# World Health Organization

# Laboratory Diagnosis and Monitoring

# of

# Diabetes Mellitus

# 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$\beta$ | = | 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 ß-cells of pancreatic islets (islets of Langerhans)
  - non-specific, termed ICA
  - glutamic acid decarboxylase ($GAD_{65}$)
  - phosphotyrosine phosphatase (IA-2, IA-2ß)
- 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 ß-chains (valine).

Islet cell antibodies (ICA): antibodies directed against different proteins in and on the islet ß-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 ($GADA_{65}$) : Auto-antibodies directed against a membrane protein of the islet ß-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 by the WHO Expert Committee on Diabetes (1980, 1985). The current WHO classification system has been established in co-operation with the National Diabetes Data Group (USA). It is mainly based on the aetiology of diabetes mellitus (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 ß-cell function |
| Genetic defects of insulin action |
| Diseases of the exocrine pancreas |
| Endocrinopathies |
| Drug- or chemical- induced diabetes |
| Infections |
| Uncommon forms of 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

(Insulin-dependent diabetes, juvenile diabetes)
Type 1 diabetes is characterized by cellular-mediated autoimmune destruction of islet ß-cells.
Markers:
- – islet cell antibodies (ICAs)
- – auto-antibodies to insulin (IAAs)
- – auto-antibodies to glutamic acid decarboxylase ($GAD_{65}$)
- – auto-antibodies to tyrosine phosphatases IA-2 and IA-2ß

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 ß-cells.

Laboratory findings:
- – Hyperglycaemia
- – Ketonuria
- – Low or undetectable serum insulin and C-peptide levels
- – Auto-antibodies against components of the islet ß-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 (≥ 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 (≥ 140/90 mmHg in adults)
- - HDL cholesterol level $\leq$1.0 mmol/L ($\leq$0.38 g/L) and/or a triglyceride level ≥ 2,3 mmol/L (≥2,0 g/L)
- - Reduced physical activity
- - History of gestational diabetes mellitus (GDM) or delivery of babies >4,5 kg
- -

MODY is a form of youth onset diabetes which is not insulin-dependent, with a strong dominant family history, and is associated with abnormal hepatic nuclear factor (HNF) or glucokinase genes.

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 ß-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)

Definition: 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
Therapy:    nutrition therapy
>insulin (glucose-lowering drugs not advised).

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.

Laboratory strategy to diagnose  GDM:
>One step approach:    OGTT (75 g glucose)

>Two step approach:    1.    First OGTT with 50 g glucose load; cut-off value after 1 hour  plasma glucose >7,8 mmol/L (>1,40 g/L)
>2.    Second OGTT with  75 g glucose load and evaluation as the standard OGTT

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

## 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 world-wide 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. Presently as many as 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. 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. These authors favour economic models which give preference to opportunistic screening rather than systematic screening. In other countries with a higher prevalence of diabetes, systematic screening may be more cost-effective.

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 ≥ 25.0 kg/m²)
- who have delivered a baby  >4.5 kg or had GDM
- who are hypertensive (≥ 140/90 mmHg)
- with raised serum triglyceride and cholesterol levels
- who were previously found to have IGT or IFG

Systematic screening: Identification of people with new diabetes will be low at follow-up examinations at regular intervals (e.g. 3 years) because the incidence of new disease is low. This will give rise to problems of specificity and motivation. For the systematic screening of diabetes the recommendation of the American Diabetes Association may be followed. In this, screening should begin at an age of 45 years and be repeated at intervals of 3 years.

The basic laboratory measures for screening are:
1. Fasting capillary blood glucose
2. Glucosuria
3. $HbA_{1c}$
4. OGTT

The common and best indicator for estimating diabetes prevalence and incidence is fasting blood glucose (FPG). 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.
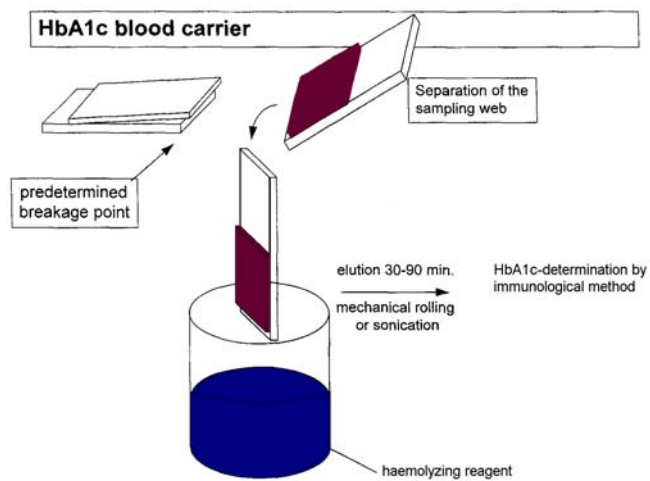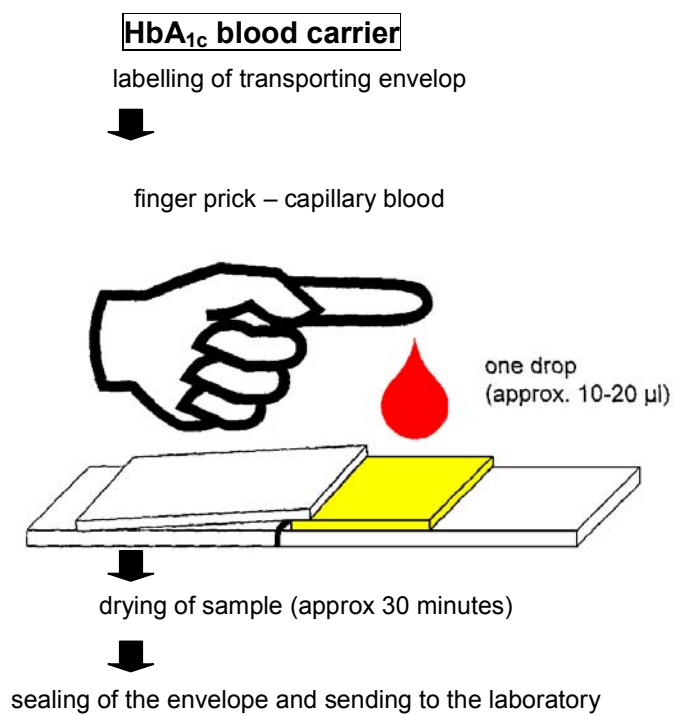
# Screening strategies from a laboratory technical perspective.

*Decentralized screening*

In decentralized screening fasting blood glucose is the appropriate analyte, followed by retesting FPG and/or by urine glucose. 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 chromatographic methods 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 easy specimen collection, 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 degraded during transportation whist 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

one drop
(approx. 10-20 µl)

drying of sample (approx 30 minutes)

sealing of the envelope and sending to the laboratory



HbA1c blood carrier

predetermined
breakage point

Separation of the
sampling web

elution 30-90 min.

mechanical rolling
or sonication

HbA1c-determination by
immunological method

haemolyzing reagent

# 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 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 (see below) 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 (or rather arterialized glucose) is the measure of preference in some 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 5–7 % 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. De-proteinized 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-methyldopa |
| | 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:

|  | Serum/plasma | Whole blood |
|---|---|---|
| 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: | $\leq$ 0,83 mmol/L ($\leq$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

($H_2O_2$, HOCl)

False-negative results by reducing substances (eg ascorbic acid)

2. Semi-quantitative tests:

Visual evaluation: by enclosed colour charts: Clinistix, Multistix

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

<u>Normal reference:</u>    undetectable

<u>Problems:</u>
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. 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) 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:

<u>Advantages</u>:
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

<u>Limitations of blood glucose meters</u>:
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 | Photometry | Plasma | Drop Non-wipe | 60 | 3 | 0,38 – 4,5 |

<u>Recommendations for glucose monitoring in diabetes:</u>

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 provides information on latent diabetes states. 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 IGT and in epidemiological population studies, but is not recommended or necessary for routine diagnostic use.

<u>Preparation of the patient:</u>
Three days unrestricted, carbohydrate rich diet and activity.
No medication on the day of the test.
12-h fast.
No smoking.
Glucose load: <u>Adults</u> 75 g in 300 – 400 mL of water.
<u>Children:</u> 1,75 g/Kg up to 75 g glucose
 Solutions containing glucose and oligosaccharides are commercially available.

<u>Plasma glucose sampling:</u>
10 min before glucose load
120 min after glucose load
Urine glucose can be additionally measured in case of hyperglycaemia.

<u>Evaluation:</u>

|  | **Fasting plasma glucose** |  | **120 min glucose** |
|---|---|---|---|
| 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) |
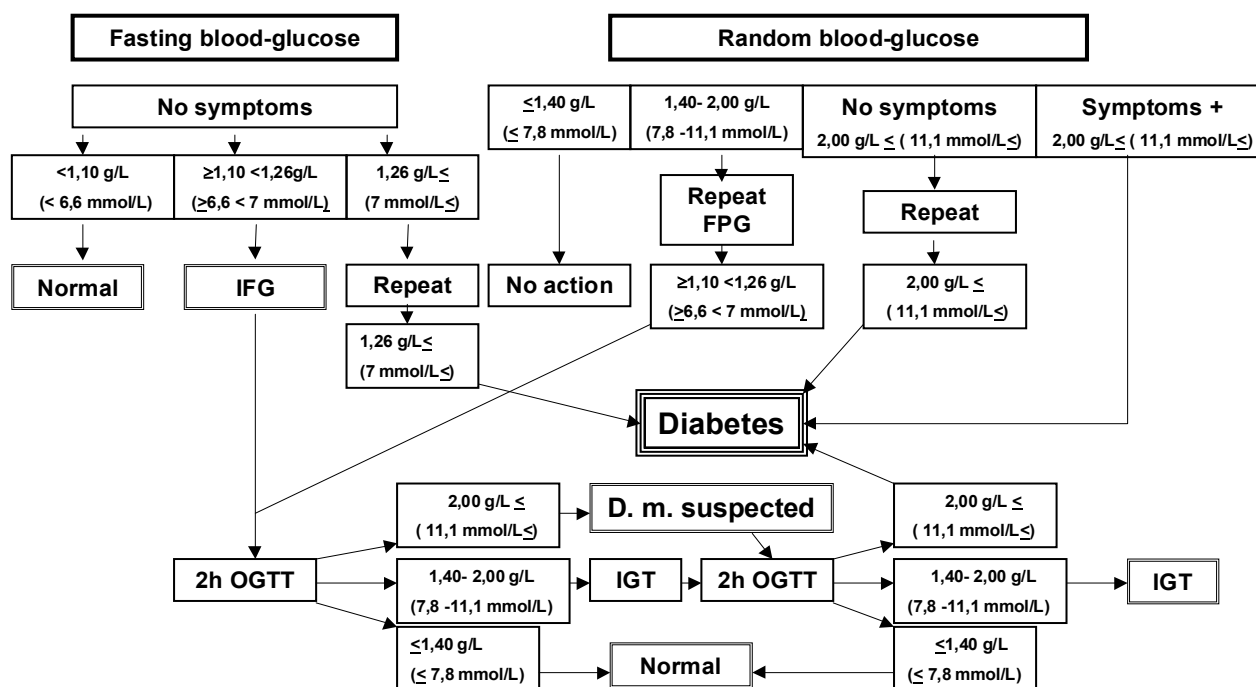
These values are for the preferred measure of plasma glucose;  different values apply to whole blood or capillary blood glucose

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
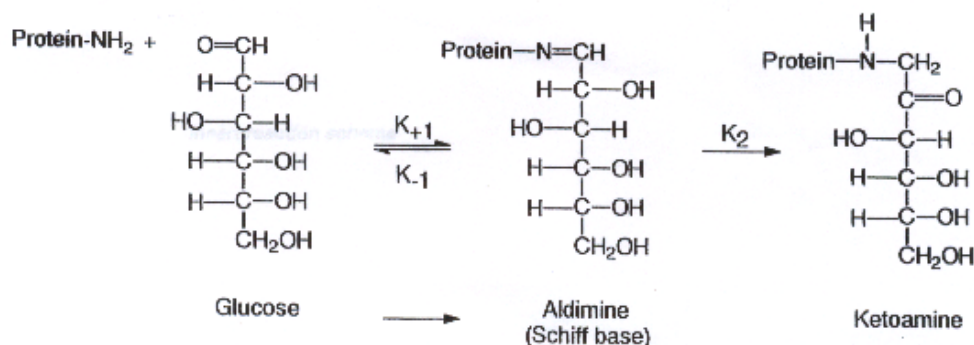
# Glycated proteins

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 to120 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.

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) 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 ß-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:
- clinicians can refer individual results to the complication rates reported from
- Type 1 diabetes and Type 2 diabetes studies, and thus determine individual patient risk;
- clinicians can communicate results between themselves and others without adjustment of results to different reference intervals and clinical trails 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 aldimine 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 aldimine; |
| Thiobarbituric acid | Hydrolytic cleavage and colourimetric determination of ketohexoses | $HbA_{1c}$ | 2-4 ml | 8 h | Only detects the ketoamine form, the aldimine is eliminated; also reacts with sialic acid |
| Electrophoresis | Electro-endosmosis | $HbA_{1c}$ | 20 µl | 35 min. | aldimine interference |
| Isoelectric focussing | pH gradient 5 – 6.5 | $HbA_{1c}$, aldimine | 10 µl | 1 h | Detection of abnormal haemoglobins |
| Affinity chromatography | Phenylboronate column | Total glycohaemo globin | 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 | $HbA_{1c}$, $HbA2_c$ | 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

| 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 |

*After: Nathan et al 1984 (blood); Rohlfing et al 2002 (plasma)*

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:

Ketoamines $\xrightarrow{\text{pH = 10,35}}$ Enaminols + NBT $\longrightarrow$ Formazane

NBT = nitroblue tetrazolium

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 glycated 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. 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 $\mu$g/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 frequency of testing for albumin excretion rate in people with diabetes is 1-2 times per year 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
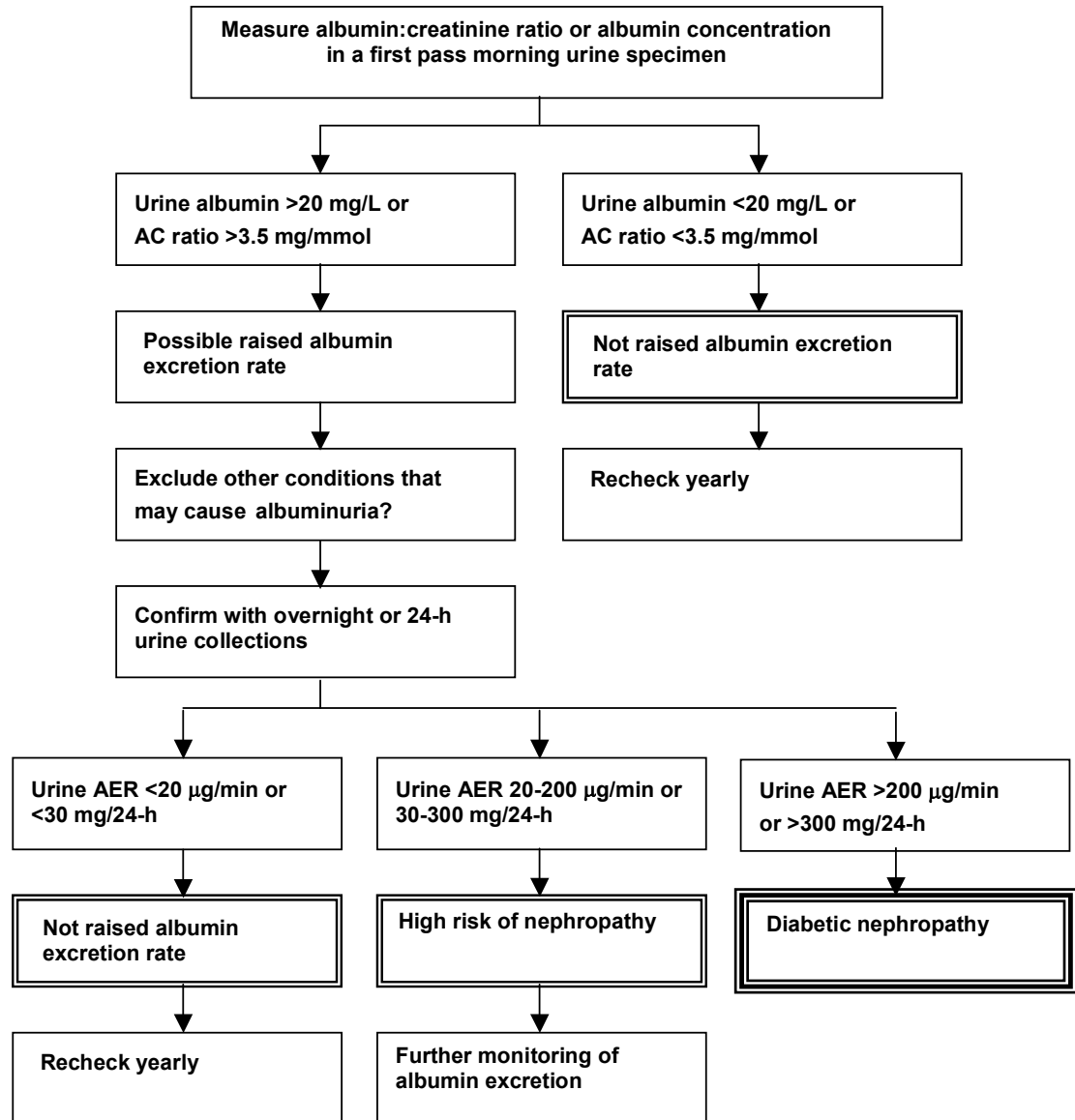
For semi-quantitative measurement the following are available:
        Gold-immunoassay
        Latex agglutination
        Silver dot blot assay
        Nigrosin assay

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

```
                ┌─────────────────────────────────────────────────┐
                │  Measure albumin:creatinine ratio or albumin     │
                │  concentration in a first pass morning urine     │
                │  specimen                                        │
                └─────────────────────────────────────────────────┘
                    │                                    │
        ┌───────────────────────┐          ┌───────────────────────┐
        │ Urine albumin >20 mg/L │          │ Urine albumin <20 mg/L │
        │ or AC ratio >3.5       │          │ or AC ratio <3.5       │
        │ mg/mmol                │          │ mg/mmol                │
        └───────────────────────┘          └───────────────────────┘
                    │                                    │
        ┌───────────────────────┐          ┌───────────────────────┐
        │ Possible raised albumin│          │ Not raised albumin     │
        │ excretion rate         │          │ excretion rate         │
        └───────────────────────┘          └───────────────────────┘
                    │                                    │
        ┌───────────────────────┐          ┌───────────────────────┐
        │ Exclude other          │          │ Recheck yearly         │
        │ conditions that may    │          └───────────────────────┘
        │ cause albuminuria?     │
        └───────────────────────┘
                    │
        ┌───────────────────────┐
        │ Confirm with overnight │
        │ or 24-h urine          │
        │ collections            │
        └───────────────────────┘
```

| Urine AER <20 μg/min or <30 mg/24-h | Urine AER 20-200 μg/min or 30-300 mg/24-h | Urine AER >200 μg/min or >300 mg/24-h |
|---|---|---|
| Not raised albumin excretion rate | High risk of nephropathy | Diabetic nephropathy |
| Recheck yearly | Further monitoring of albumin excretion | |

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