DIAGNOSTIC TESTS FOR DIABETES MELLITUS

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ABSTRACT

In this chapter, indications for screening for diabetes mellitus are reviewed. Criteria for diagnosis are fasting plasma glucose ≥ 126 mg/dl (7.0 mmol/l) or random glucose ≥200 mg/dl (11.1 mmol/l) with hyperglycemic symptoms, hemoglobin A1c (HbA1c) ≥6.5%, and oral glucose tolerance testing (OGTT) 2-h glucose ≥200 mg/dl (11.1 mmol/l) after 75 g of glucose. One-step and two-step strategies for diagnosing gestational diabetes using pregnancy-specific criteria as well as use of the 2-h 75-g OGTT for the postpartum testing of women with gestational diabetes (4-12 weeks after delivery) are described. Testing for other forms of diabetes with unique features are reviewed, including the recommendation to use the 2-h 75 g OGTT to screen for cystic fibrosis-related diabetes and post-transplantation diabetes, fasting glucose test for HIV positive individuals, and genetic testing for monogenic diabetes syndromes including neonatal diabetes and maturity-onset diabetes of the young (MODY). Elevated measurements of pancreatic islet autoantibodies (e.g., to the 65-KDa isoform of glutamic acid decarboxylase (GAD65), tyrosine phosphatase related islet antigen 2 (IA-2), insulin (IAA), and zinc transporter (ZnT8)) suggest autoimmune type 1 diabetes (vs type 2 diabetes). IAA is primarily measured in youth. The use of autoantibody testing in diabetes screening programs is recommended in first degree relatives of an individual with type 1 diabetes or in research protocols. C-peptide measurements can be helpful in identifying those who have type 1 diabetes (low or undetectable c-peptide) from those who may have type 2 or monogenic diabetes.

SCREENING FOR DIABETES MELLITUS AND PREDIABETES

Early detection and treatment of diabetes mellitus is important in preventing acute and chronic complications of this disease. Individuals with symptoms suggestive of hyperglycemia, such as polyuria, polyphagia, polydipsia, unexplained weight loss, blurred vision, excessive fatigue, or infections or wounds that heal poorly should be promptly tested. The American Diabetes Association (ADA) recommends routinely screening for type 2 diabetes in adults every three years beginning at age 45. In asymptomatic people, testing for type 2 diabetes should be considered in adults of any age if they are overweight or obese (BMI ≥ 25 kg/m^2, or ≥ 23 kg/m^2 if Asian background), planning pregnancy, and/or if they have additional risk factors as listed below in Table 1. Repeat screening should be performed at least every

three years. Patients with prediabetes should be screened yearly (1). The US Preventive Services Task Force recommends glucose screening for all asymptomatic overweight or obese adults ages 40-70 (2); the American Association of Clinical Endocrinologists recommends screening at risk individuals at any age (3).

Table 1. Risk Factors for the Development of Type 2 Diabetes
Physical inactivity
First-degree relative with diabetes
High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
Women who delivered a baby weighing >9 lb. or were diagnosed with Gestational Diabetes
Hypertension (≥130/80 mm Hg or on therapy for hypertension)
HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.8 mmol/L)
Individuals with polycystic ovary syndrome
People with prediabetes (HbA1C ≥5.7%, Impaired Glucose Tolerance (IGT), or Impaired Fasting Glucose (IFG))
Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans. Metabolic dysfunction-associated steatotic liver disease)
History of cardiovascular disease
Individuals in other high-risk groups (HIV, exposure to high-risk medicines, evidence of periodontal disease, history of pancreatitis

Type 2 diabetes is becoming a growing problem in children and adolescents in high-risk populations. To address this issue, the ADA recommends screening overweight [body mass index (BMI) ≥85th percentile] or obese (BMI ≥95th percentile) youth at least every 3 years, beginning at age 10 or at the onset of puberty, if they have 1 or more additional risk factors listed below in Table 2. Repeat testing should be done more frequently if BMI increases (1).

Table 2. Risk Factors for Type 2 Diabetes in Children and Adolescents
Family history of type 2 diabetes in first and second-degree relatives
Race and ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)
Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, small-for- gestational-age birth weight, or polycystic ovary syndrome)
Maternal history of diabetes or gestational diabetes during child's gestation

DIAGNOSING DIABETES AND PREDIABETES

The diagnosis of diabetes can be made using the fasting plasma glucose, random plasma glucose, oral glucose tolerance test, or hemoglobin A1c (HbA1c) (1). Testing should be performed on 2 separate days using one or more of the above tests, unless unequivocal hyperglycemia is present. Alternatively, in the absence of symptoms of hyperglycemia, diabetes can be diagnosed if there are two different abnormal

test results from the same sample (1). An overview of the ADA criteria is shown in Table 3.

Table 3. ADA Criteria for the Diagnosis of Diabetes
HbA1C ≥6.5%. The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.
FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.
2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an Oral Glucose Tolerance Test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
In an individual with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L). Random is any time of day without regard to time since previous meal.

HbA1c

The use of the HbA1c assay was recommended for the diagnosis of diabetes in 2009 by an International Expert Committee (4). HbA1c levels reflect overall glycemic control and correlate with the development of microvascular complications. An HbA1c ≥ 6.5% on two separate occasions can be used to diagnose diabetes. An HbA1c level of 6.0% to ≤ 6.5% identifies high risk of developing diabetes. The ADA considers individuals with a HbA1c of 5.7% to 6.4% at increased risk for developing diabetes (1). HbA1c should not be used to diagnose gestational diabetes, diabetes in HIV positive individuals, post-organ transplantation, or in people with cystic fibrosis.

Fasting and Random Plasma Glucose

Fasting plasma glucose is one method recommended by the ADA for the diagnosis of diabetes in children and non-pregnant adults (1). The interpretation of fasting glucose measures is shown in Table 4. The test should be performed after an 8 hour fast. For routine clinical practice, fasting plasma glucose may be preferred over the oral glucose tolerance test because it is rapid, easier to administer, is more convenient for patients and providers, and has a lower cost (1). A random plasma glucose level, which is obtained at any time of the day regardless of the time of the last meal, can also be used in the diagnosis of diabetes in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.

Table 4. Fasting Plasma Glucose Criteria	
	Fasting Plasma Glucose
Normal glucose tolerance	<100 mg/dl (5.6 mmol/l)
Impaired fasting glucose (pre-diabetes)	100-125 mg/dl (5.6-6.9 mmol/l)
Diabetes mellitus	≥126 mg/dl (7.0 mmol/l)

For the diagnosis of diabetes, standard venous plasma glucose specimens should be obtained. Specimens should be processed promptly, since glucose is metabolized at room temperature. This process is influenced by storage temperature, storage time as well as other factors, and is accelerated in the presence of bacteria or leukocytosis.

Whole blood glucose specimens obtained with point-of- care devices should not be used for the diagnosis of diabetes because of the inaccuracies associated with these methods. Capillary and venous whole blood glucose concentrations are approximately 15% lower than plasma glucose levels in fasting specimens. However, most devices account for this difference in their calibration.

Oral Glucose Tolerance Test (OGTT)

OGTTS FOR THE DIAGNOSIS OF DIABETES AND IMPAIRED GLUCOSE TOLERANCE IN NON-PREGNANT INDIVIDUALS

A formal OGTT can be used to establish the diagnosis of diabetes mellitus (Table 5). OGTT is more cumbersome and costlier than the fasting plasma glucose test; however, the use of only the fasting plasma glucose may not identify a proportion of individuals with impaired glucose tolerance or diabetes (5). A plasma glucose level 2-hours after a glucose challenge may identify additional individuals with abnormal glucose tolerance who are at risk for microvascular and macrovascular complications, particularly in high-risk populations in which postprandial (versus fasting) hyperglycemia is evident early in the disease (6,7).

When using an OGTT, the criteria for the diagnosis of diabetes is a 2 h glucose >200 mg/dl (11.1 mmol/l) after a 75-gram oral glucose load (ADA and WHO criteria). The 75-gram glucose load should be administered when the patient has ingested at least 150 grams of carbohydrate for the 3 days preceding the test and after an overnight fast. Dilution of the 75-gram oral glucose load (300-900 ml) may improve acceptability and palatability without compromising reproducibility (8). The patient should not be acutely ill or be taking drugs that affect glucose tolerance at the time of testing, and should abstain from tobacco, coffee, tea, food, alcohol and vigorous exercise during the test.

Table 5. Oral Glucose Tolerance Test Glucose Criteria

	2-h Plasma Glucose (after 75-gram Glucose Load)
Normal glucose tolerance	<140 mg/dl (7.8 mmol/l)
Impaired glucose tolerance(pre-diabetes)	140-199 mg/dl (7.8-11.1 mmol/l)
Diabetes mellitus	≥200 mg/dl (11.1 mmol/l)

OGTTS FOR THE DIAGNOSIS OF GESTATIONAL DIABETES

Please see the Endotext Chapter on Gestational Diabetes for additional details on the diagnosis of gestational diabetes. The prevalence of gestational diabetes (GDM) varies among racial and ethnic groups and between screening practices, testing methods, and diagnostic criteria. The overall frequency of GDM in the 15 centers participating in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was 17.8% (9), and regional estimates

may vary from 10% to 25 % depending on the population studied (10). The prevalence increases with increased number of risk factors (Tables 6 and 7), such that 33% of women with 4 or more risk factors have gestational diabetes (11). This condition is important to diagnose early because of the increased perinatal morbidity associated with poor glycemic control.

The US Preventive Task Force recommends screening for gestational diabetes in asymptomatic women after 24 weeks of gestation (12); the ADA recommends screening all pregnant women routinely between 24- and 28-weeks' gestation (Table 8). If the woman has risk factors, however, screening should be performed at the initial prenatal visit using standard criteria (1).

Table 6. Risk Factors for the Development of Gestational Diabetes
Overweight or obese
Previous history of impaired glucose tolerance, gestational diabetes, or delivery of a baby weighing >9 lb.
Glycosuria or history of abnormal glucose tolerance
Family history of diabetes (especially first degree relative)
Polycystic ovarian syndrome, hypertension, glucocorticoid use
History of poor obstetric outcome
Age (>25 years)
High risk ethnicity
Multiple gestation

Table 7. Low Risk for the Development of Gestational Diabetes
Age (< 25 years)
Normal weight pre-pregnancy
Low risk ethnicity
No first-degree relatives with diabetes
No history of abnormal glucose tolerance
No history of poor obstetric outcome

Table 8. Time of Initial Testing for Gestational Diabetes	
Risk of Development of Gestational Diabetes	**Time of Initial Testing for Gestational Diabetes**
Low risk	24-28 weeks gestation
Average risk	24-28 weeks gestation
High risk	As soon as feasible; repeat at 24-28 weeks if earlier testing normal

More than one method has been recommended for the screening and diagnosis of gestational diabetes. The criteria for the diagnosis of this condition remain controversial because the glucose thresholds for the

development of complications in pregnancies with diabetes remain poorly defined. Currently, the ADA suggests screening for GDM with either the “one-step” or “two-step” approach (1). Long term outcome studies evaluating pregnancies complicated by GDM are currently underway and hopefully a uniform approach will be adopted.

One-Step Strategy

The International Association of Diabetes and Pregnancy Study Group (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations including the ADA recommend that all women not previously known to have diabetes undergo a 75-gram 2-hour OGTT at 24-28 weeks of gestation (Table 9). This approach, which has been adopted internationally, is expected to increase the prevalence of GDM as only one abnormal value is sufficient to make the diagnosis (1,13). In 2017, the American College of Obstetricians and Gynecologists (ACOG) stated that clinicians may make the diagnosis of gestational diabetes based on only one elevated blood glucose value if warranted, based on their population, although this organization still supports the “two step” approach for diagnosis of GDM (14). These glucose thresholds were based on outcome data of the HAPO study that conveyed an odds ratio for adverse maternal, fetal, and neonatal outcomes of at least 1.75 based on fully adjusted logistic regression models (15).

Table 9. Oral Glucose Tolerance Test Glucose Criteria for the Diagnosis of GDM

75-gram 2- hour OGTT: Performed at 24-28 weeks gestation in the morning after an overnight fast of at least 8 hours. GDM is diagnosed when any of the following values are exceeded:	
Fasting	≥ 92 mg/dL (5.1 mmol/L)
One Hour	≥ 180 mg/dL (10.0 mmol/L)
Two Hour	≥ 153 mg/dL (8.5 mmol/L)

Two-Step Strategy

The American College of Obstetricians and Gynecologists (ACOG) as well as the National Institutes of Health (NIH) have been in support of the "two step" approach which consists of universal screening of all pregnant women at 24-28 weeks gestation with a 50-gram glucose challenge regardless of timing of previous meals (Table 10), followed by a 100- gram three-hour OGTT in screen positive patients (14, 16).

In the two-step approach, first a 50-gram oral glucose load is administered regardless of the timing of previous meals. The following thresholds have been defined as a positive screen: ≥130 mg/dL, ≥135 mg/dL, or ≥140 mg/dL (7.2 mmol/L, 7.5 mmol/L, or 7.8 mmol/L); the lower threshold has an estimated sensitivity and specificity of 88-99% and 66-77% compared to 70-88% and 69-89% respectively for the higher cutoff values of ≥135 mg/dL or ≥140 mg/dL (1).

Table 10. Abnormal Glucose Level on Screening Test

50-gram Glucose Load	
1-h Plasma Glucose	≥130 mg/dl (7.8 mmol/l)

If the screening test is abnormal, the diagnosis of gestational diabetes should be confirmed using a formal 100-gram OGTT (Table 11). This test should be performed after an overnight (8-14 h) fast. It is generally recommended that the woman ingest at least 150 grams of carbohydrate/day for the 3 days prior to testing to prevent false positive results; however, the necessity of this preparatory diet in normally nourished women has been challenged (17). The ADA recommends using the Carpenter/Coustan criteria (1). At least 2 of the following 4 venous plasma glucose levels must be attained or exceeded to make the diagnosis of GDM (1).

Table 11. Oral Glucose Tolerance Test Glucose Criteria for the Diagnosis of GDM

	Carpenter/Coustan	National Diabetes Data Group
Fasting	≥95 mg/dl (5.3 mmol/l)	≥105 mg/dl (5.8 mmol/l)
One Hour	≥180 mg/dl (10.0 mmol/l)	≥190 mg/dl (10.6 mmol/l)
Two Hours	≥155 mg/dl (8.6 mmol/l)	≥165 mg/dl (9.2 mmol/l)
Three Hours	≥140 mg/dl (7.8 mmol/l)	≥145 mg/dl (8.1 mmol/l)

OGTTS FOR POSTPARTUM TESTING OF WOMEN WITH GESTATIONAL DIABETES

Women with a history of GDM are at a higher risk of developing type 2 diabetes than women without GDM (18,19). Women at the highest risk are those with multiple risk factors, those who had more severe gestational diabetes, and those with poorer beta cell function (11). The ADA recommends testing women 4-12 weeks after delivery using a two-hour 75-gram OGTT. Women with normal results should be retested at least every 3 years. It is recommended that women with impaired fasting glucose or impaired glucose tolerance be retested on a yearly basis (1).

Special Populations

OGTTS FOR DIAGNOSIS OF CYSTIC FIBROSIS-RELATED DIABETES

Diabetes is common in patients with cystic fibrosis and is associated with adverse effects on nutritional status as well as pulmonary function. Annual screening for diabetes is recommended for individuals over age 10 with cystic fibrosis (1). HbA1c and fructosamine can be inaccurate in this population. In a retrospective analysis of the Toronto cystic fibrosis database, screening for diabetes using a HbA1c cutoff of 5.5% had a sensitivity of 91.8% and specificity of only 34.1% (20) but more studies need to be performed before the use of HbA1c is generally recommended for the diagnosis of diabetes in these individuals.

The use of the 2-hour 75 gm OGTT is recommended for the screening of healthy outpatients with cystic fibrosis. For patients receiving continuous drip feedings, laboratory glucose levels at the midpoint or immediately after feedings should be obtained. The diagnosis of diabetes is based on glucose levels ≥200 mg/dL on 2 separate occasions. If the patient is acutely ill or ingesting glucocorticoids, a FPG ≥126 mg/dL or 2-hour postprandial glucose ≥200 mg/dL that persists for >48 hours is sufficient to diagnose diabetes (21, 22).

FASTING GLUCOSE FOR DIAGNOSIS OF PREDIABETES AND DIABETES IN PEOPLE LIVING WITH HIV

Screening for prediabetes and diabetes by measuring fasting glucose before and 3-6 months after starting or changing antiretroviral therapy is recommended for everyone living with HIV (1). If normal, a fasting glucose test should be performed yearly. Screening using a HbA1c test is not recommended for diagnosis due to risk of inaccuracies (1, 23).

OGTTS FOR DIAGNOSIS OF POST-TRANSPLANTATION DIABETES

After an individual has had an organ transplant and is on stable immunosuppressive therapy, routine screening for diabetes is recommended. The recommended screening test is an OGTT post-transplantation (1).

TESTS USED FOR CLASSIFICATION OF DIABETES

General Approach

Other tests are used for the purpose of classifying diabetes. For details see individual chapters in Endotext:

- Diagnosis And Clinical Management Of Monogenic Diabetes
- Atypical Forms Of Diabetes
- Lipodystrophy Syndromes: Presentation And Treatment
- Fibrocalculous Pancreatic Diabetes
- Diabetes Mellitus After Solid Organ Transplantation
- Diabetes In People Living With Hiv
- Autoimmune Polyglandular Syndromes
- Etiology And Pathogenesis Of Diabetes Mellitus In Children And Adolescents

In brief, most patients with diabetes can be classified as either type 1 or type 2 diabetes using clinical judgement and simple tests if needed. However, the pathophysiology of diabetes is complex and significant overlap can exist, potentially leading to misclassification. While youth with type 1 diabetes typically present with rapid onset symptoms, adults with type 1 diabetes may have a much slower, more indolent course. While the incidence rate of type 1 diabetes is higher in youth, over half of individuals diagnosed with type 1 diabetes are adults (24). This is why ~40% of adults with new onset type 1 diabetes are initially misclassified as having type 2 diabetes (25). Another term for slowly progressing type 1 diabetes is latent autoimmune diabetes in the adult (LADA). However, the American Diabetes Association classifies LADA as type 1 diabetes. It is important to recognize these individuals because they require insulin sooner than individuals with type 2 diabetes (26) and they have a higher long-term risk of complications (27). On the other hand, type 2 diabetes can present in some populations (particularly those with Black or Latinx background) with diabetic ketoacidosis (DKA) and this is termed ketosis prone diabetes (28). The importance of this is that about half of individuals initially presenting with DKA who have normal c-peptide and negative autoantibodies may be able to come discontinue insulin therapy (1,29).

The most discriminating features of type 1 diabetes are younger age (<35 years), lower body mass index (<25 kg/m^2), unintentional weight loss, ketoacidosis, and severe hyperglycemia (>360 mg/dl) at presentation (1, 25). A helpful pneumonic is AABBCC which stands for age, autoimmunity (personal or family

history of other autoimmune disorders), body habitus, background (family history of type 1 diabetes), control (glucose), and comorbidity (such as treatment with a checkpoint inhibitor for cancer). However, these features are not absolute, and the correct classification may only become apparent over time.

An overview of the classification for suspected type 1 diabetes is shown in the Figure. For anyone with possible type 1 diabetes, testing for autoantibodies such as glutamate decarboxylase isoform 65 (GAD65A), insulin, insulinoma antigen 2, and zinc transporter isoform 8 (Znt8A) should be performed. The GAD antibody is the most prevalent autoantibody, but false positives can occur and the presence of multiple positive autoantibodies, and/or higher titers increases specificity.

The c-peptide is often normal at the time of diagnosis. Among individuals who have had diabetes for many years, it is important to note that autoantibodies may become undetectable. On the other hand, while the c-peptide is often normal at the time of diagnosis, it typically declines over time (and glucose fluctuations become more difficult to manage) making the clinical diagnosis clearer. The c-peptide should be obtained from a random (nonfasting) sample and interpreted within the context of a concomitant serum glucose level (ideally ≥144 mg/dl) (30). If normal, it should be measured periodically where the diagnosis is unclear.

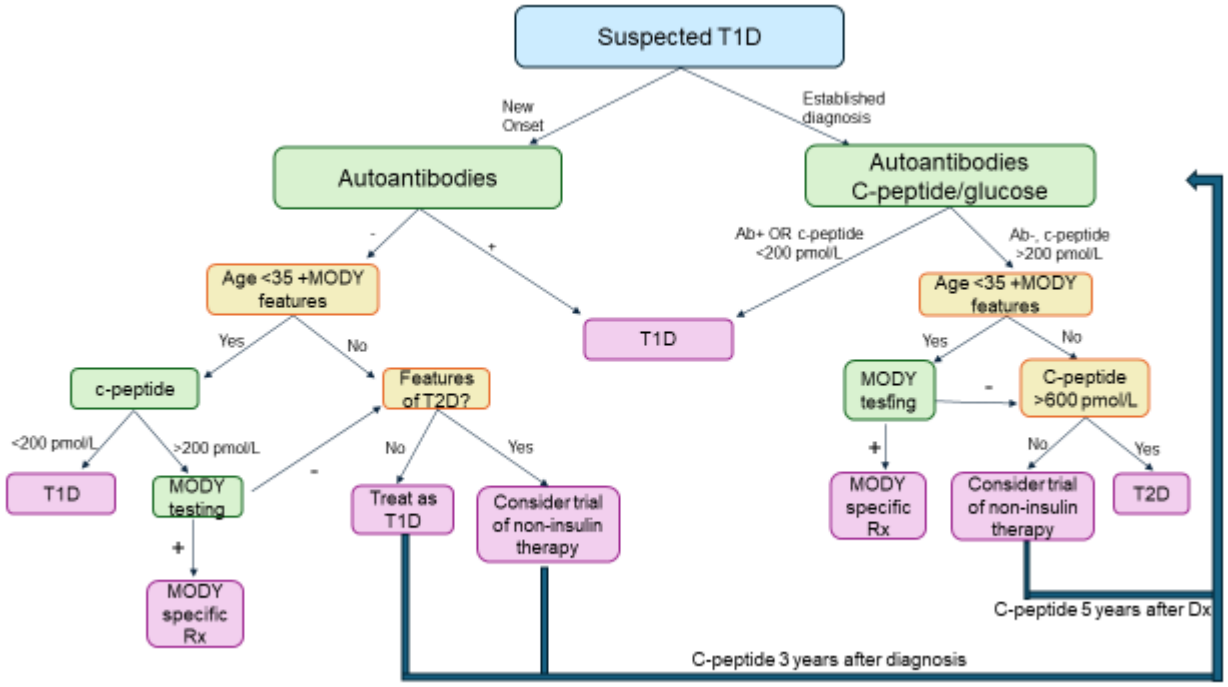

Figure. Classification of suspected type 1 diabetes (T1D). Ab=antibody, MODY=maturity onset diabetes of youth, T2D=type 2 diabetes, Rx=treatment, Dx=diagnosis.

While type 2 diabetes is considered polygenic, several forms of monogenic diabetes are well known. These are often non-syndromic and include neonatal diabetes and older onset forms that collectively were formerly known as maturity onset diabetes of youth (MODY). Monogenic diabetes is typically inherited in an autosomal dominant manner and should be suspected in individuals diagnosed as children or young adults (<25 years) with a strong family history and without other clinical features of type 1 or type 2

diabetes such as obesity or type 1 diabetes autoantibodies (1). Individuals commonly have an intact c-peptide and HbA1c <7.5% at diagnosis. When these forms are suspected, patients should be referred for genetic testing. Some mutations leading to diabetes involve multiple organ systems and can be categorized as syndromic diabetes. Syndromic features include maternally inherited deafness, renal cysts, partial lipodystrophy, or severe insulin resistance in the absence of obesity. Such individuals should also be referred for genetic testing.

A comparison of features of types of diabetes is shown in Table 12.

Table 12. Characterization of Common Types of Diabetes (1)

	T1D	"LADA"	T2D	MODY
Age	Often young	>age 25	Often adult	<age 25
Family history	Occasional	Occasional	Usually	Yes
C-peptide	Low, often undetectable	Varies	Normal or high	normal
Auto-ab	+	+	-	-
Weight	Tend to be lean	Tend to be lean	Usually overweight	Tend to be lean
Metabolic syndrome	No	Varies	Usually	No
Insulin requirement	Yes	Varies, rapid progression	Varies	Varies

C-peptide

During the processing of proinsulin to insulin in the beta cell of the pancreas, the 31 amino acid connecting peptide which connects the A and B chains, called c-peptide, is enzymatically removed and secreted into the portal vein. C-peptide circulates independently from insulin and is mainly excreted by the kidneys. Levels are elevated in renal failure. Standardization of different c-peptide assays is still suboptimal. C-peptide testing is used to examine insulin secretory reserve in people with diabetes.

At the time of type 1 diabetes diagnosis, c-peptide levels commonly overlap with those observed in type 2 diabetes and cannot reliably distinguish between

these diabetes types. With longer duration, there is progressive loss of c-peptide, and although c-peptide levels in many individuals with long-standing type 1 diabetes are extremely low or undetectable, there is heterogeneity in residual beta cell function with detectable c-peptide being more common in adult-onset type 1 diabetes (33). In type 1 diabetes, detectable c-peptide is associated with better glycemic control, less hypoglycemia, and decreased microvascular disease (34-35).

Type 2 diabetes is heterogeneous, with many individuals having progressive loss of beta cell function over many years evidenced by decreasing c-peptide levels. Fasting and glucose-stimulated c-peptide levels have been used in the past to distinguish type 1 (severe insulin deficiency) from type 2 diabetes with limited success. However, targeted testing may be more discriminatory. When random c-peptide testing was performed >3 years after clinical diagnosis of type 1 diabetes, 13% had a c-peptide ≥200 pmol/L, and after islet autoantibody and genetic testing, 6.8% of these were reclassified: 5.1% as having type 2 diabetes and 1.6% as having monogenic diabetes (36).

C-peptide stimulation using glucagon or a mixed meal such as Sustacal, has also been used to help differentiate between type 1 and type 2 diabetes, and to determine the need for insulin therapy in type 2 diabetes. In the glucagon stimulation test, glucose, insulin and c-peptide levels are measured 6 and 10 min after the intravenous injection of 1 mg of glucagon. Normal stimulation of c-peptide is a 150- 300% elevation over basal levels. In the mixed meal tolerance test, Sustacal (6 mg/kg up to a maximum or 360 ml) is ingested over 5 minutes, and glucose and c-peptide are measured 90 min after oral ingestion. These tests have had limited general clinical utility since they do not reliably discriminate between patients who require insulin therapy. They have been used in research studies and in the evaluation of patients after pancreatectomy and pancreatic transplantation. In the Diabetes Control and Complications Trial, a basal c- peptide value of <0.2 pmol/ml and stimulated level of <0.5 pmol/ml were used to confirm the presence of type 1 diabetes at entry (37). According to the ADA guidelines, a random c-peptide and concomitant glucose level obtained within 5 hours of eating is sufficient for classification.

Pancreatic Autoantibodies

Islet autoantibodies can be detected early in the development of type 1 diabetes and are considered markers of autoimmune beta cell destruction. They predict progressive beta cell destruction and ultimately beta cell failure. The autoantibodies for which specific immunoassays are available include the 65-KDa isoform of glutamic acid decarboxylase (GAD65), insulin autoantibodies (IAA), zinc transporter antibodies (ZnT8), islet cell antigen 512 autoantibodies (ICA512), and autoantibodies to the tyrosine phosphatase related antigens islet antigen 2 (IA-2) and IA-2b. Measurements of ICA512, which are autoantibodies to parts of the IA-2 antigen, are no longer recommended. The presence of high levels of 2 or more antibodies is strongly predictive of type 1 diabetes mellitus. These antibodies may be detected before the onset of type 1 diabetes, at the time of diagnosis, and for variable amounts of time after diagnosis. They have been used in screening for type 1 diabetes in first-degree relatives of an individual with type 1 diabetes or in research studies related to the early detection, treatment, and prevention of type 1 diabetes (www.diabetestrialnet.org). These measurements are not recommended for use in general screening programs in low-risk individuals. The American Diabetes Association recommends offering screening via autoantibodies in persons with a strong family history of type 1 diabetes or otherwise known risk (1). Additional information on screening for type 1 diabetes may be found in the Endotext Chapter “Changing the Course of Disease in Type 1 Diabetes”.

Commercially available assays for autoantibodies are often useful in distinguishing type 1 diabetes from type 2 diabetes. The absence of detection of these antibodies, however, does not exclude the diagnosis of type 1 diabetes. Since IAA can form in response to insulin therapy, detection can be the result of insulin injections or autoimmune insulin antibody formation. GAD65 antibodies are frequently observed early in the course of type 1 diabetes. They are also present in the rare neurological disorder, stiff-man syndrome, and in some patients with polyendocrine autoimmune disease.

In adults with newly diagnosed diabetes for whom type 1 diabetes is a possible diagnosis, GAD65 is commonly measured first, along with or followed by IA2 and ZnT8. IAA are more commonly detected in young children who develop type 1 diabetes.

Lynam and coworkers (38) developed a clinical multivariable model to help differentiate between type 1 and type 2 diabetes in adults ages 18-50 years. The model includes age at diagnosis, BMI, islet autoantibodies (GAD, IA-2), and a type 1 diabetes genetic risk score. The authors define type 1 diabetes by a non-fasting c-peptide <200 pmol/L and rapid insulin requirement within the first 3 years of diagnosis. The definition of type 2 diabetes was not requiring insulin treatment within the first 3 years after diagnosis or, if insulin was used, having a c-peptide measurement of >600 pmol/L at ≥5 years post-diagnosis. Since the measures of the genetic variants in the type 1 diabetes genetic risk score are not widely available, this model is not used clinically in the United States.

Monogenic Diabetes Syndromes

Monogenic diabetes syndromes account for 1%-5% of all individuals with diabetes and have been primarily classified as neonatal diabetes or Maturity-Onset Diabetes of the Young (MODY) based on clinical characteristics. More than 50 affected genes have been described. A *Diabetes Care* Expert Forum was assembled in 2019 to re- consider the classification of monogenic diabetes syndromes. They recommend a classification system based upon molecular genetics, listing the affected gene, inheritance/phenotype, disease mechanism/special features, and the treatment implications (39).

The ADA recommends immediate genetic testing for all infants diagnosed with diabetes within the first 6 months of life (Table 13) (1). MODY most commonly manifests before age 25 years but can be diagnosed in older individuals. The inheritance is typically autosomal dominant. Individuals who have positive islet autoantibody test results and/or low c-peptide concentrations should not be tested for monogenic diabetes syndromes (40). A MODY risk calculator is available at: https://www.diabetesgenes.org/exeter-diabetes-app/

Table 13. When to Consider Genetic Testing for Monogenic Diabetes Syndromes
Diabetes diagnosed younger than 6 months of age
Diabetes in children and young adults not characteristic of type 1 or type 2 (negative pancreatic auto-antibodies, non- obese, no features of metabolic syndrome) and with a strong family history (diabetes in successive generations suggesting dominant inheritance)
Fasting glucose 100-150 mg/dL, stable A1c (5.6-7.6%), especially if in a non-obese child or young adult

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