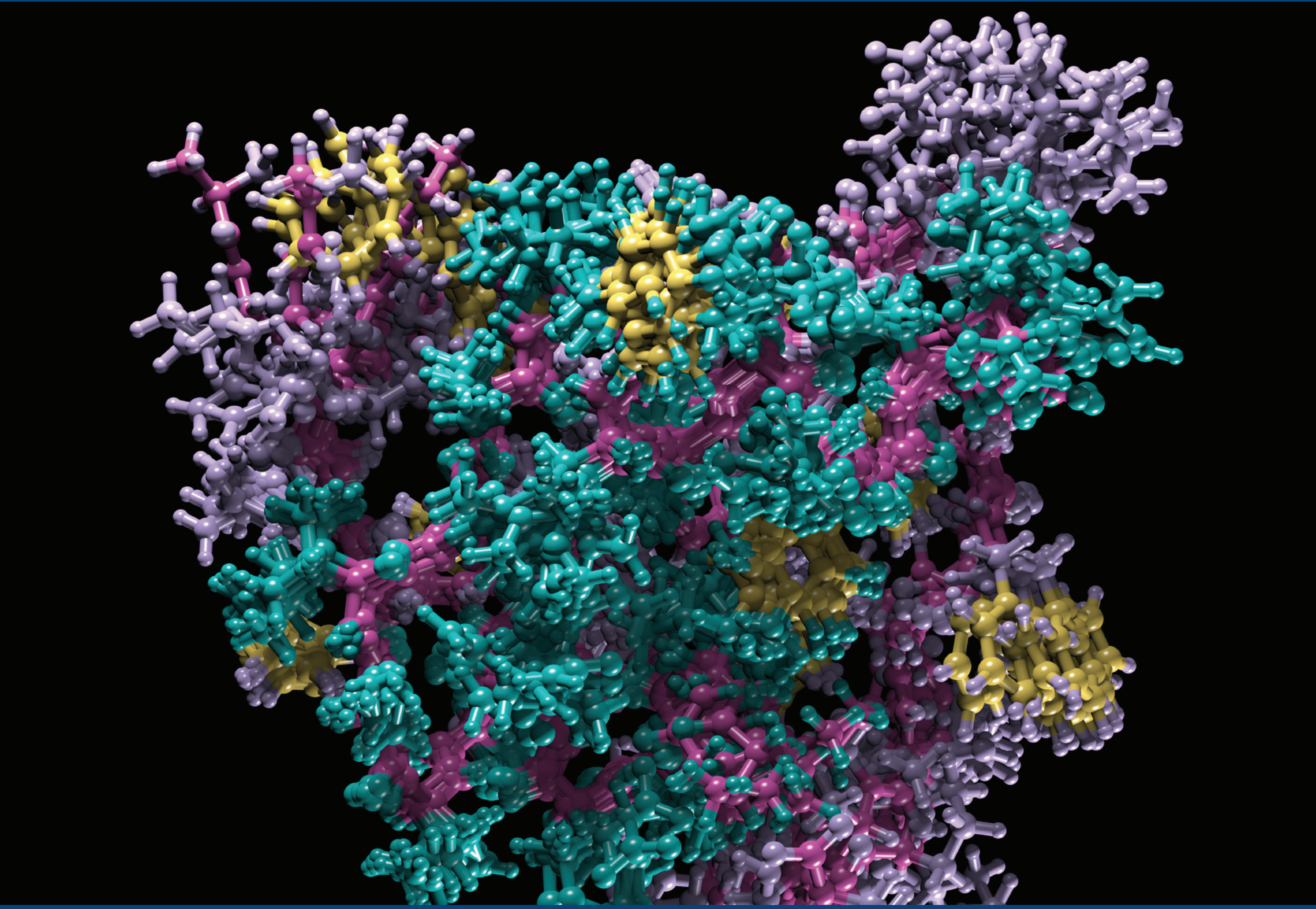
Laboratory Medicine Practice Guidelines

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

Edited by David B. Sacks



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LABORATORY MEDICINE PRACTICE GUIDELINES

GUIDELINES AND RECOMMENDATIONS FOR LABORATORY ANALYSIS IN THE DIAGNOSIS AND MANAGEMENT OF DIABETES MELLITUS

EDITED BY
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Preamble

Methods for Updating the NACB Diabetes Mellitus Laboratory Medicine Practice Guidelines

The National Academy of Clinical Biochemistry (NACB) has developed evidence-based guidelines on topics related to the practice of laboratory medicine. These guidelines are updated approximately every 5 years and are available on the NACB Web site (http://www.aacc.org/members/nacb). The NACB issued its "Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus" in 2002 *(1)*. These recommendations were reviewed and updated via an evidence-based approach, especially in areas in which new evidence has emerged since the 2002 publication. The process of updating guideline recommendations followed the standard operating procedures for preparing, publishing, and editing NACB laboratory medicine practice guidelines. The key steps are summarized in Fig. 1 and are explained below. The guideline-updating process was designed to fulfill the methodological quality criteria of the Appraisal of Guidelines for Research and Evaluation (AGREE) II Instrument *(2)*.

Figure 1: Process of updating the NACB Diabetes Mellitus guideline.

STEP 1: Determine the scope and key topics of the guideline
STEP 2: Determine the target group of the guideline and establish a multidisciplinary guideline team
STEP 3: Identify key areas for revisions and define the structure and methodology of the updated guideline
STEP 4: Define and prioritize key questions
STEP 5: Search the literature systematically for high priority questions and select relevant key publications
STEP 6: Subject selected key publications to critical expert review Extract data into evidence tables
STEP 7: Define the quality of evidence underlying each recommendation
STEP 8: Release the first draft of the guideline for public comments
STEP 9: Incorporate comments, grade recommendations and prepare the second draft of the guideline
STEP 10: Release the second draft of the guideline for public comments and submit the final draft to NACB for review and approval

STEP 1: Determine the Scope and Key Topics of the Guideline

The scope and purpose of this guideline is primarily to focus on the laboratory aspects of testing in the contexts of type 1 and type 2 diabetes mellitus (DM). It does not deal with any issues related to the clinical management of DM that are already covered in the American Diabetes Association (ADA) or WHO guidelines. In January of each year, the ADA publishes in *Diabetes Care* a supplement entitled "Clinical Practice Recommendations." This supplement, a compilation of all ADA position statements related to clinical practice, is an important resource for healthcare professionals who care for people with DM. The intention of the NACB guideline is to supplement the ADA guidelines and to avoid duplication or repetition of information. Therefore, it focuses on practical aspects of care to assist in making decisions related to the use or interpretation of laboratory tests during screening, diagnosing, or monitoring of patients with DM.

STEP 2: Determine the Target Group of the Guideline and Establish a Multidisciplinary Guideline Team

The primary target of these recommendations includes general practitioners, physicians, nurses, and other healthcare practitioners directly involved in the care of diabetic patients, as well as laboratory professionals. The guidelines can be used by patients where relevant (e.g., self-monitoring of blood glucose), policy makers, and payers for healthcare, as well as by researchers. In addition, the guidelines may advise industry/manufacturers on how to use or develop assays for the laboratory management of DM.

The guideline committee included representatives of key stakeholders to whom the recommendations are meant to apply primarily. Experts of the guideline team are listed in the guideline *(3)* and represented the NACB (D.B. Sacks, D.E. Bruns) and the ADA (M.S. Kirkman). The guideline committee included clinical experts (G.L. Bakris, A. Lernmark, B.E. Metzger, D.M. Nathan) and laboratory experts (D.B. Sacks, D.E. Bruns, M. Arnold, A.R. Horvath) whose key area of research and practice is DM. Some members of the committee provided additional support in evidence-based guideline-development methodology (D.E. Bruns, A.R. Horvath, D.B. Sacks). Members of the guideline committee were mostly from the US. The perspectives and views of various international and national organizations representing the wider laboratory and clinical professions and practice settings, as well as other potential stakeholders (including other healthcare providers, patients, policy makers, regulatory bodies, health insurance companies, researchers, and industry) were taken into account during the public-consultation process (see steps 8 and 10; see Appendix Table 1).

The guideline committee received no sponsorship, honoraria, or other direct funding related to the development of this guideline. The NACB supported the development process by providing funds to cover the expenses of meetings and consensus conferences and provided administrative support. The views of the NACB officers and staff have not influenced the content of the guideline.

All authors who contributed to the development of the recommendations of this guideline have declared (via the official disclosure form of the NACB) any financial, personal, or professional relationships that might constitute conflicts of interest with this guideline. These disclosures are part of the guideline document published on the NACB Web site.

STEP 3: Identify Key Areas for Revisions and Define the Structure and Methodology of the Updated Guideline

The chairman of the guideline committee (D.B. Sacks) acted as editor and assigned lead authors to each section. Authors reviewed the 2002 edition of the NACB DM guideline *(1)* and identified key areas for revisions and updating. The guideline team discussed the scope and methods of the updating process at a face-to-face meeting, which was followed by numerous teleconferences and e-mail exchanges among authors that were coordinated by the editor and the NACB. The guideline group decided that the structure of the guideline would remain the same as the 2002 document and that it would cover virtually all key analytes that are used primarily in the diagnosis and management of individuals with DM. As before, the testing of lipids and related cardiovascular risk factors is not covered in this update but is addressed in a separate NACB guideline *(4)*. For each area of testing discussed, the guideline highlights the clinical use and rationale for the test or tests; the preanalytical, analytical, and interpretive aspects of each test; and, where relevant, emerging considerations for future research.

STEP 4: Define and Prioritize Key Questions

The lead authors used the review process outlined above to define specific key questions to enter on a standard form developed for this process. These questions were sent to all members of the guideline committee for independent review and prioritization, a process that used preset criteria related to the relationship between testing and outcomes (see Appendix Table 2). Authors used the categories and explanatory notes provided (see Appendix Table 2) to document the rationale for prioritization or individually provided their own reasoning. Authors assigned priority scores on a scale of 1 to 4 (most important, important, moderately important, or least important, respectively). The independent replies collected from all authors were the basis for drafting a consensus priority list. Final key questions with priority scores and categories of reasoning are presented in the evidence tables (see Appendix Table 3).

STEP 5: Search the Literature Systematically for High-Priority Questions and Select Relevant Key Publications

Key questions that earned the highest priority score were covered by a more systematic approach during the search and evaluation of the evidence currently available in the literature. Other topics that were considered less important were dealt with in a less rigorous way. Because this guideline is an update of the 2002 version, authors limited their searches to the period beginning in January 2002. Guidelines related to the topic were searched in the Agency for Healthcare Research and Quality National Guideline Clearinghouse database (http://www.guideline.gov/). Systematic reviews and metaanalyses were searched by using the Clinical Queries–Find Systematic Reviews function of PubMed. If no such publications were found, PubMed, Embase, and other databases were used to search the primary literature. Because the group of authors included leading experts in their fields, the authors' personal files, communications with experts, and unpublished or ongoing-trial data were also made available to be used in the guideline-updating process. Additional literature citations were added during the comment periods (see below).

Authors selected relevant key publications for updating each section, and the editor of the guideline (D.B. Sacks) and lead authors of other sections (D.E. Bruns, M.S. Kirkman, D.M. Nathan) acted as independent expert reviewers to avoid biased selection of papers. When the guideline team retrieved and agreed with existing guideline recommendations that had already covered the key question comprehensively and had reached concordant conclusions, the guideline team simply adopted and referenced the published recommendations in order to avoid duplicate publication.

STEP 6: Subject Selected Key Publications to Critical Expert Review; Extract Data into Evidence Tables

Critical review of selected key publications formed the basis for establishing the level and quality of the evidence underlying each recommendation (see step 7 for details). Section authors and a methodology expert (A.R. Horvath) extracted data into evidence tables (see Appendix for Table 3). These tables list all key questions together with their priority scores (step 4). Related recommendations and their grades from the 2002 guideline were aligned with those of the new updated recommendations (see columns 1 and 2 in Appendix Table 3). In the updated recommendation, authors highlighted changes to the original text in boldface and provided explanation for the changes where necessary (column 3). Key references supporting the new recommendation were listed (column 4).

STEP 7: Define the Quality of Evidence Underlying Each Recommendation

To our knowledge, no uniformly accepted grading scheme exists for rating the quality of evidence and the strength recommendations when questions related to laboratory testing for the screening, diagnosis, prognosis, and monitoring of a condition are addressed *(5)*. The guideline group agreed that the grading scheme of the ADA, which was used in the 2002 version of this guideline *(1)*, is applicable predominantly to therapeutic recommendations and that its use in this diagnostic guideline was thus impracticable. Therefore, we developed a grading system by adapting the key elements of evidence-rating frameworks employed by various international guideline agencies, the US Preventive Services Task Force, and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group *(6–12)*. In this system, the overall quality of the body of evidence (step 7) and the strength of recommendations (step 9) are graded separately. Rating the quality of the *body* of evidence is based on *(a)* the level of evidence of *individual* studies defined by their study design and methodological quality; *(b)* the consistency of results across various studies; *(c)* the directness of comparisons; and *(d)* the precision-of-effect estimates. Table 1 provides a detailed explanation of evidence-level categories and these elements of the rating scheme for the quality of evidence.

Table 1. Grading the quality of evidence.

THE QUALITY OF THE BODY OF EVIDENCE IS BASED ON:
Level of evidence: This refers to the detailed study methods and the quality of their execution, i.e., the methodological quality of *individual* studies. The level of evidence can be: – *High*: if the study has an appropriate design for the question being asked and if it is well conducted in representative populations and is free from design-related biases. – *Moderate*: if the study has an appropriate design for the question being asked but suffers from some design-related biases that might influence the conclusions to a certain extent but would not affect patient-important outcomes or conclusions significantly. – *Low*: if the study is wrongly designed and conducted and there is a high likelihood that its conclusions are grossly biased and misleading.
Consistency of results across various studies: i.e., when results are heterogeneous across studies, inconsistency of results lowers the strength of evidence.
Directness of comparisons: Indirectness applies and lowers quality when, for example: – Evidence is indirectly related to the actual question; – The study population differs from that to which the study results would be applied in practice; – The test in the study differs (e.g., in its analytical performance, or a new generation of the same test has emerged) from the one commonly used or recommended in practice; – The outcome of interest for the guideline differs from the one studied in the trial.
Precision-of-effect estimates: If the study is relatively small and includes few patients or events, the confidence interval around the effect estimate is relatively large, and imprecision of results leads to downgrading the quality of evidence.
RATING SCALE FOR THE OVERALL QUALITY OF THE BODY OF EVIDENCE:
High: Further research is very unlikely to change our confidence in the estimate of effect. The body of evidence comes from high-level individual studies that are sufficiently powered and provide precise, consistent, and directly applicable results in a relevant population.
Moderate: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate and the recommendation. The body of evidence comes from high-/moderate-level individual studies that are sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the included studies; by the 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 with evidence that 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.

Members of the guideline committee received detailed explanations and guidance, as well as methodological support, on how to use the grading scheme. At this stage of the guideline-development process, section authors indicated the study design (see column 5 in Appendix Table 3) and the level of evidence (column 6) of all individual studies listed in the evidence tables. The quality of the totality of the evidence underlying each recommendation was established by means of the criteria mentioned above (column 7).

STEP 8: Release the First Draft of the Guideline for Public Comments

The first draft of the guideline was released on the NACB Web site for soliciting of public review and feedback. The still non-graded draft recommendations were sent to a number of external organizations (see Appendix Table 1) for peer review and expert comments that could be submitted either via the NACB Web site or by mail. The draft guideline was also presented at the Arnold O. Beckman consensus conference in 2007, and the discussions at this conference were recorded.

STEP 9: Incorporate Comments, Grade Recommendations, and Prepare the Second Draft of the Guideline

The guideline team reviewed and discussed the comments that were received and made many changes to the first draft to reflect the views of external peers, organizations, or individuals. The amended draft of the guideline was also presented at the 2009 AACC annual meeting and used for grading recommendations.

The grade or strength of recommendation refers to the extent of collective confidence that the desirable effects of a recommendation outweigh the potential undesirable effects. Desirable effects of a recommendation may include improved health-related, organizational, or economic outcomes or aspects of care. The quality of evidence (step 7, Table 1) is only one element in making recommendations for practice. Scientific evidence was supplemented with considered judgment that balanced the potential clinical benefits and harms with perceived patients' preferences, bioethical considerations, and organizational and economic impacts of testing *(5, 6, 9–12)*. Considered judgment therefore may have upgraded or downgraded a recommendation. Categories for grading recommendations are shown in Table 2.

During the considered-judgment process, the guideline committee was primarily driven by two core bioethical values—beneficence and nonmalevolence. The guideline group also observed the first principle of bioethics, i.e., respect for patients' autonomy and the decision-making capacities of individuals to make their own choices. The guideline group assumes that the target users will also deal with this core bioethical principle when using these guidelines in practice *(13)*. The guideline committee acknowledges that it was not able to cover universally other bioethical principles, such as justice and equity. As mentioned above, the members of the guideline team, as well as individuals who commented on the recommendations, were mostly from North America and other developed countries. Their views and experiences therefore unavoidably affected the considered-judgment and consensus processes involved in formulating recommendations. The guideline team also could not consider explicitly the cost implications of the recommendations in various resource settings, although recommendations were formulated in a generic way and in a cost-conscious manner.

Recommendations in diagnostic guidelines frequently are supported primarily by expert consensus. This reflects the often poor quality of evidence, or the lack or indirectness of evidence that the intervention is relevant to patient outcomes. To avoid the influence of dominant personalities and overrepresentation of the individual opinions or views of experts, the guideline team reached consensus when the evidence base was inconsistent, weak, or lacking. The matrix in Table 3 assisted in the assignment of final grades to recommendations. The methodology expert pregraded recommendations by using the information in columns 5, 6, and 7 of the evidence tables provided by committee members (see Appendix Table 3). Authors reviewed these grades and returned the amended evidence tables to the methodology expert for completion. Committee members added comments or explanatory notes when necessary (column 8) to enhance the transparency and reproducibility of the considered-judgment and consensus process of grading and to address the adaptability and applicability of the final recommendations. All sections were reviewed by the ADA representative (M.S. Kirkman), a clinical expert (D.M. Nathan), and a methodology expert (A.R. Horvath) and were edited by the chairman of the guideline committee (D.B. Sacks).

STEP 10: Release the Second Draft of the Guideline for Public Comments and Submit the Final Draft to the NACB for Review and Approval

The second draft of the guideline with graded recommendations was posted on the NACB Web site for a last call for public comments. The guideline recommendations were also reviewed by the Professional Practice Committee of the ADA. Several comments were received and incorporated, and the final guideline draft was submitted for review by the joint Evidence-Based Laboratory Medicine Committee of the AACC and the NACB. After addressing the reviewers' comments, the guideline committee referred the guideline to the NACB Board of Directors, which approved it before its official release for publication.

Table 2. Grading the strength of recommendations.

A. THE NACB STRONGLY RECOMMENDS ADOPTION

Strong recommendations *for* adoption 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.

Strong recommendations *against* adoption 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.

B. THE NACB RECOMMENDS ADOPTION

Recommendations *for* adoption 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 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; *or*
- There is very low–quality evidence but very strong agreement and very high level of confidence of experts that the intervention is ineffective or that benefits are closely balanced with harms, or that harms outweigh benefits.

C. THE NACB CONCLUDES THAT THERE IS INSUFFICIENT INFORMATION TO MAKE A RECOMMENDATION

Grade C is applied in the following circumstances:

- Evidence is lacking, scarce, or of very low quality, 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 information listed below and/or professional experience, or widely accepted standards of best practice. This category applies predominantly to technical (e.g., preanalytical, analytical, postanalytical), organizational, economic, or quality-management aspects of laboratory practice. In these cases, evidence often comes from observational studies, audit reports, case series or case studies, nonsystematic reviews, guidance or technical documents, non–evidence-based guidelines, personal opinions, expert consensus, or position statements. Recommendations are often based on empirical data, usual practice, quality requirements, and standards set by professional or legislative authorities or accreditation bodies, etc.

Table 3. Matrix for the assignment of grades to guideline recommendations.

Strength of recommendation (Table 2)	Quality of evidence (Table 1)	Agreement of experts
A: Strongly recommended	High Moderate	Strong–very strong
B: Recommended	Moderate Low Very low	Moderate Strong–very strong Very strong
C: Insufficient information to make recommendation	Very low Low, moderate, high	No agreement or very weak
GPP: Good practice point	Expert consensus on best practice	

Implementation and Review

To assist implementation, key recommendations of the guideline and their grades are summarized below. Key diagnostic and risk-assessment criteria are presented in tables, and a diagnostic algorithm is provided for urinary albumin testing. Most recommendations are worded to represent standards of care and thus can be easily converted to key performance indicators for local audit purposes.

Although recommendations have been developed for national and international use and are intended to be generic, certain elements of this guideline will not reflect views that are universally held, and other elements may have limited applicability in healthcare settings that lack sufficient resources for adopting the recommendations. The guideline committee advises users to adapt recommendations to their local settings. During such adaptation processes, the evidence tables provided (see Appendix Table 3) might assist users in making informed decisions.

The next review of this guideline is planned in 5 years, unless substantial new evidence emerges earlier for high-priority areas in the laboratory management of patients with DM.

Nonstandard Abbreviations

Nonstandard abbreviations throughout this document are as follows: IDDM, insulin-dependent diabetes mellitus; GDM, gestational diabetes mellitus; FPG, fasting plasma glucose; NHANES, National Health and Nutrition Examination Survey; OGTT, oral glucose tolerance test; NACB, National Academy of Clinical Biochemistry; ADA, American Diabetes Association; GPP, good practice point; IDF, International Diabetes Federation; Hb A_{1c}, hemoglobin A_{1c}; QALY, quality- adjusted life-year; UKPDS, United Kingdom Prospective Diabetes Study; DCCT, Diabetes Control and Complications Trial; CAP, College of American Pathologists; DKA, diabetic ketoacidosis; ICU, intensive care unit; SMBG, self-monitoring of blood glucose; GHb, glycated hemoglobin; DiGEM, Diabetes Glycaemic Education and Monitoring (trial); ISO, International Organization for Standardization; CGM, continuous glucose monitoring; FDA, US Food and Drug Administration; IADPSG, International Association of Diabetes and Pregnancy Study Groups; HAPO, Hyperglycemia and Adverse Pregnancy Outcome (study); AcAc, acetoacetate; βHBA, β-hydroxybutyric acid; NGSP, National Glycohemoglobin Standardization Program; eAG, estimated average glucose; ADAG, A_{1c}-Derived Average Glucose (study); ACCORD, Action to Control Cardiovascular Risk in Diabetes (study); HEDIS, Healthcare Effectiveness Data and Information Set; MODY, maturity-onset diabetes of the young; ICA, autoantibody to islet cell cytoplasm; HNF, hepatocyte nuclear factor; VNTR, variable nucleotide tandem repeat; IAA, insulin autoantibody; GAD65A, autoantibody to 65-kDa isoform of glutamic acid decarboxylase; IA-2A, autoantibody to insulinoma antigen 2; IA-2βA, autoantibody to insulinoma antigen 2β; ZnT8A, autoantibody to zinc transporter 8; LADA, latent autoimmune diabetes of adulthood; DPT-1, Diabetes Prevention Trial of Type 1 Diabetes; DASP, Diabetes Autoantibody Standardization Program; JDF, Juvenile Diabetes Foundation; JNC, Joint National Committee; NKF, National Kidney Foundation; eGFR, estimated glomerular filtration rate.

Table 4. Key Recommendations

Recommendation	Grade
Glucose	
When glucose is used to establish the diagnosis of diabetes, it should be measured in venous plasma.	A (high)
When glucose is used for screening of high-risk individuals, it should be measured in venous plasma.	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)
Blood for fasting plasma glucose analysis should be drawn in the morning after the individual has fasted overnight (at least 8 h).	B (low)
To minimize glycolysis, one should place the sample tube immediately in an ice–water slurry, and plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis.	B (moderate)

Table 4. Key Recommendations *(Cont'd)*

Recommendation	Grade
On the basis of biological variation, glucose measurement should have an analytical imprecision ≤2.9%, a bias ≤2.2%, and a total error ≤6.9%. To avoid misclassification of patients, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias.	B (low)
Glucose 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)
The imprecision of the results, coupled with the substantial differences among meters, precludes the use of glucose meters from the diagnosis of diabetes and limits their usefulness in screening for diabetes.	A (moderate)
SMBG is recommended for all insulin-treated patients with diabetes.	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)
Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. 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)
Meters should 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 SMBG and in intensive care units.	C (moderate)
Recommendations for future research: Important end-points in studies of SMBG should include, at a minimum, Hb A_{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 variation of glucose control. Ideally, outcomes (e.g., long-term complications) should also be examined.	GPP
Continuous Minimally Invasive Glucose Analyses	
Real-time CGM in conjunction with intensive insulin regimens can be a useful tool to lower Hb A_{1c} in selected adults (age >25 years) with type 1 diabetes.	A (high)
Although the evidence for lowering Hb A_{1c} is not as strong for 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 individuals 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
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)
Gestational Diabetes Mellitus	
All pregnant women not previously known to have diabetes should undergo testing for gestational diabetes mellitus at 24–28 weeks of gestation.	A (high)

Table 4. Key Recommendations

Recommendation	Grade
Gestational diabetes mellitus should be diagnosed by a 75-g oral glucose tolerance test according to the IADPSG criteria derived from the HAPO study.	A (moderate)
Urinary Glucose	
Semiquantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus.	B (low)
Ketone Testing	
Ketones measured in urine or blood in the home setting by patients with diabetes and in the clinic/hospital setting should be considered only an adjunct to the diagnosis of diabetic ketoacidosis.	GPP
Urine ketone measurements should not be used to diagnose or monitor the course of diabetic ketoacidosis.	GPP
Blood ketone determinations that rely on the nitroprusside reaction should be used only as an adjunct to diagnose diabetic ketoacidosis and should not be used to monitor diabetic ketoacidosis treatment. Specific measurement of beta-hydroxybutyric acid in blood can be used for diagnosis and monitoring of diabetic ketoacidosis.	B (moderate)
Hb A_{1c}	
Hb A_{1c} should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control.	A (moderate)
Laboratories should use only Hb A_{1c} assay methods that are certified by the NGSP as traceable to the DCCT reference. The manufacturers of Hb A_{1c} assays should also show traceability to the IFCC reference method.	GPP
Laboratories that measure Hb A_{1c} should participate in a proficiency-testing program, such as the College of American Pathologists Hb A_{1c} 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 Hb A_{1c} test results, depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population. In addition, disorders that affect erythrocyte turnover may cause spurious results, regardless of the method used.	GPP
Desirable specifications for Hb A_{1c} measurement are an intralaboratory CV<2% and an interlaboratory CV <3.5%. At least 2 control materials with different mean values should be analyzed as an independent measure of assay performance.	B (low)
Samples with Hb A_{1c} results below the lower limit of the reference interval or >15% Hb A_{1c} should be verified by repeat testing.	B (low)
Hb A_{1c} values that are inconsistent with the clinical presentation should be investigated further.	GPP
Treatment goals should be based on American Diabetes Association recommendations, which include generally maintaining Hb A_{1c} concentrations at <7% and more-stringent goals in selected individual patients if they can be achieved without significant hypoglycemia or other adverse treatment effects. Somewhat higher intervals are recommended for children and adolescents and may be appropriate for patients with limited life expectancy, extensive comorbid illnesses, a history of severe hypoglycemia, or advanced complications (note that these values are applicable only if the NGSP has certified the assay method as traceable to the DCCT reference).	A (high)
Hb A_{1c} testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or who are not meeting treatment goals.	B (low)
Hb A_{1c} may be used for the diagnosis of diabetes, with values ≥6.5% being diagnostic. An NGSP certified method should be performed in an accredited laboratory. Analogous to its use in the management of diabetes, factors that interfere with or adversely affect the Hb A_{1c} assay will preclude its use in diagnosis.	A (moderate)
Point-of-care Hb A_{1c} assays are not sufficiently accurate to use for the diagnosis of diabetes.	B (moderate)
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)

Table 4. Key Recommendations *(Cont'd)*

Recommendation	Grade
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)
Autoimmune Markers	
Islet cell autoantibodies are recommended for screening nondiabetic family members who wish to donate part of their pancreas for transplantation into 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 after HLA typing at birth.	B (low)
Screening patients with type 2 diabetes for islet cell autoantibodies 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 of treatment of type 2 diabetes.	B (low)
Screening for islet cell autoantibodies in relatives of patients with type 1 diabetes or in persons from the general population is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies.	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 in 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
Albuminuria (formerly microalbuminuria)	
Annual testing for albuminuria in patients without clinical proteinuria should begin in pubertal or postpubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment.	B (moderate)
Urine albumin at concentrations ≥30 mg/g creatinine should be considered as a continuous risk marker for cardiovascular events.	B (moderate)
The analytical CV of methods to measure albuminuria should be <15%.	B (moderate)
Semiquantitative or qualitative screening tests should be positive in >95% of patients with albuminuria to be useful for screening. Positive results must be confirmed by analysis in an accredited laboratory.	GPP
Currently available dipstick tests do not have adequate analytical sensitivity to detect albuminuria.	B (moderate)
Acceptable samples to test for increased urinary albumin excretion are timed collections (e.g., 12 or 24 h) for the measurement of albumin concentration and timed or untimed samples for measurement of the albumin–creatinine ratio.	B (moderate)
The optimal time for spot urine collection is the early morning. All collections should be at the same time of day to minimize variation. The patient should not have ingested food within the preceding 2 h, but should be well hydrated (i.e., not volume depleted).	GPP
Low urine albumin concentrations (i.e., <30 mg/g creatinine) are not associated with high cardiovascular risk if the eGFR is >60 mL $\cdot$ min^{-1} $\cdot$ $(1.73\ m^2)^{-1}$ and the patient is normotensive. If the eGFR is <60 $\cdot$ min^{-1} $\cdot$ $(1.73\ m^{2)-1}$ and/or the level of albuminuria is ≥30 mg/g creatinine on a spot urine sample, a repeat measurement should be taken within the year to assess change among people with hypertension.	A (moderate)
Miscellaneous Potentially Important Analytes	
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 be made in most cases on the basis of the clinical presentation and the subsequent course. These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis.	B (moderate)
There is no role for measurement of insulin concentration in the assessment of cardiometabolic risk, because knowledge of this value does not alter the management of these patients.	B (moderate)

Table 4. Key Recommendations

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

Abbreviations: GPP, good practice point; SMBG, self-monitoring of blood glucose; Hb A_{1c}, hemoglobin A_{1c}; NGSP, National Glycohemoglobin Standardization; DCCT, Diabetes Control and Complications Trial; CGM, continuous glucose monitoring; IADPSG, International Association of Diabetes and Pregnancy Study Groups; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; eGFR, estimated glomerular filtration rate.

REFERENCES

1. 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.
2. Appraisal of Guidelines for Research & Evaluation II. AGREE II instrument. The AGREE Next Steps Consortium, May 2009, 56 p. http://www.agreetrust.org/resource-centre/agree-ii (Accessed December 2010).
3. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:e1–e47.
4. AACC. National Academy of Clinical Biochemistry laboratory medicine practice guidelines. Emerging biomarkers for primary prevention of cardiovascular disease and stroke. Myers GL, ed. 70 p. http://www.aacc.org/members/nacb/LMPG/OnlineGuide/PublishedGuidelines/risk/Pages/toc.aspx (Accessed December 2010).
5. Horvath AR. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. Clin Chem 2009;55:853-5.
6. Scottish Intercollegiate Guidelines Network. SIGN 50: a guideline developer's handbook. Edinburgh, January 2008. 112 p. http://www.sign.ac.uk/guidelines/fulltext/50/index.html (Accessed December 2010).
7. West S, King V, Carey TS, Lohr KN, McKoy N, Sutton SF, Lux L (Research Triangle Institute – University of North Carolina Evidence-Based Practice Center, Research Triangle Park, NC). Systems to rate the strength of scientific evidence. Rockville, MD: Agency for Healthcare Research and Quality; 2002. Publication no. 02-E016 (evidence reports/technology assessment, no. 47). Contract no. 209-97-0011. p 51–63.
8. Agency for Healthcare Research and Quality. National Guideline Clearinghouse. http://www.guidelines.gov/. (Accessed December 2010).
9. Atkins D, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, et al. Grading quality of evidence and strength of recommendations. GRADE Working Group. BMJ 2004;328:1490.
10. Australian Government National Health and Medical Research Council. NHMRC levels of evidence and grades for recommendations for developers of guidelines. December 2009. http://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/evidence_statement_form.pdf (Accessed December 2010).
11. Schünemann HJ, Fretheim A, Oxman AD. Improving the use of research evidence in guideline development: 9. Grading evidence and recommendations. Health Res Policy Syst 2006;4:21.
12. Schünemann HJ, Oxman AD, Brozek J, Glasziou P, Jaeschke R, Vist GE, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ 2008;336:1106–10.
13. Watine J. What sort of bioethical values are the evidence-based medicine and the GRADE approaches willing to deal with? J Med Ethics 2010;37:184–6.

Chapter **1**

Introduction

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is under-utilized and overproduced, causing hyperglycemia. The disease is classified into several categories. The revised classification, published in 1997 *(1)*, is presented in Table 5. Type 1 diabetes mellitus, formerly known as insulin-dependent diabetes mellitus (IDDM)10 or juvenile-onset diabetes mellitus, is usually caused by autoimmune destruction of the pancreatic islet beta cells, rendering the pancreas unable to synthesize and secrete insulin *(2)*. Type 2 diabetes mellitus, formerly known as non–IDDM or adult-onset diabetes, is caused by 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 approximately 7% (range, 5%–15%) of pregnancies, usually remits after delivery, and constitutes 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)*.

Table 5. Classification of diabetes mellitus.[a]

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

[a] From the ADA *(378)*.

Diabetes is a common disease. The current worldwide prevalence is estimated to be approximately 250 x 10^6, and it is expected to reach 380 x 10^6 by 2025 *(6)*. The prevalence of diabetes [based on fasting plasma glucose (FPG) results] in US adults in 1999–2002 was 9.3%, of which 30% of the cases were undiagnosed *(7)*. The most recent data, which were derived from the 2005–2006 National Health and Nutrition Examination Survey (NHANES) with both FPG and 2-h oral glucose tolerance test (OGTT) results, show a prevalence of diabetes in US persons ≥20 years old of 12.9% (approximately 40 x 10^6) *(8)*. Of these individuals, 40% (approximately 16 million) are undiagnosed. The prevalence of diabetes has also increased in other parts of the world. For example, recent estimates suggest 110 x 10^6 diabetic individuals in Asia in 2007 *(9)*, but the true number is likely to be substantially greater, because China alone was thought to have 92.4 x 10^6 adults with diabetes in 2008 *(10)*.

The worldwide costs of diabetes were approximately $232 billion in 2007 and are likely to be $302 billion by 2025 *(6)*. In 2007, the costs of diabetes in the US were estimated to be $174 billion *(11)*. The mean annual per capita healthcare costs for an individual with diabetes are approximately 2.3-fold higher than those for individuals who do not have diabetes *(11)*. Similarly, diabetes in the UK accounts for roughly 10% of the National Health Service budget (equivalent in 2008 to £9 billion/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, they make diabetes the fourth most common cause of death in the developed world *(13)*. About 3.8 x 10^6 people worldwide were estimated to have died from diabetes-related causes in 2007 *(6)*.

The National Academy of Clinical Biochemistry (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 with an evidence-based approach, especially in key areas in which new evidence has emerged since the 2002 publication. The process of updating guideline recommendations followed the standard operating procedures for preparing, publishing, and editing NACB laboratory medicine practice guidelines, and the key steps and the grading scheme are detailed in the Preamble.

This guideline focuses primarily on the laboratory aspects of testing in diabetes.

To facilitate comprehension and assist the reader, we divide each analyte into several headings and subheadings (in parentheses), which are: use (diagnosis, screening, monitoring, and prognosis); rationale (diagnosis and screening); analytical considerations (pre-analytical, including reference intervals; and analytical, such as methods), interpretation (including frequency of measurement and turnaround time); and, where applicable, emerging considerations, which alert the reader to ongoing studies and potential future aspects relevant to that analyte.

Chapter **2**

Glucose

1. USE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2. RATIONALE

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

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

3. ANALYTICAL CONSIDERATIONS

Recommendation

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

Recommendation

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

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

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

The commonly used glycolysis inhibitors are unable to prevent short-term glycolysis. Glycolysis can be attenuated by inhibiting enolase with sodium fluoride (2.5 mg/mL of blood) or, less commonly, lithium iodoacetate (0.5 mg/mL of blood). These reagents can be used alone or, more commonly, with such anticoagulants as potassium oxalate, EDTA, citrate, or lithium heparin. Unfortunately, although fluoride helps to maintain long-term glucose stability, the rates of decline in the glucose concentration in the first hour after sample collection are virtually identical for tubes with and without fluoride, and glycolysis continues for up to 4 h in samples containing fluoride *(59)*. After 4 h, the concentration of glucose in whole blood in the presence of fluoride remains stable for 72 h at room temperature *(59)* (leukocytosis will increase glycolysis even in the presence of fluoride if the leukocyte count is very high).

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

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

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

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

Recommendation

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

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

No consensus has been achieved on the goals for glucose analysis. Numerous criteria have been proposed to establish analytical goals. These criteria include expert opinion (consensus conferences), the opinion of clinicians, regulation, the state of the art, and biological variation *(77)*. A rational and realistic recommendation that has received some support is to use biological criteria as the basis for analytical goals. It has been suggested that imprecision should not exceed one-half of the within-individual biological CV *(78, 79)*. For plasma glucose, a CV ≤2.2% has been suggested as a target for imprecision, with a 0% bias *(79)*. Although this recommendation was proposed for within-laboratory error, it would be desirable to achieve this goal for interlaboratory imprecision to minimize differences among laboratories in the diagnosis of diabetes in individuals with glucose concentrations close to the threshold value. Therefore, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias. A national or international program that uses commutable samples (e.g., fresh frozen plasma) to eliminate matrix effects and has accuracy-based grading with values derived with a reference measurement procedure should be developed to assist in achieving this objective.

4. INTERPRETATION

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

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

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

5. EMERGING CONSIDERATIONS

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

Chapter **3**

Glucose Meters

Portable meters for the measurement of blood glucose concentrations are used in 3 major settings: (*a*) in acute- and chronic-care facilities, including intensive care units (ICUs); (*b*) in physicians' offices; and (*c*) by patients at home, work, and school. Measurement in the last setting, self-monitoring of blood glucose (SMBG), was performed at least once per day by 40% and 26% of individuals with type 1 and type 2 diabetes, respectively, in the US in 1993 *(90)*. The overall rate of daily SMBG among adults with diabetes in the US increased to 40.6% in 1997 and to 63.4% in 2006 *(91)*. The ADA summarized the uses of SMBG as early as 1987 [see *(92)* and references therein] and currently recommends that SMBG be carried out ≥3 times daily by patients who use multiple insulin injections or insulin pump therapy *(92, 93)*. It is recommended that most individuals with diabetes attempt to achieve and maintain blood glucose concentrations as close to those in nondiabetic individuals as is safely possible.

1. USE

Recommendation

There are insufficient published data outcome 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)

Recommendation

The imprecision of the results, coupled with the substantial differences among meters, precludes the use of glucose meters from the diagnosis of diabetes and limits their usefulness in screening for diabetes.
A (moderate)

A. Diagnosis/screening. The glucose-based criteria for the diagnosis of diabetes are based on 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 from the diagnosis of diabetes. Similarly, screening with portable meters—although attractive because of convenience, ease, and accessibility—would generate many false positives and false negatives.

Recommendation

SMBG is recommended for all insulin-treated patients with diabetes.
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, 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)

B. Monitoring/prognosis. SMBG is recommended for all insulin-treated patients with diabetes. Intensive glycemic control can decrease microvascular complications in individuals with type 1 *(44)* or type 2 *(46)* diabetes. In the DCCT, patients with type 1 diabetes achieved intensive glycemic control by performing SMBG at least 4 times per day *(44)*. Therapy in patients with type 2 diabetes in the UKPDS *(46)* was adjusted according to FPG concentration; SMBG was not evaluated.

The role of SMBG in individuals with type 2 diabetes has generated considerable controversy *(94, 95)*. Faas et al. *(96)* reviewed 11 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 glycated Hb (GHb). The review's authors concluded that the efficacy of SMBG in type 2 diabetes is questionable *(96)*. Similar conclusions were drawn in an early (2000) metaanalysis *(97)* of a sample of patients with type 2 diabetes in the NHANES *(98)* and the Freemantle Diabetes Study *(99)*. Two early randomized trials assessed the use of glucose meters in individuals with type 2 diabetes *(100, 101)*. One of these trials *(100)* had statistical power to detect a 0.5% reduction in Hb A_{1c} but reported only a modest decrease (0.3%) in Hb A_{1c} among poorly controlled patients treated with oral agents. The second study *(101)* failed to demonstrate a significant difference in Hb A_{1c} in patients who were assigned to use meters, compared with those who were not.

For individuals with type 2 diabetes, cross-sectional and longitudinal observational studies in several countries have failed to demonstrate an improvement in glycemic control (as measured by mean Hb A_{1c} concentration) associated with the use of SMBG *(102– 104)*. This lack of effect was seen in individuals treated with insulin, oral agents, or both. Frequency of meter use did not predict Hb A_{1c}.

A 2005 Cochrane review *(105, 106)* 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 evaluate whether it was beneficial in improving quality of life, improving well-being or patient satisfaction, or decreasing the number of hypoglycemic episodes.

The randomized controlled Diabetes Glycaemic Education and Monitoring (DiGEM) trial *(107)* studied people with type 2 diabetes, a third of whom were treated with diet alone. In 2007, the investigators reported, "Evidence is not convincing of an effect of self monitoring blood glucose ... in improving glycaemic control [as assessed by Hb A_{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, "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" *(108)*.

The later ESMON study *(109)*, 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.

Two recent systematic reviews of randomized controlled studies of SMBG in people with type 2 diabetes not treated with insulin reported small but significantly greater decreases in Hb A_{1c} among patients using SMBG than in controls *(110, 111)*. In the first review *(110)*, SMBG was associated with a larger reduction in Hb A_{1c} compared with non-SMBG (weighted mean difference, −0.31%; 95% CI, −0.44 to −0.17). In the second study *(111)*, the relative decrease in Hb A_{1c} was −0.24% (95% CI, −0.34% to −0.14%). The effect of SMBG was limited to patients with Hb A_{1c} values ≥8% (64 mmol/mol).

A 2009 review of studies of patients with type 2 diabetes *(112)* addressed recent large randomized trials of tight glycemic control, a major rationale for SMBG use 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," thus raising additional uncertainty about the use of SMBG in people with type 2 diabetes.

2. RATIONALE

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 *(92)*. 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 is seen primarily in patients treated with insulin or insulin secretagogues, and it increases substantially when pharmacologic therapy is directed towards maintaining the glycemic concentrations as close to those found in nondiabetic individuals as is safely possible *(44, 46)*. The incidence of major hypoglycemic episodes—requiring third-party help or medical intervention—was 2- to 3-fold higher in the intensive-treatment group than in the conventional group in clinical trials of patients with type 1 and type 2 diabetes *(44, 46)*. Furthermore, many patients with diabetes, particularly those with type 1, lose the autonomic warning symptoms that normally precede neuroglycopenia ("hypoglycemic unawareness") *(113)*, increasing the risk of hypoglycemia. SMBG can be useful for detecting asymptomatic hypoglycemia and allowing patients to avoid major hypoglycemic episodes.

3. ANALYTICAL CONSIDERATIONS

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)

A. Preanalytical. Numerous factors can interfere with glucose analysis with portable meters. Several of these factors, such as improper application, timing, and removal of excess blood *(61)*, have been mitigated or eliminated by advances in technology. Important variables that may influence the results of bedside glucose monitoring include changes in hematocrit *(114)*, altitude, environmental temperature or humidity, hypo-tension, hypoxia and high triglyceride concentrations *(115)*, and various drugs. Furthermore, most meters are inaccurate at very high or very low glucose concentrations. Another important factor is variation in results among different glucose meters. Different assay methods and architectures lead to a lack of correlation among meters, even from a single manufacturer. In fact, 2 meters of the same brand have been observed to differ substantially in accuracy *(116, 117)*. 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 *(118)*. Thus, it is important to evaluate the patient's technique at regular intervals *(21)*. In addition to these technical issues, the anatomic site where skin-puncture samples are obtained influences results. Testing blood from so-called alternative sites may introduce a temporal lag in changes in measured blood glucose.

> ***Recommendation***
>
> Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. 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***
>
> Meters should measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories.
> GPP

B. Analytical. Virtually all glucose meters use strips that contain enzymes, such as glucose oxidase or 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 the plasma glucose concentration, irrespective of the sample type or technology *(119, 120)*. This approach can improve harmonization and allow comparison with laboratory-generated results *(121)*. The meters use reflectance photometry or electrochemistry to measure the rate of the reaction or the final concentration of the products, and they 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 improvements include automatic commencement of timing when both the sample and the strip are in the meter, smaller sample-volume requirements, an error signal if the sample volume is inadequate, "lock out" if controls are not assayed, and bar code readers to identify the lot of the strips. Moreover, meters store up to several hundred results that can subsequently be downloaded for analysis. Together, these improvements have improved the performance of new meters *(122, 123)*. Nonetheless, meter performance in the hands of patients does not equal potential performance as judged by performance in the hands of skilled medical technologists *(124)*.

Numerous analytical goals have been proposed for the performance of glucose meters. The rationale for these goals 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 *(125)*. In addition, the ADA 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 obtained by tight glucose control in the DCCT. A revised performance goal, published in 1996 *(92)*, was for a total analytical error of <5%. To our knowledge, there are no published studies of diabetes patients achieving the goal of an analytical error of <5% with any glucose meters.

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 the laboratory glucose value is >5.5 mmol/L (100 mg/dL) and (*b*) <0.83 mmol/L (15 mg/dL) of the laboratory glucose value when the glucose concentration is ≤5.5 mmol/L (100 mg/dL) *(126)*. The 2003 International Organization for Standardization (ISO) recommendations *(127)* propose that for test readings >4.2 mmol/L (75 mg/ dL), the discrepancy between meters and an accredited laboratory should be <20%; for glucose readings ≤4.2 mmol/L (75 mg/dL), the discrepancy should not exceed 0.83 mmol/L (15 mg/dL) in 95% of the samples. In both the CLSI and ISO guidelines, 5% of these results can be substantially outside these limits. 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 reliably detecting hypoglycemia. Similarly, errors of 20% can lead to errors in insulin dosing, which, when combined with other factors, can lead to hypoglycemia.

Others have proposed different approaches to establishing quality requirements. Clarke et al. *(128)* 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 *(129)*. On the basis of patients' perceptions of their needs and 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%. With hypoglycemia excluded, the analytical CV to meet the expectations of 75% of the patients was 6.4% to 9.7%. The authors recommended an analytical CV of 5% with a bias ≤5% *(129)*. A third approach used simulation modeling of errors in insulin dose *(130)*. The results revealed that meters that achieve both a CV and a bias <5% rarely lead to major errors in insulin dose. To provide the intended insulin dosage 95% of the time, however, the bias and CV needed to be <1%–2%, depending on the dosing schedule for insulin and the intervals of glucose concentrations for the individual patient *(130)*. No meters have been shown to achieve CVs of 1%–2% in routine use in the hands of patients.

The lack of consensus on quality goals for glucose meters reflects the absence of agreed objective criteria. With the same biological-variation criteria described above for glucose analysis in accredited laboratories (section 4, Interpretation), a biological goal would be a total error ≤6.9% with an imprecision (as the CV of measurements over several days or weeks) ≤2.9% and a bias ≤2.2% *(86)*. Additional studies, however, are necessary to define a goal that is related to medical needs.

Current meters exhibit performance superior to prior generations of meters *(122, 123)*. A variety of studies of newer analyzers have documented CVs 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 a single medical technologist performed all of the assays, about 50% of the analyses met the 1996 ADA criterion of <5% deviation from reference intervals *(122)*. Another study that evaluated meter performance in 226 hospitals with split samples analyzed simultaneously on meters and laboratory glucose analyzers revealed that 45.6%, 25%, and 14% of the split samples differed from each other by >10%, >15%, and >20%, respectively *(131)*. In another study, none of the meters met the 1996 ADA criterion *(132)*. In an evaluation in which "all testing was performed by trained study staff in an inpatient Clinical Research Center setting," only 81% of results with a meter that used a hexokinase method were within 10% of results obtained from an accredited laboratory *(133)*. We are aware of no studies that document patient-generated results that meet the 1996 ADA criteria. Moreover, an analysis of published studies of glucose meters demonstrated that the studies suffered from deficiencies in study design, methodology, and reporting *(134)*, raising the possibility that the reported total error underestimates the true total error of the meters. A standardized method for evaluating meters has been developed in Norway *(134)*, and the Norwegian health authorities have decided that all SMBG instruments marketed in Norway should be examined by a similar procedure *(135)*. 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 1996 ADA criteria in the hands of patients *(135)*.

Glucose meters are also used to support tight control of glucose in patients in ICU settings. A 2001 report of a seminal randomized controlled trial by van den Berghe et al. described a 34% reduction in mortality in surgical ICU patients managed according to a tight glucose-control protocol *(88)*. A metaanalysis of multiple randomized controlled trials of tight glucose control conducted 7 years later failed to identify any improved outcomes but did find an increased incidence of hypoglycemia *(136)*. A *Clinical Chemistry* Perspective article *(137)* pointed out that the study of van den Berghe et al. used a precise and accurate glucose analyzer and collected arterial blood samples, whereas subsequent studies often used glucose meters and capillary blood samples obtained by finger stick. The integrity of results obtained with fingerstick samples can be compromised by such factors as shock, hypoxia, and low hematocrit, which are common in these settings *(138)*. 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 (which include errors related to sample type and sample collection) lead to marked degradation of glycemic control in tight glucose-control protocols *(139)*. In this study, frequencies of both hyperglycemia and hypoglycemia were increased with increasing assay imprecision. In a 2005 study of ICU patients *(140)*, the agreement of meter results with accredited laboratory results was 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). Hoedemaekers et al. *(141)*, 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 the total error of meters used in ICU patients was greater than in non-ICU patients. A later report, which also studied 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 *(142)*. Similarly, a 2005 study of arterial, venous, and capillary samples from a mixed medical/surgical ICU of a tertiary care hospital in Canada found that meters did not meet proposed CLSI goals but that a blood gas analyzer did *(143)*.

Recommendation

Studies are needed to determine the analytical goals (quality specifications) for glucose meters in SMBG and in ICUs.
C (moderate)

Recommendations

For future research: important end points in studies of SMBG should include, at a minimum, Hb A_{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 variation of glucose control. Ideally, outcomes (e.g., long-term complications) should also be examined.
GPP

4. INTERPRETATION

A. 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 per day leads to deterioration in glycemic control *(92, 144, 145)*. Patients perform self-monitoring 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 by 5%–6% of patients treated with oral agents or diet alone *(98)*. 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 per day is ideal or whether a different frequency would improve glycemic control. For example, adjustment of insulin therapy in women with GDM according to the results of post-prandial, rather than preprandial, plasma glucose concentrations improved glycemic control and reduced the risk of neonatal complications *(146)*. The optimal frequency of SMBG for patients with type 2 diabetes is unknown.

The ADA recommends that patients treated with multiple daily injections of insulin perform SMBG ≥3 times per day *(21)* and states that "SMBG is useful in achieving glycemic goals" in other patients. The last statement is based on expert opinion.

Chapter 4

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 Hb A_{1c} in selected adults (age >25 years) with type 1 diabetes.
A (high)

Recommendation

Although the evidence for lowering Hb A_{1c} is not as strong for 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 individuals 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.
GPP

The development of a device for "continuous" in vivo monitoring of glucose concentrations in blood has become a very high priority as patients are required to control their plasma glucose more closely *(21, 44, 147)*. The first device approved by the US Food and Drug Administration (FDA) for minimally invasive interstitial fluid glucose sensing, the transcutaneous GlucoWatch Biographer, is no longer on the market. Several implanted-catheter systems have subsequently been approved. 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 one the patient wears for 3 days and then returns to the provider's office for its data to be downloaded for trend analyses. More recently, a number of real-time devices that allow patients to read both current glucose concentrations and trends have become commercially available. In the US, these devices include the Guardian Real-Time (Medtronic Diabetes), the Seven Plus System (DexCom), and the Freestyle Navigator (Abbott Laboratories). CGM devices require calibration and confirmation of accuracy with conventional SMBG, and the FDA advises using the latter for treatment decisions, such as calculating premeal insulin doses.

The clinical studies of these devices, generally in highly selected populations, had primarily been limited to assessments of their accuracy or to short-term trials demonstrating reductions in the time patients spend within hypo- and hyperglycemic intervals (*148*). A systematic review of trials of the non–real-time CGM system device suggests that it does not lead to significantly lower Hb A_{1c} values compared with SMBG *(149)*. In 2008, a large 26-week randomized trial of 322 type 1 diabetes patients showed that adults ≥25 years of age who used intensive insulin therapy and real-time CGM experienced a 0.5% reduction in Hb A_{1c}, from approximately 7.6% to 7.1% (approximately 60 to 54 mmol/mol), compared with the usual intensive insulin therapy with SMBG (*150*). Sensor use in children, teens, and adults to 24 years of age did not lower Hb A_{1c} significantly, and there was no significant difference in hypoglycemia for any group. The greatest predictor of Hb A_{1c} reduction in this study among all age groups was frequency of sensor use, which was lower in younger-age groups. Although CGM is an evolving technology, the emerging data suggest that it may offer benefit in appropriately selected patients who are motivated to wear it most of the time. CGM may be particularly useful for patients with hypoglycemia unawareness and/or frequent episodes of hypoglycemia; 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 *(44, 147)*. Therefore, a sensor designed to detect severe hypoglycemia alone would be of value. In contrast, a full-range, reliable continuous in vivo 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.

3. ANALYTICAL CONSIDERATIONS

The methods to sample biological fluids in a continuous and minimally invasive way vary among test systems. The underlying fundamental concept is that the concentration of glucose in the interstitial fluid correlates with blood glucose. The implanted sensors use multiple detection systems, including enzyme- (usually glucose oxidase), electrode-, and fluorescence-based techniques. Alternatives to enzymes, including artificial glucose "receptors," as glucose-recognition molecules are being developed (*151, 152*). Fluorescence technologies include the use of engineered molecules that exhibit altered fluorescence intensity or spectral characteristics on binding glucose, or the use of competitive-binding assays that use 2 fluorescent molecules in the fluorescent resonance energy transfer technique *(153–157)*.

4. INTERPRETATION

The subcutaneous sensors are generally worn for a number of days and require calibration with SMBG readings several times per day. A few small studies have examined their accuracy compared with SMBG and/or plasma glucose assays. For the Medtronic CGMS® System Gold™ device, the mean (SD) absolute difference between sensor readings and blood glucose readings was 15.0% (12.2%) for 735 paired samples, whereas the GlucoDay microdialysis device (Menarini) had a mean absolute difference of 13.6% (10.2%) for 1156 paired samples *(158)*. For both devices, accuracy was lowest in the hypoglycemic ranges. Approximately 97% of the values for both devices were within zones A and B of a Clarke error grid, with none falling in zone E (*158*). A study of 91 insulin-treated patients using the DexCom device showed that 95% of 6767 paired glucose values fell within Clarke error grid zones A and B, with a mean absolute difference of 21.2% *(148)*.

Currently, there are no analytical goals for noninvasive 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.

5. EMERGING CONSIDERATIONS

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

Chapter **5**

Noninvasive Glucose Analysis

1. USE

> ***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)

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 nonionizing 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. 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 the light passing through the sample.

A truly noninvasive method would be painless in operation and capable of continuous readings over time. In addition, noninvasive sensing technology may be less expensive to implement than 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 both low cost and painless, 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 *(159–162)*. Potential applications include discrete home glucose testing, continuous home glucose monitoring, nocturnal hypoglycemia alarm, measurements in a physician's office, point-of-care monitoring, screening for diabetes, and control of hyperglycemia in critically ill patients. To date, none of these applications has been realized.

2. RATIONALE

Indirect and direct methods are being developed for noninvasive glucose sensing. Indirect methods rely on the effect 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 (*163*). Changes in blood glucose substantially affect the difference in refractive index between skin cells and the surrounding interstitial fluid and thereby alter the scattering coefficient of skin. This parameter can be measured in a number of ways, including ocular coherent tomography. Skin impedance and the aggregation properties of erythrocytes are other indirect approaches.

Direct methods measure a property of the glucose molecule itself. Vibrational spectroscopy is the primary direct method and generally involves 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 of the analytical information must originate from the noninvasive signal. Ultimately, the 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.

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 (*164*). 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 US (*165*).

Many reports of attempts to measure glucose noninvasively lack sufficient information to judge the likelihood that glucose is actually being measured. The interpretation of such clinical data is complicated by the common use of multivariate statistical 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 (*166, 167*). Given this known limitation of these multivariate methods, care must be used in their implementation. Tests for spurious correlations *(168–170)* 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 *(171, 172)*. 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 intralaboratory comparisons.

Chapter **6**

Gestational Diabetes Mellitus

1. USE

> ***Recommendation***
>
> All pregnant women not previously known to have diabetes should undergo testing for GDM at 24–28 weeks of gestation. A (high)

GDM has been defined as any degree of glucose intolerance with onset or first recognition occurring during pregnancy *(1)*. After recent discussions, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommended that high-risk women who have diabetes established according to standard criteria (Table 6) at their initial prenatal visit receive a diagnosis of overt, not gestational, diabetes (*21*). The IADPSG recommendations are not identical to the criteria for nonpregnant individuals, in that an OGTT result with an FPG value <7.0 mmol/L (126 mg/dL) and 2-h value >11.1 mmol/L (200 mg/dL) is not called "overt diabetes." As the prevalence of obesity and type 2 diabetes has increased, the number of women with undiagnosed diabetes has risen (*173*). Therefore, the ADA now recommends that women with risk factors for type 2 diabetes be screened for diabetes according to standard diagnostic criteria (Table 6) at the first prenatal visit (*93*). Women with diabetes diagnosed with this approach should receive a diagnosis of overt diabetes.

Two randomized clinical trials have now demonstrated a 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) (*174, 175*).

Table 8. Screening for and diagnosis of GDM.

Glucose measure	Glucose concentration threshold, mmol/L (mg/dL)[a]	Percentage >threshold (cumulative)[b]
FPG	5.1 (92)	8.3%
1-h PG	10.0 (180)	14.0%
2-h PG	8.5 (153)	16.1%[c]

[a] One or more of these values from a 75-g OGTT must be equaled or exceeded for the diagnosis of GDM.
[b] Cumulative proportion of HAPO cohort equaling or exceeding those thresholds.
[c] In addition, 1.7% of the participants in the initial cohort were unblinded because of an FPG value >5.8 mmol/L (105 mg/dL) or a 2-h OGTT values >11.1 mmol/L (200 mg/dL), bringing the total to 17.8%.

2. RATIONALE

The ADA states that because of the risks of GDM to the mother and the neonate, screening and diagnosis are warranted (*21*). The screening and diagnostic criteria for GDM have recently been modified extensively. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study was a large (approximately 25 000 pregnant women) prospective, multinational epidemiologic study to assess adverse outcomes as a function of maternal glycemia (*176*). The study revealed strong, graded, predominantly linear associations between maternal glycemia and primary study outcomes, i.e., birth weight >90th percentile, delivery by cesarean section, clinical neonatal hypoglycemia, and cord serum insulin (C-peptide) concentrations >90th percentile of values in the HAPO study population. The associations remain strong after adjustments for multiple potentially confounding factors. Strong associations were also found with infant adiposity (*177*), with some secondary outcomes (including risks of shoulder dystocia and/or birth injury), and with preeclampsia (*176*). On the strength of these results, an expert consensus panel appointed by the IADPSG recommended "outcome based" criteria for the classification of glucose concentrations in pregnancy (*178*). All pregnant women not previously known to have diabetes should be evaluated by a 75-g OGTT for GDM at 24–28 weeks of gestation (*178*). Diagnostic cutpoints for fasting, 1-h, and 2-h plasma glucose concentrations have been established (Table 8). These recommendations were adopted by the ADA in 2011 *(93)* and are currently under consideration by the American College of Obstetrics and Gynecology in the US and by corresponding groups in other countries. Using the new criteria substantially increases the incidence of GDM, mainly because only 1 increased glucose value is required to diagnose GDM (prior recommendations required 2 increased glucose concentrations). Treatment will require additional resources, and outcome studies will be necessary to ascertain whether therapy is beneficial for GDM diagnosed with the new criteria; however, the 2 trials that focused on the treatment of "mild GDM" (identified with the old criteria) achieved an improvement in outcomes, with only 10%–20% of the patients requiring pharmacologic treatment in addition to medical nutritional therapy (*174, 175*).

3. ANALYTICAL CONSIDERATIONS

These considerations have been addressed earlier in the Glucose sections. Given the strict cutoffs, it is very important that close attention be paid to stringent sample-handling procedures to minimize glycolysis after phlebotomy.

4. INTERPRETATION

Recommendation

Gdm should be diagnosed by a 75-g OGTT according to the IADPSG criteria derived from the HAPO study.
A (moderate)

The ADA previously recommended that a “risk assessment” (based on age, weight, past history, and so on) be performed and that patients at average or high risk receive a glucose-challenge test. Several diagnostic strategies could be used. They were a “1-step” approach, in which an OGTT was performed initially, or a “2-step” approach, in which an administered 50-g oral glucose load (regardless of whether the patient was fasting) was followed by a plasma glucose measurement at 1 h. A plasma glucose value ≥7.8 mmol/L (140 mg/dL) indicates the need for definitive testing with an OGTT; however, a consensus was lacking as to whether a 100-g or 75-g OGTT should be performed and what cutoff values should be used for diagnosis.

Some GDM cases may represent preexisting, but undiagnosed, type 2 diabetes. Therefore, women with GDM should be screened for diabetes 6–12 weeks post-partum according to the OGTT criteria for nonpregnant women (Table 7) *(93)*. In addition, because women with GDM are at a considerably increased risk of developing diabetes later *(179)*, lifelong screening for diabetes should be performed at least every 3 years according to standard criteria for nonpregnant women (Table 6) (*93*).

Chapter 7

Urinary Glucose

1. USE

> ***Recommendation***
>
> Semiquantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus.
> B (low)

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 or refuse to perform SMBG, because the urine glucose concentration does not accurately reflect the plasma glucose concentration (*147, 180*). Notwithstanding these limitations, urine glucose monitoring is supported by the IDF in those situations in which 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 [approximately 10 mmol/L (180 mg/dL)]. This fact alone limits its usefulness for monitoring diabetes under modern care recommendations. Semiquantitative urine glucose tests also cannot distinguish between euglycemia and hypoglycemia. Furthermore, the extent to which the kidney concentrates the urine will affect urine glucose concentrations, and only mean glucose values between voidings are reflected. These facts further minimize the value of urine glucose measurements.

3. ANALYTICAL CONSIDERATIONS

Semiquantitative test-strip methods that use reactions specific for glucose are recommended. Commercially available strips use the glucose oxidase reaction (*181*). Test methods that detect reducing substances are not recommended because they are subject to numerous interferences, including numerous drugs and nonglucose sugars. When used, single voided urine samples are recommended (*147*).

4. INTERPRETATION

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

Chapter **8**

Ketone Testing

1. USE

> ***Recommendation***
>
> Ketones measured in urine or blood in the home setting by patients with diabetes and in the clinic/hospital setting should be considered only an adjunct to the diagnosis of DKA.
> GPP

The ketone bodies acetoacetate (AcAc), acetone, and β-hydroxybutyric acid (βHBA) are catabolic products of free fatty acids. Measurements of ketones in urine and blood are widely used in the management of patients with diabetes as adjuncts for both diagnosis and ongoing monitoring of 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 check urine or blood ketones in situations characterized by deterioration in glycemic control in order to detect and preempt the development of DKA (*21, 182*).

2. RATIONALE

Ketone bodies are usually present in urine and blood, but in very low concentrations (e.g., total serum ketones, <0.5 mmol/L). Increased ketone concentrations detected in patients with known diabetes or in previously undiagnosed patients presenting with hyperglycemia suggest impending or established DKA, a medical emergency. The 2 major mechanisms for high ketone concentrations in patients with diabetes are increased production from triglycerides and decreased utilization in the liver— both of which are due to an absolute or relative insulin deficiency and increased counter-regulatory hormones, including cortisol, epinephrine, glucagon, and growth hormone (*183*).

The principal ketone bodies βHBA and AcAc are typically present in approximately equimolar amounts. Acetone, usually present in only small quantities, is derived from spontaneous decarboxylation of AcAc. The equilibrium between AcAc and βHBA is shifted towards βHBA formation in any condition that alters the redox state of hepatic mitochondria to increase NADH concentrations, such as hypoxia, fasting, metabolic disorders (including DKA), and alcoholic ketoacidosis *(184–186)*. Thus, assay methods for ketones that do not include βHBA measurement may provide misleading clinical information by underestimating total ke-tone body concentration *(187)*.

3. ANALYTICAL CONSIDERATIONS

A. Urine ketone. Preanalytical. The concentrations of ketones in the urine of healthy individuals are below the detection limits of commercially available testing materials. False-positive results have been reported with highly colored urine and in the presence of several sulfhydryl-containing drugs, including angiotensin-converting enzyme inhibitors (*188*). Urine test reagents deteriorate with exposure to air, giving false-negative readings; therefore, testing material should be stored in tightly sealed containers and discarded after the expiration date on the manufacturer's label (*189*). False-negative readings have also been reported with highly acidic urine samples, such as after large intakes of ascorbic acid. Loss of ketones from urine attributable to microbial action can also cause false-negative readings. Because acetone is a highly volatile substance, samples should be kept in a closed container. For point-of-care analyses in medical facilities and for patients in the home setting, control materials (that give both negative and positive readings) are not commercially available but would be desirable to ensure accuracy of test results.

Analytical. Several assay principles have been described. Most commonly used is the colorimetric reaction that occurs between AcAc and nitroprusside (sodium nitroferricyanide) to produce a purple color (*181*). This method is widely available in the form of dipsticks and tablets and is used to measure ketones in both the urine and blood (either serum or plasma). Several manufacturers offer dipsticks for measuring 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 cannot be used to measure βHBA (*181*).

B. Blood ketones. Preanalytical. Serum/plasma ketones can be measured with the tablets or dipsticks routinely used for urine ketone measurements. Although samples can be diluted with saline to "titer" the ketone concentration (results are typically reported as "positive at a 1/*x* dilution"), βHBA, the predominant ketone body in DKA, is not detected, as with urine ketone testing.

For specific βHBA measurements, sample requirements differ among methods, as is described below. In general, blood samples can be collected into tubes containing heparin, EDTA, fluoride, citrate, or oxalate. Ascorbic acid interferes with some assay methods. AcAc interferes with some assay methods unless the samples are highly dilute. Sample stability differs among methods, but whole-blood samples are generally stable at 4 °C for up to 24 h. Serum/plasma samples are stable for up to 1 week at 4 °C and for at least several weeks at −20 °C (long-term stability data are not available for most assay methods).

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 appear to be the most widely used for the quantification of βHBA for routine clinical management *(190–192)*. The principle of the enzymatic methods is that β-hydroxybutyrate dehydrogenase in the presence of NAD+ converts βHBA to AcAc and NADH. Under alkaline conditions (pH 8.5–9.5), the reaction favors the formation of AcAc from βHBA. The NADH produced can be quantified spectrophotometrically (usually kinetically) with the use of a peroxidase reagent. Most methods permit the use of whole blood, plasma, or serum samples (required volumes are generally ≤200 μL). Some methods permit the analysis of multiple analytes; these methods are designed for point-of-care testing. Several methods are available as handheld meters, which have been FDA cleared for both laboratory use and home use by patients. These methods use 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

Recommendation

Urine ketone measurements should not be used to diagnose or monitor the course of DKA.
GPP

A. Urine ketone measurements. The presence of positive urine ketone readings in a patient with known diabetes or a patient not previously diagnosed with diabetes but who presents with typical symptoms of diabetes and hyperglycemia suggests the possibility of impending or established DKA. Although DKA is most commonly associated with type 1 diabetes, it may occur rarely in type 2 patients *(193)*. Patients with alcoholic ketoacidosis will have positive urine ketone readings, but hyperglycemia is not usually present. Positive urine ke-tone readings are found in up to 30% of first morning urine samples from pregnant women (with or without diabetes), during starvation, and after hypoglycemia *(187)*.

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 DKA treatment. Specific measurement of βHBA in blood can be used for diagnosis and monitoring of DKA.
B (moderate)

B. Blood ketone measurements. Blood ketone measurements that rely on the nitroprusside reaction should be used with caution for DKA diagnosis, because the results do not quantify βHBA, the predominant ketone in DKA. The test should not be used to monitor the course of therapy, because AcAc and acetone may increase as βHBA decreases during successful therapy *(147, 183–187)*. Blood ketone measurements that measure βHBA specifically are useful for both the diagnosis and ongoing monitoring of DKA *(194–196)*. Reference intervals for βHBA differ among assay methods, but concentrations in healthy individuals who have fasted overnight are generally <0.5 mmol/L. Patients with well-documented DKA [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 measurements by patients with diabetes are preferable (e.g., better accepted by patients, more prompt diagnosis of DKA) to urine ketone measurements. Studies are necessary to evaluate whether the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH).

Chapter **9**

Hb A_{1c}

1. USE

> ***Recommendation***
>
> Hb A_{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 Hb A_{1c}, is widely used for routine monitoring of long-term glycemic status in patients with diabetes.[11] Hb A_{1c} is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications *(147, 197)*. Hb A_{1c} testing and maintenance of specified concentrations during pregnancy in patients with preexisting type 1 or type 2 diabetes are important for maximizing the health of the newborn and decreasing perinatal risks for the mother. Specifically, stringent control of Hb A_{1c} values during pregnancy decreases the risk of congenital malformations, large-for-date infants, and the complications of pregnancy and delivery that can otherwise occur when glycemic control is not carefully managed *(198)*. A recent consensus statement *(198)* recommends an Hb A_{1c} value of <6% (42 mmol/mol) in these patients if it can be achieved without excessive hypoglycemia. Hb A_{1c} is also being used increasingly by quality-assurance programs to assess the quality of diabetes care (e.g., requiring that healthcare providers document the frequency of Hb A_{1c} testing in patients with diabetes and the proportion of patients with Hb A_{1c} values below a specified value) *(199, 200)*.

[11] The terms "glycated hemoglobin," "glycohemoglobin," "glycosylated" (which should not be used), "glucosylated hemoglobin," "Hb A_1," and "Hb A_{1c}" have all been used to refer to hemoglobin that has been modified by the nonenzymatic addition of glucose. These terms are not interchangeable, however. The current acceptable term for glycation of hemoglobin in general is "glycated hemoglobin" (GHb). Hb A_{1c} is the specific glycated species that is modified by glucose on the N terminus of the hemoglobin β chain. "Hb A_{1c}" is also the internationally accepted term for reporting all GHb results. Assay methods that measure total GHbs (e.g., boronate affinity methods) should be calibrated to report an equivalent Hb A_{1c} and be reported as Hb A_{1c} for purposes of harmonization of results. Hb A_1 is composed of Hb A_{1a}, Hb A_{1b}, and Hb A_{1c} and should not be measured or reported. The term "A_{1C} test" is used by the ADA in place of Hb A_{1c} to facilitate communication with patients. 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) involved the use of assay methods that quantified Hb A_{1c}. In this report, we use the abbreviation GHb to include all forms of glycated hemoglobin.

The ADA and other organizations that have addressed this issue recommend Hb A_{1c} measurement in both type 1 and type 2 diabetes patients to document the degree of glycemic control and to assess response to therapy *(21, 93, 201)*. The ADA has recommended specific treatment goals for Hb A_{1c} on the basis of results from prospective randomized clinical trials, most notably the DCCT for type 1 diabetes *(44, 197)* and the UKPDS for type 2 diabetes *(46)*. These trials have documented the relationship between glycemic control (as quantified by longitudinal Hb A_{1c} measurements) and the risks for the development and progression of chronic complications of diabetes. Because different GHb assays can produce different GHb values, the ADA recommends that laboratories use only assay methods that have been certified as traceable to the DCCT GHb reference *(21, 187)*; these results are reported as Hb A_{1c}. The ADA recommends that in general an Hb A_{1c} target of <7% (53 mmol/mol) is desirable for nonpregnant adults, with higher values recommended for children and adolescents *(21)*. Hb A_{1c} goals should be individualized according to the potential for benefit with regard to long-term complications and be balanced against the increased risk for the hypoglycemia that attends intensive therapy. For selected individual patients, more-stringent targets could be suggested, provided that this goal can be achieved without substantial hypoglycemia or other adverse effects of treatment. Such patients might include those with a short duration of diabetes, a long life expectancy, and no significant cardiovascular disease *(93)*. Conversely, higher Hb A_{1c} goals should be chosen for patients with a history of severe hypoglycemia, a limited life expectancy, advanced microvascular or macrovascular complications, or extensive comorbid conditions. Other clinical organizations recommend similar Hb A_{1c} targets, which range from 6.5% to 7% (48 to 53 mmol/mol) *(53, 202)*.

2. RATIONALE

Glycated proteins are formed posttranslationally from the slow, nonenzymatic reaction between glucose and free amino groups on proteins *(203)*. For Hb, the rate of GHb synthesis is principally a function of the glucose concentration 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 life span of erythrocytes *(147, 203–206)*. Several studies have demonstrated a close mathematical relationship between Hb A_{1c} con centration and mean glycemia, which should allow the expression of Hb A_{1c} as an estimated average glucose (eAG) concentration *(205, 207–209)*. Analogous to Hb (in erythrocytes), serum proteins become glycated. Commercial assays are available that measure total glycated protein (termed fructosamine) or glycated albumin in the serum. The concentrations of these glycated proteins also reflect mean glycemia, but over a much shorter time (15–30 days) than GHb (60–120 days) *(147, 203–206, 210, 211)*. The clinical utility of glycated proteins other than Hb has not been clearly established, however, and there is no convincing evidence that relates their concentrations to the chronic complications of diabetes *(147, 187)*.

3. ANALYTICAL CONSIDERATIONS

Recommendation

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

Recommendation

Laboratories that measure Hb A_{1c} should participate in a proficiency-testing program, such as the College of American Pathologists (CAP) Hb A_{1c} survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network.
GPP

Approximately 100 different GHb assay methods are in current use. They range from low-throughput research laboratory component systems and manual minicolumn methods to high-throughput automated systems dedicated to Hb A_{1c} measurements. Most methods can be classified into one of 2 groups according to assay principle *(147, 181, 204)*. The first group includes methods that quantify GHb on the basis of charge differences between glycated and nonglycated components. Examples include cation-exchange chromatography and agar-gel electrophoresis. The second group includes methods that separate components on the basis of structural differences between glycated and nonglycated components. Examples include boronate affinity chromatography and immunoassay. Most charge-based and immunoassay methods quantify Hb A_{1c}, which is defined as Hb A with glucose attached to the N-terminal valine of one or both β chains. Other methods quantify "total glycated hemoglobin," which includes both Hb A_{1c} and other Hb–glucose adducts (e.g., glucose–lysine adducts and glucose–α-chain N-terminal valine adducts). Generally, the results of methods that use different assay principles show excellent correlation, and there are no convincing data to show that any method type or analyte is clinically superior to any other. The GHb results reported for the same blood sample could differ considerably among methods, however, unless they have been standardized to a common reference [e.g., without standardization, the same blood sample could be read as 7% (42 mmol/ mol) in one laboratory and 9% (75 mmol/mol) in another] *(53, 147, 204, 212–215)*.

In 1996, the NGSP was initiated to standardize GHb test results among laboratories to DCCT-equivalent values *(215)*. The rationale for standardizing GHb test results to DCCT values was that the DCCT had determined the relationship between the results obtained for a specific GHb test (Hb A_{1c}) and long-term complications in patients with type 1 diabetes *(44, 147, 187)*. 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, 147)*. In addition, the ADA recommends that all laboratories performing GHb testing participate in the CAP proficiency-testing survey for Hb A_{1c}, which uses fresh whole-blood samples *(216)*.

The NGSP Laboratory Network includes a variety of certified assay methods, each calibrated to the DCCT reference. The DCCT reference is an HPLC cation-exchange method that quantifies Hb A_{1c}; this method is a CLSI-designated comparison method *(217)*. The assay method has been used since 1978 and has demonstrated good long-term precision (between-run CVs are consistently <3%) *(216)*. 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 1 year. An important adjunct to the program is the Hb A_{1c} 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 used 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 and between methods *(216, 218)*. 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 Web site (http://www.ngsp.org) provides detailed information on the certification process and maintains a listing of certified assay methods (updated monthly) and factors that are known to interfere with specific methods.

In 1997, the IFCC formed a committee to develop a higher-order reference method and reference materials for Hb A_{1c} analysis; the method was approved in 2001 *(219, 220)*. The analysis is performed by cleaving Hb with endoproteinase Glu-C and separating the resulting glycated and nonglycated N-terminal β-chain hexapeptides by HPLC *(220)*. The hexapeptides are quantified with electrospray ionization mass spectrometry or capillary electrophoresis. The 2 methods use the same primary reference materials, and the results are essentially identical. Hb A_{1c} is measured as the ratio of the glycated

N-terminal peptide to the nonglycated N-terminal peptide and is reported in millimoles of deoxyfructosyl Hb per mole of Hb. Of note, preparing and measuring samples with this method is laborious, very expensive, and time-consuming. The method was never envisioned 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 established a network of laboratories *(221)* (11 at the time of writing). The IFCC offers manufacturers calibrators and controls as well as a monitoring program *(221)*. Unlike the NGSP, the IFCC network does not have a certification program.

A comparison of Hb A_{1c} results obtained with pooled blood samples in the IFCC and NGSP (DCCT-aligned) networks has revealed a linear relationship (termed the "master equation"): NGSP% = (0.915 x IFCC%) + 2.15 *(220)*. Although the clinical values obtained with assays standardized with the new IFCC method correlate tightly with NGSP values, the absolute Hb A_{1c} values reported differ by 1.5%–2.0% Hb A_{1c}. Concern regarding the clinical impact of changing patients' Hb A_{1c} values led in 2007 to an agreement between the IFCC and the major diabetes organizations to report IFCC Hb A_{1c} results (in millimoles per mole) as the equivalent NGSP DCCT-aligned result (a percentage based on the master equation) and as a calculated eAG based on the A_{1c}-Derived Average Glucose (ADAG) study *(209, 222)*. In the revised agreement, published in 2010 *(223)*, both NGSP and IFCC units were recommended, but the decision to report eAG was left to the discretion of individual countries. Notwithstanding the agreement, it appears unlikely that universal reporting of Hb A_{1c} will be adopted; however, the master equation allows conversion between IFCC and NGSP numbers.

A. Preanalytical.

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

Patient variables. Hb A_{1c} results are not affected significantly by acute fluctuations in blood glucose concentrations, such as those occurring with illness or after meals; however, age and race reportedly influence Hb A_{1c}. Published data show age-related increases in Hb A_{1c} values of approximately 0.1% per decade after age 30 years *(224, 225)*. Careful phenotyping of individuals with OGTT supports an increase in Hb A_{1c} with age, even after removing from the study population patients with otherwise undiagnosed diabetes and persons with impaired glucose tolerance *(224)*. The clinical implications of the small, but statistically significant, progressive increase in "normal" Hb A_{1c} levels with aging remain to be determined *(226)*.

The effects of race on Hb A_{1c} values are controversial. Several studies have suggested a relatively higher Hb A_{1c} in African American and Hispanic populations than in Caucasian populations at the same level of glycemia *(225, 227, 228)*. The accumulated evidence suggests that there are differences in Hb A_{1c} among racial groups; however, the measurement of chronic glucose concentrations in these studies has not been sufficiently frequent to capture adequately the actual mean glycemia. Moreover, it is not clear that the differences in Hb A_{1c} have clinical significance. A recent analysis of 11 092 adults showed that blacks had mean Hb A_{1c} val ues 0.4% higher than whites *(229)*; however, race did not modify the association between Hb A_{1c} concentration and adverse cardiovascular outcomes or death *(229)*. The ADAG study, which included frequent glucose measurements, did not show a significantly different relationship between the calculated mean glucose concentration during 3 months and the Hb A_{1c} value at the end of the 3 months for Africans/African Americans and Caucasians. The relatively small size of the African/African American population, however, limits the interpretation of this finding *(209)*.

Any condition that shortens erythrocyte survival or decreases mean erythrocyte age (e.g., recovery from acute blood loss, hemolytic anemia) falsely lowers Hb A_{1c} test results, regardless of the assay method *(147)*. Vitamins C and E are reported to falsely lower test results, possibly by inhibiting Hb glycation *(230, 231)*. Iron deficiency anemia increases test results *(232)*. Food intake has no significant effect on test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, and opiate addiction reportedly interfere with some assay methods, falsely increasing results *(204, 233)*.

Several Hb variants (e.g., Hbs S, C, D, and E) and chemically modified Hb derivatives interfere with some assay methods [independently of any effects due to shortened erythrocyte survival *(234–236)*; for a review, see *(233)*]. Depending on the particular hemoglobinopathy and assay method, results can be either falsely increased or falsely decreased. Some methods may give a value in the reference interval for a nondiabetic individual with an Hb variant, but that is no assurance that no interference is present. The interference may be subtle in the reference interval but may increase steadily with increasing Hb A_{1c}. Boronate affinity chromatography assay methods are generally considered to be less affected by Hb variants than other methods. In some instances, such as with most cation-exchange HPLC methods, manual inspection of chromatograms or an automated report by the device can alert the laboratory to the presence of either a variant or a possible interference. If an appropriate method is used, Hb A_{1c} can be measured accurately in the vast majority of individuals heterozygous for Hb variants (for a summary of published studies, see http:// www.ngsp.org). If altered erythrocyte turnover interferes with the relationship between mean blood glucose and Hb A_{1c} values, or if a suitable assay method is not available for interfering Hb variants, alternative non–Hb-based methods for assessing long-term glycemic control (such as fructosamine assay) may be useful *(233)*.

Given that interferences are method specific, product instructions from the manufacturer should be reviewed before the Hb A_{1c} assay method is used. A list of interfering factors for specific assays is maintained on the NGSP Web site (http://www.ngsp.org). In selecting an assay method, a laboratory should consider characteristics of the patient population served (e.g., a high prevalence of Hb variants).

Sample collection, handling, and storage. Blood can be obtained by venipuncture or by finger-stick capillary sampling *(237, 238)*. Blood tubes should contain the anticoagulant specified by the manufacturer of the Hb A_{1c} assay method (EDTA can be used unless the manufacturer specifies otherwise). Sample stability is assay method specific *(239, 240)*. In general, whole-blood samples are stable for up to 1 week at 4 °C *(240)*. For most methods, whole-blood samples stored at −70 °C or colder are stable over the long term (at least 1 year), but samples are not as stable at −20 °C. Improper handling of samples, such as storage at high temperatures, can introduce large artifacts that may not be detectable, depending on the assay method.

Manufacturers have introduced a number of convenient blood-collection systems, including filter paper and small vials containing stabilizing/lysing reagent *(241–243)*. These systems are designed for field collection of samples and routine mailing to the laboratory and are generally matched with specific assay methods. They should be used only if studies have been performed to establish the comparability of test results for these collection systems with standard sample-collection and handling methods for the specific assay method used.

B. Analytical. Performance goals and quality control. Several expert groups have presented recommendations for assay performance. Early reports recommended that the inter-assay CV be <5% at normal and diabetic GHb concentrations *(244)*. Subsequent reports have suggested lower CVs [e.g., intralaboratory CVs <3% *(245)* or <2% *(246)*, and interlaboratory CVs <5% *(245)*]. Intraindividual CVs for healthy persons are very small (<2%), and many current assay methods can achieve intralaboratory and interlaboratory CVs of <2% and <3%, respectively *(247)*. A recent statistical analysis calculated appropriate goals for Hb A_{1c} assay performance *(218)*. If the reference change value (also termed "critical difference") is used, an analytical CV ≤2% will produce a 95% probability that a difference of ≥0.5% Hb A_{1c} between successive patient samples is due to a significant change in glycemic control [when Hb A_{1c} is 7% (53 mmol/mol)]. In addition, if a method has no bias, a CV of 3.5% is necessary to have 95% confidence that the Hb A_{1c} result for a patient with a "true" Hb A_{1c} of 7% (53 mmol/mol) will be between 6.5% and 7.5% (between 48 and 58 mmol/mol) *(218)*. We recommend an intralaboratory CV <2% and an interlaboratory CV <3.5%. For a single method, the goal should be an interlaboratory CV <3%.

A laboratory should include 2 control materials with different mean values (high and low) at both the beginning and the end of each day's run. Frozen whole-blood controls stored in single-use aliquots at −70 °C or colder 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. We recommend that a laboratory consider using both commercial and in-house controls to optimize performance monitoring.

Reference intervals. A laboratory should determine its own reference interval according to CLSI guidelines (CLSI Document C28A), even if the manufacturer has provided one. Nondiabetic test individuals should be nonobese, have an FPG concentration <5.6 mmol/L (100 mg/dL), and, ideally, have a 2-h post-OGTT plasma glucose value of <11.1 mmol/L (200 mg/dL). For NGSP-certified assay methods, reference intervals should not deviate substantially (e.g., >0.5%) from 4%–6% (20–42 mmol/mol). Note that treatment target values recommended by the ADA and other clinical organizations, not reference intervals, are used to evaluate metabolic control in patients.

Recommendation

Samples with Hb A_{1c} results below the lower limit of the reference interval or >15% Hb A_{1c} should be verified by repeat testing.
B (low)

Recommendation

Hb A_{1c} values that are inconsistent with the clinical presentation should be investigated further.
GPP

Out-of-range samples. A laboratory should repeat testing for all sample results below the lower limit of the reference interval, and if these results are confirmed, the physician should be informed to determine whether the patient has a variant Hb or shows evidence of erythrocyte destruction. If possible, the repeat Hb A_{1c} measurement should be performed with a method based on an analytical principle that is different from the initial assay. In addition, samples with results >15% Hb A_{1c} (140 mmol/mol) should be assayed a second time; if the results are confirmed, the possibility of an Hb variant should be considered *(233)*. Any result that does not correlate with the clinical impression should also be investigated.

Removal of labile GHb. The formation of Hb A_{1c} involves an intermediate Schiff base, which is called "pre-A_{1c}" or "labile A_{1c}" *(248)*. This Schiff base is formed rapidly with hyperglycemia and can interfere with some Hb A_{1c} assay methods if it is not completely removed or separated. Most currently available automated assays either remove the labile pre–Hb A_{1c} dur ing the assay process or do not measure the labile product.

4. INTERPRETATION

A. Laboratory–physician interactions. A laboratory should work closely with physicians who order Hb A_{1c} testing. Proper interpretation of test results requires an understanding of the assay method, including its known interferences. For example, if the assay method is affected by hemoglobinopathies (independently of any shortened erythrocyte survival) or uremia, the physician should be made aware of this interference.

An important advantage of using an NGSP-certified method is that the laboratory can provide specific information relating Hb A_{1c} test results to both mean glycemia and outcome risks as defined in the DCCT and UKPDS *(44, 147, 187)*. This information is available on the NGSP Web site. For example, each 1% (approximately 11 mmol/mol) change in Hb A_{1c} is related to a change in the mean plasma glucose concentration of approximately 1.6 mmol/L (29 mg/dL). Reporting Hb A_{1c} results with a calculated eAG will eliminate the need for healthcare providers or patients to perform these calculations themselves. The equation generated by the ADAG study is the most reliable to date *(209)*.

Some evidence suggests that immediate feedback of Hb A_{1c} test results to patients at the time of the clinic visit leads to an improvement in their long-term glycemic control *(249, 250)*. Not all publications have supported this observation *(251)*, however, 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 Hb A_{1c} 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 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 Hb A_{1c} concentrations at <7% and more-stringent goals in selected individual patients if they can be achieved without significant hypoglycemia or other adverse treatment effects. Somewhat higher intervals are recommended for children and adolescents and may be appropriate for patients with a limited life expectancy, extensive comorbid illnesses, a history of severe hypoglycemia, or advanced complications (note that these values are applicable only if the NGSP has certified the assay method as traceable to the DCCT reference).
> A (high)

Treatment goals. Hb A_{1c} measurements are now a routine component of the clinical management of patients with diabetes. Principally on the basis of the DCCT results, the ADA has recommended that a primary goal of therapy be an Hb A_{1c} value <7% (53 mmol/mol) *(21)*. Lower targets may be considered for individual patients, e.g., in diet-treated type 2 diabetes. Other major clinical organizations have recommended similar targets *(53)*; however, recent studies that used multiple medications to treat type 2 diabetes and aimed for Hb A_{1c} concentrations <6.5% (48 mmol/mol) have not demonstrated consistent benefits and failed to observe any benefit with regard to macrovascular disease, compared with interventions that achieved Hb A_{1c} values 0.8% to 1.1% higher *(50–52)*. The ACCORD (Action to Control Cardiovascular Risk in Diabetes) study demonstrated increased mortality with very intensive diabetes therapy [Hb A_{1c}, 6.4% vs 7.5% (46 vs 58 mmol/mol)]. These Hb A_{1c} values apply only to assay methods that have been certified as traceable to the DCCT reference, with a reference interval of approximately 4%–6% Hb A_{1c} (20–42 mmol/mol). In the DCCT, each 10% reduction in Hb A_{1c} (e.g., 12% vs 10.8% or 8% vs 7.2%) was associated with an approximately 45% lower risk for the progression of diabetic retinopathy *(42)*. Comparable risk reductions were found in the UKPDS *(197)*. Also of note is that decreases in Hb A_{1c} were associated in the DCCT and UKPDS with an increased risk for severe hypoglycemia.

> ***Recommendation***
>
> Hb A_{1c} testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or who are not meeting treatment goals.
> B (low)

Testing frequency. There is no consensus on the optimal frequency of Hb A_{1c} testing. The ADA recommends *(21)*, "For any individual patient, the frequency of A1C 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 Hb A_{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 goals" *(21)*. These testing recommendations are for nonpregnant patients with either type 1 or type 2 diabetes. In addition, all patients with diabetes who are admitted to a hospital should have Hb A_{1c} measured if the results of testing in the previous 2–3 months are not available *(21)*. Diabetes quality-assurance programs [e.g., Provider Recognition Program and HEDIS (Healthcare Effectiveness Data and Information Set) *(199, 200)*] have generally required documentation of the percentage of diabetes patients who have had at least one Hb A_{1c} measurement during the preceding year. Studies have established that serial Hb A_{1c} measurements (quarterly for 1 year) produce large improvements in Hb A_{1c} values in patients with type 1 diabetes *(252)*.

Interpretation. Hb A_{1c} values in patients with diabetes constitute 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 nondiabetic individuals, to markedly increased values (e.g., two- to threefold increases in some patients) that reflect an extreme degree of hyperglycemia. A proper interpretation of Hb A_{1c} test results requires that physicians understand the relationship between Hb A_{1c} values and mean plasma glucose, the kinetics of Hb A_{1c}, and specific assay limitations/interferences *(147)*. Small changes in Hb A_{1c} (e.g., ±0.3% Hb A_{1c}) over time may reflect assay imprecision rather than a true change in glycemic status *(218)*.

5. EMERGING CONSIDERATIONS

Recommendation

Hb A_{1c} may be used for the diagnosis of diabetes, with values >6.5% being diagnostic. An NGSP-certified method should be performed in an accredited laboratory. Analogous to its use in the management of diabetes, factors that interfere with or adversely affect the Hb A_{1c} assay will preclude its use in diagnosis.
A (moderate)

Recommendation

Point-of-care Hb A_{1c} assays are not sufficiently accurate to use for the diagnosis of diabetes.
B (moderate)

A. Use of Hb A_{1c} for diabetes screening/diagnosis. The role of Hb A_{1c} in the diagnosis of diabetes has been considered for several years (*19, 24, 37, 253*). In the past, the lack of standardization has been a major barrier. With improved standardization through the NGSP and the IFCC, and new data demonstrating the association between Hb A_{1c} concentrations and the risk for retinopathy, the International Expert Committee recommended the use of Hb A_{1c} in the diagnosis of diabetes (*20*). In making its recommendation, the Committee also considered several technical advantages of Hb A_{1c} testing compared with glucose testing, such as its preanalytical stability and decreased biological variation. Finally, the clinical convenience of the Hb A_{1c} assay, which requires no patient fasting or tolerance tests, compared with glucose-based diagnosis, convinced the Committee to recommend Hb A_{1c} testing for diagnosis. A value ≥6.5% (48 mmol/mol) was considered diagnostic on the basis of the observed relationship with retinopathy. For diagnosis, a positive test result [≥6.5% (48 mmol/mol)] should be confirmed with a repeat assay. The ADA indicates that although either an Hb A_{1c} assay or a glucose assay (FPG or OGTT) can be used as the confirmatory test, repeating the same test is preferred *(93)*. The frequency of Hb A_{1c} testing for diagnosis has not been established, but guidelines similar to those for glucose-based testing seem appropriate. Only NGSP-certified Hb A_{1c} methods should be used to diagnose (or screen for) diabetes. The ADA cautions that point-of-care devices for measuring Hb A_{1c} should not be used for diagnosis *(93)*. Although several point-of-care Hb A_{1c} assays are NGSP certified, the test is waived in the US, and proficiency testing is not necessary. Therefore, no objective information is available concerning their performance in the hands of those who measure Hb A_{1c} in patient samples. A recent evaluation revealed that few point-of- care devices that measure Hb A_{1c} met acceptable analytical performance criteria *(254)*. Absent objective—and ongoing—documentation of performance with accuracy-based proficiency testing that uses whole blood (or other suitable material that is free from matrix effects), point-of-care Hb A_{1c} devices should not be used for diabetes diagnosis or screening. The ADA has endorsed the use of Hb A_{1c} for the diagnosis of diabetes (Table 6) (*21*), as have the Endocrine Society *(255)* and the WHO. The American Association of Clinical Endocrinologists supports it in a more limited fashion. Other international organizations, including the IDF, are considering Hb A_{1c} testing for diabetes diagnosis and screening. Note that glucose-based testing for diagnosis remains valid. Analogous to the concept of impaired fasting glucose and impaired glucose tolerance, individuals with Hb A_{1c} values between 5.7% and 6.4% (39 and 46 mmol/mol) should be considered at high risk for future diabetes and should be counseled about effective measures to reduce their risk (*93*).

B. Use of other glycated proteins, including advanced glycation end products, for routine management of diabetes. Further studies are needed to determine whether other glycated proteins, such as fructosamine or glycated serum albumin, are clinically useful for routine monitoring of patients' glycemic status. Further studies are also needed to determine if measurements of advanced glycation end products are clinically useful as predictors of risk for chronic diabetes complications *(256)*. Only 1 study of a subset of DCCT patients evaluated advanced glycation end products in dermal collagen obtained with skin biopsies. Interestingly, the concentration of advanced glycation end products in dermal collagen correlated more strongly with the presence of complications than the mean Hb A_{1c} values *(257)*. The clinical role of such measurements remains undefined. Similarly, the role of noninvasive methods that use light to measure glycation transdermally is undefined.

C. Global harmonization of Hb A_{1c} testing and uniform reporting of results. As noted above, the NGSP has largely succeeded in standardizing the GHb assay across methods and laboratories. Furthermore, the IFCC standardization, which provides a chemically discrete standard, is being implemented worldwide. The reporting recommendations *(223)* need to be implemented with the education of healthcare providers and patients. Some believe that reporting eAG should complement the current reporting in NGSP/DCCT-aligned units (percentages) and the new IFCC results (millimoles per mole), because the eAG results will be in the same units (millimoles per liter or milligrams per deciliter) as patients' self-monitoring. Educational campaigns will be necessary, however, to ensure clear understanding of this assay, which is central to diabetes management.

Chapter **10**

Genetic Markers

1. USE

> ***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)

A. Diagnosis/screening. Type 1 diabetes. Genetic markers are currently of limited clinical value in evaluating and managing patients with diabetes; however, mutational analysis is rapidly emerging for classifying diabetes in the neonate *(258–260)* and in young patients with a dominant family history of diabetes, often referred to as "maturity-onset diabetes of the young" (MODY) *(261)*. Type 1 or autoimmune diabetes is strongly associated with *HLADR*[12] (major histocompatibility complex, class II, DR) and *HLA-DQ* (major histocompatibility complex, class II, DQ) genes. *HLA-DQA1* and *HLA-DQB1* genotyping can be useful to indicate the absolute risk of diabetes. The *HLA DQA1**0301–*DQB1**0302 and *DQA1**0501–*DQB1**0201 haplotypes, alone or in combination, may account for up to 90% of children and young adults with type 1 diabetes *(262)*. These 2 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 *HLA-DR* genetic factors are by far the most important determinants of type 1 diabetes risk *(263)*. HLA typing may be used in combination with islet autoantibody analyses to exclude type 1 diabetes in assisting in the diagnosis of genetic forms of diabetes.

As indicated below, HLA-DR/DQ typing can be useful to indicate a modified risk of type 1 diabetes in persons positive for islet cell autoantibodies, because 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. Some haplotypes induce susceptibility, however, whereas 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 *(264)*.

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 *(265)*. Non-HLA genetic factors include the *INS* (insulin), *PTPN22* [protein tyrosine phosphatase, non-receptortype 22 (lymphoid)], and *CTLA*4 (cytotoxic T-lymphocyte-associated protein 4) genes and several others *(263, 265)*. These additional genetic factors may assist in assigning a probability for a diagnosis of type 1 diabetes of uncertain etiology *(266)*.

It is possible to screen newborn children to identify those at increased risk for developing type 1 diabetes *(267–269)*. This strategy cannot be recommended until a proven intervention is available to delay or prevent the disease *(270)*. There is some evidence that early diagnosis may prevent hospitalization for ketoacidosis and preserve residual beta cells *(271)*. The rationale for the approach is thus discussed below under Emerging Considerations.

> ***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 patients have an autosomal dominant form of the disease or very high degrees of insulin resistance. Type 2 diabetes is a heterogeneous 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

[12] Human genes: *HLA-DR*, major histocompatibility complex, class II, DR; *HLA-DQ*, major histocompatibility complex, class II, DQ; *INS*, insulin; *PTPN22*, protein tyrosine phosphatase, non-receptor type 22 (lymphoid); *CTLA4*, cytotoxic T-lymphocyte-associated protein 4; *KCNJ11*, potassium inwardly-rectifying channel, subfamily J, member 11; *HLA-A*, major histocompatibility complex, class I, A; *HLA-B*, major histocompatibility complex, class I, B; *HLA-C*, major histocompatibility complex, class I, C; *HNF4A*, hepatocyte nuclear factor 4, alpha; *HNF1A*, *HNF1* homeobox A; *HNF1B*, *HNF1* homeobox *B; PDX1*, pancreatic and duodenal homeobox 1 (formerly known as *IPF1*); *NEUROD1*, neurogenic differentiation 1 (also known as *NeuroD* and *BETA2*); *KLF1*, Kruppel-like factor 1 (erythroid); *GCK*, glucokinase (hexokinase 4); *CEL*, carboxyl ester lipase (bile salt-stimulated lipase).

is therefore highly complex. Recent genome-wide association studies have identified >30 genetic factors that increase the risk for type 2 diabetes *(272, 273)*. The risk alleles in these loci all have rela-tively small effects (odds ratios of 1.1 to 1.3), however, and do not significantly enhance our ability to predict the risk of type 2 diabetes (*274*).

MODY. Detecting mutations in MODY patients and their relatives is technically feasible. The reduced costs of sequencing and emerging new technologies make it possible to identify mutations and toproperly classify MODY patients on the basis of specific mutations. As direct automated sequencing of genes becomes standard, it is likely that the detection of specific diabetes mutations will become routine.

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 loci for the expression of quantitative traits 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* (potassium inwardly-rectifying channel, subfamily J, member 11; also known as *KIR6.2*) gene may be treated with sulfonylurea rather than with insulin (*258*, *259*).

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 class II genes that code for several polypeptide chains *(275)*. The major (classic) class I genes are *HLA-A* (major histocompatibility complex, class I, A), *HLA-B* (major histocompatibility complex, class I, B), and *HLA-C* (major histocompatibility complex, class I, C). The loci of class II genes are designated by 3 letters: the first (D) indicates the class, the second (M, O, P, Q, or R) indicates the family, and the third (A or B) indicates the chain. Both classes of the encoded molecules are heterodimers. Class I molecules consist of an α chain and β_2-microglobulin, and class II molecules have 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 *(275)*. 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 disease, and type 1 diabetes *(275)*. Not only the disease but also autoantibodies, which are markers of the disease's pathogenesis, are often associated with HLADRB1, HLA-DQA1, and HLA-DQB1, indicating that self-peptides may also be presented to T cells *(262)*.

Genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself *(1)*. Such forms of diabetes may be secondary to the obesity associated with Prader–Willi syndrome, which maps to chromosome 15q, or to the absence of adipose tissue inherent to the recessive Seip–Berardinelli syndrome of generalized lipodystrophy, which maps to chromo-some 9q34 *(1, 276)*. More than 60 distinct genetic disorders are 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 the genetic factors that contribute to type 2 diabetes risk is substantial (*272*, *273*). 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 associated with long-term complications of diabetes, or it can be as severe as typical type 1 diabetes [e.g., hepatocyte nuclear factor (HNF) mutations] *(277)*.

Eight different MODYs have been identified. MODY-1, -3, -4, -5, -6, and -7 are all caused by mutations in the genes encoding transcription factors that regulate the expression of genes in pancreatic beta cells. These genes are *HNF4A* (hepatocyte nuclear factor 4, alpha) in MODY-1, *HNF1A* (HNF1 homeobox A) in MODY-3, *HNF1B* (HNF1 homeobox B) in MODY-5, PDX1 (pancreatic and duodenal homeobox 1; formerly known as *IPF1*) in MODY-4, *NEUROD1* (neurogenic differentiation 1; also known as *NeuroD* and *BETA2*) in MODY-6, and *KLF1* [Kruppel-like factor 1 (erythroid)] in MODY-7. Homozygous mutations of the *PDX1* gene have been shown to lead to pancreatic agenesis, and heterozygous *PDX1* mutations have been shown to cause MODY-4 *(276)*. The modes of action of the HNF lesions in MODY are still not clear. It is likely that mutations in *HNF1A*, *HNF1B*, and *HNF4A* cause diabetes because they impair insulin secretion. MODY-2 is caused by mutations in the *GCK* [glucokinase (hexokinase 4)] gene. The product of the gene is an essential enzyme in the glucose-sensing mechanism of beta cells, and mutations in this gene lead to partial deficiencies of insulin secretion. MODY-8 is due to mutations in the *CEL* [carboxyl ester lipase (bile salt-stimulated lipase)] gene.

3. ANALYTICAL CONSIDERATIONS

A detailed review of analytical issues will not be attempted here, because genetic testing for diabetes outside of a research setting is currently not recommended for clinical care. Serologic HLA typing should be replaced by molecular methods, because antibodies with a mixture of specificities and cross-reactivities have been estimated to give inaccurate results in approximately 15% of typings.

A. Preanalytical. Mutations are detected by using genomic DNA extracted from peripheral blood leukocytes. Blood samples should be drawn into test tubes containing EDTA, and the DNA should be extracted 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 100–200 μg DNA from 10 mL of whole blood. DNA samples are best kept at −80 °C in Tris-EDTA solution. These conditions maintain DNA sample integrity virtually indefinitely.

B. Analytical. Methods for the detection of mutations vary with the type of mutation. MODY mutations have substitution, deletion, or insertion of nucleotides in the coding regions of the genes. These mutations are detected by the PCR. Detailed protocols for detecting specific mutations are beyond the scope of this review.

4. INTERPRETATION

For screening 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 *(278)*. *HLA-DQ* genes appear to be central to the HLA-associated risk of type 1 diabetes, albeit *HLA-DR* genes may be independently involved [for reviews, see *(279, 280)*]. 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 transcomplementated α- and β-chain heterodimers. Thus, in any individual, 4 possible DQ dimers are encoded. Persons at the highest genetic risk for type 1 diabetes are those in whom all 4 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 absolute lifetime risk of type 1 diabetes in the general population of about 1 in 12. Persons who are protected from developing type 1 diabetes at a young age are those with HLA DRB1*15–DQA1*0201– DQB1*0602 haplotypes in particular *(281)*. 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, whereas 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 molecules are involved in antigen presentation to CD4 helper cells, and the associations outlined above are likely to be explained by defective affinities to islet cell antigenic peptides, leading to persistence of T-helper cells that escape thymic ablation. Class I HLA molecules are also implicated in type 1 diabetes. Multiple non-HLA loci also contribute to susceptibility to type 1 diabetes (*279*). For example, the variable nucleotide tandem repeat (VNTR) upstream from the INS gene on chromosome 11q is 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* and *HLADQ*—and to a lesser degree the *INS* gene—allows prediction of type 1 diabetes to better than 1 in 10 in the general population. The risk of type 1 diabetes in HLA-identical siblings of a proband with type 1 diabetes is 1 in 4, whereas siblings who have HLA haplotype identity have a 1 in 12 risk and those with no shared haplotype have a 1 in 100 risk *(280)*. Genome-wide association studies have confirmed that the following non-HLA genetic factors increase the risk for type 1 diabetes, both in first-degree relatives of type 1 diabetes patients and in the general population: *INS*, VNTR, *CTLA4*, *PTPN22*, and others *(263, 265, 282, 283)*.

5. EMERGING CONSIDERATIONS

The sequencing of the human genome and the formation of consortia have produced advances in the identification of the genetic bases for both type 1 and type 2 diabetes. This progress should ultimately lead to family counseling, prognostic information, and the selection of optimal treatments (*276, 284*).

Chapter 11

Autoimmune Markers

1. USE

> ***Recommendation***
>
> Islet cell autoantibodies are recommended for screening nondiabetic family members who wish to donate part of their pancreas for transplantation into 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 after HLA typing at birth.
> B (low)

No therapeutic intervention that will prevent diabetes has been identified *(279, 280)*. Therefore, although several islet cell autoantibodies have been detected in individuals with type 1 diabetes, their measurement has limited use outside of clinical studies. Currently, islet cell autoantibodies are not used in routine management of patients with diabetes. This section focuses on the pragmatic aspects of clinical laboratory testing for islet cell autoantibodies.

A. Diagnosis/screening. Diagnosis. In type 1 diabetes, the pancreatic islet beta cells are destroyed and lost. In the vast majority of these patients, the destruction is mediated by an autoimmune attack *(285)*. This disease is termed "type 1A" or "immune-mediated diabetes" (Table 5). Islet cell auto-antibodies comprise autoantibodies to islet cell cytoplasm (ICA), to native insulin [referred to as "insulin autoantibodies" (IAA) *(286)*], to the 65-kDa isoform of glutamic acid decarboxylase (GAD65A) *(287–289)*, to 2 insulinoma antigen 2 proteins [IA-2A *(290)* and IA-2βA (also known as phogrin) *(291)*], and to 3 variants of zinc transporter 8 (ZnT8A) *(292, 293)*. Autoantibody markers of immune destruction are usually present in 85% to 90% of individuals with type 1 diabetes when fasting hyperglycemia is initially detected *(1)*. Autoimmune destruction of beta cells has multiple genetic predispositions and is modulated by undefined environmental influences. The autoimmunity may be present for months or years before the onset of hyper-glycemia and subsequent symptoms of diabetes. After years of type 1 diabetes, some antibodies fall below detection limits, but GAD65A usually remains increased. Patients with type 1A diabetes have a significantly increased risk of other autoimmune disorders, including celiac disease, Graves disease, thyroiditis, Addison disease, and pernicious anemia *(128)*. As many as 1 in 4 females with type 1 diabetes have autoimmune thyroid disease, whereas 1 in 280 patients develop adrenal autoantibodies and adrenal insufficiency. A minority of patients with type 1 diabetes (type 1B, idiopathic) have no known etiology and no evidence of autoimmunity. Many of these patients are of African or Asian origin.

> ***Recommendation***
>
> Screening patients with type 2 diabetes for islet cell autoantibodies 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 of treatment of type 2 diabetes.
> B (low)

> ***Recommendation***
>
> Screening for islet cell autoantibodies in relatives of patients with type 1 diabetes or in persons from the general population is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies.
> B (low)

Screening. Only about 15% of patients with newly diagnosed type 1 diabetes have a first-degree relative with the disease *(294)*. The risk of developing type 1 diabetes in relatives of patients with the disease is approximately 5%, which is 15-fold higher than the risk in the general population (1 in 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 against insulin, IA-2, GAD65, or ZnT8 and are at low risk of developing type 1 diabetes *(295)*. Because of the low prevalence of type 1 diabetes (approximately 0.3% in the general population), the positive predictive value of a single islet cell autoantibody will be low *(280)*. The presence of multiple islet cell autoantibodies (IAA, GAD65A, IA-2A/IA-2βA, or ZnT8A) is associated with a >90% risk of type 1 diabetes *(292, 295, 296)*; however, until cost-effective screening strategies can be developed for young children and until effective intervention therapy to prevent or delay the onset of the disease becomes available, such testing cannot be recommended outside of a research setting.

Children with certain HLA-DR and/or HLADQB1 chains (*0602/*0603/*0301) are mostly protected from type 1 diabetes, but not from developing islet cell autoantibodies (297). Because islet cell auto-antibodies in these individuals have substantially reduced predictive significance, they are often excluded from prevention trials.

Approximately 5%–10% of adult Caucasian patients who present with a type 2 diabetes phenotype also have islet cell autoantibodies *(298)*, particularly GAD65A, which predict insulin dependency. This condition has been termed "latent autoimmune diabetes of adulthood" (LADA) *(299)*, "type 1.5 diabetes" *(300)*, or "slowly progressive IDDM" *(301)*. 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 *(300)*. Islet cell autoantibody testing in patients with type 2 diabetes has limited utility, because the institution of insulin therapy is based on glucose control.

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 in some clinical trials as surrogate end points.
B (low)

B. Monitoring/prognosis. No acceptable therapy has been demonstrated to prolong the survival of islet cells once diabetes has been diagnosed or to prevent the clinical onset of diabetes in islet cell autoantibody– positive individuals *(279)*. Thus, the use of 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 *(302*). When a partial pancreas has been transplanted from an identical twin or other HLA-identical sibling, the appearance of islet cell autoantibodies may raise consideration regarding the use of immunosuppressive agents to try to halt the 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 who have islet cell autoantibody markers of type 1 diabetes and who progress to insulin dependency *(303)*; *(b)* to screen nondiabetic 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 *(304, 305)*. For example, some pediatric diabetologists now treat children thought to have type 2 diabetes with oral medications but treat autoantibody-positive children immediately with insulin. It is possible, however, to follow patients who are islet cell autoantibody positive to the point of metabolic decompensation and then institute insulin therapy. The Diabetes Prevention Trial of Type 1 Diabetes (DPT-1) study failed to show a protective effect of parenteral insulin *(306)*.

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 lifestyle changes. There is no 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 nonautoimmune diabetes, adults with islet cell autoantibodies but clinically classified as type 2 diabetes, and children with transient hyperglycemia. The majority of nondiabetic individuals who have only 1 autoantibody may never develop diabetes. Although the production of multiple islet cell autoantibodies is associated with considerably increased diabetes risk *(295, 296)*, approximately 20% of individuals presenting with new-onset diabetes produce only a single autoantibody. Prospective studies of children reveal that islet cell autoantibodies may be transient, indicating that an islet autoantibody may have disappeared prior to the onset of hyperglycemia or diabetes symptoms *(307)*.

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.
GPP

For IAAs, a radioisotopic method that calculates the displaceable insulin radioligand binding after the addition of excess nonradiolabeled insulin *(308)* is recommended. Results are reported as positive when specific antibody binding exceeds the 99th percentile or possibly exceeds the mean plus 2 (or 3) SDs for healthy persons. Insulin autoantibody binding has been noted not to be normally distributed. Each laboratory needs to assay at least 100–200 healthy individuals to determine the distribution of binding. An important caveat concerning IAA measurement is that insulin antibodies develop after insulin therapy, even in persons who use human insulin. Data from the Diabetes Autoantibody Standardization Program (DASP) demonstrate that the interlaboratory imprecision for IAA is inappropriately large *(309)*.

GAD65A and IA-2A are measured with standardized radiobinding assays, which are performed with ^{35}S-labeled recombinant human GAD65 or IA-2 generated by coupled in vitro transcription translation with [^{35}S]methionine or other ^{35}S- or ^{3}H-labeled amino acids *(310)*. Commercially available methods for GAD65A and IA-2A are available as a radioimmunoassay with ^{125}I-labeled GAD65 (truncated at the N-terminal end to promote solubility) and IA-2, respectively. In addition, immunoassays without radio-label are commercially available for both GAD65A and IA-2A. Major efforts have been made to standardize GAD65A and IA-2A measurements *(309, 311)*. A WHO standard for both GAD65A and IA-2A has been established, and GAD65A and IA-2A amounts are expressed in international units *(312)*. The binding of labeled autoantigen to autoantibodies is normally distributed. Cutoff values should be determined from 100–200 serum samples obtained from healthy individuals. GAD65A and IA-2A results should be reported as positive when the signal exceeds the 99th percentile. Comparison of multiple laboratories worldwide is carried out in the DASP, a proficiency-testing program organized by the CDC under the auspices of the Immunology of Diabetes Society. That commercially available GAD65A and IA-2A methods are also participating in the DASP program demonstrates that it should be possible not only to harmonize participating laboratories but also eventually to standardize GAD65A and IA-2A *(311)*.

ICAs are measured by indirect immunofluorescence of frozen sections of human pancreas (*313*). ICA assays measure the degree of immunoglobulin binding to islets, and results are compared with a WHO standard serum available from the National Institute of Biological Standards and Control *(312)*. The results are reported in Juvenile Diabetes Foundation (JDF) units. Positive results depend on the study or context in which they are used, but many laboratories use 10 JDF units measured on 2 separate occasions or a single result ≥20 JDF units as titers that may indicate a significantly increased risk of type 1 diabetes. The method is cumbersome and has proved difficult to standardize. The number of laboratories that still carry out the ICA assay has decreased markedly, and the test is no longer included in the DASP program.

4. INTERPRETATION

GAD65A may be present in approximately 60%–80% of patients with newly diagnosed type 1 diabetes, but the frequency varies with sex and age. GAD65A is associated with HLA DR3–DQA1*0501–DQB1*0201 in both patients and healthy individuals. IA-2As may be present in 40%–50% of patients with newly diagnosed type 1 diabetes, but the frequency is highest in the young. The frequency decreases with increasing age. IA-2As are associated with HLA DR4– DQA1*0301–DQB1*0302. IAA positivity occurs in >70%–80% of children who develop type 1 diabetes before 5 years of age but occurs in <40% of individuals who develop diabetes after the age of 12 years. IAAs are associated with HLA DR4–DQA1*0301–DQB1*0302 and with INS VNTR *(262)*. ICA is found in about 75%–85% of new-onset patients.

The ICA assay is labor-intensive and difficult to standardize, and marked interlaboratory variation in sensitivity and specificity has been demonstrated in workshops *(284, 314)*. Few clinical laboratories are likely to implement this test. The immunoassays are more reproducible and are amenable to standardization *(309)*. Measurement of T-cell reactivity in peripheral blood is theoretically appealing, but the imprecision of such as-says precludes their use from a clinical setting *(315, 316)*. Autoantibody positivity (by definition) occurs in healthy individuals despite an absence of a 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 an individual tests positive for 2 or more autoantibodies *(306)*.

The following suggestions (*279*) have been proposed as a rational approach to the use of autoantibodies in diabetes: *(a)* Antibody assays should have a 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 false-negative results.

5. EMERGING CONSIDERATIONS

Immunoassays for IAA, GAD65A, IA-2A/IA-2βA, and ZnT8A are now available, and a panel of these autoantibodies is currently used in screening studies *(317)*. Because ICA assays are difficult to standardize, their use has declined substantially.

It is likely that other islet cell antigens will be discovered, and such discoveries could lead to additional diagnostic and predictive tests for type 1 diabetes. Autoantibody screening of dried spots obtained from finger-stick blood samples appears quite feasible in the future. For individuals who are positive for islet cell autoantibodies, *HLA-DR/HLA-DQ* genotyping will help define the absolute risk of type 1 diabetes.

Several clinical trials to prevent or intervene with type 1 diabetes are being actively pursued *(317)*. Such trials can now be done with relatives of patients with type 1 diabetes or in the general population on the basis of the islet cell autoantibody and *HLA-DR/HLA-DQ* genotype status. Risk can be assessed by islet cell auto-antibodies alone, without the need for evaluating endogenous insulin reserves, as was done for the US DPT-1 trial *(306)*. Rates of islet cell autoantibody positivity are distinctly lower in the general population than in relatives of individuals with type 1 diabetes; consequently, trials with the latter group are more economical. Potential interventional therapies (for type 1 diabetes) undergoing clinical trials include oral insulin *(317)* or nasal insulin *(318)* given to nondiabetic (but islet cell autoantibody positive) relatives of individuals with type 1 diabetes or to children with islet cell auto-antibodies and HLA genotypes conferring increased risk. Phase II clinical trials with alum-formulated GAD65 have reported no adverse events and some preservation of endogenous insulin production in GAD65A-positive diabetes patients *(319, 320)*. Additional trials of other antigen-based immunotherapies, adjuvants, cytokines, and T-cell accessory molecule– blocking agents are likely in the future *(270)*. Decreased islet cell autoimmunity will be one important outcome measure of these therapies.

Chapter **12**

Albuminuria (formerly microalbuminuria)

Albuminuria (formerly microalbuminuria) are a well-established cardiovascular risk marker, in which increases over time to macroalbuminuria (>300 mg/day) are associated with kidney disease and an increased risk for progression to end-stage renal disease. Annual testing for albuminuria is recommended by all major guidelines for patients with diabetes and/or kidney disease. To be useful, semiquantitative or qualitative screening tests must be shown to be positive in >95% of patients with albuminuria. Positive results of such tests must be confirmed by quantitative testing in an accredited laboratory.

1. USE

Recommendation

Annual testing for albuminuria in patients without clinical proteinuria should begin in pubertal or postpubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment.
B (moderate)

Recommendation

Urine albumin at concentrations ≥30 mg/g creatinine should be considered a continuous risk marker for cardiovascular events.
B (moderate)

Table 9. Review of assays to assess albuminuria.

Method	Interassay CV	Detection limit
Immunonephelometry (Beckman Coulter Array analyzer)	4.2% at 12.1 mg/L	2 mg/L
	5.3% at 45 mg/L	
Immunoturbidimetry (Dade Behring turbimeter)	4.1% at 10.6 mg/L	6 mg/L
	2.2% at 77.9 mg/L	
Hemocue (point of care)	2.2% at 77.9 mg/L	5 mg/L
	4.3% at 82 mg/L	
Radioimmunoassay	9.2% at 12.2 mg/dL	16 µg/L
	4.8% at 33 mg/L	

A. Diagnosis/screening. Diabetes is associated with a very high rate of cardiovascular events and is the leading cause of end-stage renal disease in the Western world *(321)*. Early detection of risk markers, such as albumin in the urine (formerly termed "microalbuminuria"), relies on 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 to detect albumin concentrations are used (Table 9) *(322–324)*. Low levels of albuminuria have been defined by the Joint National Committee (JNC) 7 and the ADA and have more recently been redefined by the Kidney Disease: Improving Global Outcomes Committee *(21, 325–327)* as excretion of 30–300 mg of albumin/24 h, 20–200 µg/min, or 30–300 µg/mg creatinine (Table 10) on 2 of 3 urine collections. Recent data, however, suggest that risk extends below the lower limit of 20 µg/min *(328–330)*, reinforcing the notion that this factor is a continuous variable for cardiovascular risk *(331–333)*.

The JNC 7, the National Kidney Foundation (NKF), and the ADA all recommend the use of morning spot albumin/creatinine measurement for annual quantitative testing for urine albumin in adults with diabetes *(21, 326, 327)*. Individuals should be fasting. The optimal time for spot urine collection is the early morning, but for minimizing variation, all collections should be at the same time of day; the individual preferably should not have ingested food for at least 2 h *(334)*.

Positive test results represent "albuminuria" in these guidelines, corresponding to protein excretion of >300 mg/24 h, >200 µg/min, or >300 mg/g creatinine (Table 10). In these patients, quantitative measurement of urine albumin excretion is used in assessing the severity of albuminuria and its progression, in planning treatment, and in determining the impact of therapy. To properly assess the stage of kidney disease, the estimated glomerular filtration rate (eGFR) can be calculated from the serum creatinine value, age, sex, and race of the patient (*335*). An eGFR of <60 mL/min, regardless of the presence of low levels of albuminuria, is an independent cardiovascular risk factor (*325, 327*). A urine albumin value of <30 mg/g creatinine, although considered "normal," should be reassessed annually, because values as low as 10 mg/g creatinine have been associated in some studies with an increased cardiovascular risk. If the value is ≥30 mg/g creatinine, changes should be reassessed after 6 to 12 months if antihypertensve therapy is required or annually in those who are normotensive (*326*). For children with type 1 diabetes,

Table 10. Definitions of albuminuria.[a]

	Unit of measure		
	mg/24 h	μg/min	μg/mg Creatinine
Normal	<30	<20	<30
High albuminuria (formerly microalbuminuria)	30–300	20–200	30–300
Very high albuminuria[b]	>300	>200	>300

[a] From the ADA (*21*).
[b] Also called "overt nephropathy."

testing for low levels of albuminuria is recommended to begin after puberty and after a diabetes duration of 5 years. Of note is that most longitudinal cohort studies have reported significant increases in the prevalence of low levels of albuminuria only after diabetes has been present for 5 years *(326, 336)*.

In the algorithms of both the NKF and the ADA for urine protein testing *(321)*, the diagnosis of low levels of albuminuria requires both the demonstration of increased albumin excretion (as defined above) on 2 of 3 tests repeated at intervals of 3 to 6 months and the exclusion of conditions that "invalidate" the test (Fig. 2).

B. Prognosis. Albuminuria values >30 mg/g creatinine [and lower values if the eGFR is <60 mL/min (Table 10)], has prognostic significance. Multiple epidemio-logic studies have shown it to be an independent risk marker for cardiovascular death *(325, 337, 338)*. In 80% of patients with type 1 diabetes and low levels of albuminuria, urinary albumin excretion can increase by as much as 10%–20%/year, with the development of clinical proteinuria (>300 mg albumin/day) in 10–15 years in more than half the patients. After clinical-grade proteinuria occurs, >90% of patients develop a decreased GFR and, ultimately, end-stage renal disease. In type 2 diabetes, 20%–40% of patients with stage A2 albuminuria (Table 10) progress to overt nephropathy, but by 20 years after overt nephropathy, approximately 20% develop end-stage renal disease. In addition, patients with diabetes (type 1 or type 2) and stage A2 albuminuria are at increased risk for cardiovascular disease. Of note is that low levels of albuminuria alone indicate neither an increased risk for progression to end-stage kidney disease nor kidney disease per se; hypertension needs to be present for the risk of progression *(339, 340)*. Moreover, about 20% of people progress to end-stage kidney disease without an increase in low levels of albuminuria *(341)*. Another factor that indicates progression is an increase in albuminuria from stage A2 to A3 over time despite achievement of blood pressure goals *(342)*.

C. Monitoring. The roles of routine urinalysis and albumin measurements are less clear in patients with stage A2 albuminuria. Some experts have advocated urine protein testing to monitor treatment, which may include improved glycemic control, more assiduous control of hypertension,

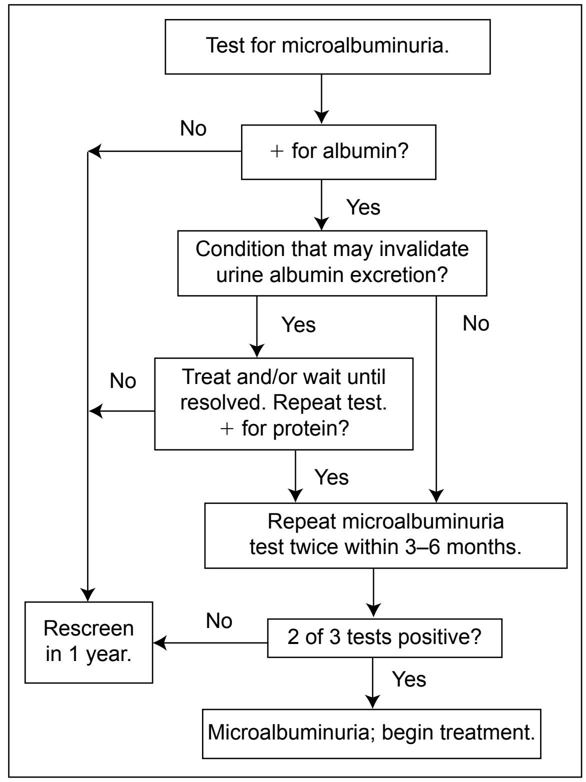

Fig. 2. Algorithm for urine protein testing.

dietary protein restriction, and therapy with blockers of the renin angiotensin system *(321)*. Several factors are known to slow the rate of urinary albumin excretion or to prevent its development. They include reducing blood pressure (with a blocker of the renin angiotensin system as part of the regimen), glycemic control, and lipid-lowering therapy *(45, 343–345)*.

2. RATIONALE

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

Albuminuria (stage A2) rarely occurs with a 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 of diagnosis of diabetes. Although older patients (age >75 years or a life expectancy <20 years) may not be at risk for clinically significant nephropathy because of a short projected life span, they will be at higher cardiovascular risk. In such patients, the role of treating albuminuria is far from clear. Published studies have demonstrated that it is cost-effective to screen all patients with diabetes and/or kidney disease for albumin-uria *(346, 347)*.

3. ANALYTICAL CONSIDERATIONS

> ***Recommendation***
>
> The analytical cv of methods to measure low levels of albuminuria should be <15%.
> B (moderate)

A. Analytical. Analytical goals can be related to the degree of biological variation, with less precision required for analytes that vary widely. Detection limits and imprecision data are summarized in Table 9. Commercially available quantitative methods for low levels of albuminuria have documented detection limits of approximately 20 μg/L or less. Within-run imprecision and day-to-day (total) imprecision are well within the analytical goal of approximately 15% and are often considerably less. Most, but not all, methods agree well and support a reference interval of 2–20 μg albumin/mg creatinine *(348)*.

The within-person variation in albumin excretion is large in people without diabetes and is even higher in patients with diabetes. Howey et al. *(349)* studied dayto-day variation, over 3–4 weeks, in the 24-h albumin excretion, the concentration of albumin, and the albumin–creatinine ratio. The last 2 variables were measured in the 24-h urine sample, the first morning void, and random untimed urine collections. In healthy volunteers, the lowest within-person CVs were obtained for the concentration of albumin in the first morning void (36%) and for the albumin–creatinine ratio in that sample (31%) (*349*). Multiple studies have evaluated the best procedure to assess albuminuria. Most studies have found that the spot urine albumin– creatinine concentration in the first morning void, rather than the 24-h urinary excretion of albumin or the timed collection, is the most practical and reliable technique (*346, 350, 351*).

To keep the analytical CV less than half the biological CV, an analytical goal of an 18% CV has been proposed *(349)*. Alternatively, if the albumin–creatinine ratio is to be used, one may calculate the need for a 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 creatinine measurement, 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 calculating either the timed excretion rate or the albumin–creatinine ratio.

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

Qualitative (or semiquantitative) assays have been proposed as screening tests for low levels of albuminuria. To be useful, screening tests must have high detection rates, 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 low levels of albuminuria, that is, an increased albumin excretion rate or its surrogate, an 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 >1 study. For example, in a large study *(352)*, the sensitivity for detection of an albumin excretion rate >30 mg/24 h 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 2 subsequent studies *(353, 354)*, the sensitivities were 67%– 86%. False-positive results also appear to be common, with rates as high as 15% *(352)*. 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. Of the available methods, the immunoturbidimetric assay is the most reliable and should be considered the standard for comparison, because it has >95% sensitivity and specificity to detect very low levels of albuminuria. Semiquantitative or qualitative screening tests should be positive in >95% of patients for the detection of albuminuria to be useful for assessment of cardiovascular risk and progression of kidney disease. Positive results obtained with such methodologies must be confirmed by an immunoturbidimetric assay in an accredited laboratory *(355)*.

> ***Recommendation***
>
> Currently available dipstick tests do not have adequate analytical sensitivity to detect low levels of albuminuria.
> B (moderate)

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

The available dipstick methods to detect low levels of albuminuria 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 also must be confirmed, a completely untenable approach. With semiquantitative tests, it may be possible (or indeed necessary) to use a cutoff <20 mg/L to ensure the detection of samples with albumin values >20 mg/L as measured by laboratory methods.

Recent studies have compared selected dipstick methods to laboratory assays. One dipstick was found to have >95% sensitivity *(322, 324)*. One such study evaluated an office-screening test that uses a monoclonal antibody against human serum albumin (Immuno-Dip; Genzyme Diagnostics) *(322)*. Screening 182 patient samples with this method with an albumin– creatinine ratio of ≥30 µg/mg as positive yielded a 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 point-of-care system for albumin compared with the Clinitek Microalbumin (Siemens) and Chemstrip Micral (Roche Diagnostics) tests, as well as with an HPLC assay, for spot albumin–creatinine ratio measurement *(324)*. Further studies are needed before the dipstick tests for low levels of albuminuria can be recommended as replacements for the quantitative tests. The use of qualitative tests at the point of care is reasonable only when it can be shown that this approach eliminates quantitative testing in a sizeable proportion of patients and detects those patients who have early renal disease.

Recommendation

Acceptable samples to test for increased urinary albumin excretion are timed collections (e.g., 12 or 24 h) for measurement of the albumin concentration and timed or untimed samples for measurement of the albumin–creatinine ratio.
B (moderate).

Recommendation

The optimal time for spot urine collection is the early morning. All collections should be at the same time of day to minimize variation. The patient should not have ingested food within the preceding 2 h but should be well hydrated (i.e., Not volume depleted).
GPP

B. Preanalytical. Collection of 24-h samples has disadvantages, specifically because many samples are collected inadequately and because total creatinine is not routinely checked to evaluate the adequacy of collection. The albumin– creatinine ratio is the superior method to predict renal events in patients with type 2 diabetes *(356)*. The ratio has a within-person biological variation similar to that of the excretion rate and correlates well with both timed excretion and the albumin concentration in a first morning void of urine *(349)*. For the ratio, a first morning void sample is preferable because this sample has a lower within-person variation than the ratio for a random urine sample taken during the day *(349)*. Although the ratio appears entirely acceptable for screening, limited data are available on 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 *(357)*. For screening, an untimed sample for albumin measurement (without creatinine) may be considered if a one uses a concentration cutoff that allows high sensitivity for detecting an increased albumin-excretion rate.

Albumin is stable in untreated urine stored at 4 °C or 20 °C for at least a week *(358)*. Neither centrifugation nor filtration appears necessary before storage at -20 °C or -80 °C *(359)*. Whether a urine sample is centrifuged, filtered, or not treated, the albumin concentration decreases by 0.27%/day at -20 °C but shows no decreases over 160 days at -80 °C *(359)*. The urinary albumin excretion rate does not show marked diurnal variation in diabetes but does so in essential hypertension *(360)*.

4. INTERPRETATION

A. Nonanalytical sources of variation. Transient increases in urinary albumin excretion have been reported with short-term hyperglycemia, exercise, uri-nary tract infections, marked hypertension, heart failure, acute febrile illness, and hyperlipidemia *(321)*.

Recommendation

Low urine albumin concentrations (i.e., <30 mg/g creatinine) are not associated with high cardiovascular risk if the eGFR is >60 ml • min^{-1} • (1.73 $m^2)^{-1}$ and the patient is normotensive. If the eGFR is <60 ml • min^{-1} • (1.73 $m^2)^{-1}$ and/or the level of albuminuria is >30 mg/g creatinine on a spot urine sample, a repeat measurement should be taken within the year to assess change among people with hypertension.
A (moderate)

B. Frequency of measurement. The NKF, the ADA, and JNC 7 recommend annual measurement in diabetic patients with albumin–creatinine ratios <30 µg/mg. After the documentation of stage A2 albuminuria (i.e., with results as defined above on 2 of 3 tests performed within 3 to 6 months), repeated testing is reasonable to determine whether a chosen therapy is effective. It may also be useful in determining the rate of disease progression and thus may 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 an early onset of diabetes, poor control, or a family history of diabetic nephropathy. The duration of diabetes prior to puberty is reportedly an important risk factor in this age group and thus can be used to support such testing in individual patients *(361)*.

Chapter 13

Miscellaneous Potentially Important Analytes

MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES. I. INSULIN AND PRECURSORS

1. USE

> ***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 be made in most cases on the basis of the clinical presentation and the subsequent course. These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis.
> B (moderate)

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

A. Diagnosis. In the last several years, interest has increased in the possibility that measurements of the concentrations of plasma insulin and its precursors might be of clinical benefit. In particular, published evidence reveals that increased concentrations of insulin and/or proinsulin in nondiabetic individuals predict the development of coronary artery disease *(362)*. Although this possibility may be scientifically valid, its clinical value is questionable. An increased insulin concentration is a surrogate marker that can be used to estimate resistance to insulin-mediated glucose disposal, and it can identify individuals at risk for developing syndrome X, also known as the insulin resistance syndrome or the metabolic syndrome *(363)*. 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 *(364, 365)*. Because of 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 with more traditional clinical and laboratory measurements *(366)* and that measures of insulin resistance (that include insulin measurements) predict the risk of diabetes or coronary artery disease only moderately well, with no threshold effects *(367)*. 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, the 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 *(366, 367)*.

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 pretreatment insulin concentration, the more appropriate might be insulin, or an insulin secretagogue, as the drug of choice to initiate treatment. Although 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 *(368)*. The diagnosis of an islet cell tumor is based on the persistence of inappropriately increased plasma insulin concentrations in the face of a low glucose concentration. In addition, an increase in the ratio of fasting pro-insulin 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)*. Even in this clinical situation, however, the response to drug therapy will provide useful information, and measurement of C-peptide may not be clinically necessary. Measurementof C-peptide is essential in the investigation of possible factitious hypoglycemiadue to surreptitious insulin administration *(369)*.

In the past, some advocated 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 by abnormalities of carbohydrate metabolism; both abnormalities may respond to treatment with metformin or thiazolidinediones. Although clinical trials have generally evaluated insulin resistance by using the hyperinsulinemic euglycemic clamp, ratios of fasting glucose to insulin, and other modalities, the optimal laboratory evaluation of these patients in routine clinical care has not been 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 *(370)*.

2. ANALYTICAL CONSIDERATIONS

Recommendation

Because 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

Although it has been assayed for >40 years, there is no standardized method available to measure serum insulin *(371)*. Attempts to harmonize insulin assays with commercial insulin reagent sets have produced greatly discordant results *(372)*. Recently, an insulin standardization workgroup of the ADA, in conjunction with the National Institute of Diabetes and Digestive and Kidney Diseases, the CDC, and European Association for the Study of Diabetes, called for harmonization of insulin assay results through traceability to an isotope-dilution liquid chromatography–tandom mass spec-trometry reference *(373)*. 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 *(374)*. Analogous to insulin, considerable imprecision among laboratories has also been observed for measurement of C-peptide. A comparison of 15 laboratories that used 9 different routine C-peptide assay methods, found within- and between-run CVs as high as >10% and 18%, respectively *(375)*. 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 US Centers for Medicare and Medicaid Services that Medicare patients have C-peptide measured in order to be eligible for coverage of insulin pumps. Initially, the requirement was that the C-peptide concentration be ≤0.5 ng/mL; however, because of the noncomparability of results from different assays, which led to denial of payment for some patients with values >0.5 ng/mL, the requirement now states that the C-peptide concentration should be ≤110% of the lower limit of the reference interval of the laboratory's measurement method *(376)*.

MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES. 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 *(371)*. 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 *(377)*. On occasion, however, the titer of insulin antibodies in the circulation can be quite high and associated with a dramatic resistance to the ability of exogenous insulin to lower plasma glucose concentrations. This clinical situation is quite rare, it 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 are 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.

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 that has accumulated in the last 7 to 8 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 nontraditional cardiovascular risk factors for routine assessment of risk in patients with diabetes. These sections have, therefore, been removed.

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REFERENCES

1. ADA. 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. IDF. Diabetes atlas. 3rd ed. Brussels: IDF; 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 2010;362:1090–101.
11. ADA. 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. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039–57.
16. WHO. 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 HbA_{1c} levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diabetes Care 1997;20:785–91.18. McCance DR, Hanson RL, Charles MA, Jacob-sson 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. WHO. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Geneva: WHO; 2006.
20. The International Expert Committee. International Expert Committee report on the role of the A_{1c} assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327–34.
21. ADA. Standards of medical care in diabetes— 2010. Diabetes Care 2010;33(Suppl 1):S11–61.
22. ADA. Tests of glycemia in diabetes. Diabetes Care 2001;24(Suppl 1):S80–2.
23. IDF. Task Force. Global guideline for type 2 diabetes. Brussels: IDF; 2005. p 1–11.
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. ADA. 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, Löwel 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. HbA_{1c} measurement improves the detection of type 2 diabetes in high-risk individuals with nondiagnostic levels of fasting plasma glucose: the Early Diabetes Intervention Program (EDIP). Diabetes Care 2001;24:465–71.
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. Diabetes Cost-Effectiveness Study Group, Centers for Disease Control and Prevention. 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. Kahn R, Alperin P, Eddy D, Borch-Johnsen K, Buse J, Feigelman J, et al. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. Lancet 2010;375:1365–74.
36. Greenberg RA, Sacks DB. Screening for diabetes: is it warranted? Clin Chim Acta 2002;315:61–9.
37. 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.
38. 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.
39. Tai ES, Goh SY, Lee JJ, Wong MS, Heng D, Hughes K, et al. Lowering the criterion for im- paired fasting glucose: impact on disease prevalence and associated risk of diabetes and ischemic heart disease. Diabetes Care 2004;27: 1728–34.
40. 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.
41. 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.
42. DCCT. The relationship of glycemic exposure (HbA_{1c}) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968–83.
43. 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.
44. 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.
45. 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.
46. UKPDS Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–53.
47. 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.
48. 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.
49. 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.
50. 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.
51. 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.
52. 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.
53. Berg AH, Sacks DB. Haemoglobin A_{1c} analysis in the management of patients with diabetes: from chaos to harmony. J Clin Pathol 2008;61: 983–7.
54. 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.
55. 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.
56. Harris MI. Undiagnosed NIDDM: clinical and public health issues. Diabetes Care 1993;16: 642–52.
57. 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.
58. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: why it matters. Clin Chem 2009;55:850–2.
59. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315–7.
60. Ladenson JH. Nonanalytical sources of variation in clinical chemistry results. In: Sonnenwirth A, Jarett L, eds. Clinical laboratory methods and diagnosis. St. Louis: C.V. Mosby Co.; 1980. p 149–92.
61. Sacks DB. Carbohydrates. In: Burtis CA, Ash-wood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. St. Louis: Elsevier Saunders; 2006. p 837–902.
62. 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.
63. 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.
64. 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.
65. 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.
66. Boyanton BL Jr, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7.
67. 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.
68. Larsson-Cohn U. Differences between capillary and venous blood glucose during oral glucose tolerance tests. Scand J Clin Lab Invest 1976; 36:805–8.
69. Lind T, de Groot HA, Brown G, Cheyne GA. Observations on blood glucose and insulin determinations. Br Med J 1972;3:320–3.
70 Sacks DB. Carbohydrates. In: Burtis CA, Ash-wood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. St. Louis: Elsevier Saunders. Accepted; forthcoming 2012.

71. Tchobroutsky G. Blood glucose levels in diabetic and non-diabetic subjects. Diabetologia 1991; 34:67–73.
72. 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.
73. 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.
74. 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.
75. 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.
76. 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.
77. Fraser CG, Petersen PH. Analytical performance characteristics should be judged against objective quality specifications. Clin Chem 1999;45: 321–3.
78. 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.
79. Fraser CG. The necessity of achieving good laboratory performance. Diabet Med 1990;7: 490–3.
80. Olefsky JM, Reaven GM. Insulin and glucose responses to identical oral glucose tolerance tests performed forty-eight hours apart. Diabetes 1974;23:449–53.
81. 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.
82. 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.
83. 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.
84. 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.
85. Lacher DA, Hughes JP, Carroll MD. Estimate of biological variation of laboratory analytes based on the Third National Health and Nutrition Ex- amination Survey. Clin Chem 2005;51:450–2.
86. Westgard QC. Desirable specifications for total error, imprecision, and bias, derived from biologic variation. http://www.westgard.com/biodatabase1. htm (Accessed July 2009).
87. 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.
88. 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.
89. ADA. Standards of medical care in diabetes— 2009. Diabetes Care 2009;32(Suppl 1):S13–61.
90. 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.
91. CDC. Self-monitoring of blood glucose among adults with diabetes—United States, 1997– 2006. MMWR Morb Mortal Wkly Rep 2007;56: 1133–7.
92. ADA. Self-monitoring of blood glucose. Diabetes Care 1996;19(Suppl 1):S62–6.
93. ADA. Standards of medical care in diabetes— 2011. Diabetes Care 2011;34(Suppl 1):S11–61.
94. 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.
95. 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.
96. 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.
97. 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.
98. Harris MI. Frequency of blood glucose monitoring in relation to glycemic control in patients with type 2 diabetes. Diabetes Care 2001;24: 979–82.
99. 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.
100. 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.
101. 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.
102. 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.
103. 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.
104. 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.
105. 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.
106. 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.
107. 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.

108. 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.
109. 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.
110. Allemann S, Houriet C, Diem P, Stettler C. Self-monitoring of blood glucose in non-insulin treated patients with type 2 diabetes: a systematic review and meta-analysis. Curr Med Res Opin 2009;25:2903–13.
111. Poolsup N, Suksomboon N, Rattanasookchit S. Meta-analysis of the benefits of self-monitoring of blood glucose on glycemic control in type 2 diabetes patients: an update. Diabetes Technol Ther 2009;11:775–84.
112. Montori VM, Fernández-Balsells M. Glycemic control in type 2 diabetes: time for an evidence-based about-face? Ann Intern Med 2009;150: 803–8.
113. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. Endocr Rev 1991;12:356–71.
114. 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.
115. ADA. Consensus statement on self-monitoring of blood glucose. Diabetes Care 1994;17:81–6.
116. Tate PF, Clements CA, Walters JE. Accuracy of home blood glucose monitors. Diabetes Care 1992;15:536–8.
117. 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.
118. 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.
119. 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.
120. 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.
121. 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.
122. Weitgasser R, Gappmayer B, Pichler M. Newer portable glucose meters—analytical improvement compared with previous generation devices? Clin Chem 1999;45:1821–5.
123. 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.
124. 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. 125. ADA. Consensus statement on self-monitoring of blood glucose. Diabetes Care 1987;10:93–9.
126. NCCLS. Ancillary (bedside) blood glucose testing in acute and chronic care facilities; approved guideline C30-A. Villanova (PA): NCCLS; 1994; 14:1–14.
127. ISO. In vitro diagnostic test systems — requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus. Geneva: ISO; 2003. ISO 15197:2003; 1st ed. 2003-05-01.
128. 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.
129. 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.
130. Boyd JC, Bruns DE. Quality specifications for glucose meters: assessment by simulation modeling of errors in insulin dose. Clin Chem 2001; 47:209–14.
131. 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.
132. 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.
133. 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 681–3.
134. Mahoney J, Ellison J. Assessing the quality of glucose monitor studies: a critical evaluation of published reports. Clin Chem 2007;53:1122–8.
135. 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.
136. 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.
137. 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.
138. Dungan K, Chapman J, Braithwaite SS, Buse J. Glucose measurement: confounding issues in setting targets for inpatient management. Diabetes Care 2007;30:403–9.
139. 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.
140. Finkielman JD, Oyen LJ, Afessa B. Agreement between bedside blood and plasma glucose measurement in the ICU setting. Chest 2005; 127:1749–51.
141. 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.
142. Meynaar IA, van Spreuwel M, Tangkau PL, Dawson L, Sleeswijk Visser S, et al. Accuracy of AccuChek glucose measurement in intensive care patients. Crit Care Med 2009;37:2691–6.
143. 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.
144. 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.

145 Nathan D. The importance of intensive supervision in determining the efficacy of insulin pump therapy. Diabetes Care 1983;6:295–7.
146. 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.
147. 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.
148. 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.
149. 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 A_{1c} (HBA_{1c}) levels in type I diabetic patients: a systematic review. Diabetes Res Clin Pract 2008;81:79–87.
150. Tamborlane WV, Beck RW, Bode BW, Bucking-ham 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.
151. Haupt K, Mosbach K. Plastic antibodies: developments and applications. Trends Biotechnol 1998;16:468–75.
152. Chen G, Guan Z, Chen CT, Fu L, Sundaresan V, Arnold FH. A glucose-sensing polymer. Nat Biotechnol 1997;15:354–7.
153. James TC, Sananayake DRAS, Shinkai S. A glucose-selective molecular fluorescence sensor. Angew Chem Int Ed Engl 1994;33:2207–9.
154. Birch DJS, Imhof RE. Time-domain fluorescence spectroscopy using time-correlated single-photon counting. Top Fluoresc Spectrosc 1994; 1:1–95.
155. 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.
156. Rolinski OJ, Birch DJS, McCartney LJ, Pickup JC. Near-infrared assay for glucose determination. Proc Soc Photo Opt Instrum Eng 1999;3602:6–14.
157. 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.
158. 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.
159. 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.
160. Arnold MA, Small GW. Noninvasive glucose sensing. Anal Chem 2005;77:5429–39.
161. 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.
162. Sieg A, Guy RH, Delgado-Charro MB. Noninvasive and minimally invasive methods for transdermal glucose monitoring. Diabetes Technol Ther 2005;7:174–97.
163. 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.
164. Heise HM, Lampen P. Transcutaneous glucose measurements using near-infrared spectroscopy: validation of statistical calibration models. Diabetes Care 2000;23:1208–10.
165. 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.
166. 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.
167. 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.
168. Small GW. Chemometrics and near-infrared spectroscopy: avoiding the pitfalls. Trends Anal Chem 2006;25:1057–66.
169. Gabrielsson J, Trygg J. Recent developments in multivariate calibration. Crit Rev Anal Chem 2006;36:243–55.
170. 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.
171. 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. Hoboken, NJ: John Wiley & Sons; 2009. p 357–90.
172. 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. Hoboken, NJ: John Wiley & Sons; 2009. p 391– 419.
173. Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. Diabetes Care 2008;31: 899–904.
174. 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.
175. 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.
176. 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.
177. HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes 2009;58:453–9.
178 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.
179. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 2002;25: 1862–8.
180. ADA. Tests of glycemia in diabetes. Diabetes Care 1999;22:S77–9. 181. Sacks DB. Diabetes mellitus. In: Burtis CA, Ash-wood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. St. Louis: Elsevier Saunders. Accepted, forthcoming 2012.
182. 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.
183. Kreisberg RA. Diabetic ketoacidosis: new concepts and trends in pathogenesis and treatment. Ann Intern Med 1978;88:681–95.

184. 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.
185 Stephens JM, Sulway MJ, Watkins PJ. Relationship of blood acetoacetate and 3-hydroxybutyrate in diabetes. Diabetes 1971;20:485–9.
186. Porter WH, Yao HH, Karounos DG. Laboratory and clinical evaluation of assays for beta-hydroxybutyrate. Am J Clin Pathol 1997;107: 353–8.
187. ADA. Tests of glycemia in diabetes [Position statement]. Diabetes Care 2000;23 Suppl 1:S80–2.
188. Csako G. Causes, consequences, and recognition of false-positive reactions for ketones. Clin Chem 1990;36:1388–9.
189. Rosenbloom AL, Malone JI. Recognition of impending ketoacidosis delayed by ketone reagent strip failure. JAMA 1978;240:2462–4.
190. McMurray CH, Blanchflower WJ, Rice DA. Automated kinetic method for D-3-hydroxybutyrate in plasma or serum. Clin Chem 1984;30:421–5.
191. Koch DD, Feldbruegge DH. Optimized kinetic method for automated determination of beta-hydroxybutyrate. Clin Chem 1987;33:1761–6.
192 D'arrigo T. Beyond blood glucose. DiabetesForecast 1999;52:37–8.
193. Westphal SA. The occurrence of diabetic ketoacidosis in non-insulin-dependent diabetes and newly diagnosed diabetic adults. Am J Med 1996;101:19–24.
194. 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.
195. 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.
196. 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.
197. ADA. Implications of the Diabetes Control and Complications Trial [Position statement]. Diabetes Care 2000;23 Suppl 1:S24–6.
198. 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.
199. Davidson MB. Diabetes research and diabetes care. Where do we stand? Diabetes Care 1998; 21:2152–60.
200. ADA. Provider Notes: the newsletter of the ADA/ NCQA Provider Recognition Program. Provid Notes 2000;1:1–4.
201. 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.
202. Qaseem A, Vijan S, Snow V, Cross JT, Weiss KB, Owens DK. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A_{1c} targets. A guidance statement from the American College of Physicians. Ann Intern Med 2007;147:417–22.
203. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med 1981; 70:325–30.
204. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Chem 1986;32:B64–70.
205. 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.
206. 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.
207. Murata GH, Hoffman RM, Duckworth WC, Wendel CS, Shah JH. Contributions of weekly mean blood glucose values to hemoglobin A_{1c} in insulin-treated type 2 diabetes: the Diabetes Outcomes in Veterans Study (DOVES). Am J Med Sci 2004;327:319–23.
208. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50: 2239–44.
209. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the hemoglobin A_{1c} assay into estimated average glucose values. Diabetes Care 2008;31:1473–8.
210. 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.
211. Tahara Y, Shima K. Kinetics of HbA_{1c}, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995;18:440–7.
212. 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.
213. Bodor GS, Little RR, Garrett N, Brown W, Goldstein DE, Nahm MH. Standardization of glyco-hemoglobin determinations in the clinical laboratory: three years of experience. Clin Chem 1992;38:2414–8.
214. 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.
215. Goldstein DE, Little RR. Bringing order to chaos: the National Glycohemoglobin Standardization Program. Contemp Int Med 1997;9:27–32.
216. 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.
217. DCCT. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. Clin Chem 1987;33:2267–71.
218. Little RR, Rohlfing CL, Sacks DB. Status of HbA_{1c} measurement and goals for improvement: from chaos to order for improving diabetes care. Clin Chem 2011;57:205–14.
219. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, et al. Approved IFCC reference method for the measurement of HbA_{1c} in human blood. Clin Chem Lab Med 2002;40:78–89.
220. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, et al. IFCC reference system for measurement of hemoglobin A_{1c} in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166–74.

221. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson J, et al. The IFCC reference measurement system for HbA_{1c}: a 6-year progress report. Clin Chem 2008;54:240–8.
222. Sacks DB. Translating hemoglobin A_{1c} into average blood glucose: Implications for clinical chemistry. Clin Chem 2008;54:1756–8.
223. Hanas R, John G. 2010 consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. Clin Chem 2010;56: 1362–4.
224. Pani LN, Korenda L, Meigs JB, Driver C, Cha-many 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.
225. Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in hemoglobin A_{1c} levels: a cross-sectional analysis of 2 studies. Ann Intern Med 2010;152:770–7.
226. Little RR, Sacks DB. HbA_{1c}: How do we measure it and what does it mean? Curr Opin Endocrinol Diabetes Obes 2009;16:113–8.
227. 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.
228. Saaddine JB, Fagot-Campagna A, Rolka D, Narayan KM, Geiss L, Eberhardt M, Flegal KM. Distribution of HbA_{1c} levels for children and young adults in the U.S.: Third National Health and Nutrition Examination Survey. Diabetes Care 2002;25:1326–30.
229. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010;362: 800–11.
230. Davie SJ, Gould BJ, Yudkin JS. Effects of vitamin C on glycosylation of proteins. Diabetes 1992; 41:167–73.
231. 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.
232. Tarim O, Kucukerdogan A, Gunay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A_{1c} in type 1 diabetes mellitus. Pediatr Int 1999;41:357–62.
233. 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.
234. 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.
235. 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.
236. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW, Krejs GJ. Evaluation of HbA_{1c} determination methods in patients with hemoglobinopathies. Diabetes Care 2000;23: 339–44.
237. Rendell M, Brannan C, Nierenberg J, Rasbold K, Hestorff T. Fingerstick glycosylated hemoglobin, plasma protein, and albumin. Diabetes Care 1987;10:629–32.
238. 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.
239. 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.
240. 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.
241. Baglin SK, Brown AS. Two capillary blood-collection techniques for estimating glycohemoglobin compared. Clin Chem 1995;41:330–2.
242. Voss EM, Cembrowski GS, Clasen BL, Spencer ML, Ainslie MB, Haig B. Evaluation of capillary collection system for HbA_{1c} specimens. Diabetes Care 1992;15:700–1.
243. Little RR, Wiedmeyer HM, Huang DH, Goldstein DE, Parson RG, Kowal R, et al. A simple blood collection device for analysis of glycohemoglobin (GHB). Clin Chem 1998;44:A139.
244. 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.
245. Marshall SM, Barth JH. Standardization of HbA_{1c} measurements: a consensus statement. Ann Clin Biochem 2000;37:45–6.
246. 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.
247. Sacks DB. CAP surveys: participant summary for glycohemoglobin survey 2010 set GH2-A. Northfield, IL: College of American Pathologists; 2010.
248. Goldstein DE, Peth SB, England JD, Hess RL, Da Costa J. Effects of acute changes in blood glucose on HbA_{1c}. Diabetes 1980;29:623–8.
249. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA_{1c} levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. Diabetes Care 1999;22: 1785–9.
250. 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 HbA_{1c} 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.
251. 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.
252. 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.
253. Davidson MB, Peters AL, Schriger DL. An alternative approach to the diagnosis of diabetes with a review of the literature. Diabetes Care 1995;18:1065–71.
254. Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A_{1c} point-of-care instruments do not meet the general accepted analytical performance criteria. Clin Chem 2010;56:44–52.
255. 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 A_{1c} for the diagnosis of diabetes. Endocr Pract 2010;16:155–6.

256. 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.
257. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, et al. Skin collagen glycation, glycoxidation, and cross-linking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA_{1c} as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes 1999;48: 870–80.
258. 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.
259. 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.
260. 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.
261. 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.
262. 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.
263. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. N Engl J Med 2009;360: 1646–54.
264. 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.
265. 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;703–7.
266. 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 U S A 2000;97: 11371–6.
267. 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.
268. 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.
269. 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.
270. Kukreja A, Maclaren NK. Autoimmunity and diabetes. J Clin Endocrinol Metab 1999;84: 4371–8.
271. 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.
272. 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.
273. 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.
274. 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.
275. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med 2000;343:702–9.
276. Taylor SI, Arioglu E. Genetically defined forms of diabetes in children. J Clin Endocrinol Metab 1999;84:4390–6.
277. Fajans SS, Bell GI, Bowden DW, Halter JB, Polonsky KS. Maturity onset diabetes of the young (MODY). Diabet Med 1996;13:S90–5.
278. Todd JA. Genetics of type 1 diabetes. Pathol Biol (Paris) 1997;45:219–27.
279. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221–9.
280. Harrison LC. Risk assessment, prediction and prevention of type 1 diabetes. Pediatr Diabetes 2001;2:71–82.
281. 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.
282. 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.
283. 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.
284. 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. p 1471–88.
285. 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.
286. 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.
287. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 1990;347: 151–6; erratum 1990;347:728.
288. 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.
289. Atkinson MA, Maclaren NK. Islet cell autoantigens in insulin-dependent diabetes. J Clin Invest 1993;92:1608–16.
290. 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 U S A 1996;93:6367–70.
291. 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 U S A 1996;93:2307–11.

292. 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.
293. 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.
294. 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.
295. 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.
296. 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.
297. 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.
298. 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;351:376]. Lancet 1997;350:1288–93.
299. 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.
300. 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.
301. 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.
302. 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.
303. 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.
304. Petersen JS, Dyrberg T, Damm P, Kuhl C, Molsted-Pedersen L, Buschard K. GAD65 auto-antibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. Diabetologia 1996;39:1329–33.
305. 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.
306. 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.
307. 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.
308. Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA. A novel micro-assay for insulin auto-antibodies. J Autoimmun 1997;10:473–8.
309. Bingley PJ, Bonifacio E, Mueller PW. Diabetes Antibody Standardization Program: first assay proficiency evaluation. Diabetes 2003;52:1128–36.
310. 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.
311. 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.
312. Mire-Sluis AR, Gaines Das R, Lernmark A. The World Health Organization International Collaborative Study for islet cell antibodies. Diabetologia 2000;43:1282–92.
313. Gleichmann H, Bottazzo GF. Progress toward standardization of cytoplasmic islet cell-antibody assay. Diabetes 1987;36:578–84.
314. 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.
315. 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.
316. 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.
317. 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.
318. 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.
319. 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.
320. Lernmark A, Agardh CD. Immunomodulation with human recombinant autoantigens. Trends Immunol 2005;26:608–12.
321. ADA. Diabetes nephropathy. Diabetes Care 1999;22(Suppl 1):S66–9.
322. 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.
323. Khosla N, Sarafidis PA, Bakris GL. Microalbuminuria. Clin Lab Med 2006;26:635–53, vi–vii.

324. 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.
325. Levey AS, de Jong PE, Coresh J, Nahas ME, Astor BC, Matsushita K, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int [Epub ahead of print 2010 Dec 8].
326. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis 2007;49:S12–154.
327. 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.
328. 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.
329. 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.
330. 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 735–6.
331. Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, et al. Albumin-uria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med 2003;139:901–6.
332. 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.
333. 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.
334. 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.
335. 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.
336. 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.
337. 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.
338. 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.
339. 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.
340. 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 466–7.
341. 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.
342. Duka I, Bakris G. Influence of microalbuminuria in achieving blood pressure goals. Curr Opin Nephrol Hypertens 2008;17:457–63.
343. 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.
344. 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.
345. 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.
346. 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.
347. 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 2104.
348. 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.
349. 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.
350. 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.
351. 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.
352. 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.
353. Fernández-Fernández I, Páez Pinto JM, Hermosín Bono T, Vázquez Garijo P, Ortiz Camuñez MA, Tarilonte Delgado MA. Rapid screening test evaluation for microalbuminuria in diabetes mellitus. Acta Diabetol 1998;35: 199–202.
354. 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.
355. Shaikh A, Seegmiller JC, Borland TM, Burns BE, Ladwig PM, Singh RJ, et al. Comparison between immunoturbidimetry, size-exclusion chromatography, and LC-MS to quantify urinary albumin. Clin Chem 2008;54:1504–10.

356. Lambers Heerspink HJ, Gansevoort RT, Brenner BM, Cooper ME, Parving HH, Shahinfar S, de Zeeuw D. Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol 2010;21:1355–60.
357. 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.
358. 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.
359. 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.
360. 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.
361. 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.
362. 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.
363. 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. p 509–19.
364. Chevenne D, Trivin F, Porquet D. Insulin assays and reference values. Diabetes Metab 1999;25: 459–76.
365. Del Prato S. Measurement of insulin resistance in vivo. Drugs 1999;58:3–6; discussion 75–82.
366. 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.
367. 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.
368. Marks V. Recognition and differential diagnosis of spontaneous hypoglycaemia. Clin Endocrinol (Oxf) 1992;37:309–16.
369. Marks V, Teale JD. Hypoglycemia: factitious and felonious. Endocrinol Metab Clin North Am 1999;28:579–601.
370. American College of Obstetrics and Gynecology. ACOG practice bulletin. Polycycstic ovary syndrome. Number 41, December 2002. Int J Gynaecol Obstet 2003;80:335–48.
371. 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.
372. 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.
373. Miller WG, Thienpont LM, Van Uytfanghe K, Clark PM, Lindstedt P, Nilsson G, Steffes MW. Toward standardization of insulin immunoas-says. Clin Chem 2009;55:1011–8.
374. 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.
375. 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.
376. Centers for Medicare and Medicaid Services. Infusion pumps: C-peptide levels as a criterion for use. https://www.cms.gov/transmittals/ downloads/R513CP.pdf (Accessed March 2008).
377. Burge MR, Schade DS. Insulins. Endocrinol Metab Clin North Am 1997;26:575–98.
378. ADA. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl 1): S62–9.

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Appendix

The organizations and individuals listed below were invited to comment on the National Academy of Clinical Biochemistry draft guidelines for laboratory testing of diabetes. We would like to acknowledge and thank those organizations and individuals who reviewed and commented on the draft guidelines. For those organizations that were able to send a representative to the Arnold O. Beckman Conference or provide written comments, the name of the representative is listed with the organization.

Appendix Table 1. Organizations and individuals participating in the public commenting of the NACB Diabetes Mellitus Guidelines

Organizations:

ARUP Laboratories William Roberts, MD, PhD http://www.aruplab.com/	Association of Public Health Laboratories www.aphl.org
Agency for Healthcare Research and Quality www.ahrq.gov	Bayer HealthCare Donald Parker, PhD http://www.bayerhealthcare.com/scripts/pages/en/index.php
American Academy of Family Physicians www.aafp.org	Centers for Disease Control and Prevention www.cdc.gov Jane Kelly, MD
American Association of Clinical Endocrinologists www.aace.com	Centers for Medicare and Medicaid Services http://www.cms.gov/
American Association of Diabetes Educators www.aadenet.org Amparo Gonzalez RN, CDE Karen Fitzner, PhD	College of American Pathologists www.cap.org Peter Howanitz, MD
American College of Obstetricians and Gynecologists www.acog.org Donald Coustan, MD	Department of Veterans Affairs www.va.gov Leonard Pogach, MD
American College of Physicians www.acponline.org Merri Pendergrass, MD	Diabetes UK www.diabetes.org.uk
American Diabetes Association www.diabetes.org M. Sue Kirkman, MD	The Endocrine Society www.endo-society.org Lisa Marlow
Association for Clinical Biochemistry www.acb.org.uk Garry John, MD	European Association for the Study of Diabetes www.easd.org Jonathan Levy, MD
Food and Drug Administration www.fda.gov Arleen Pinkos	Siemens Healthcare Diagnostics Roma Levy, MS Tricia Bal, MD Susan Selgren, PhD http://www.medical.siemens.com/webapp/wcs/stores/servlet/StoreCatalogDisplay~q_catalogId~e_-101~a_langId~e_-101~a_storeId~e_10001.htm
International Diabetes Federation www.idf.org	Lifescan Inc John Mahoney, BA http://www.lifescan.com/
International Federation of Clinical Chemistry and Laboratory Medicine www.ifcc.org Mauro Panteghini, MD	National Institute of Diabetes and Digestive and Kidney Diseases (of the National Institutes of Health) www.nih.gov

International Society of Diabetes and Vascular Disease http://www.intsocdvd.com/	National Medical Association http://www.nmanet.org
Italian SIBioC-SIMeL Study Group on Diabetes http://www.simel.it/en/ http://www.sibioc.it/	North American Nursing Diagnosis Association (NANDA-International) www.nanda.org Mary Ann Lavin, ScD, RN, FAAN
Juvenile Diabetes Research Foundation www.jdrf.org	Roche Diagnostics Theresa Bush, PhD http://www.roche.com/index.htm

Individuals:

Phillip Bach, Primary Children's Medical Center, Salt Lake City, USA	John Mahoney, Lifescan, USA
Jim Boyd, University of Virginia, USA	Matthew Meerkin, University of Notre Dame, Australia
Yu Chen, Dr. Everett Chalmers Regional Hospital/Horizon Health Network, Canada	Andrea Mosca, University of Milan, Italy
Rob Christenson, University of Maryland Medical Center, USA	Christian Perier, Hospital Nord, Saint-Etienne, France
Edgard Delvin, CHU Ste-Justine, Montreal, Canada	Leonard Pogach, VA New Jersey Healthcare System, USA
Kent Dooley, LifeLabs, British Columbia, Canada	Chris Price, University of Oxford, UK
Raymond Gambino, Quest Diagnostics Inc, USA	Kastoori Ramakrishnan, ProdConcepts, LLC
Mary Lou Gantzer, Siemens Healthcare Diagnostics, USA	Maria del Patrocinio Chueca Rodriguez, Hospital Reina Sofia, Spain
Eswari Gudipati, USA (patient view)	Kareena Schnabl, DynaLIFEDx, Canada
Trefor Higgins, DynaLifeDx, Canada	Dhastagir Sheriff, Al Arab Medical University, Benghazi, Libya
Stephen Kahn, Loyola University, USA	Robbert Slingerland, Isala Klinieken, The Netherlands
Raymond Karcher (retired), Beaumont Hospital, USA	John Tayek, Harbor UCLA Medical Center, USA
Eric Kilpatrick, Hull Royal Infirmary, UK	Joseph Watine, Hôpital de la Chartreuse, Villefranche-de-Rouergue, France
Ben Kukoyi, Houston, USA	Shirley Welch, Kaiser Permanente, USA
Phillip Lee, University of Texas Medical Branch Galveston, USA	William E. Winter, University of Florida, USA
Randie Little, University of Missouri-Columbia School of Medicine, USA	

Appendix Table 2: Criteria for prioritization of key questions

Prioritization criteria	Explanatory notes	Examples
A: The test has high impact on *clinical* outcomes (e.g. morbidity, mortality, prognosis)	**A1:** The test or its characteristics (e.g. its diagnostic or target value or range) are directly or indirectly linked to important clinical outcomes The test is a surrogate (indirect) measure of important clinical outcomes	– Glucose cut-off values for diagnosing DM, IFG or IGT – The impact of maternal glycemia on pregnancy outcomes (direct link to outcome); OGTT diagnostic criteria to detect GDM (indirect link to outcome) – HbA_{1c} is a surrogate measure of morbidity and mortality
	A2: The test and its result have a major impact on clinical management decisions	– Diagnostic criteria for DM to guide initiation of treatment – HbA_{1c} values in guiding decision on changing treatment – Albuminuria results guiding decisions on initiating therapy with ACE-inhibitors
	A3: There is current controversy on the use of the test in practice	– OGTT vs FPG for the diagnosis of DM – Diagnostic criteria for GDM
	A4: There is wide variation in practice with unfavorable outcomes (e.g. misdiagnosis of the condition)	– Differing criteria for diagnosing DM or GDM – Variations in the use of random or timed specimens and albumin concentration or albumin excretion rate *vs* ACR for diagnosing albuminuria
	A5: New and substantial evidence has emerged since the publication of the 2002 NACB guideline	– SMBG in type 2 DM – HAPO study in GDM
B: The test has high impact on *organizational* outcomes	**B1:** High volume testing with uncertain impact	SMBG in type 2 DM
	B2: There is public/commercial/ professional/ governmental pressure on testing	– Use of portable meters in groceries, by patients, etc. – Changing the expression of HbA_{1c} values due to standardization
C: The test has high impact on *economic* outcomes	**C1:** Testing is associated with high costs	SMBG
	C2: New and substantial evidence has emerged on the cost-effectiveness of the test since the publication of the 2002 NACB guideline	

Abbreviations: ACE: Angiotensin Converting Enzyme; ACR: Albumin Creatainine Ratio; DM: Diabetes Mellitus; FPG: Fasting Plasma Glucose; GDM: Gestational Diabetes Mellitus; HAPO: Hyperglycemia and Adverse Pregnancy Outcome; IFG: Impaired Fasting Glucose; IGT: Impaired Glucose Tolerance; NACB: National Academy of Clinical Biochemistry; OGTT: Oral Glucose Tolerance Test; SMBG: Self-Monitoring of Blood Glucose

Appendix Table 3: Evidence table

Chapter 1: GLUCOSE

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
DOES GLUCOSE NEED TO BE MEASURED IN PLASMA FOR THE DIAGNOSIS OF DIABETES MELLITUS?							[3]Priority: 3 (B2, C1)	
1.a	Glucose should be measured in plasma in an accredited laboratory to establish the diagnosis of diabetes ***Level A***	When glucose is used to establish the diagnosis of diabetes, it should be measured in venous plasma ***A (high)***	Clarification	American Diabetes Association. Standards of medical care in diabetes–2010. Diab Care 2010; 33 (Suppl 1):S11-61	Guideline expert opinion	Low	High	Direct relationship between glucose and complications of diabetes has been shown in earlier high quality studies incorporated in ADA and WHO guidelines. Difficult to evaluate quality of evidence as plasma glucose has been sole diagnostic criterion for diabetes for many years of clinical practice. Glucometers are not accurate enough to diagnose diabetes. This represents strong agreement of experts.
				World Health Organization, Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycermia: Report of a WHO/IDF Consultation. Geneva: World Health Organization, 2006	Guideline	Low		
				Engelgau MM, et al. Comparison of fasting and 2-hour glucose and HbA_{1c} levels for diagnosing diabetes. Diagnostic criteria and performance revisited. Diab Care 1997;20(5):785-91.	cross-sectional population-based sample	High		WHO recommends "venous plasma glucose" should be standard, but due to wide-spread use of capillary sampling (especially in under-resourced countries) capillary samples are accepted as a pragmatic solution. However, evidence does NOT support use of capillary samples.
				McCance DR, e al. Comparison of tests for glycated haemo-globin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. BMJ. 1994; 308(6940): 1323-8. Erratum in: BMJ 1994; 309(6958):841	Cross sectional and longitudinal analysis	High		Provides evidence on the relation between complications and concomitant results of the three tests. Recommendation upgraded for direct link between glucose and DM complications and outcomes.

[1] 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.

[2] Explanations for grading the level and quality of evidence and for grades of recommendations are given in Tables 1 and 2 in the Preamble.

[3] For priority codes, see Appendix Table 2

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
DOES GLUCOSE NEED TO BE MEASURED IN PLASMA FOR THE SCREENING OF DIABETES MELLITUS?							[(3)]Priority: 3 (B2, C1)	
1.b	Glucose should be measured in plasma in an accredited laboratory for screening of high-risk individuals ***Level E***	When glucose is used for screening of high-risk individuals, it should be measured in venous plasma ***B (moderate)***	Former recommendation was split for clarification and re-grading	American Diabetes Association. Standards of medical care in diabetes --2010. Diab Care 2010; 33 (Suppl 1):S11-61	Guideline expert opinion	Low	Moderate	WHO accepts glucometers for screening, for pragmatic reasons i.e., lack of access to an accredited central lab in underdeveloped countries. This represents a strong consensus view that it is "better than doing nothing".
				World Health Organization, Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycermia: Report of a WHO/IDF Consultation. Geneva: World Health Organization, 2006.	Guideline	Low		
				Jesudason DR, et al. Macro-vascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and HbA_{1c} for cost-effective screening. Diab Care 2003; 26:485-90.	Population-based analysis	Moderate - high		
				Knowler WC, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346:393-403.	RCT	High		Recommendation downgraded for indirectness – outcome was to reduce DM with treatment/lifestyle changes.
				Tuomilehto J, 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.	RCT	High		
1.c		Plasma glucose should be measured in an accredited laboratory when used for diagnosis of or screening for diabetes ***GPP***	Former recommendation was split for clarification and re-grading					Consensus of experts

Chapter 1: GLUCOSE *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
ARE SCREENING PROGRAMS FOR DIABETES MELLITUS EFFECTIVE?							[3]Priority: NOT LISTED	
1.d		Outcome studies are needed to determine the effectiveness of screening ***C (moderate)***	New recommendation based on additional evidence	Kahn R, et al. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. Lancet 2010;375:1365-74	Cost-effectiveness study	High	Moderate	No evidence so far that screening has benefit. Quality of evidence downgraded for indirectness.
				Glumer C, et al. What determines the cost-effectiveness of diabetes screening? Diabetologia 2006; 49:1536-44.	Cost-effectiveness modeling study	Moderate		
				Icks A, et al. Cost-effectiveness of type 2 diabetes screening: results from recently published studies. Gesundheitswesen 2005; 67 Suppl 1:S167-71	Review and cost-effectiveness analysis	Moderate - low		
				Hoerger TJ, et al. Screening for type 2 diabetes mellitus: a cost-effectiveness analysis. Ann Intern Med 2004; 140:689-99.	Cost-effectiveness analysis by Markov model	Moderate		
				Dallo FJ, Weller SC. Effectiveness of diabetes mellitus screening recommendations. Proc Natl Acad Sci USA 2003; 100:10574-9.	Cross-sectional analysis of population-based study	High		
				Jesudason DR, et al. Macro-vascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and HbA_{1c} for cost-effective screening. Diab Care 2003; 26:485-90.	Population-based analysis	Moderate - high		
				Perry RC, et al. HbA_{1c} measurement improves the detection of type 2 diabetes in high-risk individuals with nondiagnostic levels of fasting plasma glucose: the Early Diabetes Intervention Program (EDIP). Diab Care 2001; 24:465-71	RCT	High		

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
DOES GLUCOSE NEED TO BE MEASURED IN PLASMA FOR THE MONITORING OF DIABETES MELLITUS?							[3]Priority: 3 (B2, C1)	
1.e	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. ***Level E***	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)***	No change	American Diabetes Association. Standards of medical care in diabetes–2010. Diab Care 2010; 33 (Suppl 1):S11-61.	Guideline expert opinion	Low	***Low***	
WHAT ARE THE PRE-ANALYTICAL CONSIDERATIONS IN GLUCOSE TESTING?							[3]Priority: NOT LISTED	
1.f	Blood for fasting plasma glucose analysis should be drawn after the subject has fasted overnight (at least 8 h). ***Level B***	Blood for fasting plasma glucose analysis should be drawn in the morning after the individual has fasted overnight (at least 8 h) ***B (low)***	Clarification	WHO Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. Geneva: World Health Organization, 2006	Guideline	Low	Low	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. No RCT compared morning vs afternoon testing in terms of diagnostic accuracy or outcomes. Therefore quality of evidence is downgraded for indirectness. However, there is strong consensus of experts that a fasting plasma specimen drawn in the morning should be used.
				Troisi RJ, et al. Diurnal variation in fasting plasma glucose: implications for diagnosis of diabetes in patients examined in the afternoon. JAMA 2000; 284:3157-9.	Retrospective population-based study	High		
				American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diab Care 1997; 20:1183-97.	Guideline	Low		

Chapter 1: GLUCOSE *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
1.g	Plasma should be separated from the cells within 60 min; if this is not possible, a tube containing a glycolytic inhibitor such as sodium fluoride should be used for collecting the sample ***Level B***	To minimize glycolysis, one should place the sample tube immediately in an ice–water slurry, and the plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as sodium fluoride, should not be relied on to prevent glycolysis ***B (moderate)***	Clarification	Gambino R et al. Acidification of blood is superior to sodium fluoride alone as an inhibitor of glycolysis. Clin Chem 2009;55:1019-21.	Observational	High	Moderate	A consistent body of good evidence that delay in sample processing leads to reduction in glucose in sample, and thus strong consensus that this may alter diagnostic accuracy. However, no study is available to determine if this leads to unfavorable outcomes or increased rate of complications. Therefore quality of evidence is downgraded for indirectness.
				Bruns DE, Knowler WC. Stabilization of glucose in blood samples: Why it matters. Clin Chem [Editorial] 2009;55:850-2.	Editorial	Low		*In vitro* decrease of glucose may lead to missed diagnoses of diabetes in the large proportion of the population who have glucose concentrations near the diagnostic cut points for diabetes.
				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	Review (book chapter)	Moderate-low		
				Boyanton BL, Jr., Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002; 48:2242-7	Observational	High		
				Stahl M, et al. 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	Observational	High		

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Chan AY, et al. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989; 35:315-7.	Observational	High		
				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	Review (book chapter)	Moderate-low		
DO ANALYTICAL GOALS FOR GLUCOSE ANALYSIS NEED TO CHANGE/IMPROVE WITH THE LOWERED CUTOFF FOR IFG?							[3]Priority: 2 (A1-3, B2)	
1.h		On the basis of biological variation, glucose measurement should have an analytical imprecision ≤2.9%, a bias ≤2.2%, and a total error ≤6.9%. To avoid misclassification of patients, the goal for glucose analysis should be to minimize total analytical error, and methods should be without measurable bias ***B (low)***	New recommendation for setting analytical performance goals for achieving better diagnostic accuracy around diagnostic thresholds.	Ricos C et al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest. 1999;59:491-500	Review	Moderate	Low	Quality of evidence is downgraded for indirectness to outcomes and for lack of primary studies linking analytical performance to outcomes. However, there is strong expert consensus that analytical uncertainty of glucose measurement could result in misclassification of patients. The related recommendation therefore was upgraded to reflect this potential impact on patient centered outcomes.
				Fraser CG. The necessity of achieving good laboratory performance. Diabet Med 1990; 7:490-3.	Expert opinion	Low		

Chapter 2: GLUCOSE METERS

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
SHALL PORTABLE METERS BE USED IN DIAGNOSIS AND SCREENING OF DIABETES MELLITUS?							[3]Priority: 2 (A3-4, B2, C1)	
2.a	There are no published data to support a role for portable meters in the diagnosis of diabetes or for population screening. The imprecision of the meters, coupled with the substantial differences among meters, precludes their use in the diagnosis of diabetes and limits their usefulness in screening for diabetes ***Level E***	There are insufficient published outcome data to support a role for portable meters and skin-prick (finger-stick) blood samples in diagnosis of diabetes or for population screening ***C (moderate)***	New evidence emerged since 2002 and clarification. Prior recommendation was split into two separate recommendations for clarity and regarding.	Dungan K, et al. Glucose measurement: Confounding issues in setting targets for inpatient management. Diab Care 2007; 30(2): 403-409.	Review	Low	Moderate	WHO recommends plasma, but accepts capillary whole blood using glucometer. WHO accepts meters for screening for practical and financial reasons. This represents a strong consensus view that it is "better than doing nothing". Glucometers are not accurate enough to diagnose diabetes. This represents strong agreement of experts. Quality of evidence downgraded for inconsistency and indirectness of evidence.
				The Diabetes Research in Children Network (DirecNet) Study Group. Accuracy of newer generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) inpatient exercise study. Diabetes Technology and Therapeutics 2005; 7(5): 675-680.	Observational (Analytical evaluations)	High		
HOW SHOULD PORTABLE METERS BE USED IN MONITORING TYPE 1 DIABETES MELLITUS?							[3]Priority: NOT LISTED	
2.c	SMBG is recommended for all insulin-treated patients with diabetes. For type 1 patients, SMBG is recommended three or more times a day. SMBG may be desirable in patients treated with sulfonylureas or other insulin secretagogues and in all patients not achieving goals ***Level B***	Self-monitoring of blood glucose (SMBG) is recommended for all insulin-treated patients with diabetes ***A (high)***	Clarification	American Diabetes Association. Standards of medical care in diabetes–2010. Diab Care 2010;33 (Suppl 1):S11-61	Guideline expert opinion	Low	High	Intensive glycemic control in patients with type 1 diabetes was achieved in the DCCT by participants performing SMBG at least four times per day, hence the ADA recommendation and a strong consensus for SMBG to be performed three or more times per day in type 1 diabetes.
				DCCT Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-986.	RCT	High		

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
SHOULD PORTABLE METERS BE USED IN MONITORING TYPE 2 DM?							[(3)]Priority: 2 (A3, A5, B1-2, C1)	
2.d	In patients with type 2 diabetes, SMBG may help achieve better control, particularly when therapy is initiated or changed. However, there are no data to support this concept. The role of SMBG in patients with stable type 2 diabetes controlled by diet alone is not known ***Level C***	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)***	New evidence emerged since the 2002 publication	Allemann S, Houriet C, Diem P, Stettler C. Self-monitoring of blood glucose in non-insulin treated patients with type 2 diabetes: a systematic review and meta-analysis. Curr Med Res Opin 2009;25:2903-13	Systematic Review	High	High	In spite of the number of high quality new studies and evidence reviews, there is insufficient evidence to claim improved outcomes for SMBG in type 2 DM. Therefore clear recommendations for or against SMBG in type 2 DM cannot be made at this stage.
				Poolsup N, Suksomboon N, Rattanasookchit S. Meta-analysis of the benefits of self-monitoring of blood glucose on glycemic control in type 2 diabetes patients: an update. Diabetes Technol Ther. 2009;11:775-84	Systematic Review	High		
				Farmer 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;21;335:132	RCT	High		
				Martin S, at al. The ROSSO Study Group. Self-monitoring of blood glucose in type 2 diabetes and long-term outcome: an epidemio-logical study. Diabetologia 2006;49:271–8.	Epidemio-lo-gical cohort study	Moderate		
				Karter AJ, et al.Longitudinal study of new and prevalent use of self-monitoring of blood glucose. Diab Care 2006;29:1757–63.		High		
				Welschen LMC, et al. Self-monitoring of blood glucose in patients with type 2 diabetes mellitus who are not using insulin. Cochrane Database of Systematic Reviews 2005;Issue 2. Art. No.: CD005060.	Observational study	High		Systematic review of 6 RCTs
				Welschen LMC, et al. Self-moni-toring of blood glucose in patients with type 2 diabetes who are not using insulin: a systematic review. Diab Care 2005;28:1510–7.	Systematic review			

Chapter 2: GLUCOSE METERS *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Davidson MB. Counter-point: Self-Monitoring of Blood Glucose in Type 2 Diabetic Patients not Receiving Insulin: A waste of money. Diab Care 2005;28:1531-3.	Expert opinion	Low		
				Franciosi M, et al., the QuED Study Group. Self-monitoring of blood glucose in non-insulin-treated diabetic patients: a longitudinal evaluation of its impact on metabolic control. Diab Med 2005;22:900–6.	Observational study	High		
				Guerci B, et al., the ASIA Group. 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.	Multi-center, prospective open label, randomized trial	Moderate		
				Harris MI. Frequency of blood glucose monitoring in relation to glycemic control in patients with type 2 diabetes. Diab Care 2001;24:979-82.	Cross-sectional study	High		NHANES study
				Coster S, et al. Self-monitoring in Type 2 diabetes mellitus: a meta-analysis. Diab Med 2000;17:755-761.	Meta-analysis	High		Meta-analysis of 8 RCTs
				Faas A, et al. The efficacy of self-monitoring of blood glucose in NIDDM subjects. Diab Care 1997;20:1482-1486.	Systematic review	High		11 studies reviewed, including 6 RCTs

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
WHAT ARE THE PRE-ANALYTICAL CONSIDERATIONS FOR GLUCOSE METERS?							[3]Priority: 2 (A2-3, B1-2, C1)	
2.e	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 accuracy of patient results. ***Level B***	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)***	Clarification and new data	Kristensen GB, et al. Standardized evaluation of nine instruments for self-monitoring of blood glucose. Diab Technol and Therap 2008;10:467-77.	Observational	High	Moderate	
				Kristensen GB, et al. Standardized evaluation of instruments for self-monitoring of blood glucose by patients and a technologist. Clin Chem 2004; 50:1068-71.	Observational	High		
				Kabadi UM, et al. The effect of recurrent practice at home on the acceptability of capillary blood glucose readings. Accuracy of self blood glucose testing. Diab Care 1994;10:1110-23.	Observational	Moderate		
WHAT ARE THE ANALYTICAL CONSIDERATIONS FOR GLUCOSE METERS?							[3]Priority: 2 (A2-3, B1-2, C1)	
2.f	Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. No published study has achieved the goals proposed by the ADA. Manufacturers should work to improve the imprecision of current meters ***Level E***	Multiple performance goals for portable glucose meters have been proposed. These targets vary widely and are highly controversial. 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)***	Clarification and new data	Kristensen GB, et al. Standardized evaluation of nine instruments for self-monitoring of blood glucose. Diab Technol and Therap 2008;10:467-77.	Observational	High	Low	Performance goal targets vary widely and are highly controversial. No evidence is available that the ADA targets of less than 5% total error can be achieved in practice. Downgraded evidence for inconsistency, indirectness and lack of consensus of experts.
				The Diabetes Research in Children Network (DirecNet) Study Group. Accuracy of newer generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) Inpatient Exercise Study. Diab Technol Ther 2005;7:675-83.	Observational (Analytical evaluation)	Moderate		
				Bohme P, et al. Evolution of Analytical Performance in Portable Glucose Meters in the Last Decade Diab Care 2003;26:1170-5.	Observational	High		
				Skeie S, et al. Instruments for self-monitoring of blood glucose: comparisons of testing quality achieved by patients and a technician. Clin Chem 2002;48:994-1003.	Observational	High		

Chapter 2: GLUCOSE METERS *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Weitgasser R, et al. Newer portable glucose meters - analytical improvement compared with previous generation devices? Clin Chem 1999;45:1821-1825.	Observational	High		
				American Diabetes Association. Self-monitoring of blood glucose. Diab Care 1996;19 (S 1):S62-66.	Guideline	Low		
				Novis DA, Jones BA. Interinstitutional comparison of bedside blood glucose monitoring program characteristics, accuracy perfor-mance, and quality control documentation. Arch Pathol Lab Med 1998;122:495-502.	Observational	High		Q-probe
				Barr JT, et al. Ancillary (bedside) blood glucose testing in acute and chronic care facilities. NCCLS 1994;14:1-14.	Guideline	Low		
2.g	We recommend meters that measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories. ***Level E***	Meters should measure and report plasma glucose concentrations to facilitate comparison with assays performed in accredited laboratories ***GPP***	No change, rewording		Expert consensus	Low	Very low	

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
ARE GLUCOSE METERS ADEQUATE FOR WIDESPREAD USE IN INTENSIVE CARE UNITS?							[(3)]Priority: 2 (A1-3, B2, C1)	
2.h	Clinical studies are needed to determine the analytic goals for glucose meters. At a minimum, the end points should be glycated hemoglobin and frequency of hypoglycemic episodes. Ideally, outcomes (e.g., long-term complications and hypoglycemia) should also be examined ***Level E***	Studies are needed to determine the analytical goals (quality specifications) for glucose meters in SMBG and in intensive care units ***C (moderate)***	Clarification and expansion of scope of recommendation to intensive care setting	Meynaar IA, et al. Accuracy of AccuChek glucose measurement in intensive care patients. Crit Care Med 2009;37:2691-6.	Observational study	High	Moderate-low	
2.i		Recommendations for future research: Important end points in studies of SMBG should include, at a minimum, hemoglobin A_{1c} (Hb A_{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 variation of glucose control. Ideally, outcomes (e.g., long-term complications) should also be examined ***GPP***		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.	Simulation modeling	Moderate		
				Scott MG, et al. Tight glucose control in the intensive care unit: Are glucose meters up to the task? Clin Chem 2009; 55:18-20.	Expert opinion	Low		
				Scott MG, et al. Tight glucose control in critically ill adults [Letter]. JAMA 2008; 300(23):2726-7.	Expert opinion	Low		
				Wiener RS, et al. Benefits and risks of tight glucose control in critically ill adults. JAMA 2008;300(8):933-944.	Systematic review and meta-analysis	Moderate		
				Hoedemaekers CW, et al. Accuracy of bedside glucose measurement from three glucometers in critically ill patients. Crit Care Med 2008;36(11):3062-6.	Observational study	High		
				Dungan K, et al. Glucose measurement: confounding issues in setting targets for inpatient management. Diabetes Care 2007;30:403-9.	Narrative review	Low		
				Finkielman J, et al: Agreement between bedside blood and plasma glucose measurement in the ICU setting. Chest 2005;127:1749-51.	Observational study	Low		
				van den Berghe G, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med. 2001;345(19):1359-1367.	RCT	Moderate		

Chapter 3: CONTINUOUS MINIMALLY-INVASIVE GLUCOSE ANALYSES

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
ARE THERE ADEQUATE WELL CONTROLLED STUDIES DEMONSTRATING THE IMPACT OF CONTINUOUS GLUCOSE MONITORS ON INTERMEDIATE OUTCOMES (E.G. HbA_{1c}) TO JUSTIFY WIDESPREAD ADOPTION OF THE TECHNOLOGY?							[(3)]Priority: 2 (A1, A3, B2, C1)	
GIVEN THE HIGH COSTS OF THE TECHNOLOGY, ARE THERE EVIDENCE-BASED SELECTION CRITERIA FOR ITS USE AND POTENTIAL REIMBURSEMENT?							[(3)]Priority: 2 (A3, C1)	
3.a	Noninvasive glucose analyses cannot be recommended as replacements for SMBG or glucose measurements by an accredited laboratory. Ongoing developments in the field, such as use of the new Gluco Watch Biographer, may influence this recommendation. ***Level E***	Real-time continuous glucose monitoring (CGM) in conjunction with intensive insulin regimens can be a useful tool to lower Hb A_{1c} in selected adults (age >25 years) with type 1 diabetes ***A (high)***	Gluco Watch technology is no longer on market and has been supplanted by subcutaneous CGM devices. Additional evidence is available about effectiveness of real-time CGM.	The Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group: N.Engl.J.Med. 2008;359:1464-1476	RCT	High	High	Three age subgroups pre-specified for outcome assessment
3.b		Although the evidence for lowering Hb A_{1c} is not as strong for 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)***	New recommendation based on additional evidence	The Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group: N.Engl.J.Med. 2008;359:1464-1476	RCT	Moderate	Moderate	This was a per-protocol post-hoc analysis of the relationship between HbA_{1c} lowering and days per week of use, not an intention-to-treat analysis or the primary outcome. Therefore the quality of evidence and the strength of recommendation were downgraded.

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
3.c		Real-time CGM may be a supplemental tool to SMBG in individuals with hypoglycemia unawareness and/or frequent episodes of hypoglycemia ***B (low)***	New recommendation based on additional evidence	Garg S, et al. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor - a randomized controlled trial. Diab Care 2006;29:44-50	RCT	Moderate	Low	Comparison of real-time vs. blinded CGM (outcomes were patients' time in hyper-glycemic and hypoglycemic ranges). Evidence is indirect as the outcome was a surrogate biochemical marker (although patient-related), i.e. not clinical episodes of hypoglycemia.
ARE CONTINUOUS GLUCOSE MONITORS SUFFICIENTLY ACCURATE FOR CLINICAL USE BY PATIENTS?							[(3)]Priority: 1 (A1-4, B1-2, C1)	
3.d		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***	New recommendation		Clinical experience and FDA labeling of the device	Low	Very low	FDA labeling of the device (for trend assessment, not treatment decisions - use SMBG for insulin dosing)

Chapter 4: NON-INVASIVE GLUCOSE ANALYSIS

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
SHOULD PRESENT NON-INVASIVE GLUCOSE SENSING TECHNOLOGY BE RECOMMENDED FOR MONITORING GLYCEMIA?							[(3)]Priority: 3 (A3, A5, B2)	
4.a	Noninvasive glucose analyses cannot be recommended as replacements for SMBG or glucose measurements by an accredited laboratory. Ongoing developments in the field, such as use of the new Gluco Watch Biographer may influence this recommendation. ***Level E***	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)***	New recommendation and clarification	Arnold MA, et al. Selectivity assessment of noninvasive glucose measurements based on analysis of multivariate calibration vectors. J Diabetes Sci Technol 2007;1:454-62.	Animal model	Low	Very low	Demonstration of selectivity issues. Downgraded for indirectness
				Tura A, et al. Non-invasive glucose monitoring: assessment of technologies and devices according to quantitative criteria. Diabetes Res Clin Pract 2007;77:16-40.	Review of technologies	Low		Review with assessment of feasibility of each approach
				Arnold MA, Small GW. Noninvasive glucose sensing. Anal Chem 2005;77:4529-39.	Review of technologies	Low		Review with listing of critical analytical parameters
				Khalil OS. Non-invasive glucose measurements at the dawn of the new millennium: An update. Diabetes Technol Ther 2004;6:660-697.	Review of technologies	Low		Review with assessment of feasibility of each approach
				Gutman S, et al. Regulatory aspects of noninvasive glucose measurements. Diabetes Technol Ther 2002;4:779-81.	Consensus statement	Low		Listing of anticipant FDA requirements for approval of any future non-invasive sensing technology.

Chapter 5: GESTATIONAL DIABETES MELLITUS (GDM)

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
WHAT ARE THE STRATEGIES FOR DETECTION AND DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS?							[3]Priority: 1 (A5, B2)	
5.a		All pregnant women not previously known to have diabetes should undergo testing for gestational diabetes mellitus (GDM) at 24–28 weeks of gestation ***A (high)***	New recommendation based on additional evidence of associations of maternal glycemia and perinatal outcome and RCT results showing benefit from treating mild GDM and expert consensus.	American Diabetes Association. Standards of medical care in diabetes–2011. Diab Care 2011;34 (Suppl 1):S11-61	Guideline, position statement	High	High	Based on the HAPO study and the IADPS criteria, ADA recommends that women with risk factors for type 2 diabetes are screened for diabetes at the first prenatal visit.
				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. Diab Care 2010;33:676-82.	Guideline, expert consensus	High		Expert Consensus Panel appointed by IADPSG recommended “outcome based” criteria for the classification of glucose concentrations in pregnancy.
				Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group: Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: Associations with neonatal anthropometrics. Diabetes 2009;58:453-459.	Prospective observational study of a multicenter cohort	High		
				Landon MB, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med 2009;361:1339	RCT	High		This RCT does not deal with the diagnosis of GDM directly but provides evidence that treating mild GDM improves outcome.
				Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Cooperative Research Group (Metzger BE, HAPO Study PI). Hyperglycemia and Adverse Pregnancy Outcomes. N Engl J Med 2008;358:1991-2002	Prospective observational study of multicenter cohort	High		Strong evidence for continuous association between maternal glucose levels and pregnancy outcome
				Crowther CA, et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477	RCT	High		This RCT does not deal with the diagnosis of GDM directly but provides evidence that treating mild GDM improves outcome.

Chapter 5: GESTATIONAL DIABETES MELLITUS (GDM) *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
5.b		GDM should be diagnosed by a 75-g OGTT according to the IADPSG criteria derived from the HAPO study ***A (moderate)***	New recommendation based on additional evidence and expert consensus.	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. Diab Care 2010;33:676-82.	Guideline, expert consensus	High	Moderate*	This guideline was based on the HAPO study and on the opinions of the IADPSG Consensus Panel members because associations between maternal glycemia and clinical outcomes were continuous with no obvious thresholds at which risks increased. Therefore a consensus was required to translate these results into clinical practice.
				Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. Diabetes 2009;58:453	Prospective multi-national epidemiologic study	High		The study of 25,000 participants revealed strong, graded, predominantly linear and continuous associations between maternal glycemia and primary study outcomes
				Metzger, et al. Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diab Care 2007;30:S251-S260.	Conference review	Moderate-low		Opinion of world-wide experts based on findings of the HAPO outcome study.

*NB: The HAPO study and the subsequent guideline published suggest setting diagnostic thresholds at OR 1.75, but OR 1.5 and 2.0 were also considered.
The authors themselves suggest the followings:

It is likely that additional well-designed randomized controlled trials and other clinical studies will be needed to determine

[1] cost-effective therapeutic strategies for treatment of GDM diagnosed by the IADPSG Consensus Panel–recommended criteria;
[2] optimal glycemic treatment targets;
[3] appropriate follow-up of mothers to determine risks for later development of diabetes, other metabolic disorders, or CVD risk factors; and
[4] follow-up of children to assess potential associations of maternal glycemia with long-term risks of obesity, altered glucose metabolism, and CVD risk factors.

Therefore recommendations are likely to change as more evidence becomes available or modified locally for resource considerations. Therefore the quality of evidence is downgraded to moderate but, due to strong consensus on the current criteria, the strength of recommendation is A.

Chapter 6: URINARY GLUCOSE

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR URINE GLUCOSE TESTING IN THE MANAGEMENT OF DIABETES MELLITUS?							[3]Priority: NOT LISTED	
6.a	Semi-quantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus ***Level C***	Semiquantitative urine glucose testing is not recommended for routine care of patients with diabetes mellitus ***B (low)***	No change	Goldstein DE, et al. Tests of glycemia in diabetes. Diab Care 2004;27:1761-73.	Guideline	Low	Low	Downgraded for low quality and indirectness of evidence. However, consensus is strong against the use of this test. IDF supports urine glucose monitoring where blood glucose is not available or affordable.
				American Diabetes Association. Tests of glycemia in diabetes. Diab Care 1999;22:S77-9.	Guideline	Low		

Chapter 7: KETONE TESTING

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
WHICH PATIENTS SHOULD BE ADVISED TO MEASURE URINE OR BLOOD KETONES AT HOME, AND UNDER WHAT CIRCUMSTANCES?							[3]Priority: 2 (A2-4)	
7.a	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 ***Level E***	Ketones measured in urine or blood in the home setting by patients with diabetes and in the clinic/hospital setting should be considered only an adjunct to the diagnosis of diabetic ketoacidosis (DKA) ***GPP***	No change	ADA: Standards of Medical Care in Diabetes–2009; Diab Care 2009; 32 (Suppl 1):S13-S61	Guideline expert opinion	Low	Very low	Expert opinion, clinical experience
				ADA: Hyperglycemic crises in diabetes (position statement). Diab Care 2004; 27 (Suppl 1):S94-102	Guideline expert opinion	Low		
7.b	Urine ketone determinations should not be used to diagnose or monitor the course of DKA ***Level A***	Urine ketone measurements should not be used to diagnose or monitor the course of DKA ***GPP***	No change	ADA Tests of glycemia position statement, DiaB Care 2001; 23 (Suppl 1):S80-82).	Guideline expert opinion	Low	Very low	Based on lack of measurement of beta-hydroxybutyrate by nitroprusside
ARE DIRECT MEASUREMENTS OF βHBA PREFERABLE TO NITROPRUSSIDE MEASUREMENTS OF KETONES?							[3]Priority: 3 (A2)	
7.c	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. Further studies are needed to determine if the test offers any clinical advantage over more traditional management approaches (e.g., measurements of serum CO_2, anion gap, or pH). ***Level E***	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 DKA treatment. Specific measurement of β-hydroxybutiric acid in blood can be used for diagnosis and monitoring of DKA ***B (moderate)***	No change	Wiggam MI, et al. Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxybutyrate concentration as the end point of emergency management. A randomized controlled study. Diabetes Care 1997;20:1347-52.	RCT	Moderate	Moderate	Outcome not clinically meaningful Downgraded for indirectness of evidence
				Umpierrez GE, et al. Clinical utility of beta-hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diab Care 1995;18:137-8.	Observational cohort study	Moderate		Comparison of two strategies of monitoring DKA
				Noyes KJ, et al. Hydroxybutyrate near-patient testing to evaluate a new endpoint for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. Pediatr Diabetes 2007;8:150-156	Observational cohort study	Moderate		Comparison of two strategies of monitoring DKA

Chapter 8: HEMOGLOBIN A_{1c}

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
HOW GLYCATED HEMOGLOBIN SHOULD BE USED IN MONITORING DIABETES MELLITUS?							[(3)]Priority: NOT LISTED	
8.a	Glycated hemoglobin (GHb) should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control. ***Level A***	Hb A_{1c} should be measured routinely in all patients with diabetes mellitus to document their degree of glycemic control ***A (moderate)***	Clarification	American Diabetes Association. Standards of medical care in diabetes–2010. Diab Care 2011;34 (Suppl 1):S11-61.	Guideline	Moderate	Moderate	The DCCT and UKPDS had determined the relationship between the results of a specific GHb test (HbA_{1c}) and long-term complications in patients with type 1 and type 2 diabetes, respectively HbA_{1c} has become a surrogate outcome measure in DM but this represents indirect evidence and therefore of moderate quality. However there is strong consensus for measuring HbA_{1c} routinely in DM monitoring. Therefore the recommendation is upgraded.
				Nathan DM, et al. Management of hyperglycaemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia 2006;49:1711-21.	Consensus statement	Low		
				U.K. Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonyl-ureas 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	RCT	High		
				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.	RCT	High		

Chapter 8: HEMOGLOBIN A_{1c} *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
WHAT ARE THE ANALYTICAL CONSIDERATIONS AND GOALS FOR HbA_{1c} MEASUREMENT?							[(3)]Priority: 2 (A1)	
8.b	Laboratories should use only GHb assay methods that are certified by the National Glycohemoglobin Standardization Program as traceable to the DCCT reference. In addition, laboratories that measure GHb should participate in a proficiency-testing program, such as the CAP Glycohemoglobin Survey, that uses fresh blood samples with targets set by the National Glycohemoglobin Standardization Program Laboratory Network ***Level B***	Laboratories should use only Hb A_{1c} assay methods that are certified by the National Glycohemoglobin Standardization Program (NGSP) as traceable to the DCCT reference. The manufacturers of Hb A_{1c} assays should also show traceability to the IFCC reference method ***GPP***	Clarification and addition of new recommendation based on expert consensus	Hanas R, John G. 2010 consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. Clin Chem 2010;56:1362-4	Consensus statement	Moderate	Low	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 and as the equivalent NGSP DCCT-aligned result. Some, but not all, organizations have agreed to report HbA_{1c} as the DCCT-aligned percentage and the IFCC value.
				Weykamp C, et al. The IFCC reference measurement system for HbA_{1c}: a 6-year progress report. Clin Chem 2008;54:240-8	Progress report	Moderate		
				Goldstein DE, et al. Tests of glycemia in diabetes. Diab Care 2004;27:1761-73	Positions statement	Low		
				Hoelzel W, et al. IFCC reference system for measurement of hemoglobin A_{1c} in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166-74.	Method-comparison study	High		Impact on patient outcomes is unknown and indirect, therefore quality of evidence is downgraded. However, there is strong consensus of experts on HbA_{1c} reporting.
8.c		Laboratories that measure Hb A_{1c} should participate in a proficiency-testing program, such as the College of American Pathologists (CAP) Hb A_{1c} survey, that uses fresh blood samples with targets set by the NGSP Laboratory Network ***GPP***		Jeppsson JO, et al. Approved IFCC reference method for the measurement of HbA_{1c} in human blood. Clin Chem Lab Med 2002;40:78-89.	Method development	High		
				Little RR, et al. The national glycohemoglobin standardization program: a five-year progress re-port. Clin Chem 2001;47:1985-92.	Analytical study	Moderate		Retrospective analysis of analytical performance of the NGSP network and clinical labs in HbA_{1c} measurement
				Little RR, Goldstein DE. Standardization of glycohemoglobin measurements. AACC Endo 1995;13:109-24	Analytical study	Low		

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
8.d	Laboratories should be aware of potential interferences, including hemoglobinopathies that may affect GHb test results. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population ***Level A***	Laboratories should be aware of potential interferences, including hemoglobinopathies,that may affect Hb A_{1c} test results, depending on the method used. In selecting assay methods, laboratories should consider the potential for interferences in their particular patient population. In addition, disorders that affect erythrocyte turnover may cause spurious results, regardless of the method used ***GPP***	Clarification and new recommendation based on experience and published reports.	Ziemer DC, et al. Glucose-independent, black-white differences in hemoglobin A_{1c} levels: a cross-sectional analysis of 2 studies. Ann Intern Med 2010;152:770-7	Cross-sectional study	Moderate	Low	Quality of evidence downgraded for indirectness
				Selvin E, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010;362:800-11	Observational cohort study	High		
				Bry L, et al. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin [Review]. Clin Chem 2001;47:153-63.	Review	Low		
				Schnedl WJ, et al. Evaluation of HbA_{1c} determination methods in patients with hemoglobinopathies. Diab Care 2000;23:339-44.	Test comparison study	Moderate		
				Roberts WL, et al. Glycohemo-globin results in samples with hemoglobin C or S trait: a comparison of four test systems. Clin Chem 1999;45:906-9	Test comparison study	Moderate		
				Weykamp CW, et al. Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 methods. Clin Chem 1993;39:1717-23.	Multi/center method comparison study	Moderate		

Chapter 8: HEMOGLOBIN A_{1c} *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
8.e	Laboratories should use GHb assay methods with an interassay CV<5% (ideally <3%). At least two control materials with different mean values should be analyzed as an independent measure of assay performance. Laboratories should verify specimens below the lower limit of the reference interval or greater than 15% by repeat testing. If Schiff base (labile pre-HbA_{1c}) interferes with the assay method, it should be removed prior to assay	Desirable specifications for Hb A_{1c} measurement are an intralaboratory CV <2% and an interlaboratory CV <3.5%. At least 2 control materials with different mean values should be analyzed as an independent measure of assay performance ***B (low)***	Clarification and rewording of recommendations	Little RR, et al. Status of HbA_{1c} measurement and goals for improvement: From chaos to order for improving diabetes care. Clin Chem 2011;in press Sacks DB. CAP Surveys: Participant Summary for Glycohemoglobin Survey 2010 Set GH2-A. Northfield, IL: College of American Pathologists, 2010.	Review National survey (<10% from outside US)	Moderate Moderate	Low	This study used the reference change value (also called critical difference) to calculate an appropriate analytical goal The body of evidence is of low quality for indirectness of the data to clinical outcomes, but there is strong consensus of experts for appropriate analytical specifications to avoid unfavorable outcomes of misclassifications and mismanagement of patients. Therefore the recommendation was upgraded.
8.f	***Level C***	Samples with Hb A_{1c} results below the lower limit of the reference interval or >15% Hb A_{1c} should be verified by repeat testing ***B (low)***		Goodall I, et al. 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.	Consensus statement	Low		
8.g		Hb A_{1c} values that are inconsistent with the clinical presentation should be investigated further ***GPP***		Bry L, et al. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin [Review]. Clin Chem 2001;47:153-63 Marshall SM, Barth JH. Standardization of HbA_{1c} measurements: a consensus statement. Ann Clin Biochem 2000;37:45-6	Review Consensus statement	Low Low		

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
WHAT ARE THE HbA_{1c} TREATMENT GOALS IN DIABETES MELLITUS?							[(3)]Priority: 2 (A1, A2)	
8.h	Treatment goals should be based on ADA recommendations which include maintaining GHb concentrations <7% and reevaluation of the treatment regimen for GHb values > 8%. (Note that these values are applicable only if the assay method is certified as traceable to the DCCT reference.) ***Level B***	Treatment goals should be based on American Diabetes Association recommendations, which include generally maintaining Hb A_{1c} concentrations at <7% and more-stringent goals in selected individual patients if they can be achieved without significant hypoglycemia or other adverse treatment effects. Somewhat higher intervals are recommended for children and adolescents and may be appropriate for patients with a limited life expectancy, extensive comorbid illnesses, a history of severe hypoglycemia, or advanced complications (note that these values are applicable only if the NGSP has certified the assay method as traceable to the DCCT reference) ***A (high)***	Clarification	ADA. Standards of medical care in diabetes–2010. Diab Care 2010;33 (Suppl 1):S11-61.	Guideline	Moderate	High	Converging validity of several controlled clinical trials on patient-centered outcomes in type 1 and type 2 diabetes. Upgraded for directness and consistency and strong consensus of experts and several clinical organizations.
				Duckworth W, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360:129-39	RCT	High		
				Gerstein HC, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59	RCT	High		
				Patel A, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-72.	RCT	High		
				Berg AH, Sacks DB. Haemoglobin A_{1c} analysis in the management of patients with diabetes: from chaos to harmony. J Clin Pathol 2008;61:983-7.	Review	Low		
				Qaseem A, et al. Glycemic control and type 2 diabetes mellitus: the optimal hemoglobin A_{1c} targets. A guidance statement from the American College of Physicians. Ann Intern Med 2007;147:417-22	Guideline, consensus statement	Moderate		
				ADA. Implications of the Diabetes Control and Complications Trial (position statement). Diab Care 2000;23 (Suppl 1):S24-6	Position statement	Low		
				DCCT. The relationship of glycemic exposure (HbA_{1c}) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968-83	RCT	High		

Chapter 8: HEMOGLOBIN A_{1c} *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
WHAT SHOULD BE THE FREQUENCY OF HbA_{1c} MONITORING IN DIABETES MELLITUS?							[3]Priority: NOT LISTED	
8.i	GHb testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or are not meeting treatment goals ***Level B***	Hb A_{1c} testing should be performed at least biannually in all patients and quarterly for patients whose therapy has changed or who are not meeting treatment goals ***B (low)***	No change	ADA. Standards of medical care in diabetes–2010. Diab Care 2010;33 Suppl 1:S11-61.	Guideline	Moderate	Low	240 patients; followed x1 year; 50% had HbA_{1c} measured every 3 months; 50% no HbA_{1c} measured. Does not directly evaluate frequency – only testing vs no testing. Moreover, the best correlations of HbA_{1c} with complications have been based on quarterly HbA_{1c} testing for capturing overall glycemic exposure. However, there is no consensus on the optimal frequency of HbA_{1c} testing. Most recommendations are based on strong expert consensus.
				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	RCT	Moderate		
SHOULD HbA_{1c} BE USED FOR SCREENING AND DIAGNOSIS OF DIABETES MELLITUS?							[3]Priority: 1 (A1-5, B2, C1)	
8.j		Hb A_{1c} may be used for the diagnosis of diabetes, with values ≥6.5% being diagnostic. An NGSP-certified method should be performed in an accredited laboratory. Analogous to its use in the management of diabetes, factors that interfere with or adversely affect the Hb A_{1c} assay will preclude its use in diagnosis ***A (moderate)***	New recommendation based on additional evidence and consensus of experts	ADA. Standards of medical care in diabetes–2010. Diab Care 2010;33 (Suppl 1):S11-61.	Guideline	Moderate	Moderate	The data supporting the use of HbA_{1c}, i.e. its relationship with risk of retinopathy, is similar to the data that support glucose testing as the means of diagnosis. These are definitional issues. Both the ADA and the American Endocrinology societies endorsed the HbA_{1c} test for diagnosis. Other international organizations, including the WHO and IDF, are considering HbA_{1c} for diabetes diagnosis and screening, therefore there is an emerging strong consensus on the topic, which resulted in upgrading the recommendation.
				American Association of Clinical Endocrinologists/American College of Endocrinology statement on the use of hemoglobin A_{1c} for the diagnosis of diabetes. Endocr Pract 2010;16:155-6	Guideline	Moderate		
				Cheng YJ, et al. Association of A1C and fasting plasma glucose levels with diabetic retinopathy prevalence in the U.S. population: Implications for diabetes diagnostic thresholds. Diab Care 2009;32(11): 2027-32	Population-based cross sectional	High		

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Nathan DM et al. for the International Expert Committee on the Diagnosis of Diabetes. Report on the Role of the Glycated Hemoglobin (A1C) Assay in the Diagnosis of Diabetes. Diab Care 2009;32:1327-34	Expert consensus	Low		A HbA_{1c} value of 6.5% or greater was considered diagnostic based on the observed relationship with retinopathy in more than 28,000 persons. This represents direct relationship to outcomes and thus quality of evidence is upgraded.
				Sabanayagam C, et al. Relationship between glycated haemoglobin and microvascular complications: is there a natural cut-off point for the diagnosis of diabetes? Diabetologia 2009;52(7):1279-89.	Population-based cross sectional	High		
				Ito C, et al. Importance of OGTT for diagnosing diabetes mellitus based on prevalence and incidence of retinopathy. Diab Res Clin Pract. 2000;49(2-3): 181-6	Population-based cross sectional	High		
8.k		Point-of-care Hb A_{1c} assays are not sufficiently accurate to use for the diagnosis of diabetes ***B (moderate)***	New recommendation	American Diabetes Association. Standards of medical care in diabetes –2011. Diab Care 2011;34 (Suppl 1):S11-61	Guideline	Moderate	Moderate	The ADA cautions that POCT devices for HbA_{1c} should not be used for diagnosis.
				Lenters-Westra E, Slingerland RJ. Six of eight hemoglobin A_{1c} point-of-care instruments do not meet the general accepted analytical performance criteria. Clin Chem 2010;56:44-52	Analytical study	Moderate		

Chapter 9: GENETIC MARKERS

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR GENETIC TESTING IN TYPE 1 DIABETES MELLITUS?							[3]Priority: NOT LISTED	
9.a	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, valuable information can be obtained with definition of diabetes-associated mutations ***Level E***	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)***	New information is available on mutations in the proinsulin and other genes that are linked to neonatal diabetes	Concannon P, et al. Genetics of type 1A diabetes. N Engl J Med 2009;360:1646	Review	Moderate	Moderate	Useful review of genetic factors outside the HLA region.
				Murphy R, et al. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab 2008;4:200-13.	Linkage analyses	High		Monogenic diabetes below the age of six needs to be considered for monogenic diabetes
				Edghill EL, 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	Linkage analyses in multiple familes	High		Many mutations than known hitherto affect the human preproinsulin gene
				Støy J, et al. Neonatal Diabetes International Collaborative Group. Insulin gene mutations as a cause of permanent neonatal diabetes. Proc Natl Acad Sci USA. 2007;104(38):15040-4	Linkage analyses	Moderate		Diabetes below the age of six months needs to be considered for monogenic diabetes.
				Hagopian WA, et al. TEDDY–The Environ-mental Determinants of Diabetes in the Young: an observational clinical trial. Ann N Y Acad Sci 2006;1079:320-6.	Observational study	High		In contrast to other studies, the TEDDY study has sufficient statistical power to answer questions related to environmental triggers for islet autoimmunity and type 1 diabetes.
				Barker JM, et al. Clinical characteristics of children diagnosed with type 1 diabetes through intensive screening and follow-up. Diab Care 2004;27:1399-404.	Screening study of children at risk for type 1 diabetes	Moderate		Early diagnosis may prevent hospitalization with ketoacidosis and preserve residual beta cells. More outcome studies are needed to prove this.

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Graham J, et al. Genetic effects on age-dependent onset and islet cell auto- antibody markers in type 1 diabetes. Diabetes 2002;51:1346-55	Population-based case-control study	Moderate		First time INS VNTR were found to be associated with INS VNTR.
				Fajans SS, et al. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345:971-80	Review	Low		Careful analysis of family history of diabetes is important to the detection of monogenic diabetes.
				Kukreja A, Maclaren NK. Auto-immunity and diabetes. J Clin Endocrinol Metab 1999;84:4371	Review	Moderate		
				Rewers M, et al. Newborn scree-ning for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia 1996;39:807	Screening study of children at risk for type 1 diabetes	Moderate		It is possible to screen new-born children to identify those at increased risk for developing type 1 diabetes. This strategy cannot be recommended until there is a proven intervention available to delay or prevent the disease.
				Ziegler AG, et al. Prophylactic insulin treatment in relatives at high risk for type 1 diabetes. Diabetes Metab Rev 1993;9:289	Review	Moderate		
IS THERE A ROLE FOR GENETIC TESTING IN TYPE 2 DIABETES MELLITUS?							[3]Priority: NOT LISTED	
9.b	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 ***Level E***	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)***	No change	Meigs JB, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208-19.	Genome wide association case-control study	Moderate	Moderate	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
				Scott LJ, et al. A genome- wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341	Genome wide association case-control study	Moderate		
				Saxena R, et al. Genome wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316: 1331	Genome wide association case-control study	Moderate		

Chapter 10: AUTOIMMUNE MARKERS

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
SHOULD GAD65, IA-2 OR INSULIN AUTOANTIBODIES BE USED FOR THE DIAGNOSIS, SCREENING, MONITORING OF TYPE 1 AND TYPE 2 DIABETES?							[3]Priority: 1.5 (A1-5, C1) [3]Priority: 3 (A3-4, C1)	
10.a	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, immune-mediated (type 1) diabetes. Islet cell autoantibodies are not recommended for routine diagnosis of diabetes nor for screening ***Level E***	Islet cell autoantibodies are recommended for screening nondiabetic family members who wish to donate part of their pancreas for transplantation into a relative with end-stage type 1 diabetes ***B (low)***	Considerable progress has been made to standardize islet cell autoantibody tests.	Bingley PJ,et al. Measurement of islet cell antibodies in the Type 1 Diabetes Genetics Consortium: efforts to harmonize procedures among the laboratories. Clin Trials. 2010;7(1 Suppl):S56-64.	Analytical test evaluation	Moderate	Low	International workshops using serum exchange exercises provide measures of inter-laboratory variation. Quality of evidence is downgraded for indirectness.
10.b		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 after HLA typing at birth ***B (low)***		Törn C, et al. Participating Laboratories. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia. 2008;51(5):846-52.	Analytical test evaluation	Moderate		
10.c	Screening from GAD65 antibodies in patients diagnosed with type 2 diabetes is not recommended at present to be reclassified with type 1 diabetes. ***Level E***	Screening patients with type 2 diabetes for islet cell autoantibodies 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 of treatment of type 2 diabetes ***B (low)***	Considerable progress has been made to standardize islet autoantibody tests. It is not clear to what extent a positive islet autoantibody test would suffice to alter diagnostic criteria.	Rolandsson O, Palmer JP. Latent autoimmune diabetes in adults (LADA) is dead: long live autoimmune diabetes! Diabetologia. 2010;53(7):1250-3.	Review	Low	Low	Review suggesting that islet autoantibody positivity should suffice to classify adult diabetes patients with "autoimmune diabetes" is GAD65 autoantibody positive. Strength of recommendation is upgraded for strong consensus

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
10.d	Screening of relatives of patients with type 1 diabetes or of persons in the general population for islet cell autoantibodies is not recommended at present ***Level E***	Screening for islet cell autoantibodies in relatives of patients with type 1 diabetes or in persons from the general population is not recommended at present. Standardized islet cell autoantibodies are tested in prospective clinical studies ***B (low)***	Clarification and addition of new recommendation based on new evidence	Patterson CC, et al. 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	Multicentre prospective registration study	Moderate	Low	Epidemiology data
				Maclaren N, 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	Review	Low		
				Verge CF, 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.	Multicentre prospective registration study	Moderate		Data only applicable to first degree relatives who comprise only 10-15% of newly diagnosed type 1 diabetes children. Quality of the overall body of evidence was downgraded for lack of suitably powered studies or RCTs investigating the value of islet cell autoantibody testing for screening purposes
10.e	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 endpoints ***Level E***	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 in some clinical trials as surrogate end points ***B (low)***	No change	Sosenko JM et al. Glucose excursions between states of glycemia with progression to type 1 diabetes in the diabetes prevention trial-type 1 (DPT-1). Diabetes Prevention Trial-Type 1 Study Group. Diabetes. 2010;59(10):2386-9.	Prospective family study of islet autoantibody positive subjects	Moderate	Low	Data on first degree relatives suggest an important contribution of insulin sensitivity on glucose tolerwance. Quality of the overall body of evidence was downgraded for lack of sufficient data from multiple studies

Chapter 10: AUTOIMMUNE MARKERS

No	1. NACB 2002 recommendation and its grade(1)	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence(2)	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence(2) (high-moderate-low)	7. Quality of evidence(2) (high-moderate-low-very low)	8. Comments
10.f	It is important that autoantibodies be measured only in an accredited laboratory with an established quality control program and participation in a proficiency testing program ***Level E***	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***	Clarification, but no change	Bonifacio E, et al Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocrinol Metab. 2010;95(7):3360-7.	Analytical test evaluation	Moderate	Moderate	Standardization was possible between three expert laboratories.

Chapter 11: LOW LEVELS OF ALBUMINURIA (FORMERLY MICROALBUMINURIA)

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
WHEN TESTING FOR LOW LEVELS OF ALBUMINURIA IS INDICATED?							[(3)]Priority: 1 (A5, A1-2)	
11.a	Annual microalbumin testing of patients without 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. The role of testing is unclear in patients under treatment with angiotensin-converting enzyme inhibitors and in those with short life expectancy. ***Level E***	Annual testing for albuminuria in patients without clinical proteinuria should begin in pubertal or postpubertal individuals 5 years after diagnosis of type 1 diabetes and at the time of diagnosis of type 2 diabetes, regardless of treatment ***B (moderate)***	Clarification	American Diabetes Association. Standards of medical care in diabetes –2010. Diab Care 2010; 33 (Suppl 1):S11-61.	Guideline expert opinion	Low	Moderate	There is a higher incidence of obesity and metabolic derangements that accompany this problem including an increase in cardiovascular risk. Low levels of albuminuria is a risk marker for cardiovascular events and predictive of cardiovascular events. This is especially true in diabetes.
				Vassalotti JA, et al. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. Am J Kidney Dis 2007;50 (2):169-180	Position statement	Low		
				KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. Am J Kidney Dis 2007;49 (2 Suppl 2):S12-154	Guideline	Moderate		
				Klausen KP, et al. Very low level of microalbumin-uria is associated with increased risk of death in subjects with cardio-vascular or cerebro-vascular diseases. J Intern.Med. 2006;260 (3):231-237	Cohort study	Low		
				Klausen KP, et al. New definition of microalbuminuria in hyper-tensive subjects: association with incident coronary heart disease and death. Hypertension 2005;46 (1):33-37	Observational study	Low		
				Kistorp K, et al. N-terminal pro-brain natriuretic peptide, C-reactive protein, and urinary albumin levels as predictors of mortality and cardiovascular events in older adults. JAMA 2005;293:1609-1616.	Meta-analysis	Moderate		
				Gansevoort RT, et al. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int.Suppl 2005; (94):S28-S35	Observational study	Moderate		Study in the Netherlands of more than 30,000 people

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Ibsen H, et al. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for end point reduction in hypertension study. Hyper-tension 2005;45 (2):198-202	Post hoc analysis	Moderate		Post hoc analysis of clinical cardiovascular outcome trials
				Arnlov J, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. Circulation 2005;112 (7):969-975	Observational study	Moderate		Study of cardiovascular outcomes
				Chobanian AV, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42 (6):1206-1252	Guideline statement from NIH	Moderate		
				Lepore G, et al. Cost-effectiveness of two screening programs for microalbuminuria in type 2 diabetes. Diab Care 2002;25 (11):2103-2104	Cost-effectiveness analysis	Moderate		
WHAT IS THE RELATIONSHIP BETWEEN ALBUMINURIA AND CARDIOVASCULAR OUTCOMES?							[3]Priority: 1 (A5, A1-2)	
11.b		Urine albumin at concentrations ≥30 mg/g creatinine should be considered a continuous risk marker for cardiovascular events ***B (moderate)***	New recommendation	G. Pambianco, et al. 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. Diab Care 2007;30(5):1248-1254.	Observational cohort study	Moderate	Moderate	This was an observational study in patients with type 1 diabetes followed for 12 years.
				Klausen KP, et al. Very low level of microalbuminuria is associated with increased risk of death in subjects with cardiovascular or cerebro-vascular diseases. J Intern Med 2006;260 (3):231-237.	Cohort study	Moderate		

Chapter 11: LOW LEVELS OF ALBUMINURIA (FORMERLY MICROALBUMINURIA) *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Ratto E, et al. Microalbuminuria and cardiovascular risk assessment in primary hypertension: should threshold levels be revised? Am J Hypertension 2006;19 (7):728-734	Observational cohort study	Low		The study evaluated level of microalbuminuria relative to development of left ventricular hypertrophy; not cardiovascular outcome
				Klausen KP, et al. New definition of microalbuminuria in hyperten-sive subjects: association with incident coronary heart disease and death. Hypertension 2005;46 (1):33-37	Observational cohort study	Low		
				K. Wachtell, et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann.Intern.Med. 2003;139 (11):901-906.	Prospective randomized trial	High		This clinical trial evaluated changes in albuminuria over a 5 year period in high risk patients for cardiovascular events all of whom had left ventricular hypertrophy.
				R. Rachmani, et al. Considerations about the threshold value of microalbuminuria in patients with diabetes mellitus: lessons from an 8-year follow-up study of 599 patients. Diab. Res.Clin. Pract. 2000;49 (2-3):187-194.	Observational cohort study	Moderate		This was an 8 year follow-up of 599 people with diabetes evaluating changes in cardiovascular risk markers including microalbuminuria
WHAT ARE THE ANALYTICAL CONSIDERATIONS WHEN TESTING FOR LOW LEVELS OF ALBUMINURIA?							[3]Priority: NOT LISTED	
11.c	The analytical CV of methods to measure micro-albuminuria should be <15% ***Level E***	The analytical CV of methods to measure albuminuria should be <15% ***B (moderate)***	No change	Sarafidis PA, et al. A comparative evaluation of various methods for microalbuminuria screening. Am.J Nephrol. 2008;28 (2):324-329.	Randomized study	Moderate	Moderate	Comparative studies of different validated assays
				Gansevoort RT, et al. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int.Suppl 2005;(94):S28-S35	Observational study	Moderate		
				Incerti J, et al. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial. Transplant. 2005;20 (11):2402-2407	Observational study	Moderate		

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Meinhardt U, et al. Microalbumin-uria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collection J Diab Compli-cations 2003;17 (5):254-257	Observa-tional study	Moderate		
11.d	Semiquantitative or qualitative screening tests for microalbumin-uria 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 ***Level E***	Semiquantitative or qualitative screen-ing tests should be positive in >95% of patients with albuminuria to be useful for screen-ing. Positive results must be confirmed by analysis in an ac-credited laboratory ***GPP***	No change	Sarafidis PA,et al. A comparative evalu-ation of various methods for microal-buminuria screening. Am.J Nephrol. 2008;28 (2):324-329.	Randomized study	Moderate	Moderate	Most recent studies do have >95% for Hemocue and Im-munodip but only one study confirmed against standard lab for Hemocue
				Shaikh A, et al. Comparison between immunoturbidimetry, size-exclusion chromatography, and LC-MS to quantify urinary albumin. Clin Chem 2008;54 (9):1504-1510	Analytical study	Moderate		Recommendation down-graded for indirectness of analytical data to clinical outcomes
11.e		Currently available dipstick tests do not have adequate ana-lytical sensitivity to detect albuminuria ***B (moderate)***	New recom-mendation according to recent literature on the topic	Gansevoort RT, et al. The validity of screening based on spot morning urine samples to detect subjects with micro-albuminuria in the general population. Kidney Int.Suppl 2005; (94):S28-S35.	Observa-tional study	Moderate	Moderate	There is no convincing evidence in multiple studies for any specific test achieving >95% diagnostic sensitivity in two or more different studies.
				Incerti J, et al. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial. Transplant. 2005;20(11):2402-2407.	Observa-tional study	Moderate		Due to this, no specific screening test can be recom-mended. “Dipstick” tests for microalbuminuria cannot be recommended as replace-ment for the quantitative tests.
				Davidson MB, et al. ImmunoDip: an improved screening method for microalbuminuria. Am J Nephrol 2004;24:284-8.	Observa-tional study	Moderate		
				Meinhardt U, et al. Microalbumin-uria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collection. J Diab Comp-lications 2003;17 (5): 254-257.	Observa-tional study	Moderate		
				Fernandez Fernandez I, et al. Rapid screening test evaluation for microal-buminuria in diabetes mellitus. Acta Diabetol 1998; 35:199-202	Observa-tional study	Moderate		

Chapter 11: LOW LEVELS OF ALBUMINURIA (FORMERLY MICROALBUMINURIA) ***(Cont'd)***

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Leong SO, et al. 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.	Randomized trial	Moderate		
				Poulsen PL, et al. Evaluation of a dipstick test for micro-albuminuria in three different clinical settings, including the correlation with urinary albumin excretion rate. Diabetes Metab 1992;18:395-400.	Observational study	Low		
WHAT ARE THE PREANALYTICAL CONSIDERATIONS WHEN TESTING FOR LOW LEVELS OF ALBUMINURIA?							[3]Priority: 3 (A3-4)	
11.f	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. ***Level E***	Acceptable samples to test for increased urinary albumin excretion are timed collections (e.g., 12 or 24 h) for measurement of the albumin concentration and timed or untimed samples for measurement of the albumin–creatinine ratio B (moderate)	No change, but new evidence supports recommendation	Lambers Heerspink HJ, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. J Am Soc Nephrol 2010;21:1355-60	Prospective cohort	High	Moderate	The albumin:creatinine ratio is the superior method to predict renal events in patients with type 2 diabetes
				Ibsen H, et al. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for end point reduction in hypertension study. Hypertension 2005;45:198-202.	Observational study	Moderate		
				Gansevoort RT, et al. The validity of screening based on spot morning urine samples to detect subjects with microalbuminuria in the general population. Kidney Int.Suppl 2005;(94):S28-S35	Observational study	Moderate		
				Meinhardt U, et al. Microalbumin-uria in diabetes mellitus: efficacy of a new screening method in comparison with timed overnight urine collectionJ Diabetes Compli-cations 2003;17 (5):254-257	Observational study	Moderate		
				Hishiki S, et al. Circadian variation of urinary microalbumin excretion and ambulatory blood pressure in patients with essential hypertension. J Hypertens 1998;16:2101-8.	Observational study	Low		

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
				Howey JE, et al. Biologic variation of urinary albumin: consequences for analysis, specimen collection, interpretation of results, and screening programs. Am J Kidney Dis 1989;13:35-7.	Observational study	Moderate		
WHAT IS THE OPTIMAL TIME OF DAY TO MEASURE ALBUMINURIA?							[3]Priority: 2 (A2-4)	
11.g		The optimal time for spot urine collection is the early morning. All collections should be at the same time of day to minimize variation. The patient should not have ingested food within the preceding 2 h but should be well hydrated (i.e., not volume depleted). ***GPP***	New recommendation	Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, and Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am.Soc. Nephrol. 2009;20 (2):436-443.	Prospective non-randomized	Moderate	Low	Collected three different urines and analyzed in three different ways. One study only that investigates this topic. Recommendation downgraded for indirectness of evidence and lack of more data.
HOW FREQUENTLY ALBUMINURIA SHOULD BE MEASURED?							[3]Priority: 1 (A5, A1-2)	
11.h		Low urine albumin concentrations (i.e., <30 mg/g creatinine) are not associated with high cardiovascular risk if the eGFR is >60 mL . min^{-1}. $(1.73\ m^2)^{-1}$ and the patient is normotensive. If the eGFR is <60 mL . min^{-1}. $(1.73\ m^2)^{-1}$ and/or the level of albuminuria is ≥30 mg/g creatinine on a spot urine sample, a repeat measurement should be taken within the year to assess change among people with hypertension ***A (moderate)***	New recommendation	Levey AS, et al. The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011;*in press*	Consensus report	Moderate	Moderate	Strong consensus of experts upgraded the recommendation
				Yuyun MF, et al. Micro-albuminuria 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 (1):189-198	Prospective cohort	Moderate		

Chapter 12: MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR MEASUREMENT OF INSULIN AND C-PEPTIDE CONCENTRATIONS TO DISTINGUISH TYPE 1 FROM TYPE 2 DIABETES MELLITUS?							(3)Priority: 2 (A3-4)	
12.a	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. There is no role for measurement of insulin concentration in the diagnosis of the metabolic syndrome because knowledge of this value does not alter the management of these patients. ***Level E***	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 be made in most cases on the basis of the clinical presentation and the subsequent course. These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis ***B (moderate***)	Changed wording. Many groups, including ADA, are moving beyond the categorical concept ("diagnosis") of metabolic syndrome to that of continuous and more global measures of risk for diabetes and cardiovascular disease.	Rutter MD, et al. Use of Alternative thresholds defining insulin resistance to predict incident type 2 diabetes and cardiovascular disease. Circulation. 2008;117:1003-1009.	Cohort study	Moderate	Moderate	Models of predictive baseline measures of insulin resistance (which include measures of insulin) in a large population. Surrogate IR measures (which all included measures of insulin) had modest performance at the 76th centile, with no threshold effects. Prediction was particularly poor for CVD.
				Wilson PW et al. Prediction of incident diabetes mellitus in middle-aged adults: The Framingham Offspring Study. Arch Intern Med 2007;167:1068-74.	Cohort study	Moderate		Models of predictive baseline values in a large population. Factors easily obtainable on history, exam, or standard lab tests (glucose, lipids) predicted incident DM strongly. Addition of more complex factors, including fasting insulin, did not add significantly.
				Despres J-P et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952-7.	Case-control study	Moderate		Case-control study looking at baseline fasting insulin levels in Quebec Heart Study. High fasting insulin levels appeared to be an independent risk factor for IHD. However, only excluded clinically diagnosed DM (in early 1990s, probably many undiagnosed) and did not adjust for any measures of glycemia or BMI

Chapter 12: MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[1]	2. NACB 2011 updated/ new recommendation with its grade and quality of evidence[2]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[2] (high-moderate-low)	7. Quality of evidence[2] (high-moderate-low-very low)	8. Comments
12.a	These assays are useful primarily for research purposes and, in rare cases, to identify patients with an absolute requirement for insulin before switching to oral agents, or to assist patients in obtaining insurance coverage for continuous subcutaneous infusion pumps. ***Level E***	These assays are useful primarily for research purposes. Occasionally, C-peptide measurements may help distinguish type 1 from type 2 diabetes in ambiguous cases, such as patients who have a type 2 phenotype but present in ketoacidosis. ***B (moderate)***	New evidence regarding using C-peptide to clarify diagnosis	Balasubramanyam A et al. Accuracy and predictive value of classification schemes for ketosis-prone diabetes. Diab Care 2006; 29:2575-9.	Observational prognostic/ diagnostic study	Moderate	Moderate-low	Investigation of patients presenting with ketosis, with absent or preserved C-peptide function at one year the outcome. Unclear how direct the outcome is, whether this is better than current care
	A possible role for measurement of fasting insulin or the assessment of insulin resistance is in the evaluation of patients with polycystic ovary syndrome who may be candidates for treatment aimed at lowering insulin resistance in the absence of overt diabetes or glucose intolerance ***Level E***	None	Prior recommendation deleted. No evidence that this is better than clinical evaluation for signs of insulin resistance; not recommended by ACOG or other groups.	American College of Obstetrics and Gynecology. ACOG practice bulletin. Polycycstic ovary syndrome. Number 41, December 2002. Int J Gynecol Obstet 2003; 80:335-48	Guideline/ Expert consensus	Low	Very low	Prior recommendation was also supported by expert opinion only

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR MEASUREMENT OF INSULIN CONCENTRATIONS OR INDIRECT MEASURES OF INSULIN RESISTANCE IN THE ASSESSMENT OF PATIENTS' CARDIOMETABOLIC RISK OR TO DETERMINE USE OF INSULIN SENSITIZING DRUGS IN DIABETIC OR NON-DIABETIC PATIENTS?							[(3)]Priority: 2 (A3)	
12.b		There is no role for measurement of insulin concentration in the assessment of cardiometabolic risk, because knowledge of this value does not alter the management of these patients ***B (moderate)***	New recommendation	Rutter MD, et al. Use of Alternative thresholds defining insulin resistance to predict incident type 2 diabetes and cardiovascular disease. Circulation. 2008;117:1003-9.	Cohort study	Moderate	Moderate	
				Wilson PW et al. Prediction of incident diabetes mellitus in middle-aged adults: The Framingham Offspring Study. Arch Intern Med 2007;167:1068-74.	Cohort study	Moderate		
				Despres J-P et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952-7.	Case-control study	Moderate		
DO INSULIN MEASUREMENTS NEED TO BE HARMONIZED?							[(3)]Priority: 2 (A3)	
12.c		Because 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***		Staten M, et al, for the Insulin Standardization Workgroup. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diab Care 2010;33:205-6	Expert consensus	Low	Low	Commentary summarizes the above papers and calls for a standardized insulin assay based on above.
				Miller WG, et al for the Insulin Standardization Work Group. Toward standardization of insulin immunoassays. Clin Chem 2009;55:1011-8	Investigation of alternate preparation for insulin reference materials	Moderate		Most assays can achieve consistent performance with calibration traceability based on individual serum samples with insulin concentrations set by isotope dilution mass spectrometry.
				Marcovina S, et al. Standardization of insulin immunoassays: report of the American Diabetes Association Workgroup. Clin Chem 2007; 53:711-6	Comparison of different insulin assays currently on the market in the US.	Moderate		Current FDA-approved commercially available insulin assays provide a wide range of values for the same samples. There clearly is a need to standardize the reference system and protocols to enable all assays to achieve consistent and uniform results and to report insulin in identical units.

Chapter 12: MISCELLANEOUS POTENTIALLY IMPORTANT ANALYTES *(Cont'd)*

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR INSULIN AUTOANTIBODY TESTING IN MANAGING PATIENTS WITH DIABETES MELLITUS?							[(3)]Priority: NOT LISTED	
12.d	There is no published evidence to support the use of insulin antibody testing for routine care of patients with diabetes ***Level E***	There is no published evidence to support the use of insulin antibody testing for routine care of patients with diabetes. ***C (very low)***	No change	Bingley PJ,et al. Measurement of islet cell antibodies in the Type 1 Diabetes Genetics Consortium: efforts to harmonize procedures among the laboratories. Clin Trials 2010;7(1 Suppl):S56-64.	Analytical test evaluation	Moderate	Very low	International workshops using serum exchange exercises provide measures of inter-laboratory variation. Standardization was possible between three expert laboratories. Quality of evidence and strength of recommendation are downgraded for indirectness.
				Bonifacio E, et al Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. J Clin Endocri-nol Metab. 2010;95(7):3360-7.	Analytical test evaluation	Moderate		
				Törn C, et al. Participating Laboratories. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia. 2008;51(5):846-52.	Analytical test evaluation	Moderate		

No	1. NACB 2002 recommendation and its grade[(1)]	2. NACB 2011 updated/new recommendation with its grade and quality of evidence[(2)]	3. Why was it necessary to modify the recommendation?	4. Key references supporting the new recommendation	5. Study design	6. Level of evidence[(2)] (high-moderate-low)	7. Quality of evidence[(2)] (high-moderate-low-very low)	8. Comments
IS THERE A ROLE FOR AMYLIN AND LEPTIN TESTING IN MANAGING PATIENTS WITH DIABETES MELLITUS?							[(3)]Priority: NOT LISTED	
	Assays for amylin are not clinically useful in the management of diabetes. These studies should be confined to the research setting ***Level E***	None	The evidence accumulated in the last six to seven years has failed to identify any clinical value in measuring these analytes in patients with diabetes.					
	Routine measurement of plasma leptin concentrations is not of value at this time for the evaluation or management of patients with diabetes or obesity ***Level E***	None	Recommendation removed for reasons mentioned above					