



## **Role of Clinical Laboratory in Diagnosis and Management of Diabetes Mellitus- Review Article**

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### **Abstract**

The aim of the clinical laboratory in endocrine disease diagnosis and management is detecting either the hormones or their downstream reaction metabolites or some other related substances. In the case of hormone measurement almost all the routine methods are based on immunoassay with different labels (radioimmunoassay, enzyme linked immunosorbant assay, chemiluminescence assays ...) and different sensitivity and specificity. But their related metabolites can be measured with different methods from simple biochemical to highly sophisticated methods. These tests are used either for diagnosis or monitoring. With respect to diabetes the tests are categorized to biochemical, immunological and genetic. In this paper we will describe the most common tests, but the genetic tests that are not used in routine investigations are out of the scope of this paper.

**Keywords:** Laboratory, Diagnosis, Diabetes

### **Introduction**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

The clinical laboratories play an essential role in both the diagnosis and management of diabetes. There are different laboratory tests from simple biochemical markers such as FPG, HbA1c and urine albumin, to more sophisticated tests for hormones or their downstream reaction metabolites

or some other related substances measurements that can help physician in different stages.

### ***Preclinical stages***

Autoimmune type 1 diabetes is associated with some immunological markers. Measurements of islet autoantibodies can help in the diagnosis of autoimmune diabetes. Presence of islet autoantibodies in nondiabetic people signifies an increased risk for the development of type 1 diabetes. Islet cell autoantibodies are not recommended for routine diagnosis of diabetes now. The most helpful autoimmune markers which are described below are: ICAs, IAAs, GADAs and IA-2As (1).

### Islet cells Antibodies (ICAs)

ICAs are polyclonal autoantibodies react with all of the islet cells. The presence of ICAs is an important marker of  $\beta$ -cell autoimmunity, but their role in disease etiology is not clear (2-3).

Frequency of ICAs in community is low (0.1-3%) (4), but they can be detected in 70-80% of newly diagnosed type 1 diabetes patients. ICAs reactivity is often decreased after diagnosis and after 10 years, only about 5-10% of type 1 diabetic patients remain ICAs positive. Approximately 2-3% of first-degree relatives of type 1 diabetes patients are ICAs-positive.

In adults with non-insulin-dependent diabetes, the detection of ICAs is in favor of existence of latent autoimmune diabetes of adulthood (LADA). Based on the presence of islet autoantibodies, 4-17% of patients diagnosed as type 2 diabetic, have LADA. A higher ICAs titer in prediabetic subjects is associated with a higher risk for type 1 diabetes (3).

These antibodies are entirely IgG, and frequently complement fixing. In healthy relatives of diabetic children, complement-fixing ICAs are shown to be more strongly predictive of disease compared with non-complement-fixing ICAs (4).

### Insulin Autoantibodies (IAAs)

Insulin was the first reported islet autoantigen and  $\beta$ -cell-specific autoantigen. It is important to search insulin autoantibodies before the administration of exogenous insulin because after 5-7 days of exogenous insulin treatment, insulin antibodies will arise. IAAs are more frequent in individuals with HLA-DR4 (2). Taking numerous

studies together, IAAs positivity is more frequent in children and decrease with aging (2-4).

IAAs may be detected in many other autoimmune diseases including autoimmune thyroid disease, Addison's disease, chronic hepatitis, pernicious anemia, systemic lupus erythematosus and rheumatoid arthritis (2-3).

### GADAs

GADAs target glutamic acid decarboxylase which is expressed predominantly in the nervous system. Because GADAs are more persistent than ICAs after the diagnosis of type 1 diabetes, they may be more often positive than ICAs in LADA. By using GADAs as the autoimmune marker (instead of ICAs), LADA prevalence in type 2 diabetes seems to be greater. GADAs are detected in 60% or more of new-onset cases of type 1 diabetes and 3-5% of their relatives (2).

### Insulinoma-associated antigens (IA-2A and IA-2 $\beta$ A)

Insulinoma-associated proteins are two members of the protein tyrosine phosphatase (PTP) families, which are found in nervous tissue and other endocrine tissues.

IA-2A and IA-2 $\beta$ A are detected in about 60% or more of new-onset type 1 diabetes compared with for ~2-3% of general population.

### Clinical stages

Diabetes is diagnosed solely by demonstration of hyperglycemia. In 2010, ADA diagnostic criteria were modified to able to identify individuals at risk of retinopathy and nephropathy better (Table 1).

**Table 1:** Criteria for diagnosis diabetes mellitus

Any of the following is diagnostic:

1. HbA1C $\geq$ 6.5%. The test should be performed in a laboratory using a method that is NGSP\* certified and standardized to DCCT\*\* assay
2. Fasting plasma glucose  $\geq$ 126mg/dL (7 mmol/L)
3. 2hour postload plasma glucose concentration  $\geq$ 200mg/dL (11.1mmol/L) during the OGTT. The test should be performed as described by WHO using glucose load containing the equivalent of 75 gr anhydrous glucose dissolved in water
4. Classic symptoms of diabetes and random plasma glucose concentration  $\geq$ 200mg/dL (11.1mmol/L)

\*NGSP: National Glycohemoglobin Standardization Program/ \*\*DCCT: Diabetes Control and Complications Trial

In the absence of unequivocal hyperglycemia criteria 1-3 results should be confirmed by repeated analysis on a subsequent day to establish the diagnosis (5).

Population screening for type 2 diabetes is now recommended for those at increased risk of developing the disease (5) (Table 2).

Blood should be drawn in the morning after 8 hour fasting. Because of diurnal variation in FPG, (higher in the morning than in the afternoon), in-

dicating if current diabetes diagnostic tests used in the afternoon, approximately half of all cases of undiagnosed diabetes will be missed (6).

In healthy people glucose concentrations vary with age. From the third to the sixth decade of life, mean fasting plasma glucose increases, but does not significantly change after age 60. After a glucose challenge, concentration is considerably higher in older persons (1).

**Table 2:** Categories of increased risk for diabetes

1. IFG: Fasting plasma glucose between 100 and 125 mg/dL (5.6 – 6.9 mmol/L)
2. IGT: 2-hour plasma glucose concentration in the 75 gr OGTT is between 140 and 199 mg/dL (7.8 - 11 mmol/L)
3. HbA1C 5.7-6.4%

FPG

### **Oral Glucose Tolerance Test (OGTT)**

Glucose measurement before and after giving defined amount of glucose orally, should provide a standard method to evaluate individuals and establish values for healthy and diabetic subjects, but many factors can affect OGTT results and in many studies reproducibility of the OGTT in classifying patients is reported as 50–58% (7-8). It is not recommended by ADA for diabetes diagnosis in routine conditions but it is recommended in limited situations by the WHO (5, 9).

OGTT is recommended in following conditions:

1. Diagnosis of GDM
2. Diagnosis of IGT. This remains contentious.
3. Evaluation of a patient with unexplained nephropathy, neuropathy, or retinopathy, with random glucose concentration less than 140 mg/dL.
4. Population studies for epidemiological data (9).

According to WHO criteria when the FPG concentration is in the IFG range (110–126 mg/dL), an OGTT is recommended (10). After 3 days of unrestricted diet and an overnight fast (8–14 h), FPG is measured, followed by the oral ingestion of 75 g of anhydrous glucose or partial hydroly-

sates of starch of the equivalent carbohydrate content) in 250–300 mL of water over 5 min. For children, the dose is 1.75 g glucose/kg up to 75 g of glucose. Blood samples are collected 2 h after the load, and plasma glucose is analyzed. Results are interpreted as detailed in Table 3.

**Table 3:** WHO criteria for interpreting 2-h OGTT (9)

	Plasma glucose concentration, mmol/L (mg/dL)	
	0h	2h
IFG	≥ 6.1 (110) < 7.0 (126)	<7.8 (140)
IGT	< 7.0 (126)	≥ 7.8 to < 11.1 (140-200)
Diabetes	≥ 7.0 (126)	≥ 11.1 (200)

### **HbA1c**

Glycated hemoglobin is formed posttranslationally by nonenzymatic addition of glucose to hemoglobin molecule. Total glycohemoglobin includes all glycated fractions, comprising HbA<sub>1c</sub> and hemoglobin glycated at sites other than the N-terminus of the β chain. HbA<sub>1c</sub> is a GHb that results from the condensation of glucose to the N-terminal valine of the hemoglobin β-chain. The concentration of HbA<sub>1c</sub> depends on both the concentration

of glucose in the blood and the lifespan of the erythrocyte. Because erythrocytes are in the circulation for approximately 4 months, HbA<sub>1c</sub> represents the integrated glucose concentration over the preceding 2 to 3 months (11).

There are no significant effects of sex, ethnicity, season or even acute illness on HbA<sub>1c</sub> results. However there are evidences that black people have higher HbA<sub>1c</sub> levels than whites across the complete range of glycemia, and the differences increase as glucose intolerance worsens (12).

The effects of age on GHb are controversial. Some studies reveal that there is no relationship between age and HbA<sub>1c</sub> (13) and the some studies have shown that there is a positive relationship between age and HbA<sub>1c</sub> (14-15).

Although in adults, intraindividual variation of HbA<sub>1c</sub> is minimal, some studies have revealed that in pediatrics population, it is higher and should be considered in interpretation of results (16).

In hemolytic anemia or recent blood loss, HbA<sub>1c</sub> is falsely decreased regardless of the assay method due to shortening of RBC lifespan (9). Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin, but vitamin C may increase values with some assays (17). Several hemoglobin variant (e.g, Hb S, C, F) and chemically modified derivatives of hemoglobin interfere with some assay methods and falsely increase or decrease the results (18-19). Boronate affinity chromatographic assay methods are generally less affected by hemoglobinopathies than methods that are charge difference based methods (19-20).

Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, and chronic ingestion of salicylates interfere with some assay methods, falsely increasing results (21).

No patient preparation is needed. Venous blood should be taken in tubes with EDTA (otherwise specified by the manufacturer). Heparin is not suitable for some methods. The specimen can be stored one week at 4 °C and 18 month in -70 °C (22).

Although the results of methods using different assay principles show good correlation, the re-

ported GHb results from the same blood sample could be significantly different (23-25).

In 1996, the National Glycohemoglobin Standardization Program (NGSP) was initiated to standardize GHb test results among laboratories to Diabetes Control and Complications Trial DCCT equivalent values (24, 26). The NGSP Laboratory Network includes a variety of assay methods, each calibrated to the DCCT reference. The DCCT reference is a HPLC cation-exchange method that quantifies HbA<sub>1c</sub>. The laboratories in the network interact with manufacturers of GHb methods to help them first in calibrating their methods and then in providing comparison data for certification of traceability to the DCCT. Certification is valid for one year.

#### *Use of GHb for diabetes screening/diagnosis*

With review of epidemiological evidence, expert Committee recommended the use of the HbA<sub>1c</sub> test to diagnose diabetes, with a threshold of 6.5%, and ADA confirm this decision. The diagnostic A1C cut point of 6.5% is associated with an inflection point for retinopathy prevalence, as are the diagnostic thresholds for FPG and 2-h PG (5). The diagnostic test should be performed using a method that is certified by the NGSP and standardized or traceable to the DCCT reference assay. Point-of-care A1C assays are not sufficiently accurate to use for diagnostic purposes now.

A1C range of 5.7 to 6.4% is used to identify individuals with high risk for future diabetes and to whom the term pre-diabetes may be applied.

Use of A1C has many advantages, patients do not need to fast and specimen can be drawn in any time and is less affected by recent physical activities or stresses. But there are some limitations to use HbA<sub>1c</sub> measurement instead of fasting plasma glucose or the OGTT. As the two tests detect different people, some individuals with diabetes detected on OGTT cannot be diagnosed if using HbA<sub>1c</sub> ≥6.5% criteria. Furthermore, some conditions (described before) can affect A1C results.

For NGSP-certified assay methods, reference intervals should not deviate significantly (e.g., >0.5%) from the 4–6% range. Note that for patients follow up ADA target values derived from

the DCCT and United Kingdom Prospective Diabetes Study (UKPDS) are used (not the reference intervals) (27).

### **Urine albumin**

Microalbuminuria is defined as excretion of 30–300 mg of albumin/24 h (or 20–200 µg/min or 30–300 µg/mg of creatinine; on two of three urine collections repeated at intervals of 3–6 months (1) (Table 4).

According to ADA recommendation for adult diabetic patients, periodic qualitative (dipstick) testing of urine albumin should be done. Positive tests represent "clinical albuminuria" or "overt nephropathy" in the ADA recommendations, and protein excretion >300 mg/24 h (>200 µg/min or >300 µg/mg of creatinine). Quantitative measurement of urine protein excretion should be of a part of patient care to plan treatment. Measurement of creatinine clearance as an index of glomerular filtration rate can be performed on the same timed (usually 12-h or 24-h) urine collection.

Negative qualitative tests for "clinical proteinuria" (albumin excretion <300 mg/day) should be followed with a test for microalbuminuria. For children with type 1 diabetes, testing for microalbuminuria is recommended to begin after puberty and after duration of diabetes for 5 years.

Annual microalbumin testing of 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. The role of testing is unclear in patients under treatment with angiotensin-converting enzyme inhibitors and in those with a short life expectancy (such as very old patients).

Microalbuminuria rarely occurs with a short duration of type 1 diabetes or before puberty. Thus, testing is less urgent in these situations. Albumin is stable in untreated urine at least 1 week at 4 or 20 °C (9) and over 160 days at -80 °C.

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

In diabetic patients the estimated within subject variation (CV), for the albumin concentration in the first morning void and for the albumin:creatinine ratio were 61% and 39% respectively. Thus a goal of 15% appears reasonable to accommodate use of the measured albumin concentration for calculation of either the timed excretion rate or the albumin:creatinine ratio (5, 9).

### **Insulin, Proinsulin, C-Peptide, and Glucagon**

There is no role for routine testing for insulin, C-peptide, proinsulin and glucagon in most patients with diabetes but the other utilities are demonstrated in Table 5.

**Table 4:** Definitions of microalbuminuria and clinical albuminuria

	Albumin excretion		
	mg/24h	µg/min	µg/mg of creatinine
Normal	<30	<20	<30
microalbuminuria	30-300	20-200	30-300
albuminuria	>300	>200	>300

### **Conclusion**

Despite of different sophisticated tests for Diagnosis and management of Diabetes, it seems that

the most informative tests stills are the relatively simple and cost effective tests such as FPG and HbA1c that are recommended in both diagnosis and follow up.

**Table 5:** Clinical utility of Insulin, Proinsulin, C-Peptide, and Glucagon (2)

<b>Insulin</b>	
	Evaluation of fasting hypoglycemia
	Evaluation of the polycystic ovary syndrome (PCOS)
	Classification of diabetes mellitus
	Predict diabetes mellitus
	Assessment of $\beta$ -cell activity
	Select optimal therapy for diabetes
	Investigation of insulin resistance
	Predict the development of coronary artery disease
<b>Proinsulin</b>	
	Diagnosis of $\beta$ cell tumors
	Familial hyperproinsulinemia
	Cross-reactivity of insulin assays
<b>C-Peptide</b>	
	Evaluation of fasting hypoglycemia
	$\beta$ cell tumors
	Factitious
	Classification of diabetes mellitus
	Assessment of $\beta$ cell activity
	Obtain insurance coverage for insulin pump
	Monitoring therapy
	Pancreatectomy
	Transplant (pancreas-islet cell)
<b>Glucagon</b>	
	Diagnosis of $\alpha$ -cell tumors

## Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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