



World Health Organization

Laboratory Diagnosis and Monitoring

of

Diabetes Mellitus

2002

WHO Library Cataloguing-in-Publication Data

Laboratory diagnosis and monitoring of diabetes mellitus / Hans Reinauer ... [et al.]

1.Diabetes mellitus - diagnosis 2.Blood glucose - analysis 3.Blood glucose self-monitoring 4.Glucose tolerance test 5.Hemoglobin A, Glycosylated - analysis 6.Urine - chemistry 7.Manuals I.Reinauer, Hans. II.World Health Organization.

ISBN 92 4 159048 3

(NLM classification: WK 810)

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Printed by the WHO Documents Production Services, Geneva, Switzerland

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2002

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Abbreviations

AC ratio	=	albumin/creatinine ratio
BMI	=	body mass index
CEN	=	Comité Européen de Normatisation
CV	=	coefficient of variation
DCCT	=	Diabetes Control and Complication Trial
EDTA	=	ethylenediamine tetra acetic acid
FPG	=	fasting plasma glucose
FPLC	=	fast high pressure liquid chromatography
GADA	=	glutamic acid decarboxylase auto-antibodies
GDM	=	gestational diabetes mellitus
GFR	=	glomerular filtration rate
HPLC	=	high pressure liquid chromatography
IA-2, IA-2 β	=	protein-tyrosine phosphatase auto-antibodies
ICA	=	islet cell auto-antibodies
IFG	=	impaired fasting glycaemia
IGT	=	impaired glucose tolerance
ISO	=	International Organization for Standardisation
LADA	=	latent autoimmune diabetes in adults
MODY	=	maturity onset diabetes of the young
OGTT	=	oral glucose tolerance test
POCT	=	point of care testing (= testing near to the patient, bedside testing)
RIA	=	radioimmunoassay
SMBG	=	self monitoring of blood glucose
UAE	=	urinary albumin excretion
WHO	=	World Health Organization

Glossary

Accuracy of measurement (analytical accuracy): Closeness of the agreement between the result of a measurement and a true value of the analyte

Auto-antibodies: Antibodies directed against the patient's own proteins, cells or tissues. In Type 1 diabetes antibodies are directed against

- components of β -cells of pancreatic islets (islets of Langerhans)
 - non-specific, termed ICA
 - glutamic acid decarboxylase (GAD₆₅)
 - phosphotyrosine phosphatase (IA-2, IA-2 β)
- circulating proteins
 - insulin/proinsulin auto-antibodies (IAA)

Fructosamine: Generic name for plasma protein ketoamines resulting from glycation of proteins, mainly of albumin and immunoglobulins.

Haemoglobin A_{1c} (HbA_{1c}): The main fraction of glycated haemoglobin A composed of covalently bound glucose at the amino-end of the haemoglobin β -chains (valine).

Islet cell antibodies (ICA): antibodies directed against different proteins in and on the islet β -cells. ICA are determined by immunofluorescence technology. The results are given in JDF-units. The cut-off value is >10 JDF units.

Glutamic acid decarboxylase antibodies (GADA₆₅): Auto-antibodies directed against a membrane protein of the islet β -cell which is a glutamate decarboxylase. GADA are determined by RIA. The results are given in units, with a cut-off value of 1.9 units/mL.

IA-2A antibodies: Auto-antibodies to protein-tyrosine phosphatase which is expressed on the secretory granules of islets and neuro-endocrine tissues. The IA-2A results are presented as an index. An index of > 1.1 is taken as abnormal.

Diagnostic sensitivity: The ability of a test to give positive results for individuals who have the particular disease or condition for which they are being tested; it is measured as the ratio of positive tests to the total number of tests in those that have the disease (expressed as a percentage). It is the percentage of true-positive results.

Diagnostic specificity: The ability of a test to give a negative result for individuals who do not have the disease or condition for which they are being tested. It is measured as the ratio of negative tests to the total number of tests in those that do not have the disease or condition (expressed as a percentage). It is the percentage of true-negative results.

Precision (of measurement): Closeness of agreement between independent test results obtained under stipulated conditions. Precision depends only on the distribution of random errors and does not relate to the true value or the specified value. The measure of precision usually is expressed in terms of imprecision and computed as a standard deviation or the coefficient of variation of the test results. Lower precision is reflected by a larger standard deviation.

Screening: The process of identifying those individuals who are at sufficiently high risk of a specific disorder to warrant further investigation or direct action. Screening is systematically offered to a population of people who have not sought medical attention on account of symptoms of the disease for which screening is being offered and is normally initiated by medical authorities and not by a patient's request for help on account of a specific complaint. The purpose of screening is to benefit the individuals being screened:

- Selective or targeted screening performed in a subgroup of subjects who have already been identified as being at relatively high risk in relation to age, body weight, ethnic origin etc.
- Opportunistic screening carried out at a time when people are seen, by health care professionals, for a reason other than the disorder in question.

Note: 'selective or targeted screening' and 'opportunistic screening' are not mutually exclusive.

Traceability: Property of the result of a measurement or the value of a standard, whereby it can be related to stated references, usually a national or international standard through an unbroken chain of comparisons all having stated uncertainties.

Uncertainty (of measurement): Parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could be reasonably attributed to the measurand.

The parameter may be, for example, a standard deviation (or a given multiple of it), or the half-width of an interval having a stated level of confidence.

Introduction

Diabetes mellitus is a group of diseases characterized by an elevated blood glucose level (hyperglycaemia) resulting from defects in insulin secretion, in insulin action, or both. Diabetes mellitus is not a pathogenic entity but a group of aetiologically different metabolic defects. Common symptoms of diabetes are lethargy from marked hyperglycaemia, polyuria, polydipsia, weight loss, blurred vision and susceptibility to certain infections. Severe hyperglycaemia may lead to hyperosmolar syndrome and insulin deficiency to life-threatening ketoacidosis. Chronic hyperglycaemia causes long-term damage, dysfunction and failures of various cells, tissues and organs. Long-term complications of diabetes are:

- Macroangiopathy: ischaemic heart disease (IHD), stroke, peripheral vascular disease (PVD)
- Microangiopathy: retinopathy, nephropathy
- Neuropathy: peripheral neuropathy, autonomic neuropathy
- Cataract
- Diabetic foot
- Diabetic heart

Classification of diabetes mellitus

There were several classification systems established for diabetes mellitus. The current WHO classification is mainly based on the aetiology of diabetes mellitus (1, 20) (Table 1).

Table 1: Classification of diabetes mellitus

Type 1 diabetes mellitus
Immune mediated
Idiopathic
Type 2 diabetes mellitus
Other specific types of diabetes
Genetic defects of islet β -cell function (e.g. MODY)
Genetic defects in insulin action
Diseases of the exocrine pancreas
Endocrinopathies
Drug- or chemical- induced diabetes
Infections
Uncommon forms of immune-mediated diabetes
Other genetic syndromes
Gestational diabetes mellitus

The terms IDDM (insulin dependent diabetes mellitus) and NIDDM (non-insulin dependent diabetes mellitus) were used previously but have now been abandoned. Presently, the terms "Type 1" and "Type 2" diabetes are used. The more prevalent form is Type 2 diabetes.

Type 1 diabetes

(Former: Insulin-dependent diabetes, juvenile-onset diabetes)

Type 1 diabetes is characterized by cellular-mediated autoimmune destruction of the pancreatic β -cells.

Markers:

- islet cell antibodies (ICAs)
- auto-antibodies to insulin (IAAs)
- auto-antibodies to glutamic acid decarboxylase (GAD₆₅)
- auto-antibodies to tyrosine phosphatases IA-2 and IA-2 β

Association with HLA: DQA and DQB genes:HLA-DR/DQ alleles may be protective

Environmental factors are poorly defined. Virus infectious and nutritional factors are discussed.

Age: Onset predominantly in childhood and adolescence, but occurs at any age

Idiopathic diabetes in African or Asian people. This form of diabetes is strongly inherited, has permanent insulinopenia, is prone to ketoacidosis without antibodies to β -cells.

Laboratory findings:

- Hyperglycaemia
- Ketonuria
- Low or undetectable serum insulin and C-peptide levels
- Auto-antibodies against components of the islet β -cells

Type 2 diabetes

(Maturity-onset diabetes, non-insulin dependent diabetes).

Type 2 diabetes is due to insulin insensitivity combined with a failure of insulin secretion to overcome this by hypersecretion, resulting in relative insulin deficiency. There is a strong genetic predisposition. Type 2 diabetes is more common in individuals with family history of the disease, in individuals with hypertension or dyslipidaemia and in certain ethnic groups.

The risk of developing Type 2 diabetes increases with:

- Family history of diabetes (in particular parents or siblings with diabetes)
- Obesity ($\geq 20\%$ over ideal body weight or BMI ≥ 25.0 kg/m²)
- Membership of some ethnic groups
- Age ≥ 45 years
- Previously identified IFG or IGT
- Hypertension ($\geq 140/90$ mmHg in adults)
- HDL cholesterol level ≤ 1.0 mmol/L (≤ 0.38 g/L) and/or a triglyceride level $\geq 2,3$ mmol/L ($\geq 2,0$ g/L)
- Reduced physical activity
- History of gestational diabetes mellitus (GDM) or delivery of babies $>4,5$ kg

The characteristic features of Type 1 and Type 2 diabetes are contrasted in Table 2.

Table 2: General characteristics Type 1 and Type 2 diabetes

Characteristics	Type 1 diabetes	Type 2 diabetes
Typical age of onset (years)	< 35	> 35
Genetic predisposition	low	high
Antibodies to β -cells	yes (90 – 95%)	no
Body habitus	normal/ wasted	obese
Plasma insulin/C-peptide	low/absent	high
Main metabolic feature	insulin deficiency	metabolic syndrome with insulin insensitivity
Insulin therapy	responsive	high doses required
Insulin secretagogue drugs	unresponsive	responsive

Laboratory findings:

- hyperglycaemia
- hyperlipidaemia
- high serum insulin/C-peptide level
- defective insulin secretion
- insulin resistance

Gestational diabetes mellitus (GDM)

All pregnancies should be screened for GDM except women with low risk of diabetes.

Definition: GDM is any degree of clinical glucose intolerance with onset or first recognition during pregnancy.

GDM complicates the pregnancy: The following problems may develop with GDM:

- altered duration of pregnancy
- placental failure
- hypertension / pre-eclampsia
- high birth weight of the newborn**

Diagnosis of GDM:

Fasting plasma glucose level $>7,0$ mmol/L ($>1,26$ g/L) or casual plasma glucose $>11,1$ mmol/L ($>2,00$ g/L), confirmed on a subsequent day.

Additional strategy to diagnose GDM:

- One step approach: OGTT (75 g glucose)
- Two step approach:
 1. First OGTT with 50 g glucose load (glucose challenge test GCT); cut-off value after 1 hour plasma glucose $\geq 7,8$ mmol/L ($>1,40$ g/L)
 2. When the glucose level after 1 hour exceeds the threshold value of GCT an OGTT with 75 g glucose load should follow. The evaluation should consider that the criteria of IFG, IGT and diabetes indicate GDM.

Six weeks after pregnancy or later the woman should be re-examined for the presence of diabetes mellitus, IGT or IFG.

Note: in some countries, only the one-step approach using 75 g glucose load is preferred.

Prevalence of diabetes

The prevalence of diabetes in Western life-style countries is estimated to be between 6,0 and 7,6%. In some developing countries the prevalence is more than 6% (Middle East, Western Pacific). The mean percentage prevalence varies between ethnic groups (American Indians, Hispanics, and others). Between 1995 and 2025 there is predicted to be a 35% increase in the worldwide prevalence of diabetes. The rising number of people with diabetes will occur mainly in populations of developing countries, leading to more than 300 million people with diabetes globally by 2025 because of the size of the population. As many as 30-50% of people with diabetes are undiagnosed. Since therapeutic intervention can reduce complications of the disease, there is a need to detect diabetes early in its course. The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity.

Screening for diabetes

Screening for diabetes is an analytical, organizational, and financial challenge (10-12, 18, 19). The organizational and financial aspects are the biggest limiting factors. Several strategies have been suggested and evaluated for community screening. If possible community screening should occur within the local health-care system so that individuals with positive findings get appropriate follow-up investigations and treatment.

Screening strategy will depend on the underlying prevalence of diabetes, structure of the local health-care system, and the economic condition of the country. The aim of screening is to identify asymptomatic individuals who are likely to have diabetes. There are two strategies that may be applied for screening

1. Detect all people with diabetes in a population.
2. Detect diabetes amongst those people who are mostly likely to have diabetes (selective, or opportunistic screening)

In a recent Danish study the authors stated that no randomized control trials are available to advise on the question of opportunistic versus systematic screening (15). These authors favour economic models which give preference to opportunistic screening rather than community screening for which the scientific evidence is not yet given.

Opportunistic screening:

Detection of people with diabetes who contact health services for other reasons, by physical and laboratory examination.

Selective screening: A verbal or written questionnaire is distributed in the population. This questionnaire should identify those individuals who are at high risk of having diabetes. They should be referred to a physician for consideration of diagnosis.

Selective screening should consider individuals :

- with typical symptoms of diabetes
- with a first-degree relative with diabetes
- who are members of a high risk ethnic group
- who are overweight (BMI \geq 25.0 kg/m²)
- who have delivered a baby >4.5 kg or had GDM
- who are hypertensive (\geq 140/90 mmHg)
- with raised serum triglyceride and cholesterol levels
- who were previously found to have IGT or IFG

The basic laboratory measures for screening are:

1. Fasting capillary blood glucose
2. 2 hours plasma glucose (OGTT)
3. HbA_{1c}

The best indicator for estimating diabetes prevalence and incidence is fasting blood glucose (FPG) in combination with 2 h plasma glucose (19). FPG concentration of $>7,0$ mmol/L ($>1,26$ g/L) is an indication for retesting. For centralized screening the analysis of glycated haemoglobin (HbA_{1c}) from a blood drop is recommended, though this approach is more expensive than FPG. Glycosuric tests are less recommended in population screening (17).

Screening strategies from a laboratory technical perspective

Decentralized screening

In decentralized screening fasting blood glucose is the appropriate analyte, followed by retesting FPG and/or by OGTT. The comparability of glucose analyses must be verified by internal and external quality control. HbA_{1c} may also be used in decentralized screening although the results may vary when different analytical procedures are used. The OGTT is not recommended as the first step of screening but rather as a confirmation test.

Centralized screening

This is dependent on appropriate specimen collection facilities, specimen stability and specimen transport. These conditions are met by capillary blood collection, preservation of the specimen as dry blood on a filter paper and HbA_{1c} analysis by an immunological procedure at a central laboratory (see Fig. 1). Chromatographic methods are less suitable for HbA_{1c} measurement in dried blood samples since some HbA_{1c} may be partially altered during transportation while still having preserved its antigenicity.

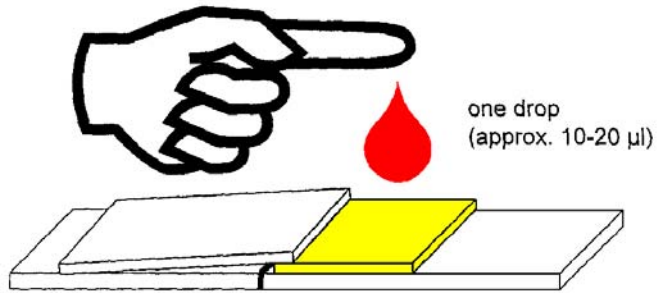
Fig. 1: Specimen collection device for centralized analysis of HbA_{1c}

HbA_{1c} blood carrier

labelling of transporting envelop



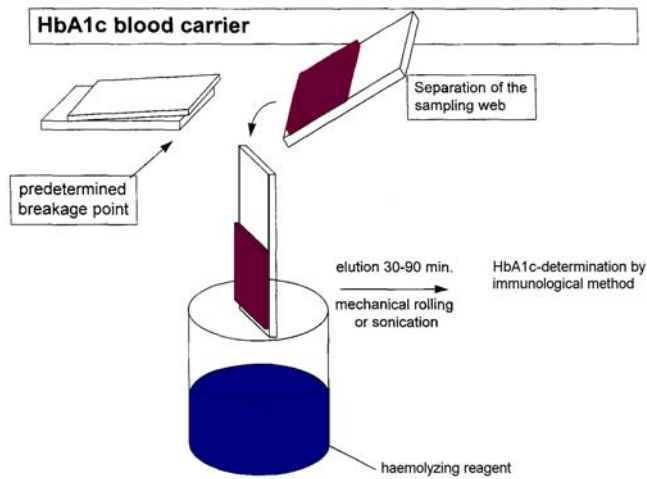
finger prick – capillary blood



drying of sample (approx 30 minutes)



sealing of the envelope and sending to the laboratory



Role of the medical laboratory in diabetes mellitus

The laboratory has an essential role in the diagnosis and management of diabetes mellitus. The laboratory indicators for the diagnosis and management of diabetes are listed in Table 3:

Table 3: Routine laboratory indicators for the control of management of diabetes

Glucose (blood, urine)	HbA _{1c}
Ketones (urine)	Fructosamine
OGTT	Urinary albumin excretion
	Creatinine / urea
	Proteinuria
	Plasma lipid profile

Advanced laboratories may use more sophisticated indicators for clinical studies that are listed in table 4.

Table 4: Advanced techniques for the assessment and control of diabetes and glucose metabolism

ICA
GADA
IA-2A
IAA
Insulin
C-peptide
IV-glucose load
clamp (euglycaemic-hyperinsulinaemic clamp)

Glucose determination

The simplest indicator of the adequacy of carbohydrate metabolism of a patient is the blood glucose concentration. However glucose is rapidly metabolized in the body. Therefore, the glucose concentration reflects the immediate status of carbohydrate metabolism, and does not allow a retrospective or prospective evaluation of glucose metabolism.

Glucose is measured in different specimens, including

- whole blood (capillary or venous blood)
- haemolysate
- plasma
- serum
- de-proteinized blood
- urine
- CSF

Blood Glucose

The pathological entity of blood glucose is the plasma glucose concentration, that is the glucose to which organ systems are exposed. Some glucose measurements detect plasma glucose directly (by electrode) and do not rely on a precise volume of plasma being applied. Plasma can also be prepared from whole blood by centrifugation, but erythrocytes will continue to metabolize glucose thus lowering the concentration measurable unless glycolysis is inhibited. As fluoride takes time to diffuse into erythrocytes, some glycolysis will continue unless the fluoridated sample is cooled in ice-water from the time of venepuncture; although the size of this effect in fluoridated is not large and is generally regarded as relevant only to research studies. This problem will particularly affect serum glucose measurement, as such samples are generally left at room temperature to enhance clot formation. An alternative approach, immediate haemolysis + glycolysis inhibition, is occasionally used.

Whole blood glucose concentration is also affected by the concentration of protein (mainly haemoglobin - 8-18 %) in the sample. For this reason whole blood concentrations are 12 to 15 % lower than plasma concentrations by a variable amount, and plasma glucose is the preferred measure. Finger prick blood samples used for immediate testing on reagent strips or electrode sensors depend on the concentration of glucose in the plasma fraction, but such systems may be calibrated by the manufacturer to plasma or whole blood standards.

The plasma glucose concentration of importance at peripheral organ systems is the arterial concentration, and this is the measure of preference in research studies. Capillary blood glucose concentrations will be a good approximation to this provided tissue perfusion is good. Venous blood will have lower glucose concentrations than arterial blood (and thus capillary blood), but the effect is not large except where glucose disposal from the blood is high (after a meal due to insulin, or during exercise) and the sample is taken proximal to a muscle bed (eg from the ante-cubital fossa).

Analytical systems are calibrated to whole blood or serum glucose.

In post-prandial state or during glucose load (OGTT) capillary blood glucose levels are approximately 1,0 mmol/L (approximately 0,20 g/L) higher than in whole venous blood. In whole blood glycolysis decreases the glucose concentration by 10-15% per hour at room temperature. Serum glucose once separated from erythrocytes remains stable at room temperature up to 8 h, or for up to 72 h at 4°C.

When collecting and transporting blood for glucose analysis it is important to inhibit enzymatic degradation of blood glucose. Glycolysis in whole blood is inhibited by sodium fluoride (6 g/L blood) or maleinimide (0,1 g/L blood). As anticoagulant EDTA (1,2-2 g/L blood) is used. Cerebrospinal fluid (CSF) should be analyzed for glucose as soon as possible.

Blood glucose collection and stability

- Collection :
1. Capillary blood
 2. Venous blood
 3. Plasma
 4. Deproteinized blood
 5. Haemolysate (digitonin, maleinimide)

Stability of specimen:	Venous blood:	at 20°C: decrease of 10-15 %/h at 4°C: decrease of 20 % in 24 h
	Stabilizer:	NaF (6 g/L) + Maleinimide (0.1 g/L blood) EDTA (1,2-2 g/L) or EDTA + maleinimide
	Plasma/serum:	at 20 °C: decrease of 15 % in 24 h Deproteinized serum: stable over days and weeks
	Interferences:	Anticoagulants, drugs, glutathione, ascorbic acid, α-methyl dopa
	Pre-analytical effects:	Posture, exercise, food ingestion, smoking, transport/preservation of specimen

Methods for blood glucose determination

Several methods are available for glucose determination. The methods for glucose analysis are the following:

Chemical methods

- ortho-toluidine
- neocuproine
- ferricyanide

Enzymatic methods

- hexokinase-G6PDH
- glucose dehydrogenase

glucose oxidase-peroxidase (ABTS)
glucose oxidase (GOD) with other indicator reactions

For the chemical oxidation/reduction methods (neocuproine method, ferricyanide method) and the o-toluidine method the reagent costs are low. Although these methods are less specific they are still useful and valid. The enzymatic analysis of glucose is more specific. However the enzymatic methods are also more expensive.

The enzymatic reference method for glucose is the hexokinase/G6PDH method. The glucose dehydrogenase method has comparable analytical performance. The glucose oxidase methods performing slightly less well, since reducing substances may interfere with the peroxidase step. Nevertheless the GOD methods are most frequently used for convenience and economic reasons.

The reference intervals of the three enzymatic methods for glucose in blood of fasting adults are (16):

	<u>Serum/plasma</u>	<u>Whole blood</u>
Hexokinase/G6PDH:	4,4 – 5,5 mmol/L (0,80–1,00 g/L)	3,6 – 5,3 mmol/L (0,65 – 0,95 g/L)
Glucokinase:	4,4 – 5,5 mmol/L (0,80–1,00 g/L)	3,6 – 5,3 mmol/L (0,65 – 0,95 g/L)
GOD/POD:	5,0 – 6,1 mmol/L (0,90–1,10 g/L)	2,9 – 5,5 mmol/L (0,70 –1,00 g/L)
CSF:	2,2 – 3,9 mmol/L (0,40–0,70 g/L)	
Urine:	≤ 0,83 mmol/L (≤0,15 g/L)	

The concentration of glucose in cerebrospinal fluid is about 60 % of the plasma value. If CSF is contaminated with bacteria or additional cells, the glucose concentration may be much lower.

Urine glucose

Urine fractions should be analysed immediately or preserved at pH <5 to inhibit bacterial metabolism of glucose or should be stored at 4 °C before analysis. Convenient paper test strips are available from manufacturers.

Advantages: rapid
inexpensive
non-invasive
qualitative tests or semi-quantitative tests

Instruments: 1. Qualitative paper test strips:
Diabur, Diastix, Glucostix, others
Enzymes: Glucose oxidase/Peroxidase
Detection limit: 5,5 mmol/L (1,0 g/L)
Problems: False-positive results by oxidizing agents (H₂O₂, HOCl)
False-negative results by reducing substances (eg ascorbic acid)

2. Semi-quantitative tests:
Visual evaluation: by enclosed colour charts: Clinistix, Multistix

3. Quantitative tests:
Because of interfering substances hexokinase and glucose dehydrogenase methods are recommended. The o-toluidine procedure is an acceptable and non-expensive method.

Normal reference: undetectable

- Problems:
1. Poor reflection of changing levels of hyperglycaemia
 2. Renal threshold varies among individuals
 3. Lack of sensitivity and specificity of the qualitative and semi-quantitative procedures.

Quality control of glucose determination

The reliability of the method used should be evaluated by analysing

- trueness
- accuracy
- precision

The uncertainty for glucose determination is found to be about 5% during serial measurement (13).

For evaluation of accuracy and trueness within series appropriate certified control material should be used. The maximal allowable deviation must be given and should be less than 15%. The precision of measurement in series and between series should be quantitatively determined. The maximal allowable imprecision in series should not exceed 5 %. Icteric, turbid and/or haemolysed sera should be used to examine interferences during glucose determination.

Self-monitoring of blood glucose

Self-monitoring of blood glucose by people with diabetes has improved the management of diabetes. The DCCT (Diabetes Control and Complications Trial) (2) and UKPDS (3) clearly demonstrated the benefits of normal or near-normal blood glucose levels. There are a variety of blood glucose meters on the market based on different principles of measurement (photometry and potentiometry) (table 5). It is almost impossible to describe the main features, the analytical reliability in different concentration ranges of all available devices. Health authorities and standardizing organizations (ISO, CEN) have defined essential requirements for these instruments which are used by patients and also non-educated personnel.

The advantages and limitations of blood glucose meters for self-monitoring are the following:

Advantages:

1. High precision (CV 3,0 – 7,1 %)
2. No need for pipettes
3. Capillary blood
4. Low price of instrument
5. Easy to use
6. Overcome colour blindness and illumination problems

Limitations of blood glucose meters:

1. Limited analytical measurement interval
2. Inaccuracy of measurement
3. Lack of compatibility with control samples
4. Matrix effects
5. Temperature effects causing false results
6. Higher costs of consumables

Table 5: Blood glucose monitors

Glucometer	Manufacturer	Principle	Calibrated for	Sampling method	Test time sec.	Sample size µl	Test interval g/L
Accu-Chek Sensor	Roche Diagnostics	Sensor	Blood	Sip-in	12	11	0,1 – 6,0
Accu-Chek Comfort	Roche Diagnostics	Photometry	Blood	Drop	12	11	0,1 – 6,0
Accu-Chek Compact	Roche Diagnostics	Photometry	Blood	Drop	40	15	0,1 – 6,0
Glucometer Elite XL	Bayer	Sensor	Plasma	Sip-in	30	2	0,4 – 5,0
Glucometer Dex 2	Bayer	Sensor	Plasma	Sip-in	30	3 - 4	0,4 – 5,0
One Touch Sure Step	LifeScan	Photometry	Plasma	Drop	15 - 30	10 - 30	0,2 – 5,0
One Touch Profile	LifeScan	Photometry	Blood	Drop	45	10	0,2 – 6,0
One Touch Ultra	LifeScan	Sensor	Plasma	Sip-in	5	1	0,2 – 6,0
Precision PCx	Abbott/Medisense	Sensor	Plasma	Sip-in	20	2 - 3	0,2 – 6,0
Precision Xtra	Abbott/Medisense	Sensor	Plasma	Sip-in	20	3.5	0,2 – 5,0
B-Glucose Analyser	HemoCue	Photometry	Blood	Sip-in	40 - 240	5	0 – 4,0
GlucoMen Glyco	Menarini	Sensor	Blood	Sip-in	30	3 - 5	0,2 – 6,0
Omnitest Sensor	Braun	Sensor	Plasma	Sip-in	15	5	0,2 – 6,0
Freestyle	TheraSense	Sensor	Plasma	Sip-in	15	0.3	0,2 – 5,0
Supreme II	Hypoguard Medisys Group	Photometry	Plasma	Drop Non-wipe	60	3	0,38 – 4,5

Recommendations for glucose monitoring in diabetes:

- 1) Individuals with diabetes should maintain blood glucose levels as close to normal as is safely possible. People with Type 1 diabetes (and others using insulin therapy) can only achieve this goal by self-monitoring of blood glucose.
- 2) The use of calibration and control solutions by the patients shall assure accuracy of results.
- 3) The user should know whether the instrument is calibrated to whole blood or plasma glucose.
- 4) People should be taught how to use and maintain the instruments, and how to interpret the data.
- 5) Health professionals should assess the performance of the patient's glucometer and the ability of the patient to use the data at regular intervals by comparative measurement of blood glucose using a method of higher reliability.
- 6) When using enzyme impregnated strips for glucose measurement it is imperative that the strips are properly stored airtight in the screw cap container provided until use for maximum shelf life.

The oral glucose tolerance test (OGTT)

The OGTT is a provocation test to examine the efficiency of the body to metabolise glucose. The OGTT distinguishes metabolically healthy individuals from people with impaired glucose tolerance and those with diabetes. The OGTT is more sensitive than FPG for the diagnosis of diabetes. Nevertheless the final diagnosis of diabetes should not be based on a single 2 h post-load glucose $\geq 11,1$ mmol/L ($\geq 2,00$ g/L) but should be confirmed in subsequent days (FPG and/or casual glucose estimation).

The OGTT is more sensitive for the diagnosis of diabetes than fasting plasma glucose. The OGTT is not used for the monitoring of day to day blood glucose control, which is done by HbA_{1c}, and repeated glucose measurement. The OGTT is used mainly for diagnosis of IFG, IGT, GDM and in certain studies with patients, but is not recommended or necessary for routine diagnostic use.

Preparation of the patient:

Three days unrestricted, carbohydrate rich diet and activity.

No medication on the day of the test.

8 to 14 h fast.

No smoking.

Glucose load: Adults 75 g anhydrous glucose in 300 – 400 mL of water.

Children: 1,75 g/Kg up to 75 g glucose

Solutions containing glucose and oligosaccharides are commercially available.

Plasma glucose sampling:

10 min before glucose load

120 min after glucose load

Urine glucose can be additionally measured in case of hyperglycaemia.

Evaluation:

	<u>Fasting plasma glucose</u>		<u>120 min glucose</u>
IFG	6,1-6,9 mmol/L (1,10-1,25 g/L)		
IGT	$\leq 7,0$ mmol/L ($\leq 1,26$ g/L)	and	7,8-11,0 mmol/L (1,40-1,99 g/L)
Diabetes	$\geq 7,0$ mmol/L ($\geq 1,26$ g/L)	or	$\geq 11,1$ mmol/L ($\geq 2,00$ g/L)

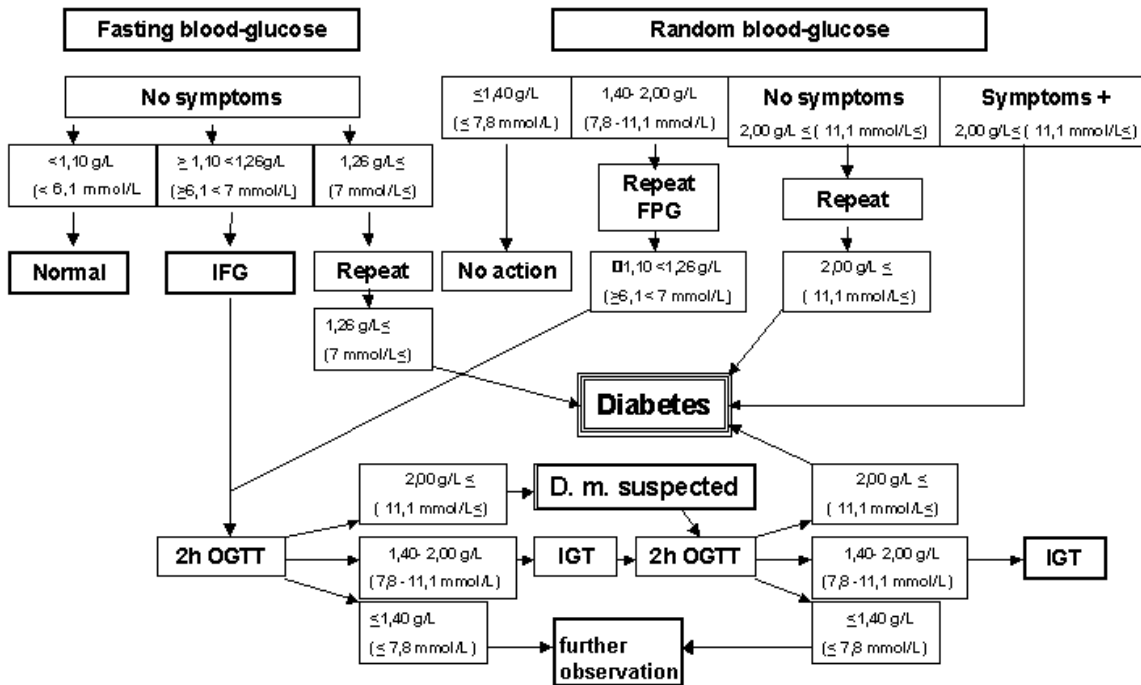
These values apply to venous plasma glucose.

Comments:

The OGTT is affected by metabolic stress from a number of clinical conditions and drug treatments, such as:

- Major surgery
- Myocardial infarction, stroke, infections, etc
- Malabsorption
- Drugs (steroids, thiazides, phenytoin, oestrogens, thyroxine)
- Stress, nausea
- Caffeine, smoking

Fig.2 Diagnostic Strategy for Diabetes



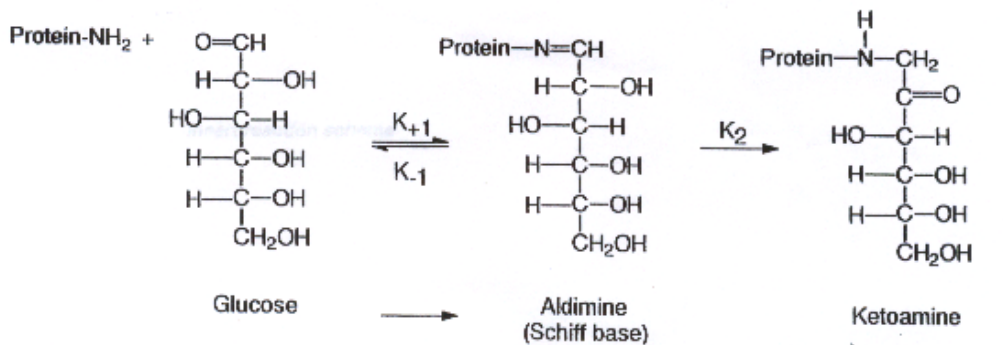
Glycated proteins

Proteins react spontaneously in blood with glucose to form glycated derivatives. This reaction occurs slowly under physiological conditions and without the involvement of enzymes. The extent of glycation of proteins is controlled by the concentration of glucose in blood and by the number of reactive amino groups present in the protein that are accessible to glucose for reaction. All proteins with reactive sites can be glycated and the concentration of the glycated proteins that can be measured in blood is a marker for the fluctuation of blood glucose concentrations during a certain period. From a clinical diagnostic point glycated proteins with a longer life time in blood are of interest, since they reflect the exposure of these proteins to glucose for longer periods.

Glycated haemoglobin

The life span of haemoglobin in vivo is 90 to 120 days. During this time glycated haemoglobin A forms, being the ketoamine compound formed by combination of haemoglobin A and glucose. Several subfractions of glycated haemoglobins have been isolated. Of these, glycated haemoglobin A fraction HbA_{1c} is of most interest serving as a retrospective indicator of the average glucose concentration over the previous 8 to 10 weeks (7, 8).

The reaction of the non-enzymatic glycation of proteins is as follows:



Analysis of HbA_{1c}

There are a variety of commercial tests systems for measuring HbA_{1c} (Table 7). The majority of commercial tests separate HbA_{1c} from non-glycated haemoglobin by chromatography. HbA_{1c} can also directly be measured in blood by immuno-chemical techniques without being separated from non-glycated haemoglobin. While it is true that there is no biochemical interference from haemoglobin variants for the affinity and immunochemical methods, there may be a biological interference in certain conditions where the haemoglobin (erythrocyte) turnover in the blood is high.

Specimen: Whole blood is used for analysis.

Blood+EDTA	100 µl
Heparinized blood	100 µl
Capillary blood	one drop on special filter paper

The specimen should be analyzed as soon as possible. In haemolysates adducts of haemoglobin with glutathione may be formed. Grossly hyperlipidaemic samples may give erroneous results by all methods except some immunological methods.

Indication:

Determination of HbA_{1c} is used as a retrospective estimate of the average blood glucose level over a period of 8 to 10 weeks. Therefore HbA_{1c} is a long term measure of glucose metabolism. HbA_{1c} is recommended as an essential indicator for the monitoring of blood glucose control.

Standardization of HbA_{1c}

Comparability of methods of measuring glycated haemoglobin has been poor for most of the time since the assays were first developed in the late 1970s. However the needs of multicentre studies of blood glucose control and complications, and in particular the Diabetes Control and Complications Trial (DCCT) (2) drove a system of harmonization of laboratory and manufacturers' methods to a standard referenced to a single laboratory's column separation method. In some countries the HbA_{1c} results are now reported as 'DCCT standardized'.

However the reference column method includes non-specific interferences of the order of 2.0 % by other haemoglobin fractions. Thus the values determined are not anchored to a single specific analyte. After considerable effort a mass spectrometric method has been developed as a reference method under the auspices of the International Federation of Clinical Chemistry (IFCC). The principle is the measurement of the β -terminal hexapeptide of haemoglobin A with or without covalently linked glucose. Manufacturers are being encouraged to reference the results of their systems to this reference method. A Certified reference material for HbA_{1c} is now available for distribution.

The major advantages of harmonization are that:

- patient understanding and self care
- clinicians can communicate results between themselves and others without adjustment of results to different reference intervals and clinical trials can be directly compared for experimental and regulatory purposes;
- standards for diabetes management can be set in clinical guidelines.

The clinical and research community is continuing to ask that results from the reference systems must become comparable with each other. Harmonization and standardization as above is therefore strongly encouraged and is currently being implemented. Where this is not the case the different reference intervals of the assays must be given.

Table 6: Analytical procedures for glycated haemoglobins

Procedure	Principle	Analyte	Sample (blood)	Analysis time	Comment
Column chromatography (macro-column)	Ion exchange chromatography	HbA _{1a} , HbA _{1b} , HbA _{1c}	100 µl	8-18 h	The aldime form is partially determined, interference by HbF, HbS, HbC, and acetaldehyde adducts
Micro-column	Ion exchange chromatography	HbA ₁	100 µl	20 min.	Interference by Hb variants, temperature and pH sensitive
HPLC	Ion exchange chromatography	HbA _{1a} , HbA _{1b} , HbA _{1c}	10 - 400 µl	3-8 min.	Interference by Hb variants, temperature and pH sensitive
FPLC	Ion exchange chromatography	HbA _{1a} , HbA _{1b} , HbA _{1c} , aldimine	20- 100 µl	5 min.	Best separation of HbA _{1c} and the aldime;
Thiobarbituric acid	Hydrolytic cleavage and colourimetric determination of ketohexoses	HbA _{1c}	2-4 ml	8 h	Only detects the ketoamine form, the aldime is eliminated; also reacts with sialic acid
Electrophoresis	Electro-endosmosis	HbA _{1c}	20 µl	35 min.	aldime interference
Isoelectric focussing	pH gradient 5 – 6.5	HbA _{1c} , aldimine	10 µl	1 h	Detection of abnormal haemoglobins
Affinity chromatography	Phenylboronate column	Total glycohaemoglobin	150 µl	Up to 20 samples per 1 h	HbF, HbS, HbC, and post-translational modifications do not interfere
Immunochemical methods	Specific antibodies (monoclonal, polyclonal) in EIA, immunoturbidimetry	HbA _{1c} , HbA _{2c}	10-50µl	Up to 250 samples per 1 h	No interference by Hb variants; glycated HbA ₂ , HbS _{1c} , are detected but not HbF _{1c}

It is recommended to measure HbA_{1c} at regular intervals four times per year for monitoring of blood glucose control.

Table 7: Reference intervals for glycohaemoglobins

Method	Brand name	Indicator	Reference interval (%)
Affinity chromatography	GHb Imx	tHb	tHb 4,8 – 7,8
		HbA _{1c}	HbA _{1c} 4,4 – 6,4
Affinity chromatography	Glyc-Affin	tHb	tHb 4,0 – 8,0
Affinity chromatography	BM HbA ₁	HbA ₁	HbA ₁ 5,0 – 8,0
Affinity chromatography	Glyc-Hb	tHb	tHb 5,0 – 8,0
Affinity chromatography (minicolumns)	HbA ₁ mini column test	HbA ₁	HbA ₁ 3,4 – 6,1
Agarose gel electrophoresis	DIATRAC	HbA _{1c}	HbA _{1c} 3,3 – 5,6
Immunoturbidimetry, polyclonal antibody	TinaQuant HbA _{1c}	HbA _{1c}	HbA _{1c} 4,3 – 5,8*
			HbA _{1c} 3,6 – 5,3*
EIA, monoclonal antibody	DAKO HbA _{1c}	HbA _{1c}	HbA _{1c} 2,8 – 4,9*
			HbA _{1c} 4,5 – 5,9*
Immunoturbidimetry, monoclonal antibody	DCA 2000	HbA _{1c}	HbA _{1c} 4,2 – 6,3
Immunoturbidimetry, monoclonal antibody	Unimate	HbA _{1c}	HbA _{1c} 4,5 – 5,7
Ion exchange chromatography (microcolumns)*	HbA _{1c} microcolumn test	HbA _{1c}	HbA _{1c} 4,2 – 5,9
HPLC ion exchange chromatography	DIAMAT	HbA ₁	HbA ₁ 5,1 – 7,3
		HbA _{1c}	HbA _{1c} 4,3 – 6,1
HPLC ion exchange chromatography	HS-8	HbA ₁	HbA ₁ 5,0 – 7,8
		HbA _{1c}	HbA _{1c} 4,4 – 5,7
HPLC ion exchange chromatography	L-9100	HbA ₁	HbA ₁ 4,5 – 6,0
		HbA _{1c}	HbA _{1c} 3,4 – 4,7

Values according to package inserts or references ,

* different standardization available,

tHb = total glycohaemoglobin

The relationship between average blood or plasma glucose and HbA_{1c} is shown in Table 8.

Table 8. Relationship between HbA_{1c} (DCCT standardized or equivalent) and average plasma or whole blood glucose concentrations from 7-point self-monitored profiles (12, 14).

HbA _{1c} (%)	glucose (mmol/L)	
	plasma	blood
4,0	3,6	2,6
5,0	5,6	4,5
6,0	7,6	6,3
7,0	9,6	8,2
8,0	11,5	10,0
9,0	13,5	11,8
10,0	15,5	13,7
11,0	17,5	15,6
12,0	19,5	17,4

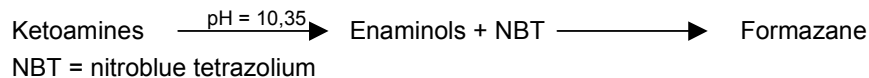
The average self-monitored pre-prandial glucose will be 0,7-1,0 mmol/L lower than from 7-point profiles.

Special analytical problems may arise in the presence of abnormal haemoglobins. Unrealistically high HbA_{1c} values (>18.0 %) may be measured with some methods. Falsely low HbA_{1c} results may be seen in haematological disorders and renal failure. Spurious elevation has been reported in hypertriglyceridaemia, hyperbilirubinaemia, alcohol abuse and treatment with aspirin.

Fructosamine test

Albumin is the main component of plasma proteins. As albumin also contains free amino groups, non-enzymatic reaction with glucose in plasma occurs. Therefore glycated albumin can similarly serve as a marker to monitor blood glucose. Glycated albumin is usually taken to provide a retrospective measure of average blood glucose concentration over a period of 1 to 3 weeks.

Under alkaline conditions (pH: 10.35) glycated proteins (ketoamine) reduce nitroblue tetrazolium (NBT) to formazane. In the fructosamine test the absorption of formazane at 530 nm is photometrically measured and compared with appropriate standards to determine the concentration of glycated proteins in plasma, the major part being contributed by albumin. The principle of reaction is as follows:



The absorbance of formazane is measured at 530 nm after 10 and 15 min. The absorbance change is proportional to the concentration of ketoamines in the plasma. The pre-incubation is necessary to eliminate fast-reacting reducing substances which may interfere.

For the standardization of the fructosamine test the following calibrators may be used:

- glycated polylysine
- glycated serum proteins

Reference interval: 205- 285 µmol/L

The following substances interfere in the photometric method of measurement:

- Ascorbic acid
- Uric acid
- Bilirubin
- Methyldopa

The interpretation of this measure will depend on the rate of turnover of glycosylated albumin. This is altered in a number of medical conditions, notably those involving liver and renal dysfunction.

Urinary albumin excretion

Diabetic patients are at high risk of developing renal insufficiency years after the onset of diabetes (8). Diabetes is the most common cause of renal failure. In one third of patients with Type 1 diabetes diabetic nephropathy leads to end-stage renal disease requiring dialysis. In Type 2 diabetes renal failure is less frequent due to earlier death from vascular disease, but, since this type of diabetes is more prevalent, about half of the cases of diabetic nephropathy occur in these patients.

The early signs of diabetic nephropathy cannot be detected by the routine screening tests for proteinuria, so that more sensitive methods for detecting abnormal albumin excretion must be used. The early stage of albuminuria is clinically defined as an albumin excretion rate of 30-300 mg/24 hours (20-200 µg/min), although true normal renal albumin excretion is lower than this. The small amount of albumin secreted in urine in early diabetic renal disease led to the misleading term "microalbuminuria", which is still widely used but should be avoided. Raised albumin excretion rate is a cardiovascular risk factor in people with Type 2 diabetes (and indeed in the non-diabetic population), in whom it should be regarded as a predictor of both increased macro- and micro-vascular risk. A classification of albuminuria is outlined in Table 9:

Table 9: A classification of abnormal renal albumin excretion rate

Albumin excretion rate	µg/min	mg/24-h	mg/L	mg/mmol creatinine	mg/g creatinine
normal	< 11	< 15	< 15	<1,5	<12
clinically abnormal	20 – 200	30 – 300	30-300	>3,5	>24
clinical nephropathy	> 200	> 300	> 300		

The albumin excretion rate in people with diabetes should be examined annually for screening. Monitoring of known abnormal albumin excretion rate should be more often. Self-monitoring is not yet available at reasonable costs.

The following procedure is suggested for the routine analysis of albuminuria in diabetes.

Begin: Type 1 diabetes after 5 years of the disease
 Type 2 diabetes with diagnosis of the disease

Commonly employed screening tests are spot urinary albumin:creatinine ratio or spot urine albumin concentration. Both are done on first pass morning urine samples to avoid the effects of activity and posture. False positive results can occur in urinary tract infection. If spot sample results suggest an abnormality, it is usually recommended to confirm the result with 2-3 overnight or 24-hour urine collections. Urinary albumin excretion varies considerably even within the same person on consecutive days.

Quantitative and semi-quantitative test systems are used to determine low rates of abnormal albumin excretion. For quantitative measurement the following principles are applied:

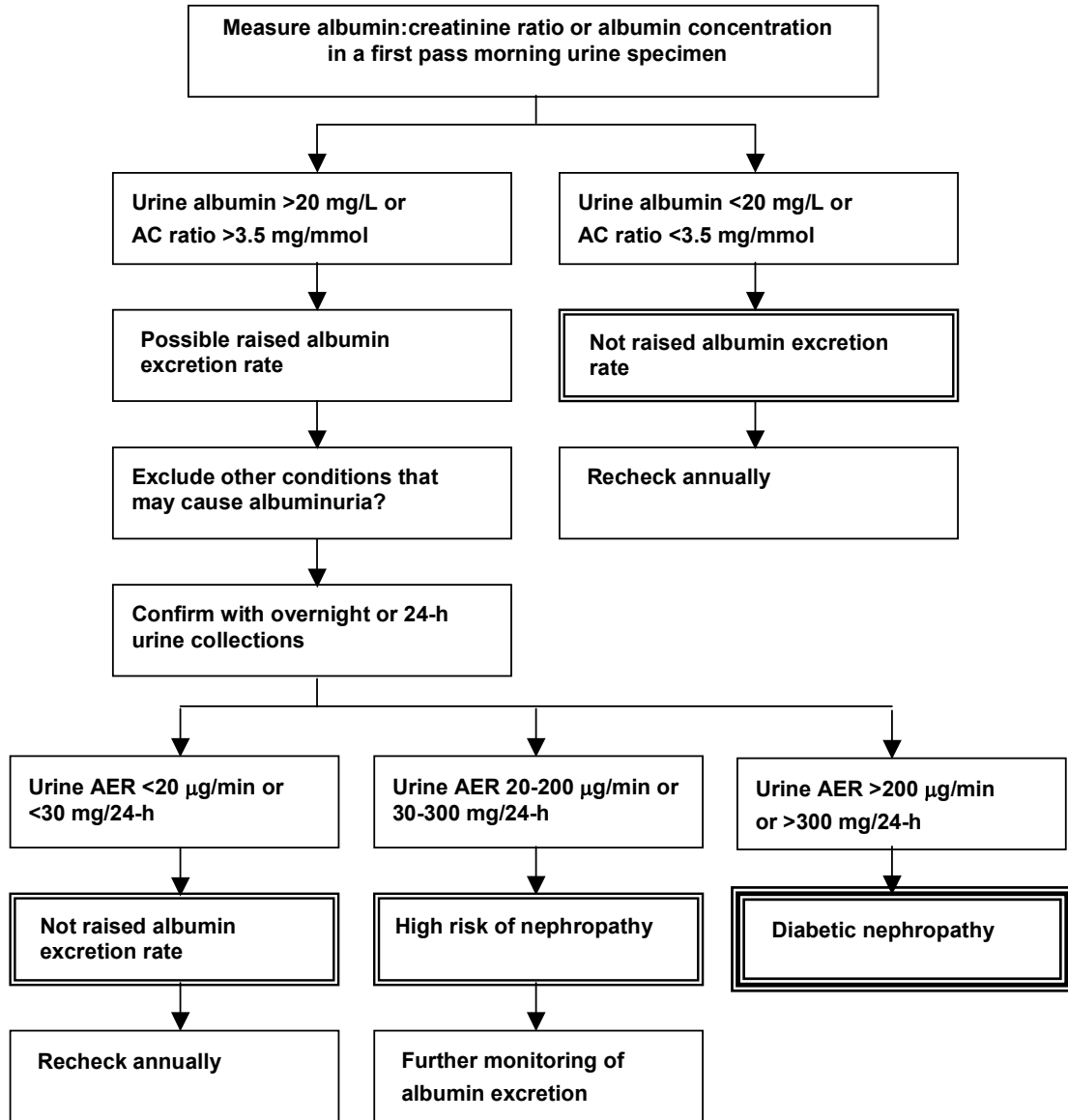
- Radioimmunoassay
- Enzyme-linked immunoassay
- Immunoturbidimetric assay
- Nephelometric assay

For semi-quantitative measurement the following are available:

- Gold-immunoassay
- Latex agglutination
- Silver dot blot assay (6)
- Nigrosin assay (5)

The semi-quantitative tests should have a sensitivity to detect 20 mg/L albumin in urine. However, the semi-quantitative nigrosin assay is an inexpensive screening test with a cut-off point at 50 mg/L of albumin.

Figure 3 Algorithm for the interpretation of albumin excretion rate in people with diabetes



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ISBN 92 4 159048 3



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