Factors influencing laboratory tests

Enzyme assays in clinical chemistry

Seminar No. 2

Q. 1

A. 1

- No physical activity
- Fasting 10-12 hours
- The day before blood collection - limited fats in diet
- Ample drinking simple water (prevention of dehydration)
- No smoking
- No alcohol
- No drugs if possible
- No stress if possible

Q. 2

A. 2

after the change of position: lying → sitting

intravasal fluid (= ECF) moves partially to interstitial space

as **the consequence of gravitation**

⇒ the ECF becomes more concentrated

⇒ **protein-bound analytes exhibit higher concentration**

Q. 3

A. 3

- Dehydration ⇒ concentrated blood ⇒ elevated Prot + Hb
- Hypoglycemia – most probably from not sufficient food intake before match
- Insufficient supply of oxygen into muscles ⇒ anaerobic glycolysis ⇒ production and export of lactate ⇒ accumulation of lactate in ECF ⇒ acidosis

A. 4

A. 4

The albumin concentration decreases

Q. 5

A. 5

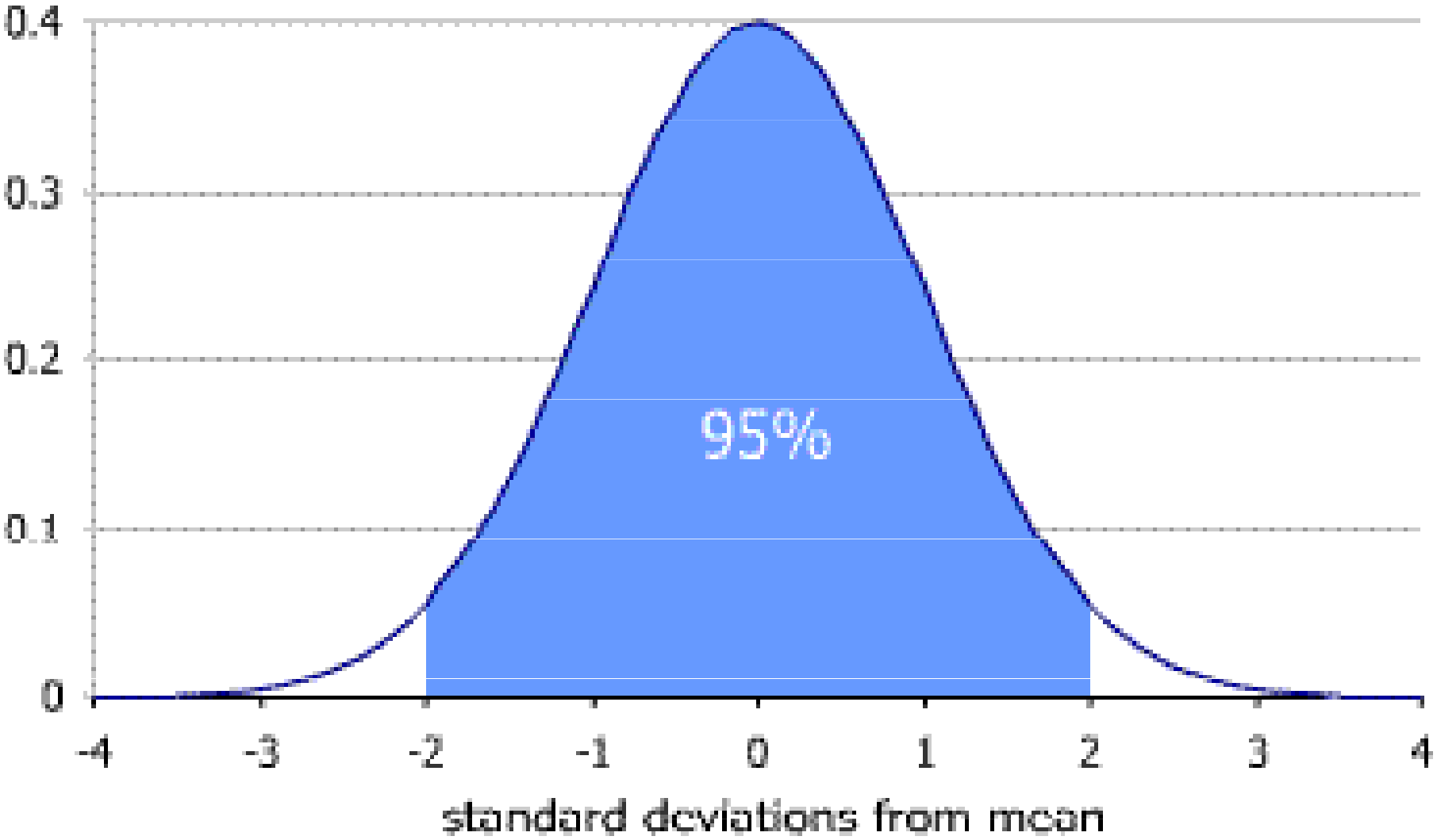
Hemolysis = disintegration of erythrocytes

Causes:

- Improper needle size
- Rapid evacuation of syringe
- Shaking blood
- Moisture and/or detergents in test tube
- Improper centrifugation

Q. 9

A. 9



Confident interval = $x \pm 2\,s = 40 \pm 2 \times 5 = 40 \pm 10$

Confident interval = 30 - 50

Q. 10

A. 10

The same as the unit of quantity measured

Q. 11

A. 11

The range: 55 – 36 = 19 g/l

The mean: 45 g/l

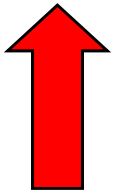
95 % confident interval: $x \pm 2\,s = 45 \pm 2 \times 6.3 = 32.4 – 57.6$ g/l

Q. 13

A. 13

Data are ordered from minimal value to maximal value:

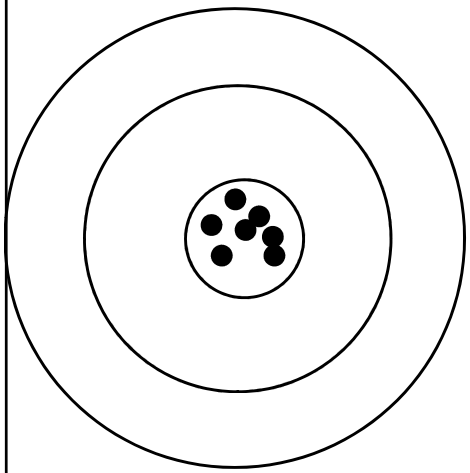
3.8, 3.9, 4.1, 4.5, 5.4, 5.5, 5.6, 6.1, 8.2

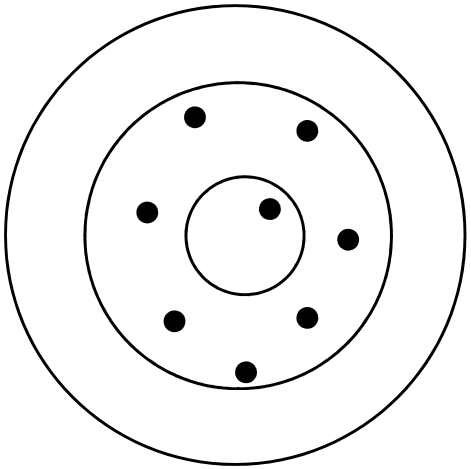


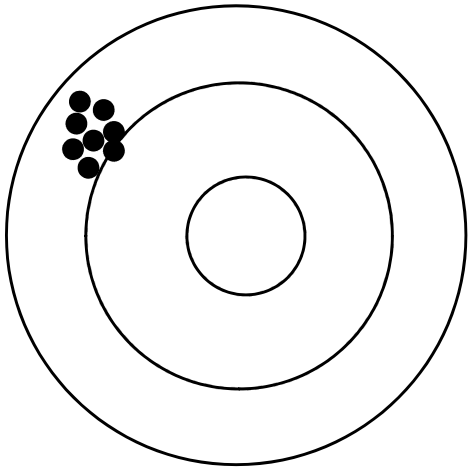
If odd number of data – the middle value is median

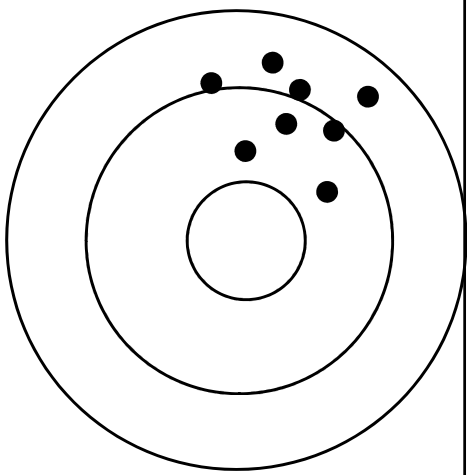
If even number of data – the average of two middle values

Q. 14





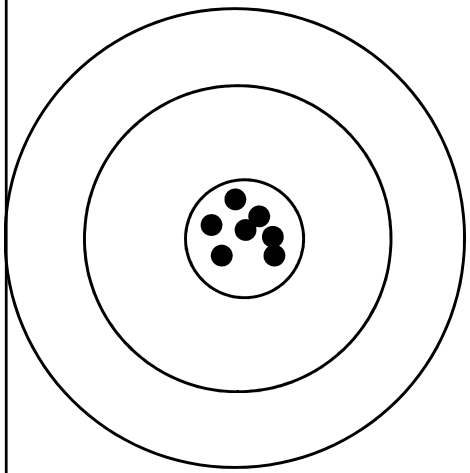




correct

precise

A. 14



correct

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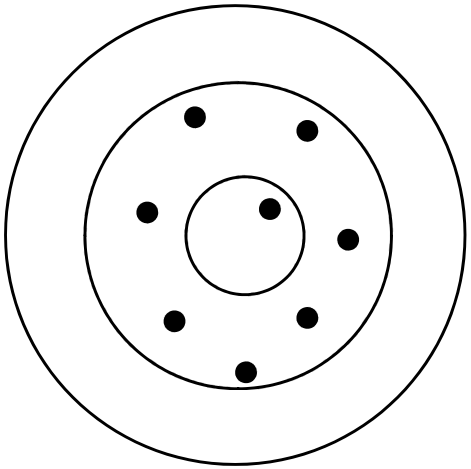
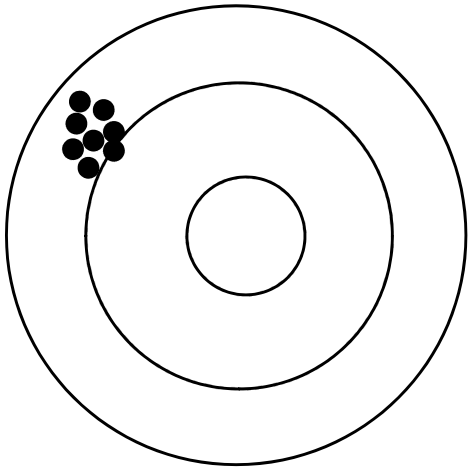


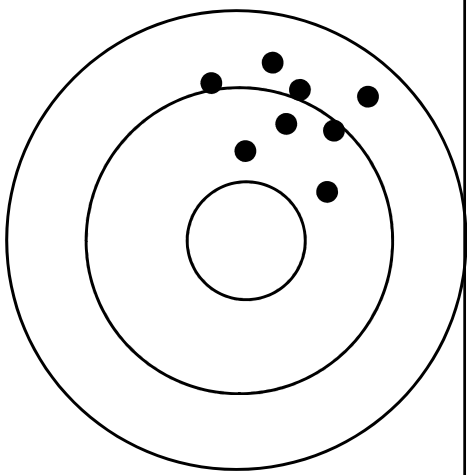
correct

imprecise



incorrect

precise



incorrect

imprecise

Q. 15

calculator at

www.cskb.cz

Analyte	**CV_i (%)**	**CV_a (%)**	**CD (%)**
pH of blood	0,2	0,1	0,62
S-Natrium	0,7	0,4	2,23
S-Albumin	3,1	0,8	8,87
U-Albumin	36	18	111,5
S-Glucose	5,7	2,9	17,72
S-Cholesterol total	6,0	3,0	18,6
S-ALT, catal. conc.	24,3	0,9	67,36
S-Cortisol	21	11	65,67
S-Adrenaline	48	24	148,65

Q. 16

A. 16

The parameter CV_i

Intra-individual biological variation varies the most

Q. 17

A. 17

7 ..... 100 %

1.33 ..... 19 % = CD

Significant difference:

7 ± 19 % = 7 ± 1.33 = 5.67 - 8.33 mmol/l

The cholesterol concentration over 8.33 mmol/l
or lower than 5.67 mmol/l means a clinical change.

Q. 18

A. 18

2,8 mmol/l ..... 100 %

x .....26 %

x = CD = 0,73

the difference between two tests: 3,2 – 2,8 = 0,4

Conclusion:

0,4 < 0,73 (CD) ⇒ no change in clinical status

Enzymes of clinical significance

You are supposed to know

- Enzymes – main features, properties, coenzymes
- Enzyme kinetics, enzyme activity

Q. 19

A. 19

Indirect determination

- catalytic concentration
- **µkat/l**
- product of enzyme reaction is determined
- most enzymes (ALT, AST)

Direct determination

- mass concentration
- **µg/l**
- enzyme molecules are determined as antigens (immunochemical assays)
- only few enzymes,
  e.g. tumour markers (PSA)

Q. 21

A. 21

- Temperature
- pH
- The presence of all necessary activators
- The absence of inhibitors
- Concentration of substrate – 0. order kinetics, the great excess of substrate so that the enzyme is saturated – the reaction proceeds with maximal velocity

How do you set constant pH 7.4 of enzyme solution at laboratory conditions?

- Hydrogen phosphate buffer is the best choice
- Corresponds to physiological conditions
- Solutions of Na_2HPO_4 and NaH_2PO_4 of the same concentration (e.g. 0.1 mol/l) are mixed together
- Their ratio is calculated from H.-H. equation:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{A} + \log \frac{[\text{hydrogenphosphate}]}{[\text{dihydrogenphosphate}]}$$

Enzymes in Blood

Feature	Plasmatic enzymes	Secretory enzymes	Intracellular enzymes
Example	coag. factors	amylase, lipase	AST, …
Source organ	liver	pancreas	various
Site of action	blood	GIT	cells
Enzyme activity in blood after source organ damage			

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Q. 24

Why are low activities of cellular enzymes detected even in serum of healthy people?

A. 24

Low activities of **intracellular** enzymes

in **extracellular** fluid (blood serum)

are the consequence

of physiological cell disintegration.

Tissue distribution of important enzymes

AST	liver, myocard
ALT	**liver**
LD	not specific
CK	**myocard, muscles**
GMT	**liver**
ALP	biliary tract, bones
ACP	**prostate**
AMS	**pancreas**
LPS	**pancreas**
CHS	**liver**

Q. 26

A. 26 Isoenzymes

- Genetically determined differences in primary structure
- Catalyze the same reaction
- May have **different subcellular distribution**
  (cytoplasm × mitochondria)
- May have **different tissue distribution**
- May be combined from more subunits (quarternary structure)
- May differ in kinetic properties (K_M)
- Usually are determined by electrophoresis
- **Elevated blood values – specific markers of tissue damages**

Intracellular location of enzymes

Intracellular Location	Enzymes
Cytoplasm	LD, **ALT**, **30 % AST**
Mitochondria	**70 % AST**
Golgi complex, ER	CHS, AMS
Lysosome	ACP
Membrane	**GMT, ALP**

Q. 28

What enzymes might appear in blood:

a) in mild hepatocellular damage

b) in serious hepatocellular damage

A. 28

a) Mild hepatocellular damage:

enzymes from **cytoplasm and/or membrane** are released into ECF – ALT, GMT, ALP

b) Severe hepatocellular damage:

enzymes from **mitochondria** are released into ECF – AST, GMD

Q. 29

A. 29

- AST/ALT > 1 ……. severe liver damage

- AST/ALT < 1 ……. mild liver damage

Q. 31+32

The levels of most blood enzymes are increased in newborns and infants. What enzyme persists elevated till puberty?

A. 31+32

ALP – the bone isoenzyme activity persists till puberty

Q. 34

Write equations of reactions catalyzed by:

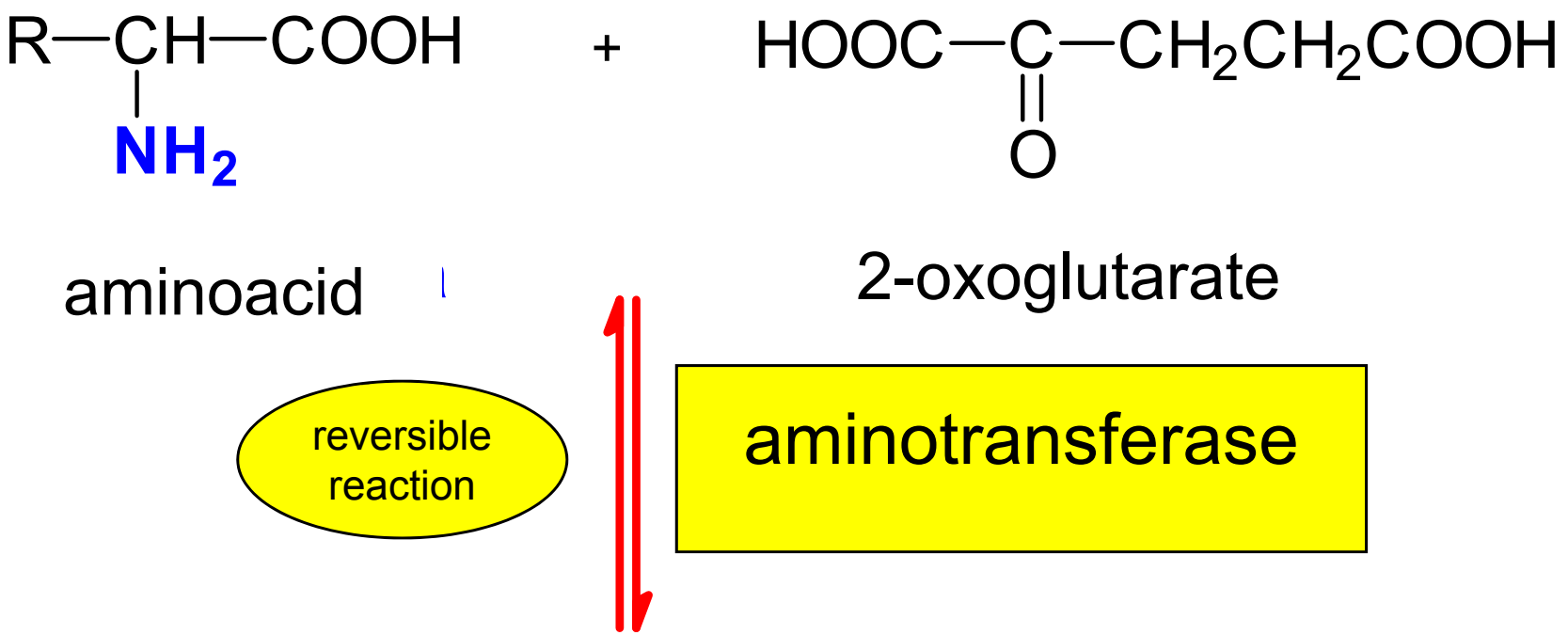
ALT

AST

LD

ALT Reaction

alanine + 2-oxoglutarate ⇆ pyruvate + glutamate


R—CH—COOH
NH2
+
HOOC—C—CH2CH2COOH
O
aminoacid
2-oxoglutarate
reversible reaction
aminotransferase
R—C—COOH
O
+
HOOC—CH—CH2CH2COOH
NH2
2-oxoacid
glutamate

AST reaction

aspartate + 2-oxoglutarate ⇆ oxaloacetate + glutamate

LD reaction

$$\text{lactate} + NAD^+ \leftrightarrows \text{pyruvate} + NADH + H^+$$

Q. 35

Lactate dehydrogenase (LD)

- **Tetramer**, two different chains (H - heart, M - muscle)
- Five isoenzymes:

  LD_1 (H_4), **LD_2** (H_3M), **LD_3** (H_2M_2), **LD_4** (HM_3), **LD_5** (M_4)
- Widely distributed in body
- Total activity determination – nonspecific finding
- $LD_1 + LD_2$ ….. marker of myocardial infarction (MI)
- Today is LD assay considered out-of-date

Creatine kinase (CK)

- **Dimer**, two different chains (M – muscle, B – brain)
- Three isoenzymes: **MM** (muscle), **MB** (heart), **BB** (brain)
- Major isoenzyme in blood is MM (95 %)
- MB form in blood: 0 – 6 %
- BB in blood: traces (BB cannot pass across blood-brain barrier)
- MB isoenzyme …. marker of myocardial infarction

Q. 37

A. 37

Amylase (AMS)

Q. 38

A. 38

- Osteoblasts – ALP
- Osteoclasts – ACP
- Prostate – ACP
- Cardiomyocytes – CK-MB
- Liver – ALT, CHS, GMT

Enzymes of Clinical Significance

Enzyme	Source of blood elevation
ALT	hepatopathy
AST	MI, hepatopathy
GMT	hepatopathy (alcohol, drugs)
ALP	biliary tract diseases, bone diseases
ACP	prostatic cancer
CK	MI (CK-MB), muscle diseases
AMS	pancreatitis
LPS	pancreatitis
CHS	hepatopathy (alcohol, drugs) – **decreased values**