Lab tests for the investigation of major human metabolic disorders, part I:

Diabetes

Disturbed glucose homeostasis

Hypoglycemia (under 2.5 mmol/l)	**Hyperglycemia (above 10 mmol/l)**
Endocrine disorders	Diabetes,
(adrenal insufficiency	Hyperthyreosis,
hypophysis insufficiency),	Overproduction of cortisone / GH / glucagone
Glycogen storage disorders,	
insulinoma,	stress
Alcoholism, liver disorders, cirrhosis	
Severe medical conditions (sepsis, uremia), fasting	
Drug-induced hypoglycemia	
Preanalytical error (use of serum/plasma unseparated from cell compartment).	

Epidemiology of diabetes (WHO)

1994: 100 millions
2010: 250 millions
2030: 350 millions of diabetics worldwide
(90-95%: Type 2 DM)

Diabetes is the cause of

50% of end-stage renal disorders
50% of cases with blindness in the elderly
risk factor for stroke (3x), infarction (3-5x)
peripheral artery disease (15-30x)

Classification of Diabetes Mellitus

1. Type 1 diabetes: autoimmune origin; the destruction of beta cells of the pancreas

(6-7%)

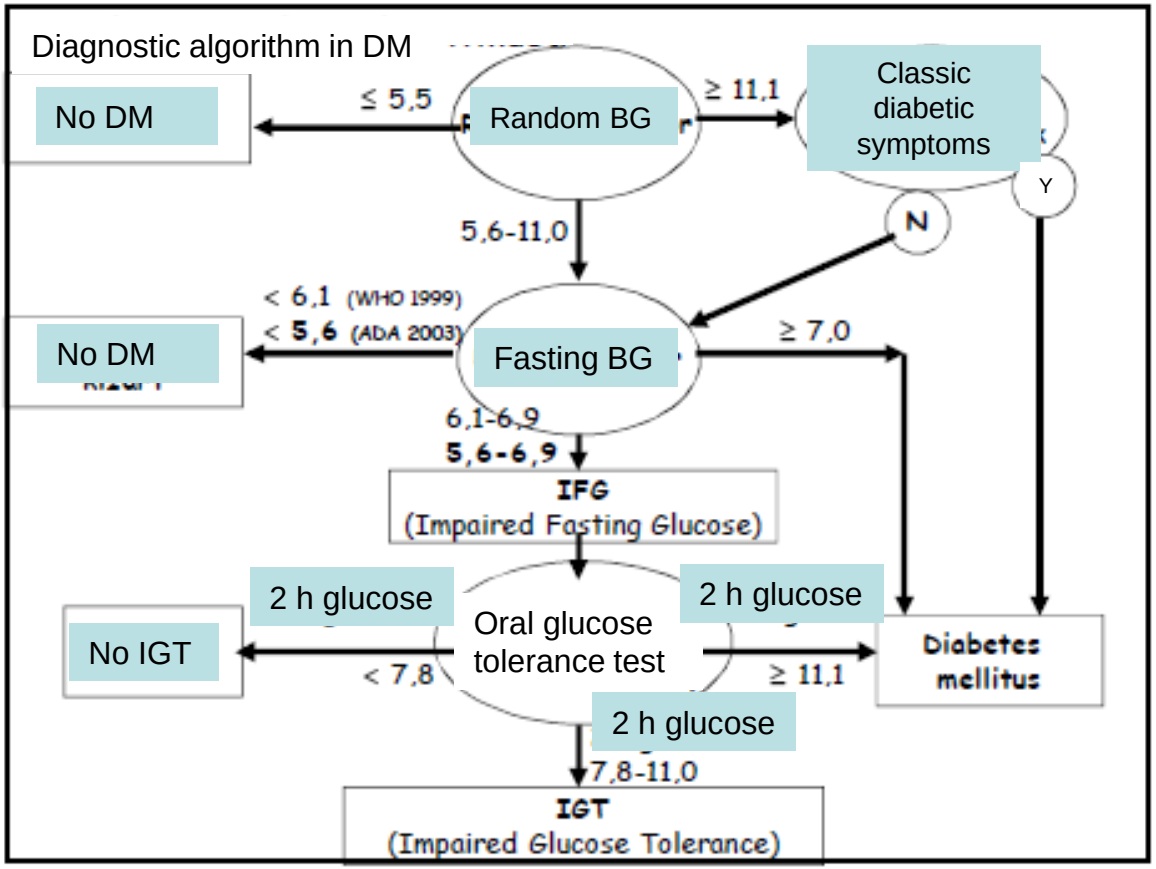
2. Type 2 diabetes: relative insulin deficiency / insulin resistance (90%)

3. Other subgroups (3-4%)

PROBLEM

- It is characteristic for T2DM that symptoms of disease (thirst, excessive urination, weakness, weight loss etc) occur just in advanced stage if ever
- Hyperglycemia, the leading symptom of diabetes does not cause any complaint

SCREENING IS OF OUTMOST IMPORTANCE



NOTE:

Formula for calculation of mg/dl from mmol/l: **mg/dl = 18 × mmol/l**

Formula for calculation of mmol/l from mg/dl: **mmol/l = mg/dl / 18**

HOW TO PERFORM OGTT

- THE test should be performed in the morning, at fasting state (at least 10 hours after a meal)
- DURING 3 days prior to OGTT the diet should contain at least 150 gram carbohydrate per day.
- PHYSICAL ACTIVITY should be as usual.
- SOME FACTORS (infection, drug, anxiety, stress, smoking) may affect the results
- 75 g (or 1.75 g/kg bw) glucose dissolved in 250 – 300 ml glucose should be ingested during 5 minutes.
- Glucose levels should be measured baseline and in the 120th minute.
- FOR non-cooperating patients: IV glucose 0,5 g/kg bw (up to 35 g), during 3 min; sampling in every 10th min, for 1 hour

GLUCOSE MEASUREMENT

- SAMPLE: recommended: NaF containing tube

WHY?

- FLUORIDE inhibits the enolase enzyme, hence inhibits the consumption of glucose by cells present in sample
- In general, glucose levels decrease by 0.5 mmol/l per hour for 3 hours, then stabilise in the presence of fluoride for 3 days.

Analytical approach for glucose level measurement

Glucose-oxidase technique:

Alfa-D-glucose ⟶ beta-D-glucose

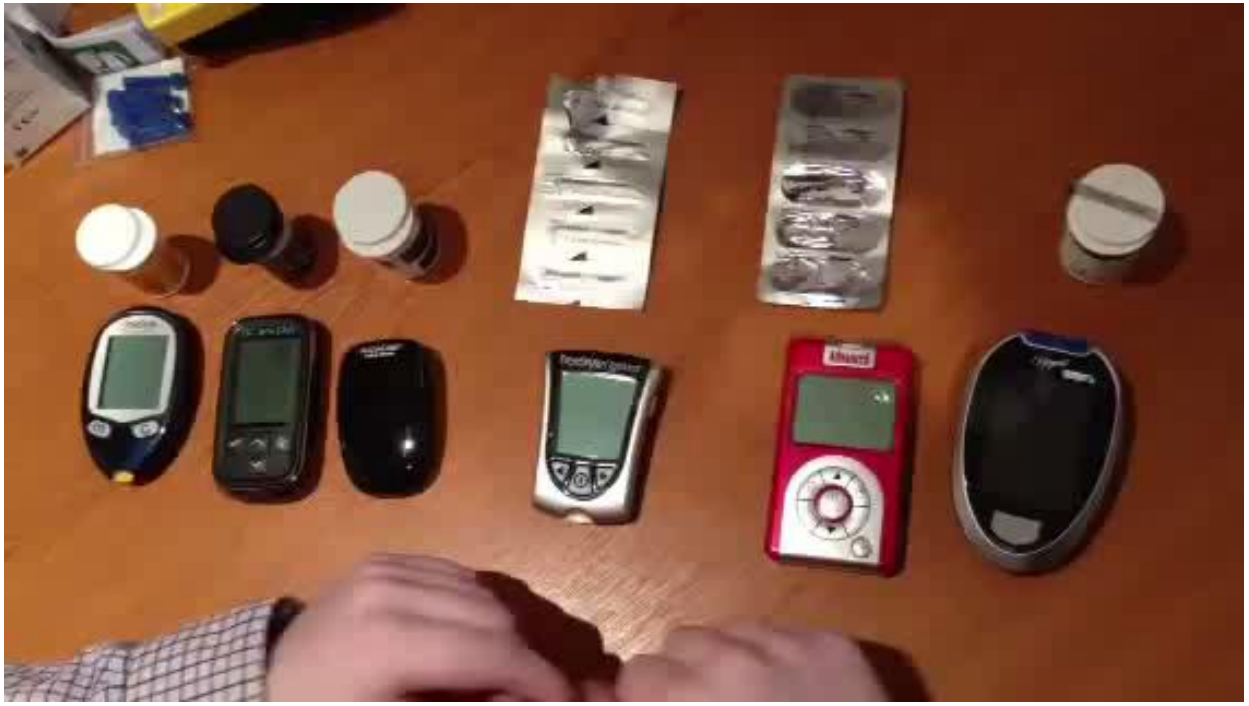
beta-D-glucose +H2O2 + O2 —(glucose oxidase)→ gluconate + H2O2

Reduced chromogen (not coloured) +H2O2 —(peroxidase)→ **Oxidised chromogen (coloured)** + 2H2O

Warning: cannot be used in reducing environment. Vitamin C decreases by 50% the level.

Pprinciple for glucose selfmonitoring devices

POCT glucose monitoring devices: do not use for diagnosis



Analysis of glucose levels in the Lab

Hexokinase method (reference method):

D-glucose $\xrightarrow{\text{Hexokinase +Mg}}$ D-glucose-6 phosphate +ADP

D-glucose-6 phosphate + NADP $\xrightarrow{\text{G-6-PD}}$ 6-phosphogluconate **+NADPH + H**

(measured near-UV, 340 nm)

Warning: EDTA plasma cannot be used

Important:

- Total blood: Capillary glucose level is lower by 5-10% than that in the venous blood
- Venous plasma glucose: equal to that in capillary whole blood
- Capillary blood: sampling site (finger tip) should be prewarmed before sampling

Urinary glucose levels

- Clinically not sound
- Methods based on the reducing capacity of glucose

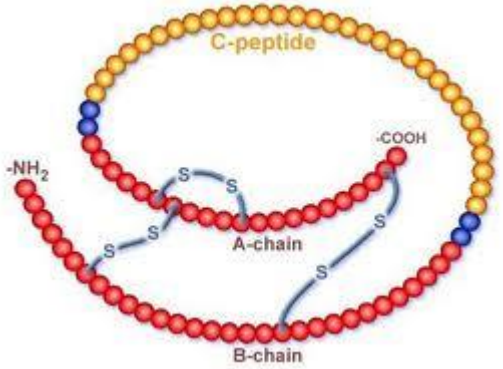
(interferring: uric acid, fructose, lactose, ketone bodies, sulfonamide, cystein, creatinine, salicilate etc.)

- Methods using glucose-oxidase approach

(false positive: hypochloric acid, expired test

false negative: vitamin C, antibiotics, salicylate, ketone bodies)

ADDITIONAL TESTS

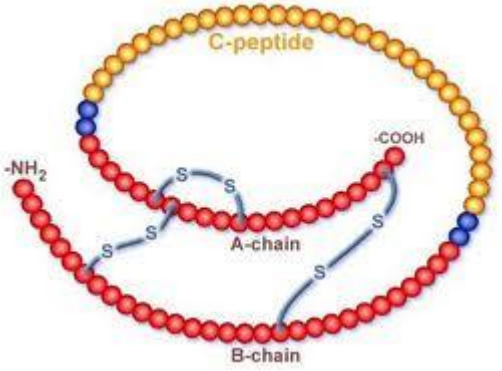


Peptide C

Reference range: 0,3-1,4 nmol/l (0,8-4,2 ng/ml).

- Pro-insuline molecule is cleaved for insulin and peptide-C before secretion.
- The quantity of secreted peptide C is identical to that of insulin.
- Indicates endogenous insulin production.
- Indication: diagnosis of islet cell tumors, pre-diabetes, hypoglycemia, insulinoma.
- Increased levels: insulinoma, type 2 DM, renal failure
- Decreased levels: type 1 DM

ADDITIONAL TESTS



INSULIN

Reference range: 18-170 pmol/l (2,6-25 mU/l) in fasting state.

- Classification / prediction of DM
- Beta-cell activity assessment
- Increased levels: insulinoma, insulin resistance / early phase of T2DM, polycystic ovary syndrome, exogenous insulin therapy, sulphanylurea therapy
- Decreased levels: T1DM

HOMA-INDEX CALCULATION (homeostasis model assessment)

Fasting INSULIN * fasting GLUCOSE

Reference range: <4.4

- High values: Indication for insulin resistance

AUTOANTIBODIES

- **Identification of patients at risk**

T1DM: 75-85%+ (otherwise: 0,5%)

Islet cell autoantibodies: ICA
Glutamate acid decarboxilase autoantibodies: GADA
Tirosin-phosphatase IA-2 autoantibodies: IA-2A
Insulin autoantibodies : IAA

DETECTION: with immunoassay / IIF

Genetic testing

HLA-DR3 (30%)

HLA-DR4 (95%) histocompatibility genes
(also present in 40% of non-diabetics)

OTHER hormones

Glucagone, IGF, epinephrine, GH, thyroxin, somatostatin

DIABETIC COMPLICATIONS

EARLY onset:
Ketoacidosis, lactic acidosis, hyper (hypo) glycemia
– blood glucose tests, blood gases, electrolytes

LABORATORY TESTS ARE ESSENTIALLY NEEDED

LATE onset:

- Microvascular
- Macrovascular
- Retinopathy
- Neuropathy
- Nephropathy --- microalbuminuria (30 – 300 mg/day)

NO test for detection , just for risk assessment (EXCEPT: nephropathy)

SHORT TERM COMPLICATION: DIABETIC KETOACIDOSIS (KETONE BODIES)

Reference range: serum 20-40 µmol/l (0,2-0,4 mg/dl)

β-hydroxy-butyrate, acetic acid, acetone.

Under normal conditions: peripheral tissues consume acetic acetate and beta-hydroxi-butyrate. Acetone is produced from acetacetate by decarboxylation.

In fasting, disturbed carbohydrate or lipid metabolism: 3-5 mmol/l, increased urinary excretion. In practice, semiquantitative assessment is adequate.

Urinary strips: acetic acetate and acetone produce violet complex with nitroprussid-sodium.

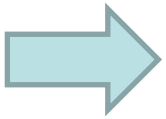
LONG-TERM COMPLICATION: CAD, nephropathy, stroke etc.

- Proteins are glycated in a non-enzymatic manner.

Connective tissue proteins

Serum proteins

Proteins in red cells



Advanced glycation end products

Fructose-amine

Glycated hemoglobin

AGE-generation

Vascular complications are localized to vessel walls.

AGE (advanced glycation end products) play a central role in vascular complications.

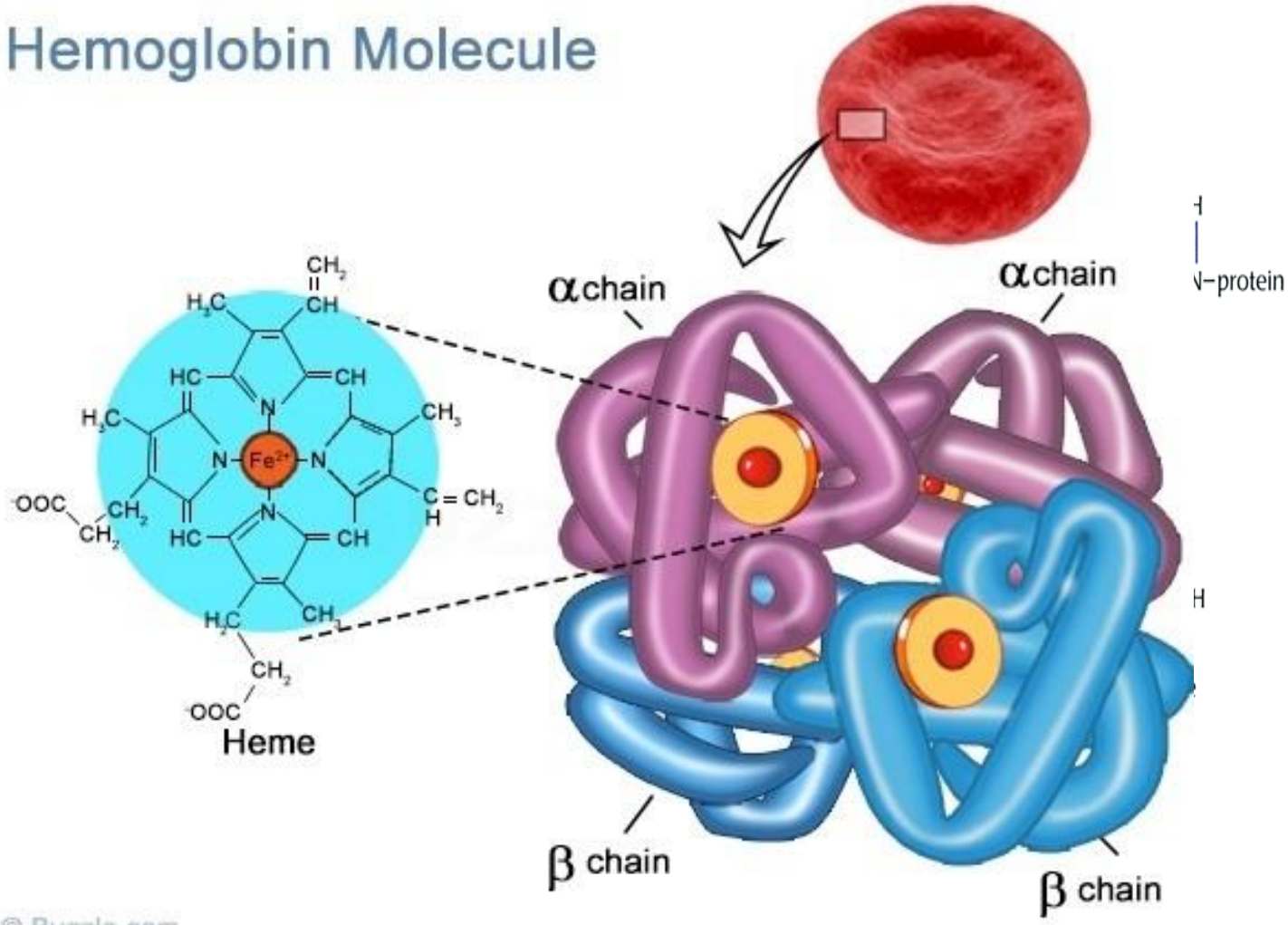
AGE-measurement

Skin autofluorescence = SAF

Unfortunately, AGEs cannot used for monitoring / diagnosis

- Lack of standardization
- Interferring factors
- Association with clinical outcome / parameters is less known, therefore surrogate marker is required

This marker is the glycated hemoglobin



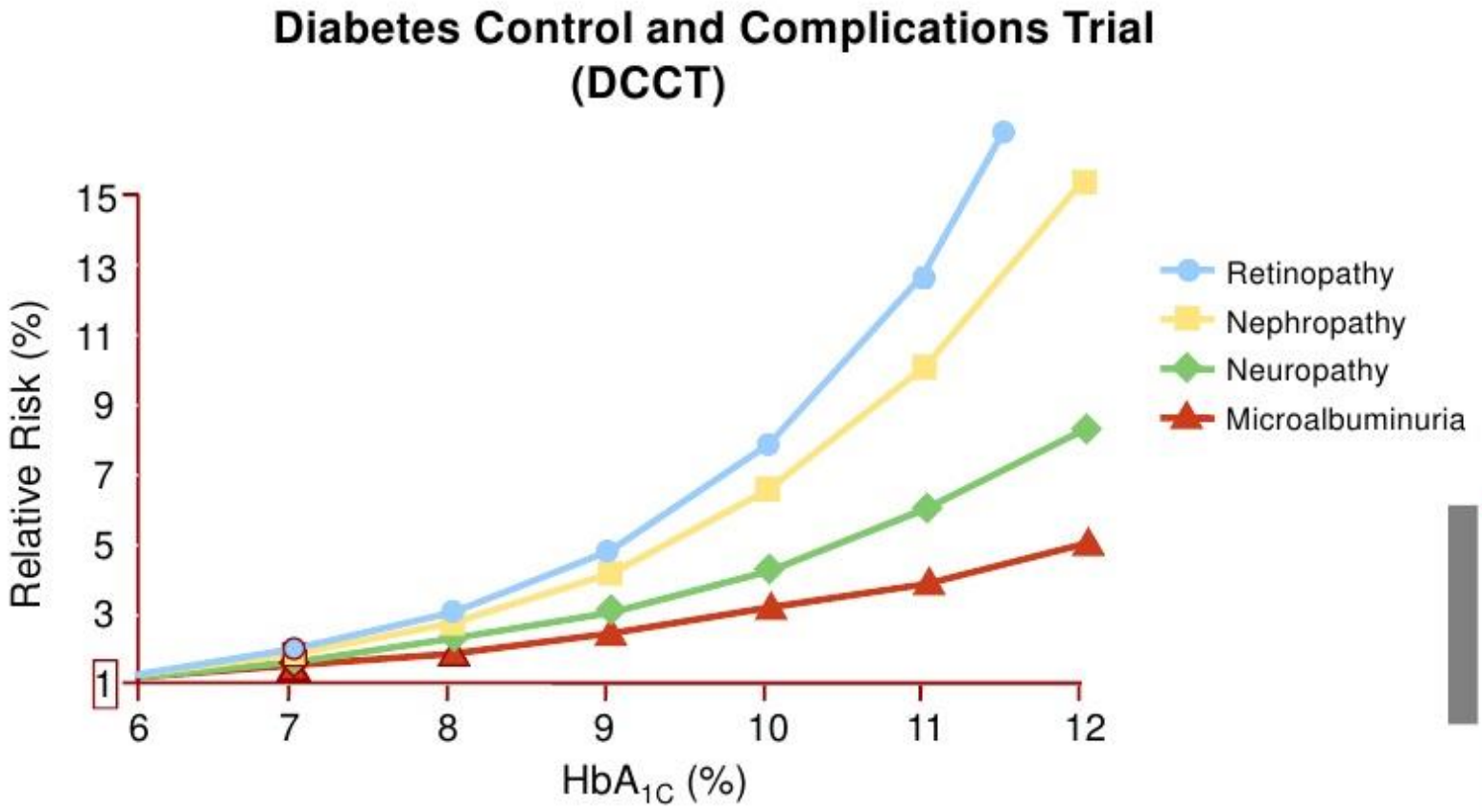
HbA1c assessment: indicator of long-term metabolic state

Self-monitoring of blood glucose levels support the appropriate dosing of insulin and the dietary modifications.

To assess the long-term efficacy of therapy HbA1c determination is needed (reflects the blood glucose levels of the last 2-3 months)

HbA1c helps to decide the appropriateness of therapy and to assess patient's compliance with therapy. More the average glucose higher the HbA1c

Relationship of HbA_{1C} to Risk of Microvascular Complications

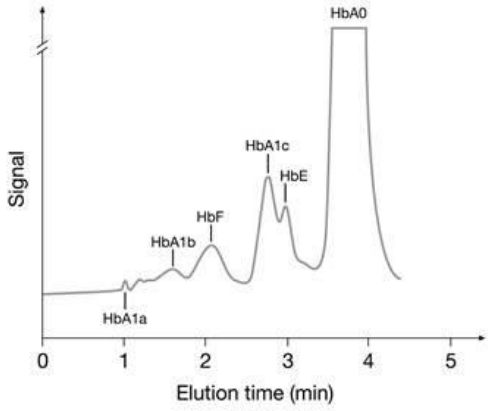


Skyler JS. Endocrinol Metab Clin North Am. 1996;25:243-254.

IMPORTANT

- 1% increase in Hb A1c: 18% and 28% increase in risk of cardiovascular and peripheral artery disease, respectively
- Appropriate blood glucose control is required
- Hgb A1c is recommended to be <7% in DM

What is measured?



HEMOGLOBIN:
Consists of 4 chains (2 alpha and 2 beta), hem
About cca 250 – 300 million Hb molecules /red cells
Valine on beta chains binds glucose in a non-enzymatic way

HbA0 (a2ß2): 90%
HbA1
- HbA1: N terminal valine, glycated by different sugars
- HbA1a1: fructose 1,6 diphosphate N terminal valine
- HbA1a2: glucose 6 phosphate N terminal valine
- HbA1b: N-terminal valine binding unidentified CHO
- **HbA1c (60-80% within HbA1): glucose bound to N terminal valine**
  (beta-N-1-deoxy-fructosyl component)

HbA2 (α2δ2)
HbF (α2γ2)

Total glycated hemoglobin: HbA1c + Hb glycated on non-N-terminel sites (affecting up 15 other sites; responsible for 40-50%)

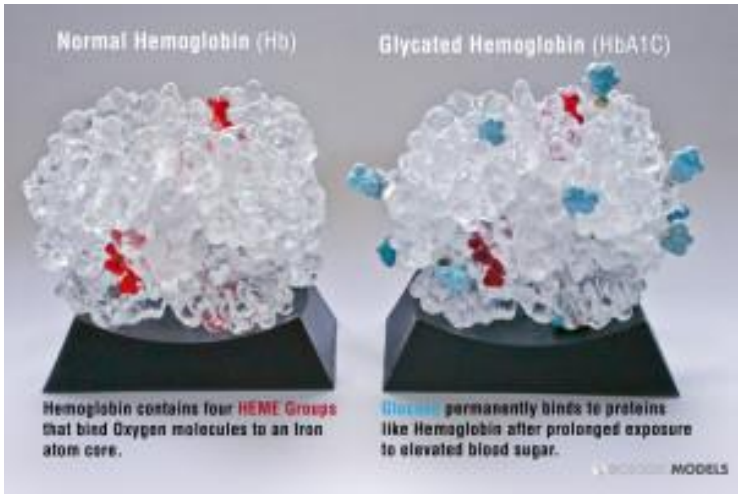
What is used?

- EDTA (purple capped) tube
- Venous / capillary blood
- In some cases: dried blood samples on filter paper

Quite stable:

- 1 week at 2 – 8 °C
- 1 year at -70 °C.
- Avoid storage at -20 °C

Principles of measurement

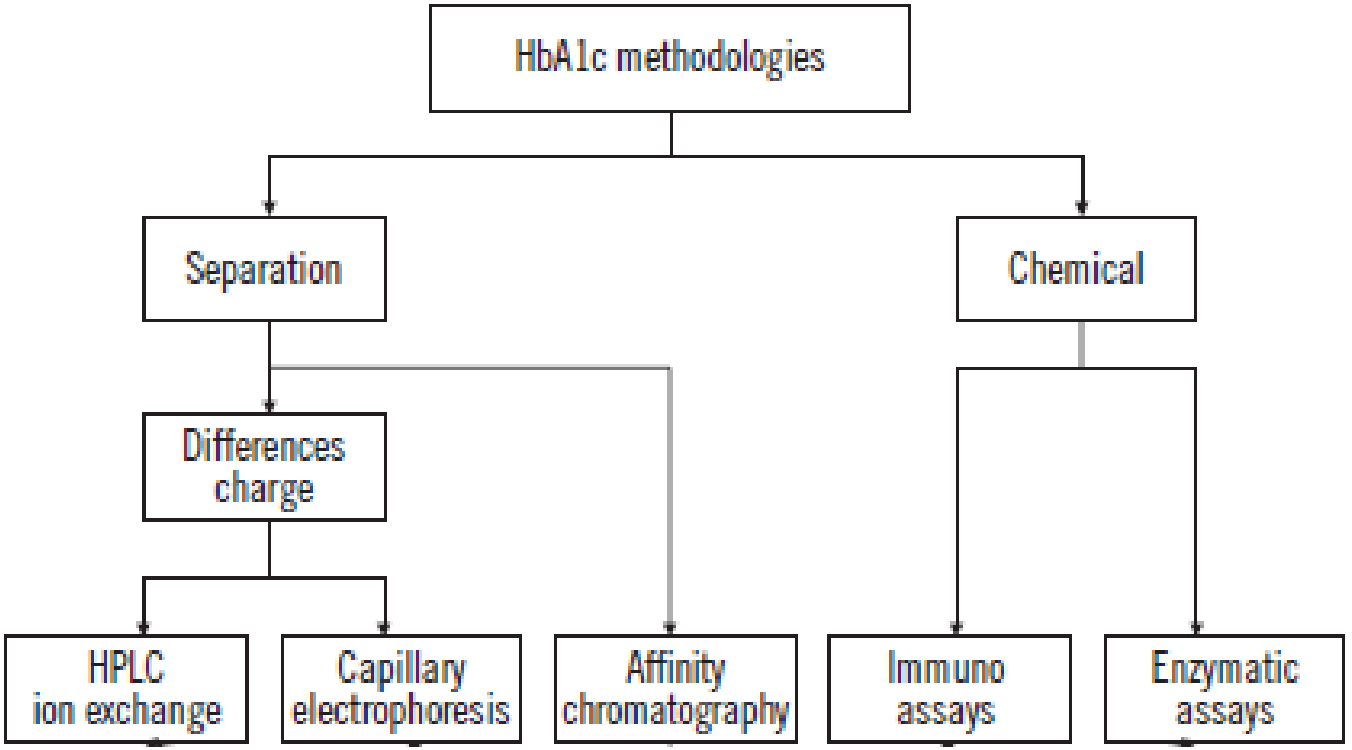


Due to glycation:

1. Charge alters
2. Antigenity changes
3. Structure changes

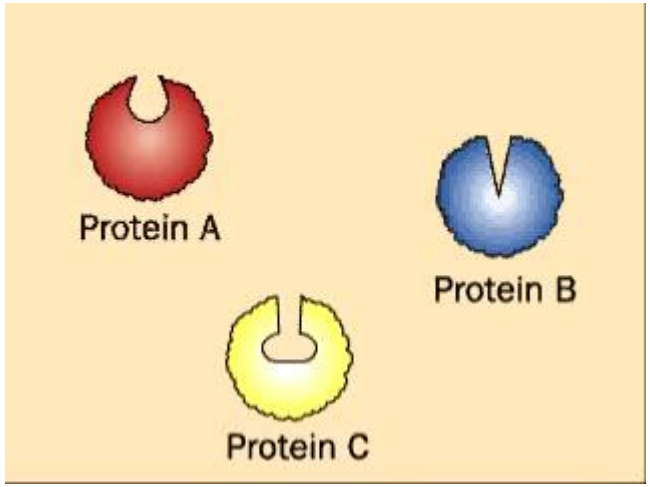
More than 100 methods for HbA1c measurement

Major methods of determination



Approaches used for HbA1c determination: separation techniques

HPLC ion exchanging chromatography



Affinity chromatography

Bio-Rad CDM System **PATIENT REPORT**
CDM 5.1 VII TURBO Instrument **V2TURBO_A1c_2.0**

Patient Data
Sample ID: **Unknown-1-39**
Patient ID:
Name:
Physician:
Sex:
DOB:

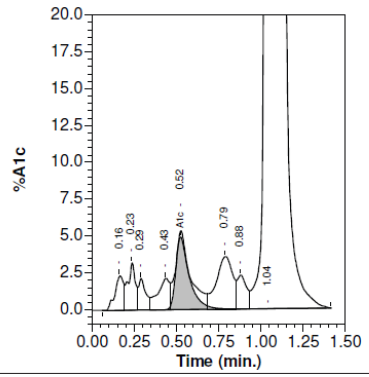
Comments:

Analysis Data
Analysis Performed: 09/06/2010 18:55:19
Injection Number: 39
Run Number: 11
Rack ID: 0006
Tube Number: 2
Report Generated: 11/06/2010 14:35:46
Operator ID:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
A1a	---	---	1.0	0.161	32941
A1b	---	---	1.2	0.232	39666
F	---	---	0.8	0.287	25659
LA1c	---	---	1.3	0.433	42133
A1c	30	4.9	---	0.523	109277
P3	---	---	3.0	0.786	95651
P4	---	---	1.1	0.877	35576
Ao	---	---	88.1	1.040	2814708

Total Area: 3,195,611

HbA1c (IFCC) = 30 mmol/mol HbA1c (NGSP) = 4.9 %



3. ábra: *Nem diabetikus (normál) minta*

Bio-Rad CDM System **PATIENT REPORT**
CDM 5.1 VII TURBO Instrument **V2TURBO_A1c_2.0**

Patient Data
Sample ID: **Unknown-1-38**
Patient ID:
Name:
Physician:
Sex:
DOB:

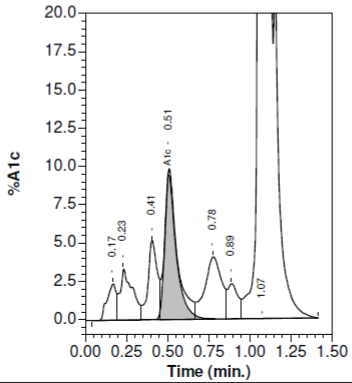
Comments:

Analysis Data
Analysis Performed: 09/06/2010 18:53:42
Injection Number: 38
Run Number: 11
Rack ID: 0006
Tube Number: 1
Report Generated: 11/06/2010 14:35:37
Operator ID:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
A1a	---	---	1.4	0.165	31282
A1b	---	---	2.7	0.230	59971
LA1c	---	---	3.4	0.406	76905
A1c	80*	9.5*	---	0.508	159876
P3	---	---	4.4	0.775	99359
P4	---	---	1.5	0.887	33611
Ao	---	---	79.6	1.073	1797385

*Values outside of expected ranges Total Area: 2,258,389

HbA1c (IFCC) = 80* mmol/mol HbA1c (NGSP) = 9.5* %



4. ábra: *Diabetikus minta, emelkedett HbA_{1c} szinttel*

Bio-Rad CDM System
CDM 5.1 VII TURBO Instrument

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data

Sample ID: **Unknown-1-39**
Patient ID:
Name:
Physician:
Sex:
DOB:

Comments:

Analysis Data

Analysis Performed: 09/06/2010 18:55:19
Injection Number: 39
Run Number: 11
Rack ID: 0006
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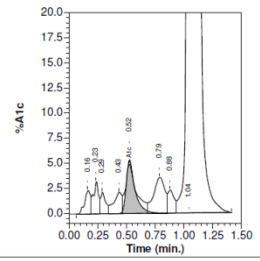

3. ábra: Nem diabetikus (normál) minta

Bio-Rad CDM System
CDM 5.1 VII TURBO Instrument

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data

Sample ID: **Unknown-1-38**
Patient ID:
Name:
Physician:
Sex:
DOB:

Comments:

Analysis Data

Analysis Performed: 09/06/2010 18:53:42
Injection Number: 38
Run Number: 11
Rack ID: 0006
Tube Number: 1
Report Generated: 11/06/2010 14:35:37
Operator ID:

Peak Name	IFCC mmol/mol	NGSP %	Area %	Retention Time (min)	Peak Area
A1a	---	---	1.4	0.165	31282
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LA1c	---	---	3.4	0.406	76905
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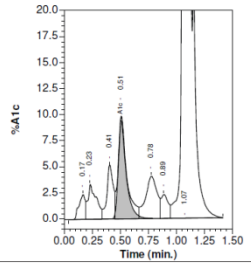


4. ábra: Diabetikus minta, emelkedett HbA_{1c} szinttel

Bio-Rad CDM System
VARIANT II TURBO Instrument

PATIENT REPORT
V2TURBO_A1c_2.0

Patient Data

Sample ID: Unknown-1-4127
Patient ID:
Name:
Physician:
Sex:
DOB:

Comments:

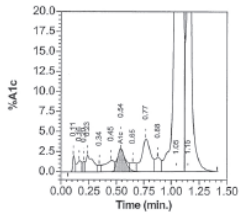
Analysis Data

Analysis Performed: 12/08/2008 16:39:46
Injection Number: 4127
Run Number: 118
Rack ID:
Tube Number: 10
Report Generated: 12/08/2008 16:46:02
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.5	0.110	13060
A1a	---	0.7	0.164	17811
Unknown	---	0.3	0.202	8393
A1b	---	1.7	0.234	41421
Unknown	---	0.3	0.341	7591
LA1c	---	1.2	0.448	30371
A1c	5.2	---	0.538	59572
Unknown	---	0.7	0.646	17213
P3	---	4.1	0.766	101523
P4	---	1.2	0.877	28772
Ao	---	50.2	1.046	1243857
Variant Window	---	36.7	1.150	909532

Total Area: 2,479,116

A1c Concentration = **5.2 %**



5. ábra: Több kisebb mennyiségű komponens (ismeretlen csúcsok) integrálva
MEGJEGYZÉS: *A CHB az LA1c adatokban tilda.*

Methods of HbA1c measurements: general clinical chemistry analyzers

Immune complex testing

SAMPLE

Digestion with proteases

fructosyl valine oxidase (FVO) enzyme
Oxidises glycated valine

H_2O_2 is generated

Color product with peroxidase

Enzymatic test

Methods: benefits and risks

Separation techniques
(CV%: <2-3%)

Benefit: reliable, Hb variants are seen,

Drawback: specific resort, specific device, more expensive

Immune analytics/ enzymatic assay
(CV%: 5-6%):

Benefit: large throughput, quick, cheap, no interference with Hb variants

Drawback: Hb variant is not seen, less precise due to two simultaneous tests

Problems with HbA1c measurements:

ABNORMALITIES WITH THE QUANTITY / TURNOVER OF HB

- Hemolytic anemia → age of red cells decreases → HbA1c levels may decrease
- Higher age of red cells (iron deficient anemias) → HbA1c levels may increase

ABNORMALITIES WITH THE QUALITY OF HB

- Hemoglobinopathies (Hgb F,Hgb C) → falsely low HbA1c (cave: neonates)

- Severe renal and liver disorders may interfere (posttranslational modifications)

Other interferring factors

Increases	Decreases
Hypertriglyceridemia (IEC+)	E/C-vitamin
Jaundice (IEC+)	pregnancy
aspirin	Acute/chronic blood loss
Uremia	malaria
Aplastic anemia	
Age (0,1% per decade)	

Evaluation of test results

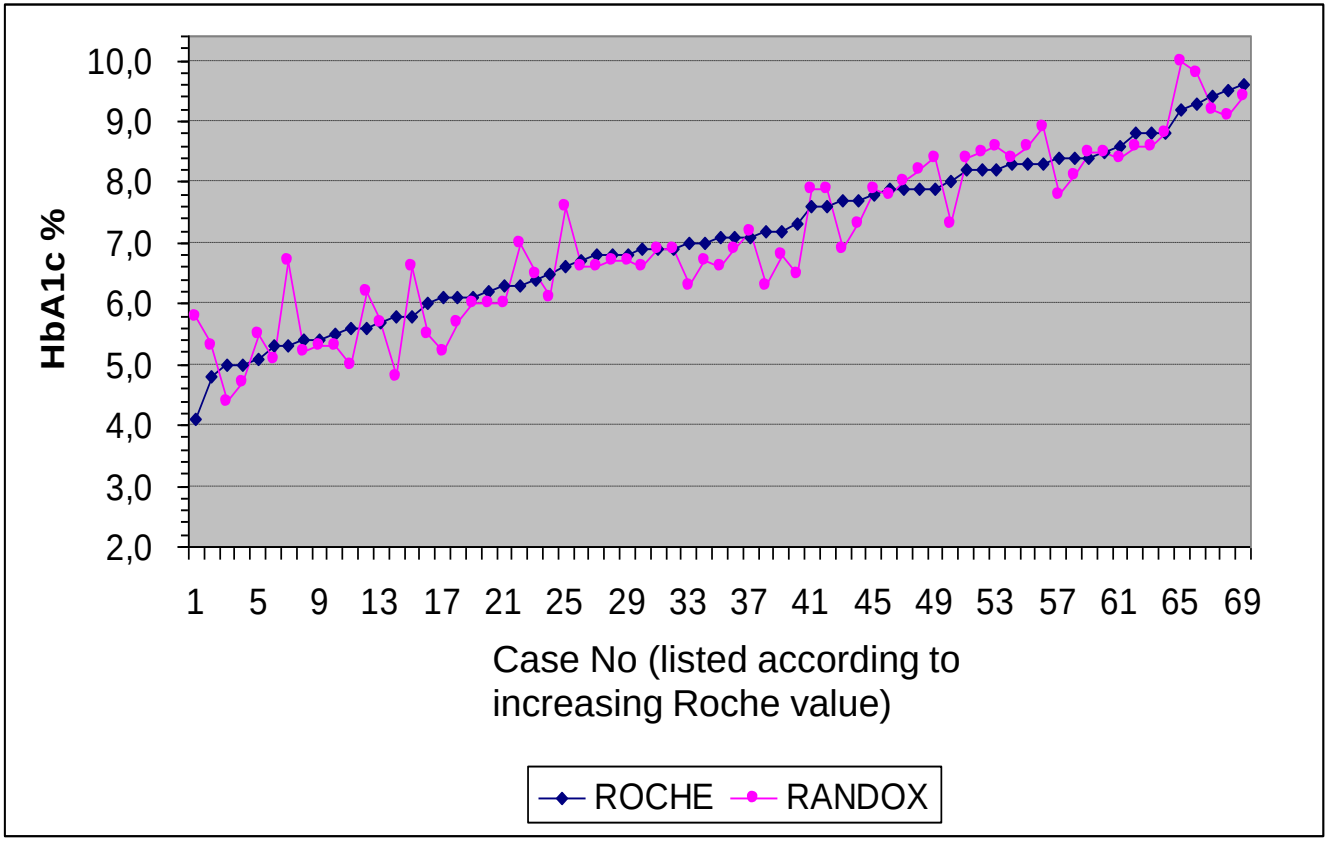
Clinically significant change: 0.5% change in consecutive samples.

Prerequisite:
Relative SD (CV%) should be

- Intralab: <3%
- Between lab, between method <5%;
- For one method <3%

This is not natural

Own study: Tina-quant and Randox HbA1c immunassay



Beko G [Introducing the new laboratory standard for HbA1c determination in Hungary]. Orv Hetil. 2011 Apr 3;152(14):555-8.

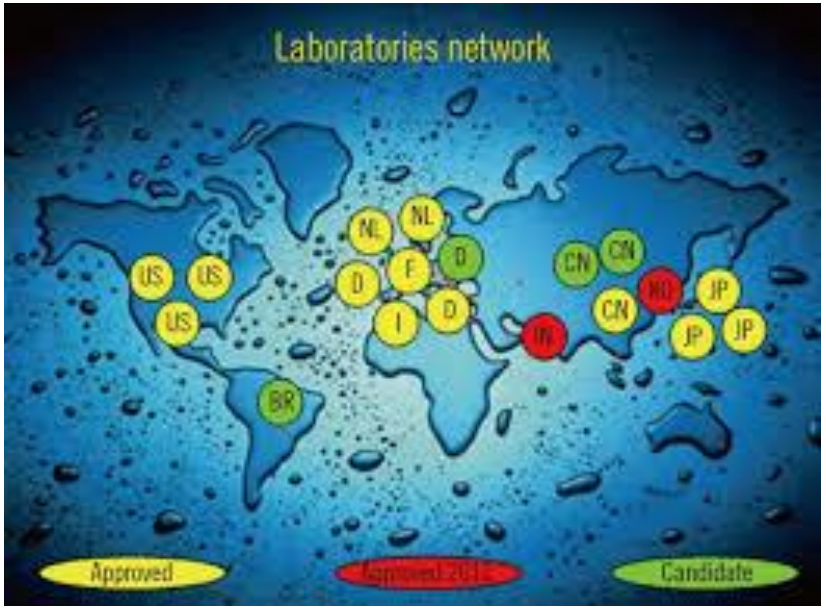
Solution: standardisation

Worldwide base of comparison: a reference method (IFCC)

Development of a ref materia: defined mixture of pure HbA1c standard and HbA0.

Then proteinase is used for digestion. Tested with HPLC-CE or HPLC-MSk.

Globally IFCC- reference labs. Manufacturers' standards are measured here.



http://www.ngsp.org/

As a result

1. Different results obtained with different methods can be compared
2. Patients can be monitored
3. Possibility to switch to SI units (mmol/mol)

Unit

mmol/mol or %?

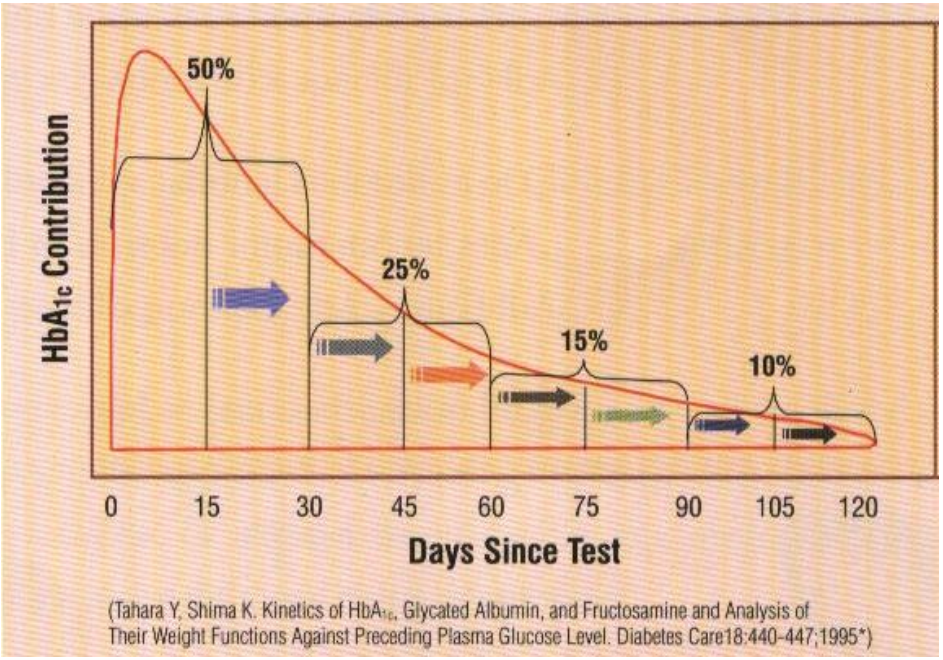
NGSP% = 0.0915 * IFCC mmol/l + 2,15

IFCC mmol/l = 10.93 NGSP% - 23,5

HbA1c (%)	HbA1c (mmol/mol)
13	119
12	108
11	97
10	86
9	75
8	64
7	53
6	42
5	31

What the result can be used for?

Monitoring the metabolic state: HbA1c indicates average glucose levels during the last 2-3 months



50%, 40% and 10 % of HbA1c are derived from days before 1-30, 31-90 and 91-120.

HbA1c and eAG

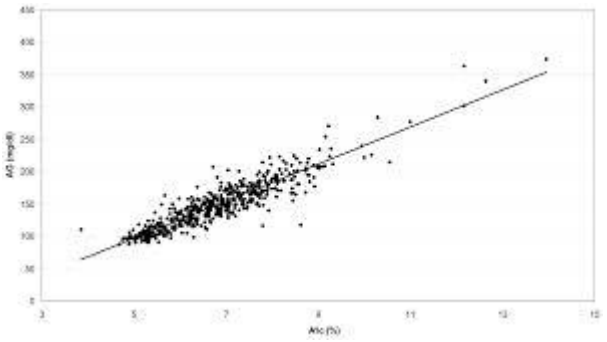
Estimated average glucose: eAG:

ADAG study (supported by ADA, EASD & IDF)
determined regularly blood glucose levels of 507 T1DM, T2DM and controls
(for at least two days CGM, 4 times, at least 3 tests per week at home vércukorszint-ellenőrzés

ADAG Study: "Translation" of HbA1c into eAG

HbA1c (%)	eAG (mg/dl)	eAG (mmol/l)
5	97	5.4
6	126	7.0
7	154	8.6
8	183	10.2
9	212	11.8
10	240	13.4

www.diabetes.org 1-800-DIABETES

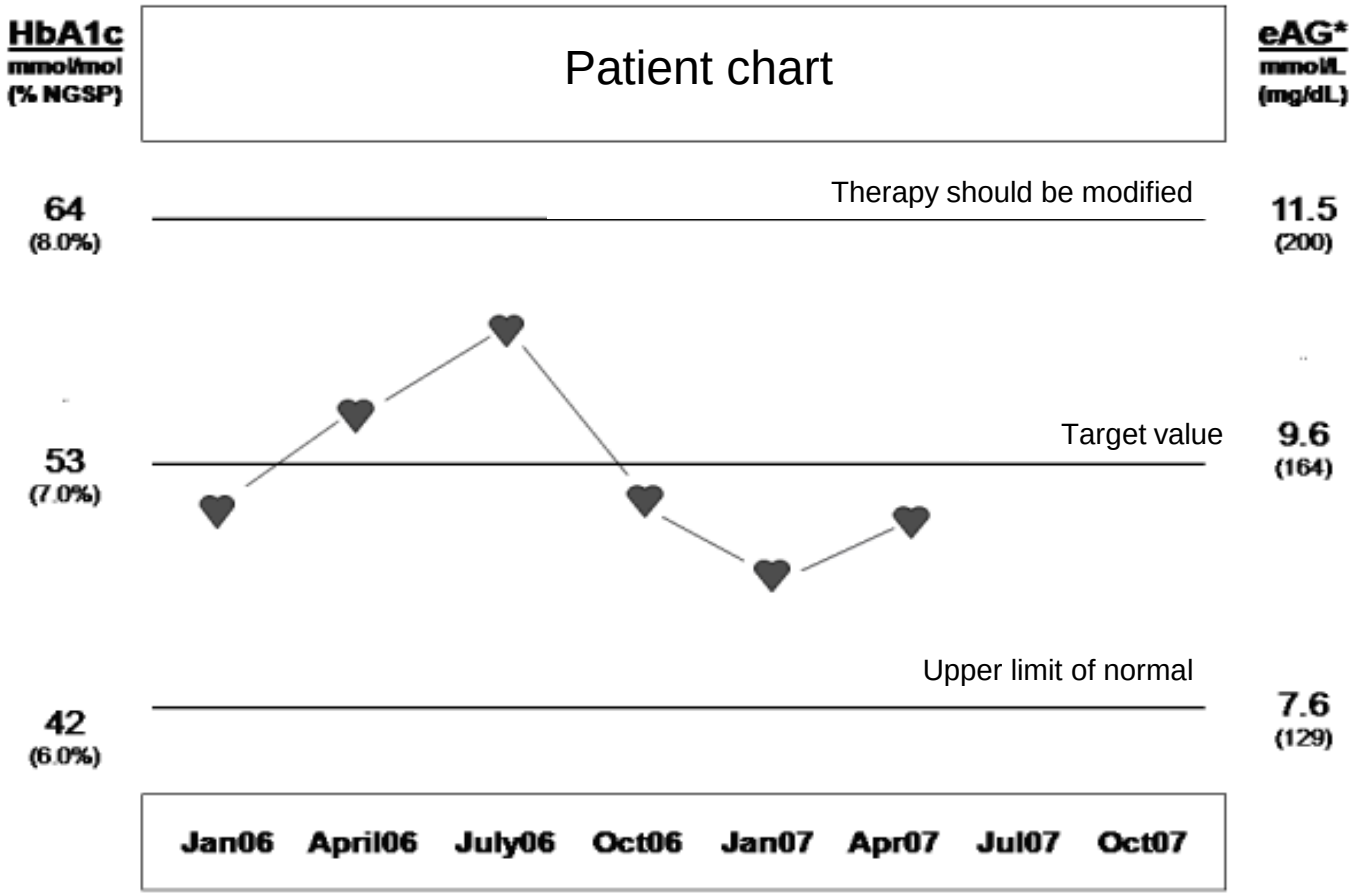


eAG(mg/dl)= (28.7*HbA1c)-46.7, r2=0.84

Limitation: few clinical data, particularly in subgroups

Diabetes Care 2008;31:1-6

The information provided by the eAG



IMPORTANT: eAG reflects the average blood glucose level representative for a given period and it is not the same as that measured at home

HbA1c: target values and DM diagnostics

Standard interpretation norm*	IFCC (mmol/mol)	NGSP (%)
Normal reference range	20-42	4-6

HbA1c or glucose levels in DM diagnostics

HbA1c benefits:

1. Stable after sampling
2. Intraindividual – between day variation: <2%
   (fasting glucose: 10-15%)
3. stress, acute disease has less impact
4. Easy sampling

General target: <7%
<5,8%: low risk of DM
>6,4%: definitive DM
5,8 – 6,4%: increasing risk of DM

Questions regarding diagnostic use of HbA1c

Standard interpretation norm*			IFCC (mmol/mol)	NGSP (%)
Normal reference range			20-42	4-6
Decision limits	Monitoring therapy	Target treatment	53	7
		Limit change therapy	64	8
	Diagnosis	Low risk	<40	<5.8
		Increasing risk future diabetes	40-46	5.8-6.4
		Diabetes	>46	>6.4

1. CUTOFF values: clearly the same?
2. Imprecision of methodology?
3. What is to be done with grey zone?
4. Impact of other conditions?
5. How the classification is affected (IGT, prediabetes)?
6. Relationship to OGTT?

<u>Currently: rather risk assessment than diagnosis</u>

HbA1c: POCT

ISSUE of quality

POCT tools can be used only when their performance fulfills inner / outer QC program criteria (CV% values)

Diagnostic value of HbA1c is limited under the following conditions

Can be used for diagnosis in the absence of following conditions:

- pregnancy
- Type 1 diabetes
- Newly developed diabetes
- acute pancreas disorder
- Drug induces hyperglycemia
- hemoglobinopathies
- Severe anemia
- Renal failure
- Liver failure
- dialysis
- HIV infection

HbA1c target values are not universal

Target values should be defined individually.

- Lower target: long life expectancy, less intentsive therapy
- High target: for patients with bad conditions, hypglycemic tendencia, childhood and adolescence, advanced vascular complications
- Very low target: the vascular complications are increased / risk of hypoglycemia

How often should it be tested?

Recommended frequency in diabetics:
Twice per year for stable disease;
4 times per year for patients with bad control, / after switch in therapy.
Hospitalization (provided there is no result from the past 3 months)

Test should be repeated (with another method) :
When results do not fit to clinical condition
Non-diabetic disorders affecting HbA1c levels

Hungarian National Health Fund:
Costs: about 4 million Eur/ year
Tests repeated within 3 months are not reimbursed

Fructose-amine: when HbA1c test is contraindicated

FRUCTOSE-AMINE, the indicator of short-term metabolic condition

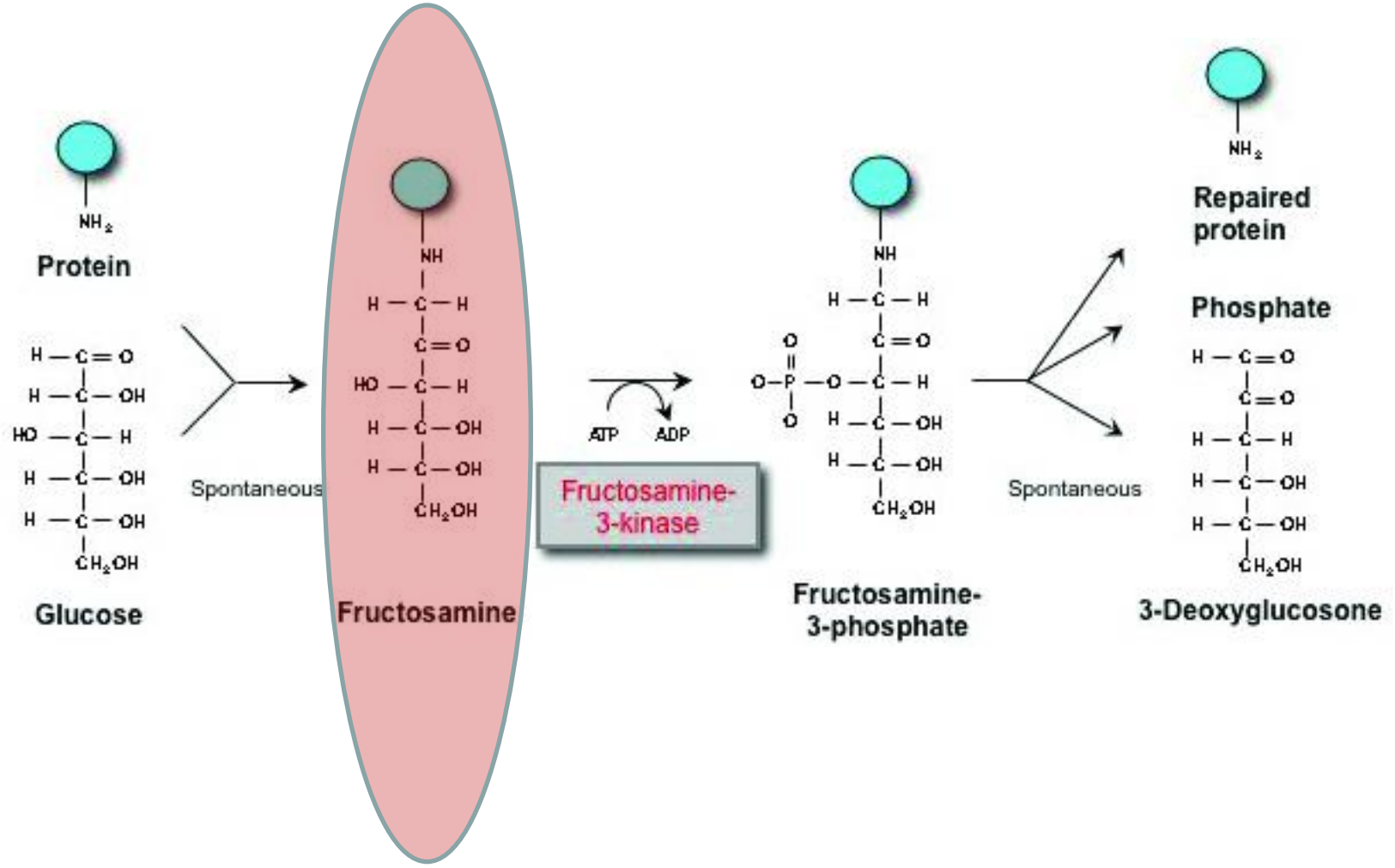
Reference range: 200-285 µmol/l.

Fructose-amine is a protein-ketoamine complex.
It is produced when glucose is attached to albumin.

It indicates the amount of glucose in µmol that binds to albumin in 1 litre of serum.
Half-life is short, it is appropriate to characterize blood glucose levels during the last 2-3 weeks

Useful when HbA1c levels cannot be interpreted.

Fructose-amine



Fructose amine

Several tests for its testing

Lack of standardization
Currently: colorimetric approach, based on chemical reactivity
Association between fructoseamine and HbA1c:

HbA1c=0,017 * fruktózamin (µmol/L)+ 1,61

Necessity for adjustment to protein /albumin levels is not clear

Sample: serum
Analyte is stable for 7 days at 18–25°C, 14 days at 2- 8 °C and 30 days at -20°C.
Hemolyzed sample: falsely increased values

Result is affected by: altered protein turnover
(liver disease, nephrosis
thyroid disease
Paraproteinemia
High vitamin C levels)

Fructoseamine

Reference range: depends on age and gender

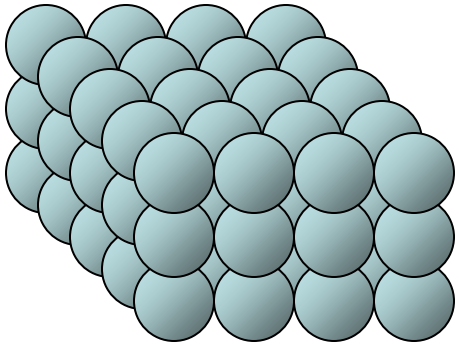
In non-diabetics: 175-280 µmol/L
Adjusted for albumin levels: 4,7-6,5 µmol/g albumin
Lower albumin levels- lower fructose amine levels

Controlled diabetes: 210-421 µmol/L
Uncontrolled diabetes: 268-870 µmol/L

Regular measurement is required

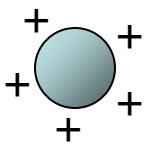
Mass spectrometry for detection

Material



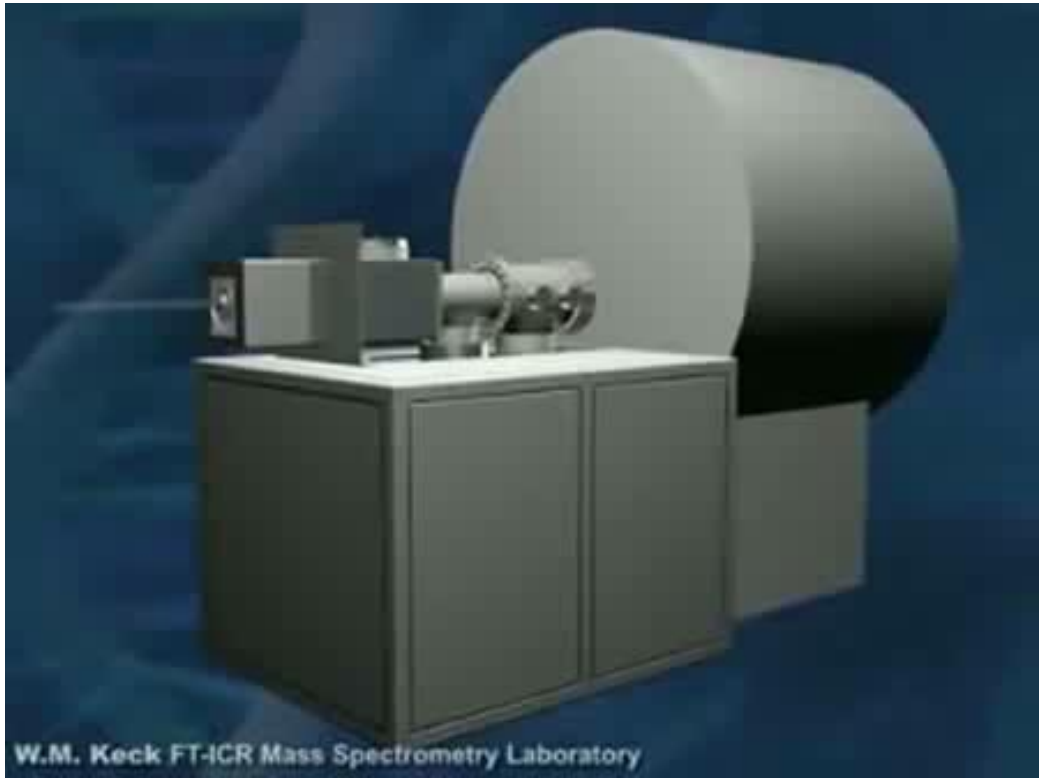
Molecule
•Molecular mass- **m**

Ionisation

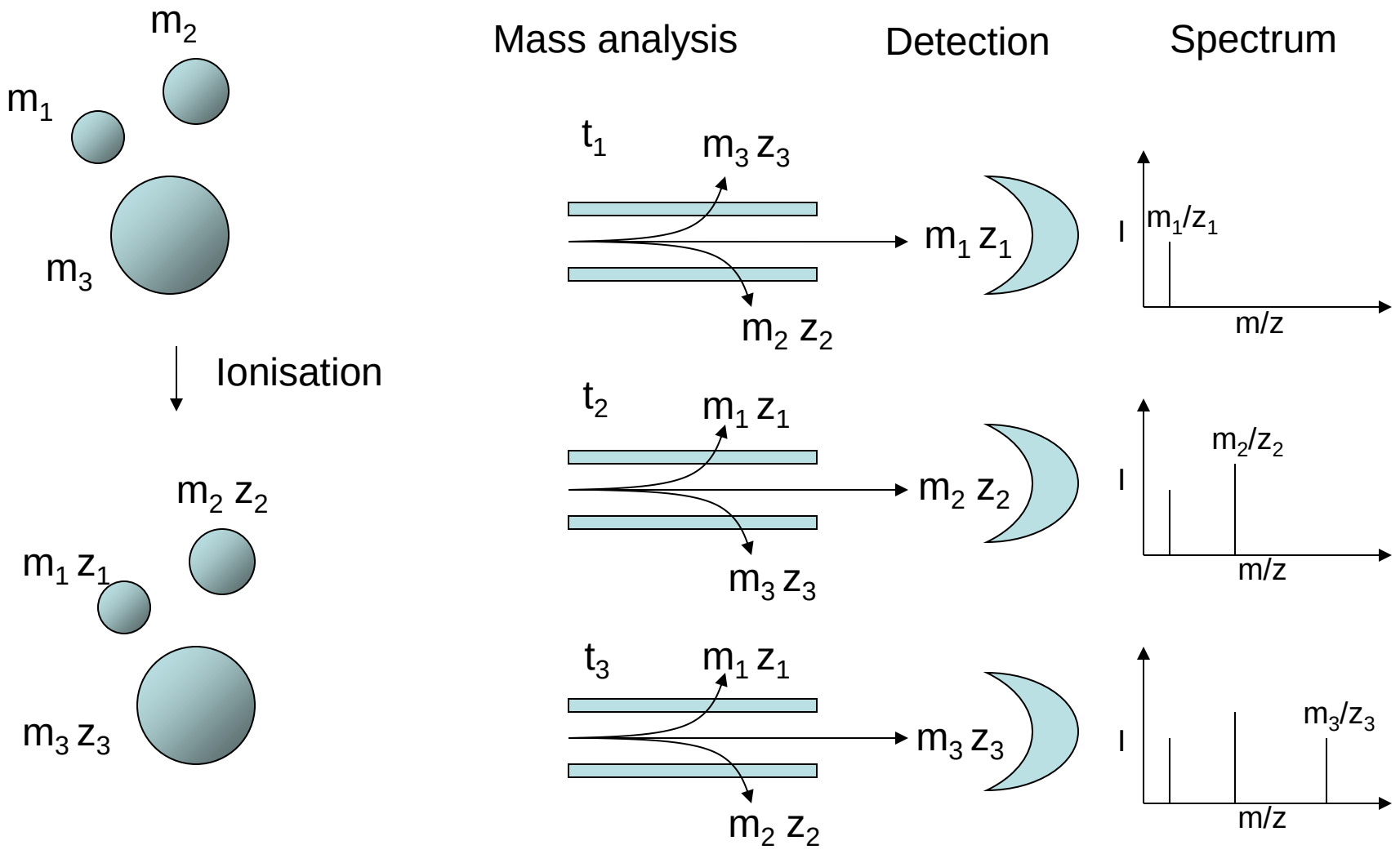


Ion
•Molecular mass– **m**
•Charge – **z**

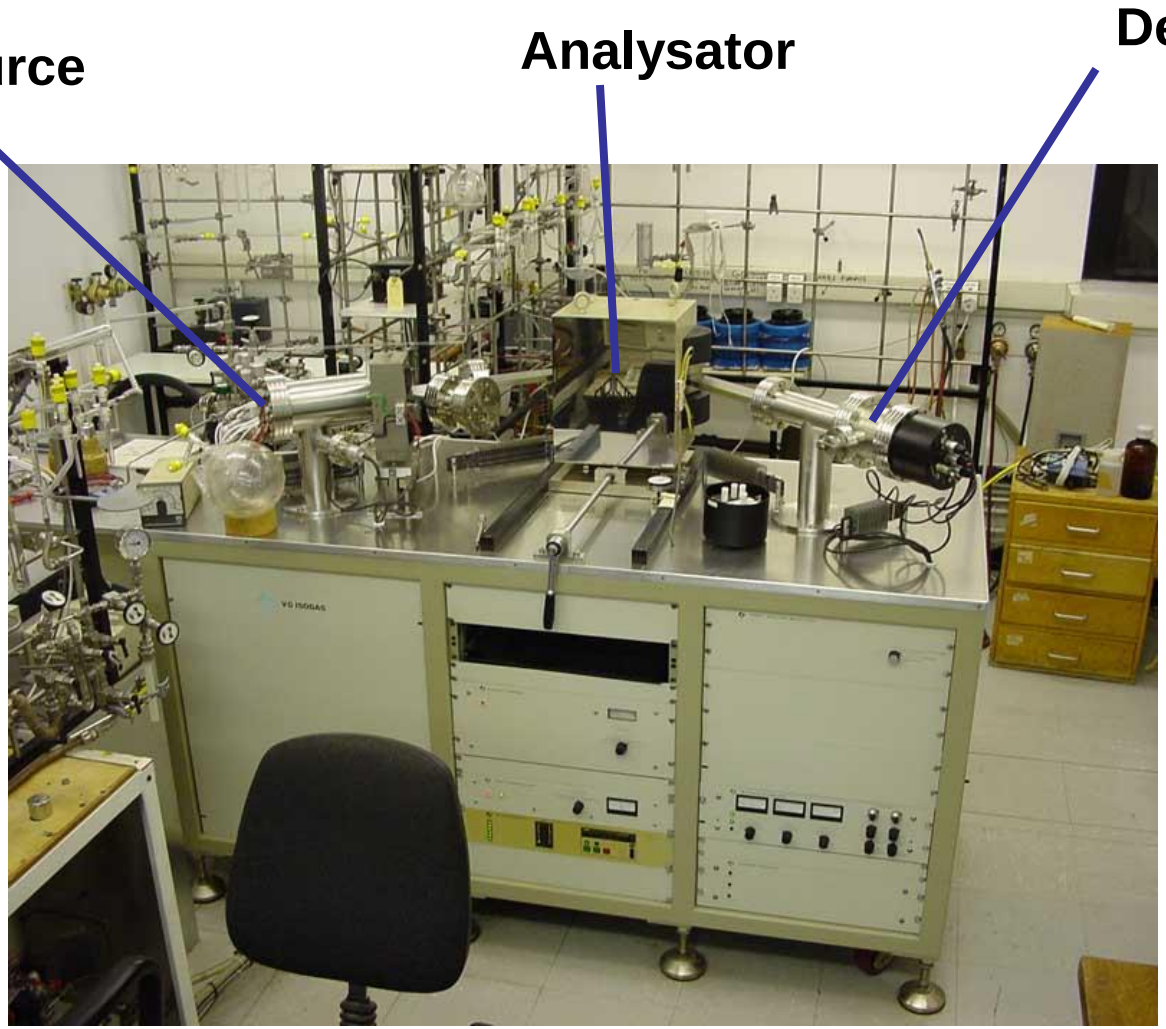
Mass spectrometry
Detection of ions according to m/z values


W.M. Keck FT-ICR Mass Spectrometry Laboratory

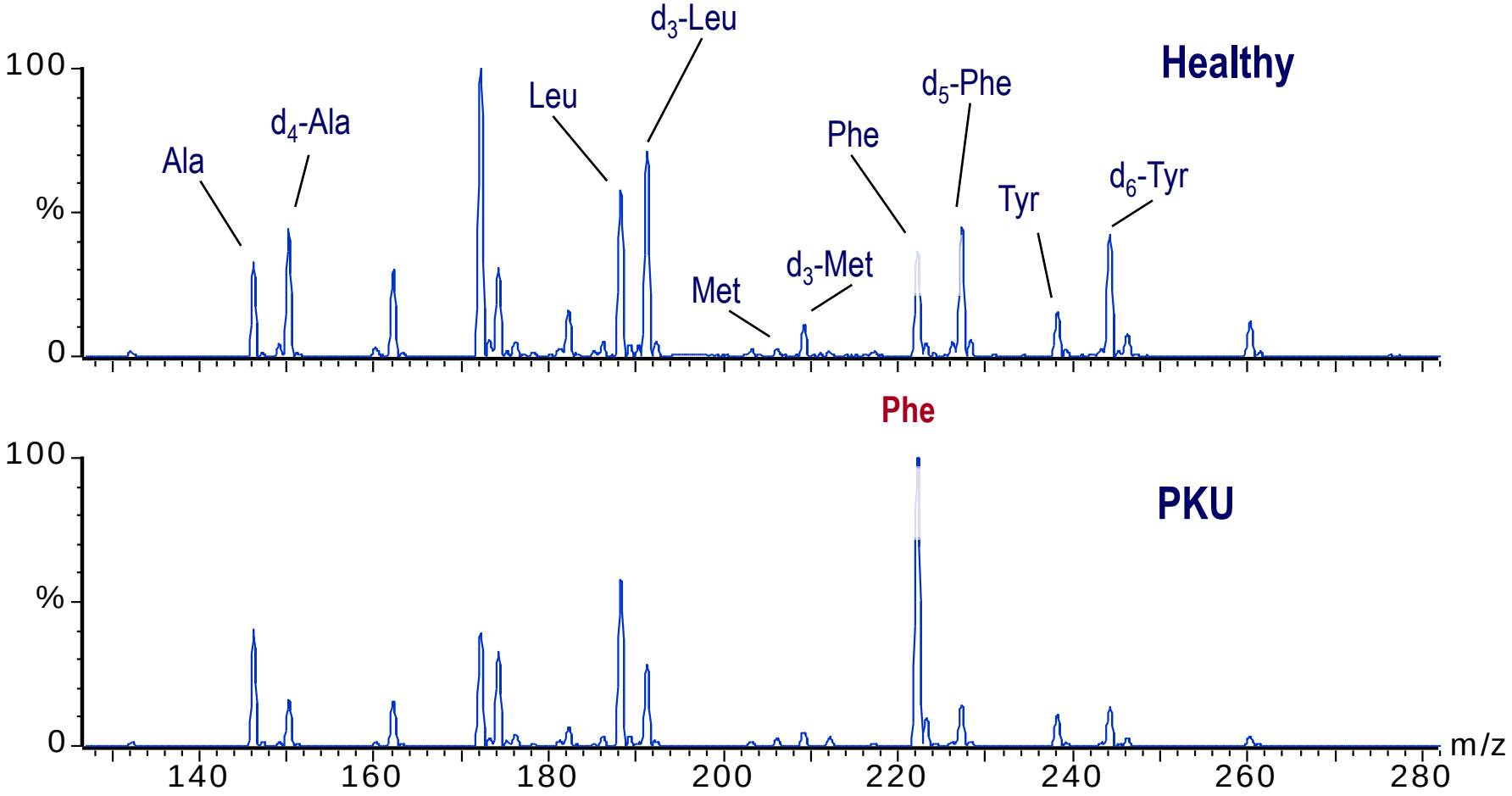
Mass spectrometry

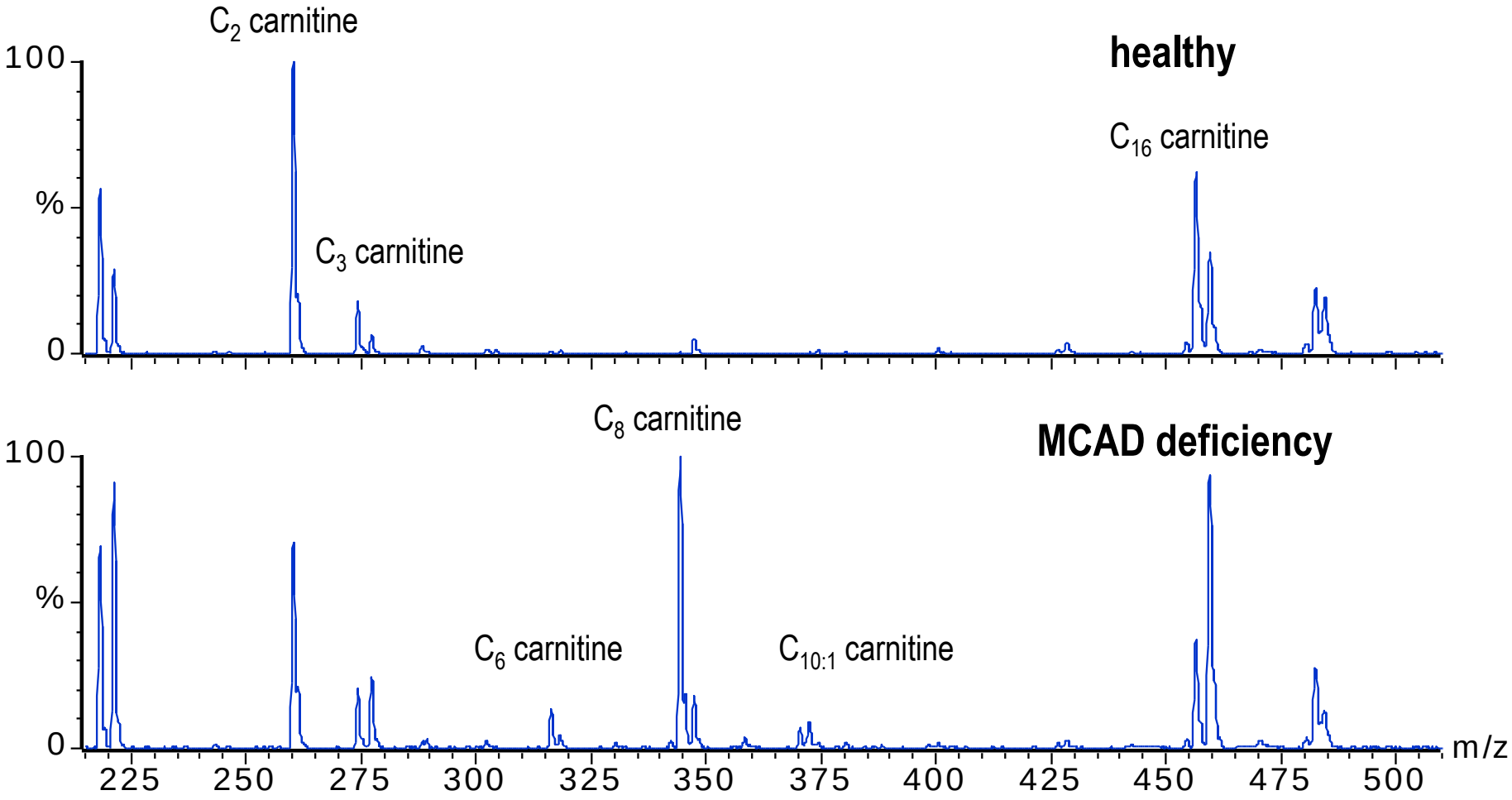


Mass spectrometer



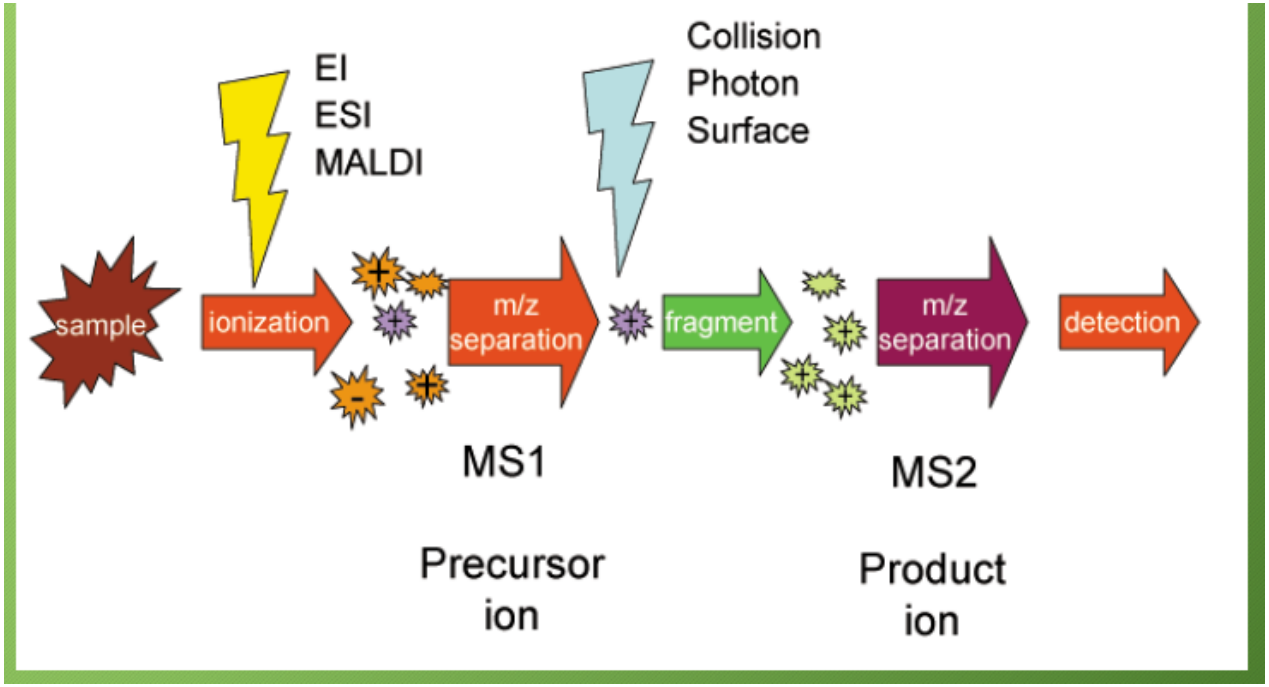
Mass spectrum analysis for newborn screening

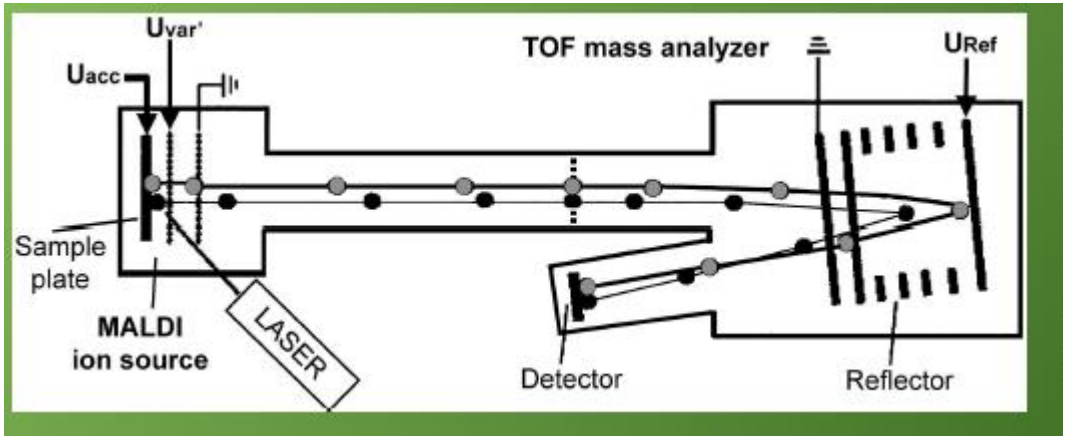



C2 carnitine
healthy
C16 carnitine
C3 carnitine
100
%
0
C8 carnitine
MCAD deficiency
C6 carnitine
C10:1 carnitine
100
%
0
m/z
225
250
275
300
325
350
375
400
425
450
475
500

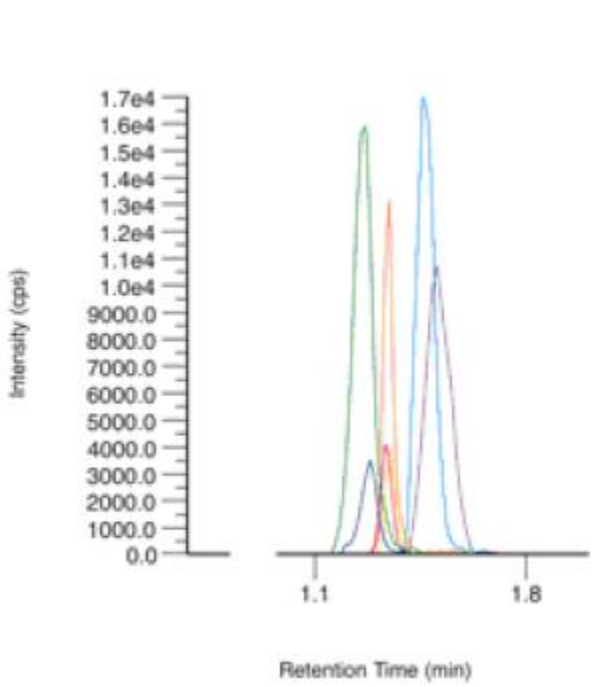
OTHER APPLICATION AREAS FOR MASS SPECTROMETRY

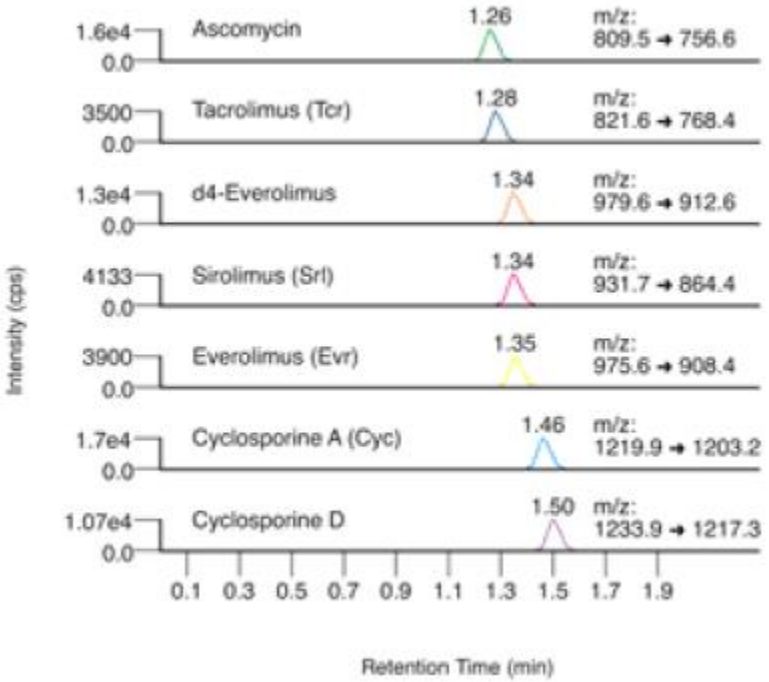
Tandem MS




Uvar'
Uacc
TOF mass analyzer
URef
Sample plate
MALDI ion source
LASER
Detector
Reflector

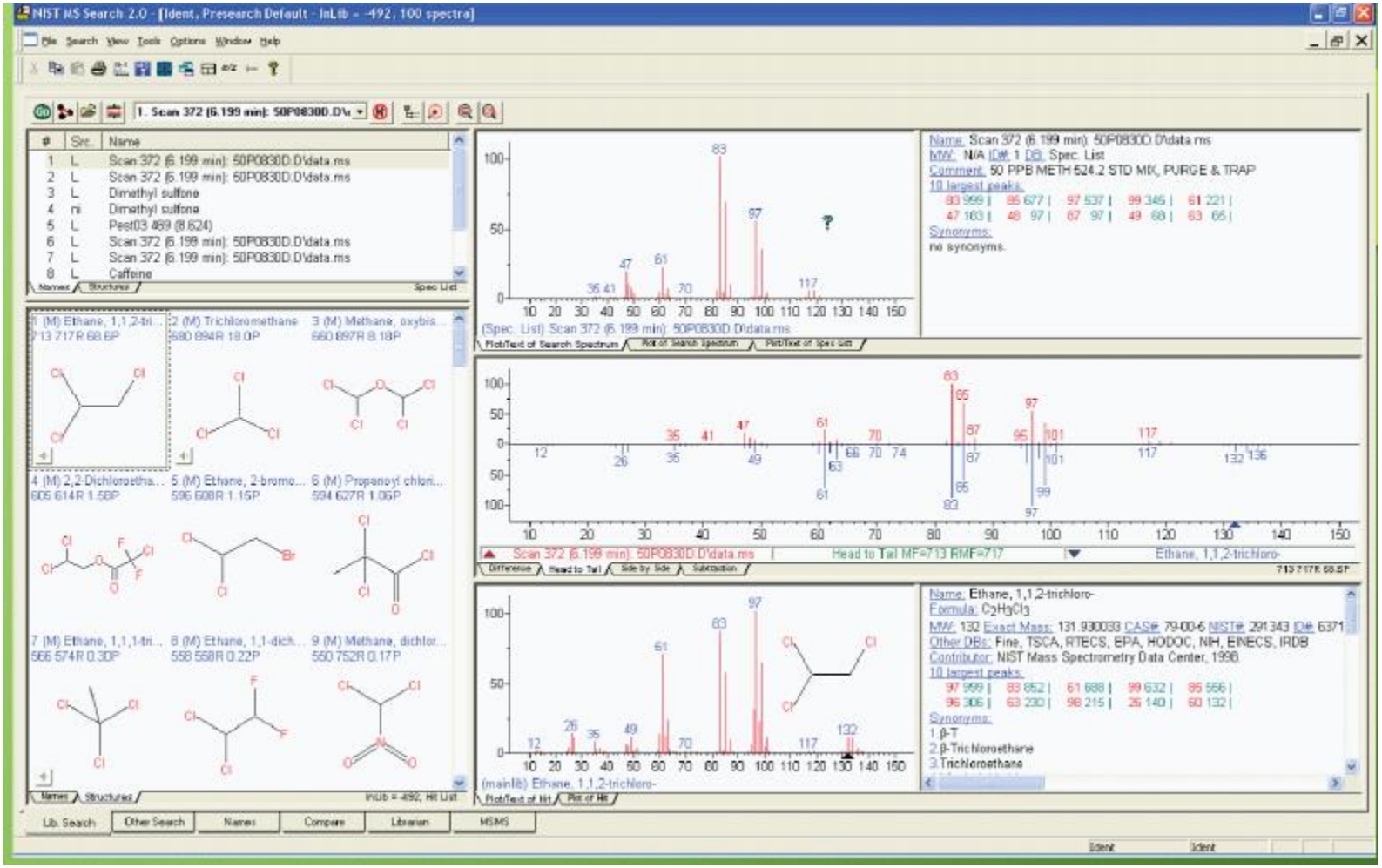
Use in labs: THERAPEUTIC DRUG MONITORING



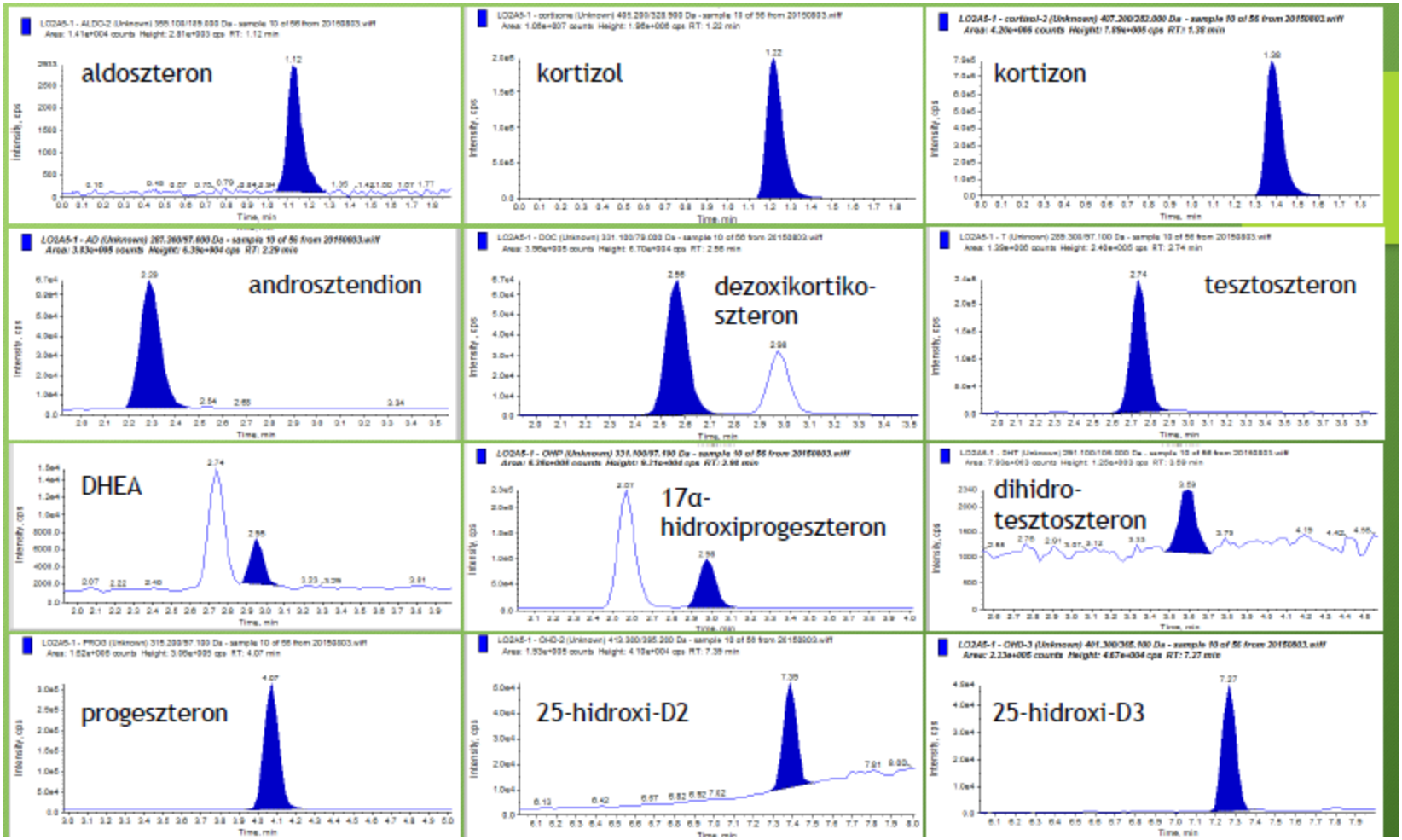


Use in labs: CLINICAL TOXICOLOGY

- Low mol weight substance detection
- Different approaches (modification of separation techniques BEFORE MS)



Use in labs: ENDOCRINOLOGICAL TESTS



COMPARISON OF MS WITH IMMUNOASSAYS

Parameter	MS	IA
Sensitivity	<1 nmol/L	<1 nmol/l
Adjustment of method	possible	cannot be performed
IVD kits	usually NO	usually YES
reliability	can be controlled	black box
maintenance cost	HIGH	moderate
Cost per sample	low	high
professional is required	yes	no
cost of machine	high	medium

Use in labs: IDENTIFICATION IN MICROBIOLOGY

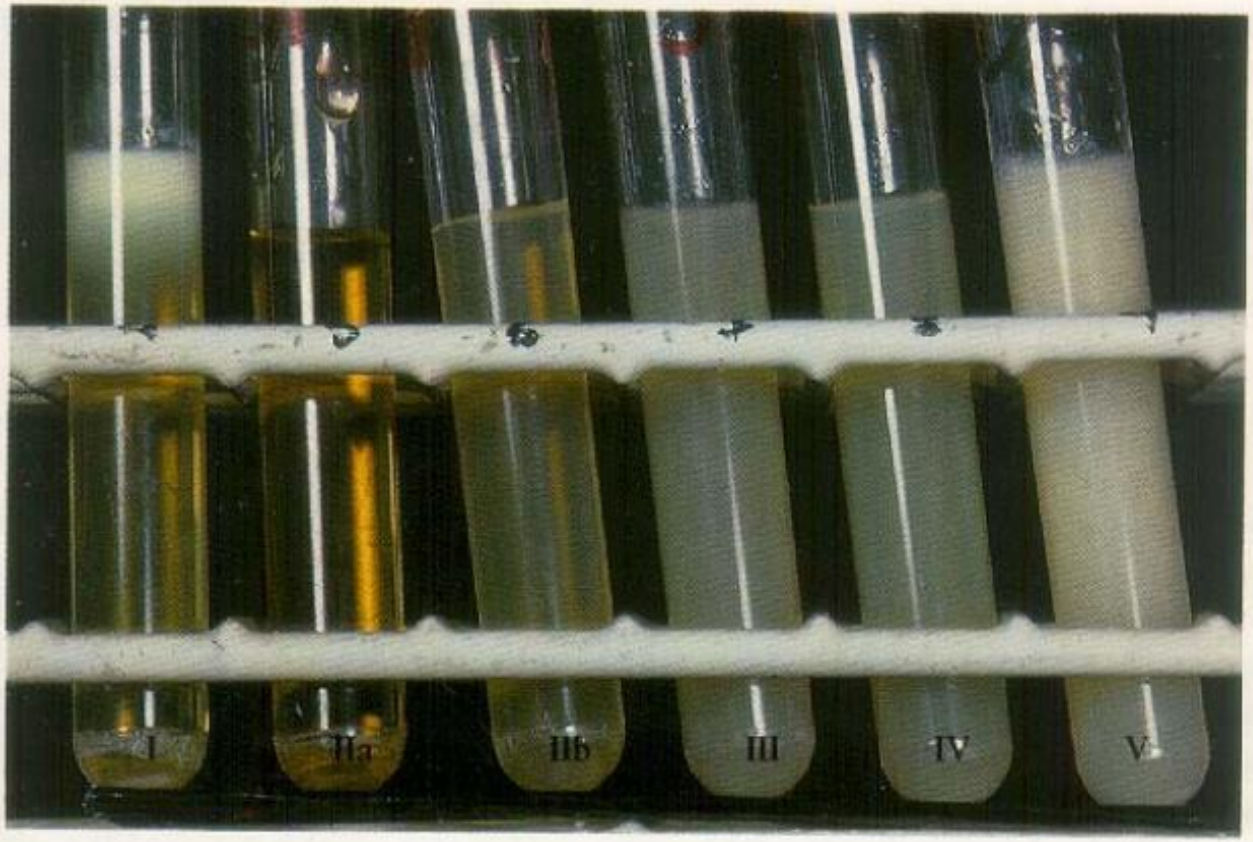
Detection and identification of microorganisms at the level of species

MALDI-TOF techniques

Patterns represent ribosomal protein fragmentation

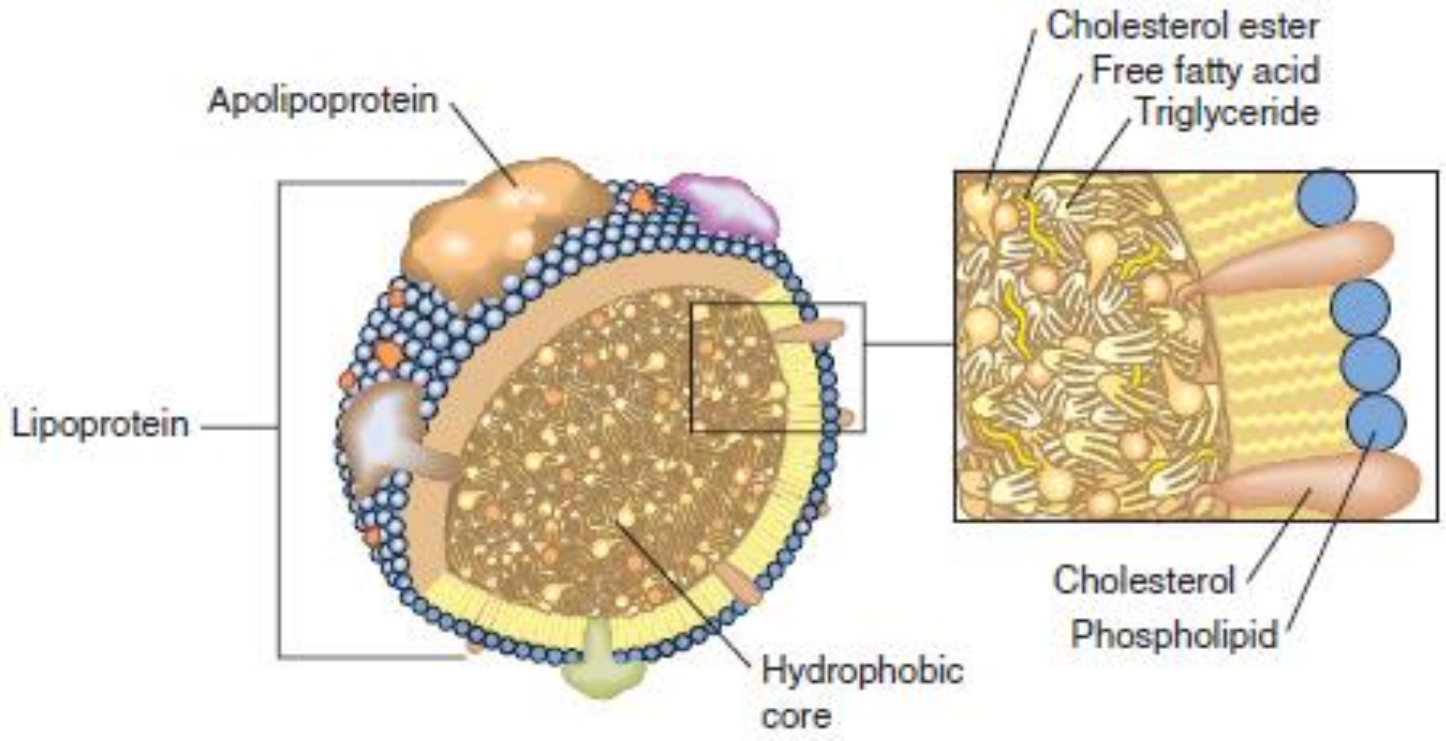
High throughput (10 sec per sample)

DISORDERS OF FAT METABOLISM



12 **Hyperlipoproteinaemic plasmas.** The appearance of fresh plasma from patients with various hyperlipoproteinaemias after 16 hours at 4°C.

Structure of lipoproteins = apoprotein + lipid



HDL & LDL

Disordered fat metabolism

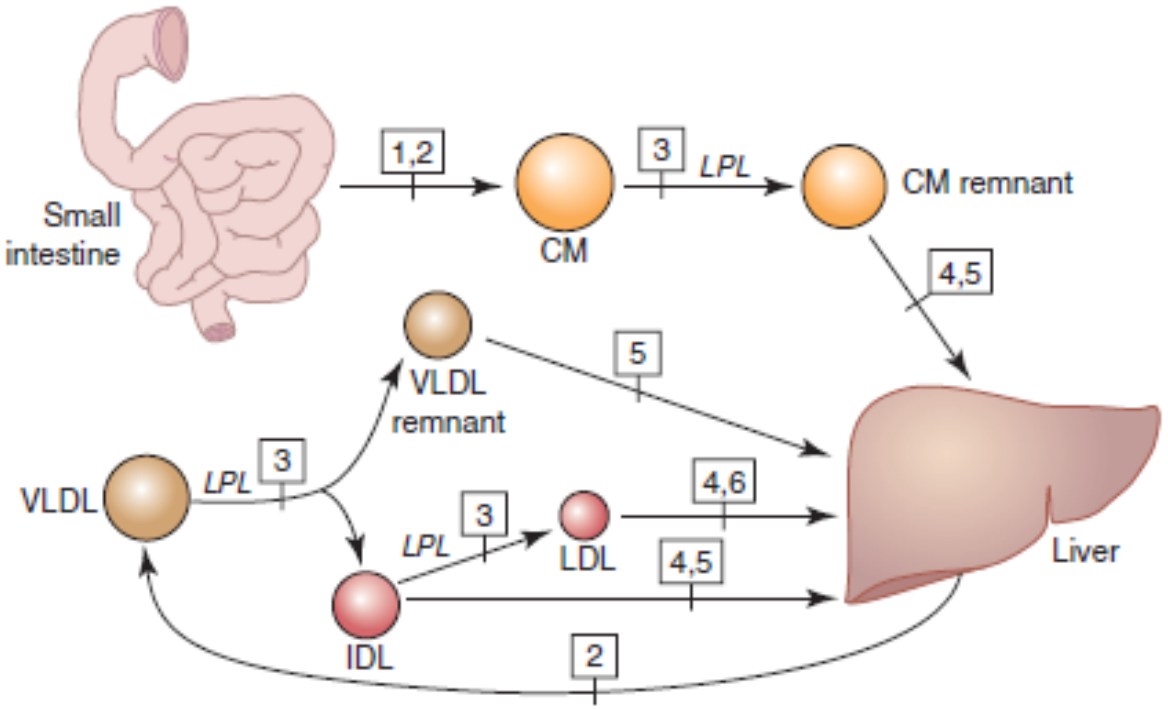
Friedrichsen-type classification (primary hyperlipidemias)

Phenotype	Chol	Tg	Chy	VLDL	IDL	LDL	Genetic cause
I	+	+++	++			low	LpL deficiency, ApoC-II deficiency
IIa	++	normal		normal		++	familiary hyperchol
IIb	++	++		++	normal or +	++	familiary combined hyperlipemia
III	++	++	+	+	++	low	familiary III típusú hyperlipemia
IV	+	++		++		normal	familiary combined HPL familiary hypertg.
V	+	++	++	++		low	familiary hypertg ApoC-II deficiency

Secondary hyperlipoproteinaemia:

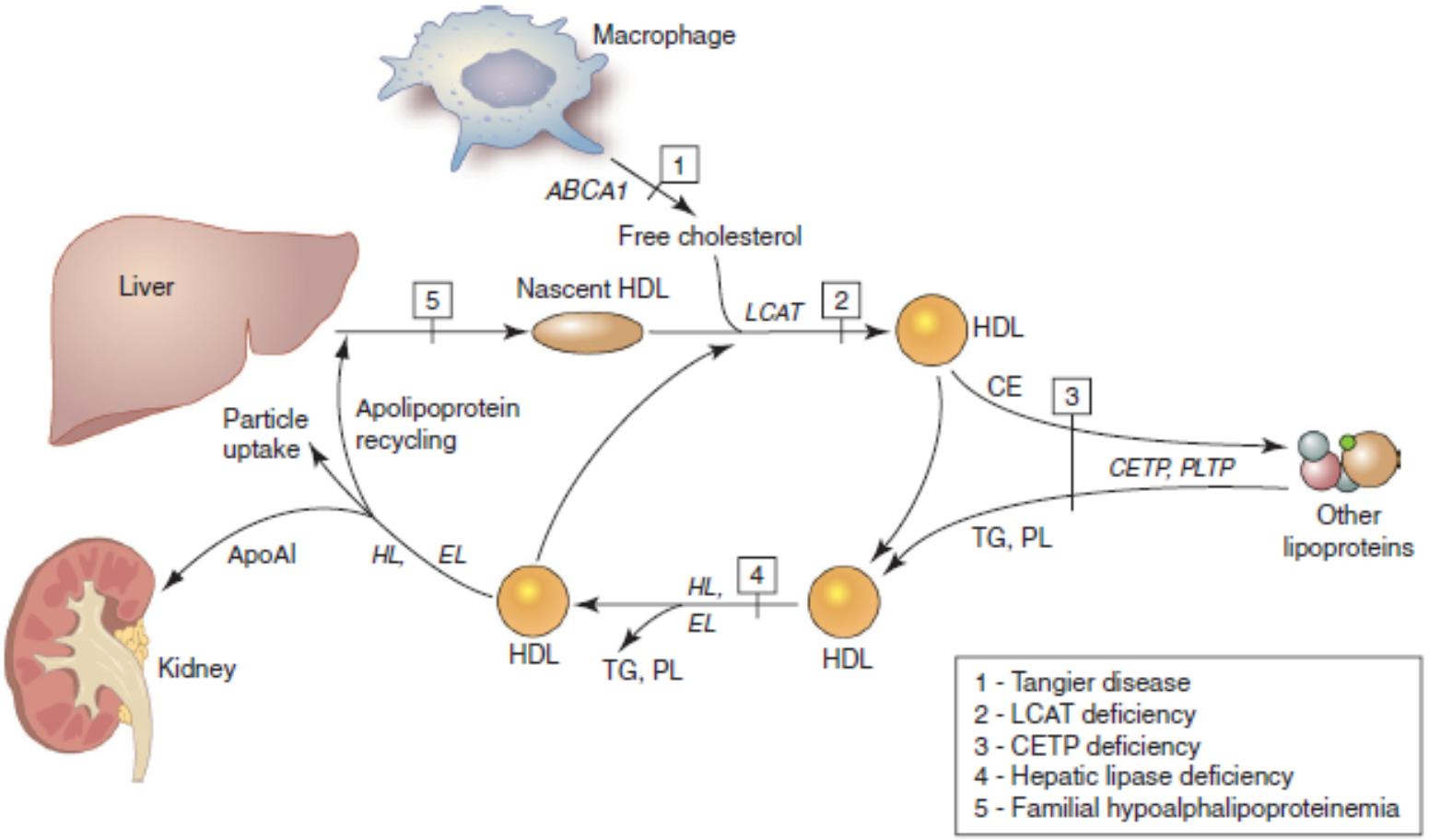
diabetes mellitus
metabolic syndrome
gout
obesity
hypothyreosis
pregnancy
estrogen, steroid therapy
nephrosis syndrome
alcoholism
Drug adverse effect
Obstructive liver disease

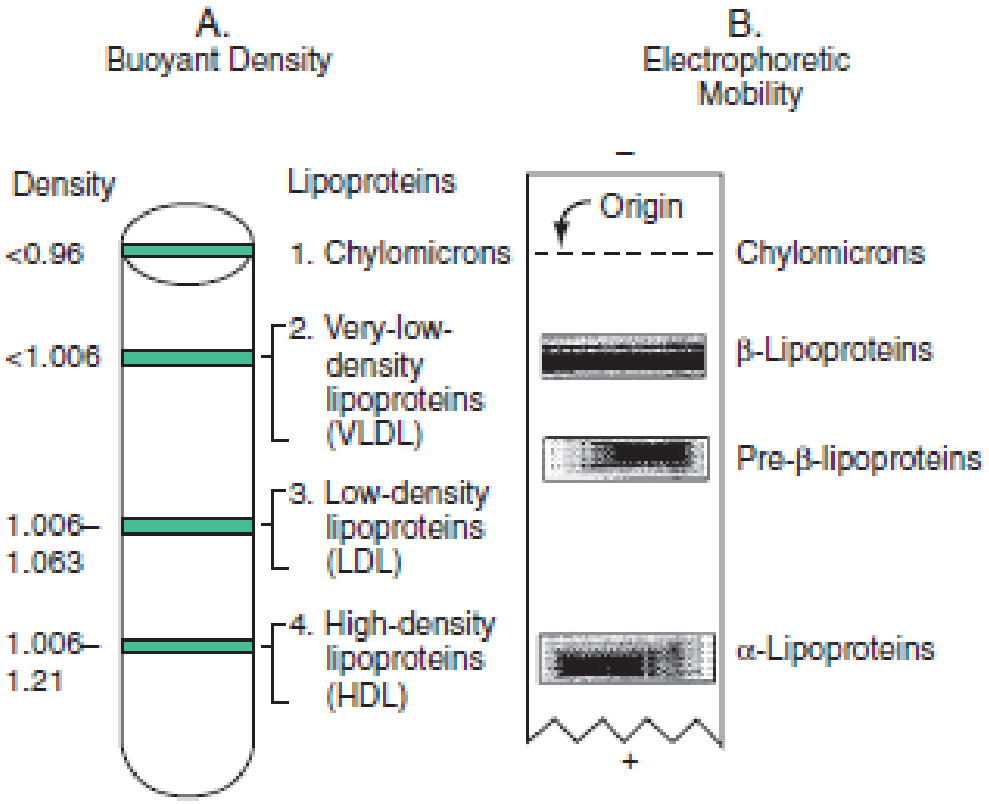
Lipid transport disorders



1 - Chylomicron retention (Apo B-48 defect)
2 - Hypobetalipoproteinemia/Abetalipoproteinemia
3 - LPL deficiency/Apo C II deficiency
4 - Familial hypercholesterolemia
5 - Dysbetalipoproteinemia (Type III hyperlipoproteinemia, associated with Apo-E-2)
6 - Familial defective Apo B

Reverse transport disorders





Chemical Composition of Major Classes of Plasma Lipoproteins

	Protein (%)*	Free cholesterol (%)	Cholesterol esters (%)	Triglyceride (%)	Phospholipid (%)
Chylomicrons	1–2	1–3	2–4	80–95	3–6
VLDL	6–10	4–8	16–22	45–65	15–20
IDL	Intermediate between VLDL and LDL				
LDL	18–22	6–8	45–50	4–8	18–24
HDL	45–55	3–5	15–20	2–7	26–32

Data from Albers (1974), Fless (1984), Gaubatz (1983), Gotto (1986), Gries (1988), and Hegele (2009).
HDL, High-density lipoprotein; *IDL*, Intermediate-density lipoprotein; *LDL*, low-density lipoprotein; *VLDL*, very-low-density lipoprotein.
*Percentage of dry weight.

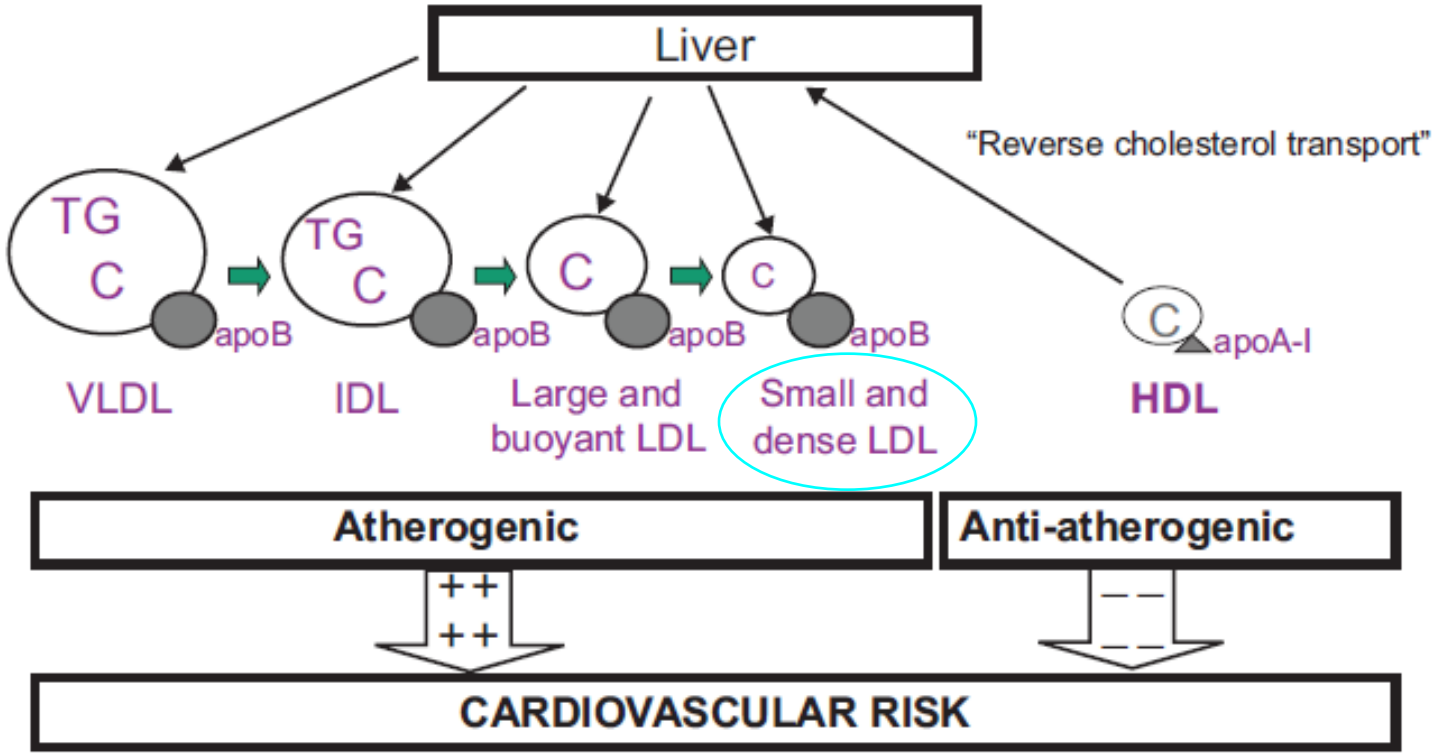


Figure 1 Atherogenic and anti-atherogenic lipoproteins. This diagram shows that there is one single apolipoprotein B (apoB) molecule in each large, buoyant or small, dense particle of very-low-density (VLDL), intermediate-density (IDL), and low-density lipoproteins (LDL). Therefore, apoB represents the total number of potentially atherogenic particles. Apolipoprotein A-I (apo A-I) is the principal protein component in high-density lipoproteins (HDL) and is responsible for starting reverse cholesterol transport. The balance between apoB and apoA-I is indicative of cardiovascular risk: the greater the ratio, the greater the risk.

Abbreviations: TG, triglycerides; C, cholesterol; + +, increased risk; – –, reduced risk.

Millán J et al: Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manag. 2009; 5: 757–765.

Preanalytics

- 12 h fasting before sampling for TG
- Postprandially high TG
- Postprandially transiently ↓ LDL-CHOL
- Alcohol consumption: TG ↑
- Total CHOL and HDL-CHOL: can be detected from non-fasting sample either
- Repeated measurements before treatment
- AMI, stroke: wait for 3 months
- Avoid: hemodilution, strangulation
- Avoid: hemolysis, ascorbic acid

4 major parameters:

Parameter	Normal	Intermediate	Pathologic
total cholesterol (mmol/l)	<5,2	5,2-6,2	6,2<
LDL cholesterol (mmol/l)	<3,4	3,4-4,2	4,2<
HDL cholesterol (mmol/l)	>1,6	1 – 1,6	1>
Triglyceride (mmol/l)	<0,9	0,9-1,42	1,42<

Other risk factors should be always considered

Target value depends on risk:

Risk category	**Target**	**LDL-cholesterol**	**total cholesterol**
I.	IHD or equivalent, Risk >20%	2,6	4,0
II.	At least 2 risk factors, risk < 20%	3,4	5,2
III.	0-1 risk factors	4,1	6,5

cholesterol

- 25-40% ‘free’ (unbound)
- 60-75% estherified with fatty acids
- Summa: ‘total cholesterol’
- Circulates exclusively with apolipoproteins

- Sample: serum or plasma (fasting)

Cholesterol: principle of analysis

cholesterol ester + free cholesterol

cholesterol esterase

free cholesterol + free fatty acids

cholesterol oxidase

cholestenone + H2O2

H2O2: colourful complex with phenol and 4-aminoantipyrin

Triglycerid level analysis

- Free glicerol levels after treatment of the specimen with lipase
- Several techniques

Preanalytical errors:

- Strangulation increases triglyceride levels
- Long-term storage on clotted sample.
- Free glycerol present in the sample.
- Haemolysis; high ascorbate levels: falsely low triglyceride levels.

LDL-CHOL determination

- Friedewald-equation: if TG < 4,5 mmol/l and there is no Type III hyperlipoproteinaemia :

LDL-CHOL = totalCHOL - TG/2,2 - HDL-CHOL (mmol/l)

TG/2,2 = VLDL

- Direct immunseparation method: apoA1 and apoE antibodies are used for binding LDL Chol
- Blocking selectively HDL, VLDL with surfactant ; selective detergents are used for the solubilization of LDL

When should one measure the cholesterol / triglyceride levels?

Guidelines recommend the measurement of lipid panel before 40 years of age as a part of routine lab investigation.

Total cholesterol, triglyceride and HDL-cholesterol levels should be measured in each people with any of the following conditions:

- Known risk factor for CVD (diabetes, hypertension)
- CVD in history
- High prevalence of CVD in young age among relatives)
- the patient or their first level relative have xanthelasma or arcus corneaej
- The serum is lipaemic in fasting state (this time triglyceride should be also measured)
- obese

Adult dosing, side effects, and drug interactions of lipid-lowering drugs

Drug class	Dose	Dosing	Major side effects and drug interactions
Statins			
Atorvastatin	10 to 80 mg/day		Headache; nausea; sleep disturbance; elevations in hepatocellular enzymes and alkaline phosphatase. Myositis and rhabdomyolysis, primarily when given with gemfibrozil or cyclosporine; myositis is also seen with severe renal insufficiency (CrCl <30 mL/min). Lovastatin, atorvastatin, rosuvastatin, and simvastatin potentiate effect of warfarin; this interaction is not seen with pravastatin, fluvastatin, or pitavastatin. Most statins can also affect digoxin metabolism and levels.
Fluvastatin	IR: 20 to 80 mg/day	IR take in the evening. Divide dose twice per day (morning and evening) if dose >40 mg/day.	
	XR: 80 mg/day	XR take any time	
Lovastatin	IR: 20 to 80 mg/day	IR take with evening meal. Divide dose twice per day with meals if dose >20 mg/day.	
	XR: 20 to 60 mg/day	XR take any time	
Pitavastatin	1 to 4 mg/day		
Pravastatin	10 to 80 mg/day		
Rosuvastatin	5 to 40 mg/day		
Simvastatin	5 to 40 mg/day	Take in the evening	

PCSK9 inhibitors : proprotein convertase subtilisin kexin type 9 inhibitors			
Alirocumab	75 to 150 mg every two weeks	Subcutaneous injections	Injection site reactions
Evolocumab	140 mg every two weeks or 420 mg every month Homozygous familial hypercholesterolemia: 420 mg every month to 420 mg every two weeks		
Fibric acid derivatives			
Fenofibrate	Nanocrystal 145 mg/day Micronized 160 to 200 mg/day	Micronized taken with meals. Use lower doses with renal insufficiency.	Skin rash, gastrointestinal (nausea, bloating, cramping) myalgia; lowers blood cyclosporine levels; potentially nephrotoxic in cyclosporine treated patients. Avoid in patients with CrCl <30 mL/min.
Gemfibrozil	600 mg twice per day	30 to 60 minutes before meals	Potentiates warfarin action. Absorption of gemfibrozil diminished by bile acid sequestrants.
Nicotinic acid (niacin)	IR: 1 to 6 g/day	IR: Taken with meals. Start with 100 mg twice per day and titrate to 500 mg three times per day. After six weeks, check lipids, glucose, liver function, and uric acid. Increase dose as needed.	Prostaglandin-mediated cutaneous flushing, headache, warm sensation, and pruritus; hyperpigmentation (particularly in intertriginous regions); acanthosis nigricans; dry skin; nausea; vomiting; diarrhea; and myositis
	XR (Niaspan): 0.5 to 2 g/day	XR: Taken at bedtime; adjust dose every four weeks as needed.	

Bile acid sequestrants			
Cholestyramine	4 to 24 g/day	Take within 30 minutes of a meal. A double dose with dinner produces same lipid-lowering effect as twice per day dosing.	Nausea, bloating, cramping, and constipation; elevations in hepatic transaminases and alkaline phosphatase. Impaired absorption of fat soluble vitamins and co-administered medications including: Amiodarone, digoxin, warfarin, thiazides, beta blockers, levothyroxine, others; interaction can be minimized by taking other medications at least 1 hour before or 4 hours after bile acid sequestrant.
Colestipol	5 to 30 g/day		
Colesevelam	3.75 g/day	Take with meals once daily or in two divided doses.	Similar
Cholesterol absorption inhibitors			
Ezetimibe	10 mg/day		Increased transaminases in combination with statins
Neomycin	1 g twice per day		Ototoxicity; nephrotoxicity
Probucol (not available in United States)	500 mg twice per day		Loose stools; eosinophilia; QT prolongation; angioneurotic edema

+ LDL apheresis: pl .homozigóta familiáris hypercholesterinaemia esetén

UpToDate ®

METABOLIC SYNDROME (if exists at all)

- Hypertension
- Hypertriglyceridemia
- low HDL-cholesterol
- Obesity
- Impaired glucose tolerance
- Microalbuminuria (WHO)

(an entity of lab disturbances)

Conditions frequently associated with metabolic syndrome

- Impaired glucose tolerance
- Atherogen dyslipidemia
- Endothel dysfunction
- Prothrombotic state
- Hemodynamic changes
- Proinflammatory state
- Increased ovarian testosterone production
- Sleeping apnoe

Disorders frequently associated to metabolic syndrome

- diabetes
- hypertension
- polycystic ovary syndrome (PCOS)
- non-alcoholic fatty acid
- sleeping apnoe
- CVD (infarction, PAD, Stroke)
- Cancer (breast, prostata, colorectal, liver)

The diagnosis is largely based on lab tests