. Mire-Sluis AR, Gaines Das R, Lernmark A. The World Health Organization International Collaborative Study for islet cell antibodies. Diabetologia 2000;43:1282-92.
325. Gleichmann H, Bottazzo GF. Progress toward standardization of cytoplasmic islet cell-antibody assay. Diabetes 1987;36:578-84.
326. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS. Combined use of autoantibodies (IA-2 autoantibody, GAD autoantibody, insulin autoantibody, cytoplasmic islet cell antibodies) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. Diabetes 1998;47:1857-66.
327. Ellis TM, Schatz DA, Ottendorfer EW, Lan MS, Wasserfall C, Salisbury PJ, et al. The relationship between humoral and cellular immunity to IA-2 in IDDM. Diabetes 1998;47:566-9.
328. Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK. Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes [see comments]. J Clin Invest 1994;94:2125-9.
329. 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;28:1068-76.
330. Nanto-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkala A, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. Lancet 2008;372:1746-55.
331. Agardh CD, Cilio CM, Lethagen A, Lynch K, Leslie RD, Palmer M, et al. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. J Diabetes Complications 2005;19:238-46.
332. Lernmark A, Agardh CD. Immunomodulation with human recombinant autoantigens. Trends Immunol 2005;26:608-12.
333. American Diabetes Association. Diabetes nephropathy. Diabetes Care 1999;22 (Supp 1):S66-9.
334. Davidson MB, Bazargan M, Bakris G, Peters Harmel A, Campese V, Basta E. ImmunoDip: an improved screening method for microalbuminuria. Am J Nephrol 2004;24:284-8.
335. Khosla N, Sarafidis PA, Bakris GL. Microalbuminuria. Clin Lab Med 2006;26:635-53, vi-vii.
336. Sarafidis PA, Riehle J, Bogojevic Z, Basta E, Chugh A, Bakris GL. A comparative evaluation of various methods for microalbuminuria screening. Am J Nephrol 2008;28:324-9.
337. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis 2007;49:S12-154.
338. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.
339. Klausen KP, Scharling H, Jensen JS. Very low level of microalbuminuria is associated with increased risk of death in subjects with cardiovascular or cerebrovascular diseases. J Intern Med 2006;260:231-7.
340. Klausen KP, Scharling H, Jensen G, Jensen JS. New definition of microalbuminuria in hypertensive subjects: association with incident coronary heart disease and death. Hypertension 2005;46:33-7.
341. Ratto E, Leoncini G, Viazzi F, Vaccaro V, Parodi A, Falqui V, et al. Microalbuminuria and cardiovascular risk assessment in primary hypertension: should threshold levels be revised? Am J Hypertens 2006;19:728-34; discussion 35-6.
342. 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;139:901-6.

DRAFT

343. Rachmani R, Levi Z, Lidar M, Slavachevski I, Half-Onn E, Ravid M. Considerations about the threshold value of microalbuminuria in patients with diabetes mellitus: lessons from an 8-year follow-up study of 599 patients. Diabetes Res Clin Pract 2000;49:187-94.
344. Pambianco G, Costacou T, Orchard TJ. The prediction of major outcomes of type 1 diabetes: a 12-year prospective evaluation of three separate definitions of the metabolic syndrome and their components and estimated glucose disposal rate: the Pittsburgh Epidemiology of Diabetes Complications Study experience. Diabetes Care 2007;30:1248-54.
345. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am Soc Nephrol 2009;20:436-43.
346. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766-72.
347. Vassalotti JA, Stevens LA, Levey AS. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. Am J Kidney Dis 2007;50:169-80.
348. Kistorp C, Raymond I, Pedersen F, Gustafsson F, Faber J, Hildebrandt P. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. Jama 2005;293:1609-16.
349. Yuyun MF, Khaw KT, Luben R, Welch A, Bingham S, Day NE, Wareham NJ. Microalbuminuria independently predicts all-cause and cardiovascular mortality in a British population: The European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) population study. Int J Epidemiol 2004;33:189-98.
350. Weir MR, Bakris GL. Editorial perspective. Should microalbuminuria ever be considered as a renal endpoint in any clinical trial? Am J Nephrol 2010;31:469-70.
351. Glassock RJ. Debate: CON position. Should microalbuminuria ever be considered as a renal endpoint in any clinical trial? Am J Nephrol 2010;31:462-5; discussion 6-7.
352. Steinke JM, Sinaiko AR, Kramer MS, Suissa S, Chavers BM, Mauer M. The early natural history of nephropathy in Type 1 Diabetes: III. Predictors of 5-year urinary albumin excretion rate patterns in initially normoalbuminuric patients. Diabetes 2005;54:2164-71.
353. Duka I, Bakris G. Influence of microalbuminuria in achieving blood pressure goals. Curr Opin Nephrol Hypertens 2008;17:457-63.
354. Schrier RW, Estacio RO, Mehler PS, Hiatt WR. Appropriate blood pressure control in hypertensive and normotensive type 2 diabetes mellitus: a summary of the ABCD trial. Nat Clin Pract Nephrol 2007;3:428-38.
355. Ruggenenti P, Fassi A, Ilieva AP, Bruno S, Iliev IP, Brusegan V, et al. Preventing microalbuminuria in type 2 diabetes. N Engl J Med 2004;351:1941-51.
356. Sinzinger H, Kritz H, Furberg CD. Atorvastatin reduces microalbuminuria in patients with familial hypercholesterolemia and normal glucose tolerance. Med Sci Monit 2003;9:PI88-92.
357. Incerti J, Zelmanovitz T, Camargo JL, Gross JL, de Azevedo MJ. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial Transplant 2005;20:2402-7.
358. Lepore G, Maglio ML, Nosari I, Dodesini AR, Trevisan R. Cost-effectiveness of two screening programs for microalbuminuria in type 2 diabetes. Diabetes Care 2002;25:2103-4; author reply 4.
359. Roberts WL, Calcote CB, Cook CB, Gordon DL, Moore ML, Moore S, et al. Comparison of four commercial urinary albumin (microalbumin) methods: implications for detecting diabetic nephropathy using random urine specimens. Clin Chim Acta 1998;273:21-33.
360. Howey JE, Browning MC, Fraser CG. Biologic variation of urinary albumin: consequences for analysis, specimen collection, interpretation of results, and screening programs. Am J Kidney Dis 1989;13:35-7.

DRAFT

361. Gansevoort RT, Verhave JC, Hillege HL, Burgerhof JG, Bakker SJ, de Zeeuw D, de Jong PE. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int Suppl 2005:S28-35.
362. Meinhardt U, Ammann RA, Fluck C, Diem P, Mullis PE. Microalbuminuria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collection. J Diabetes Complications 2003;17:254-7.
363. Poulsen PL, Hansen B, Amby T, Terkelsen T, Mogensen CE. Evaluation of a dipstick test for microalbuminuria in three different clinical settings, including the correlation with urinary albumin excretion rate. Diabetes Metab 1992;18:395-400.
364. Fernandez Fernandez I, Paez Pinto JM, Hermosin Bono T, Vazquez Garijo P, Ortiz Camunez MA, Tarilonte Delgado MA. Rapid screening test evaluation for microalbuminuria in diabetes mellitus. Acta Diabetol 1998;35:199-202.
365. Leong SO, Lui KF, Ng WY, Thai AC. The use of semi-quantitative urine test-strip (Micral Test) for microalbuminuria screening in patients with diabetes mellitus. Singapore Med J 1998;39:101-3.
366. 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;45:198-202.
367. Collins AC, Sethi M, MacDonald FA, Brown D, Viberti GC. Storage temperature and differing methods of sample preparation in the measurement of urinary albumin. Diabetologia 1993;36:993-7.
368. MacNeil ML, Mueller PW, Caudill SP, Steinberg KK. Considerations when measuring urinary albumin: precision, substances that may interfere, and conditions for sample storage. Clin Chem 1991;37:2120-3.
369. Hishiki S, Tochikubo O, Miyajima E, Ishii M. Circadian variation of urinary microalbumin excretion and ambulatory blood pressure in patients with essential hypertension. J Hypertens 1998;16:2101-8.
370. Holl RW, Grabert M, Thon A, Heinze E. Urinary excretion of albumin in adolescents with type 1 diabetes: persistent versus intermittent microalbuminuria and relationship to duration of diabetes, sex, and metabolic control. Diabetes Care 1999;22:1555-60.
371. Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952-7.
372. Reaven GM. Insulin resistance and its consequences; non-insulin dependent diabetes mellitus and coronary heart disease. In: LeRoith D, Taylor SI, Olefsky JM, eds. Diabetes Mellitus: A Fundamental Clinical Text. Philadelphia: Lippincott-Raven, 1996:509-19.
373. Chevenne D, Trivin F, Porquet D. Insulin assays and reference values. Diabetes Metab 1999;25:459-76.
374. Del Prato S. Measurement of insulin resistance in vivo. Drugs 1999;58:3-6; discussion 75-82.
375. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB, Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007;167:1068-74.
376. Rutter MK, Wilson PW, Sullivan LM, Fox CS, D'Agostino RB, Sr., Meigs JB. Use of alternative thresholds defining insulin resistance to predict incident type 2 diabetes mellitus and cardiovascular disease. Circulation 2008;117:1003-9.
377. Marks V. Recognition and differential diagnosis of spontaneous hypoglycaemia. Clin Endocrinol (Oxf) 1992;37:309-16.

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378. Marks V, Teale JD. Hypoglycemia: factitious and felonious. Endocrinol Metab Clin North Am 1999;28:579-601.
379. American College of Obstetrics and Gynecology. ACOG practice bulletin. Polycycstic ovary syndrome. Number 41, December 2002. Int J Gynaecol Obstet 2003;80:335-48.
380. Robbins DC, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, et al. Report of the American Diabetes Association's Task Force on standardization of the insulin assay. Diabetes 1996;45:242-56.
381. 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;53:711-6.
382. Miller WG, Thienpont LM, Van Uytfanghe K, Clark PM, Lindstedt P, Nilsson G, Steffes MW. Toward standardization of insulin immunoassays. Clin Chem 2009;55:1011-8.
383. Staten MA, Stern MP, Miller WG, Steffes MW, Campbell SE. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diabetes Care 2010;33:205-6.
384. Little RR, Rohlfing CL, Tennill AL, Madsen RW, Polonsky KS, Myers GL, et al. Standardization of C-peptide measurements. Clin Chem 2008;54:1023-6.
385. Centers for Medicare and Medicaid Services. Infusion pumps: C-peptide levels as a criterion for use. https://www.cms.gov/transmittals/downloads/R513CP.pdf (March 30, 2005).
386. Burge MR, Schade DS. Insulins. Endocrinol Metab Clin North Am 1997;26:575-98.
387. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33 Suppl 1:S62-9.
388. Pickup J, Rolinski O, Birch D. In vivo glucose sensing for diabetes management: progress towards non-invasive monitoring [interview by Judy Jones]. BMJ 1999;319:1289-92.

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