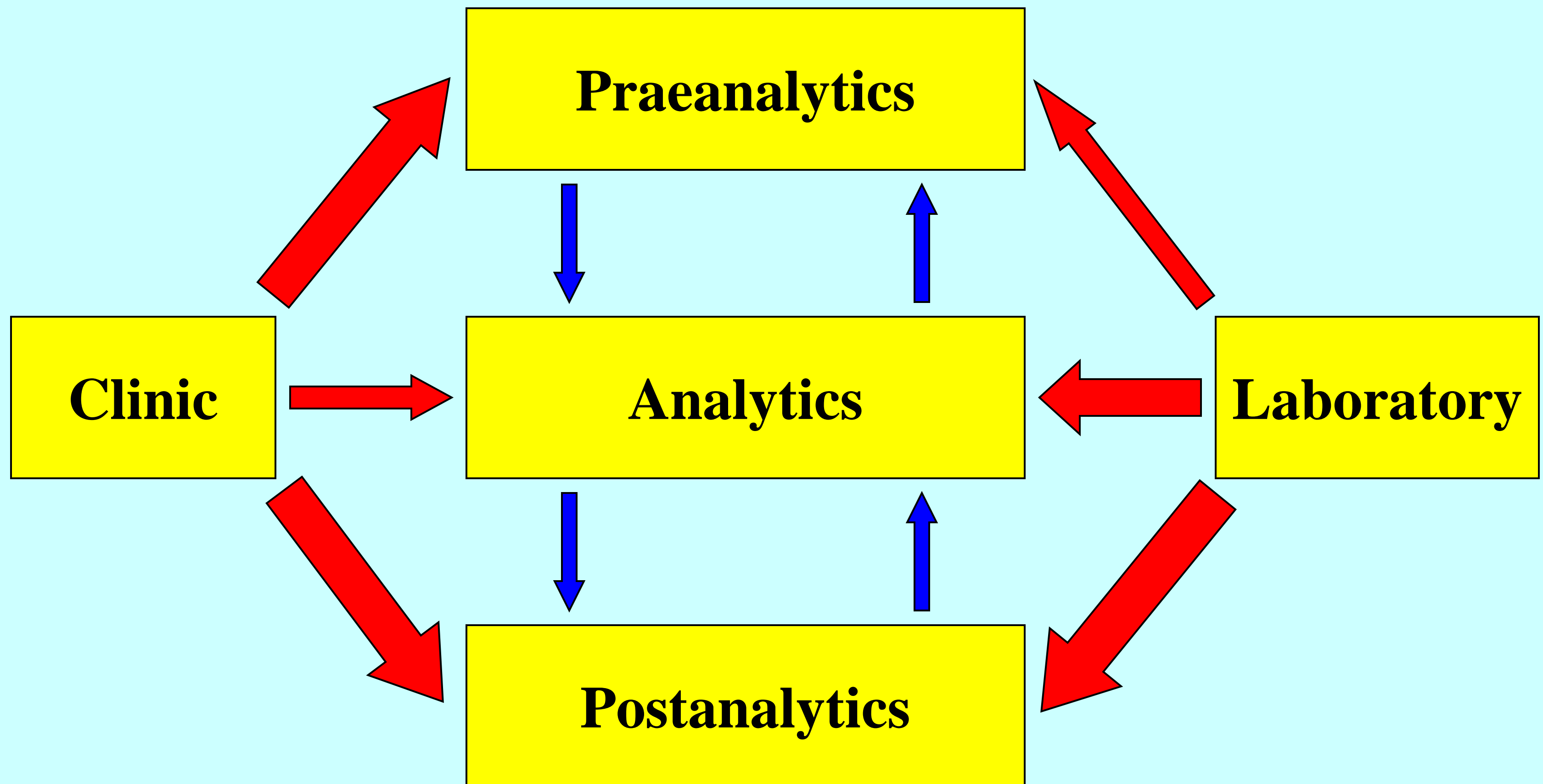
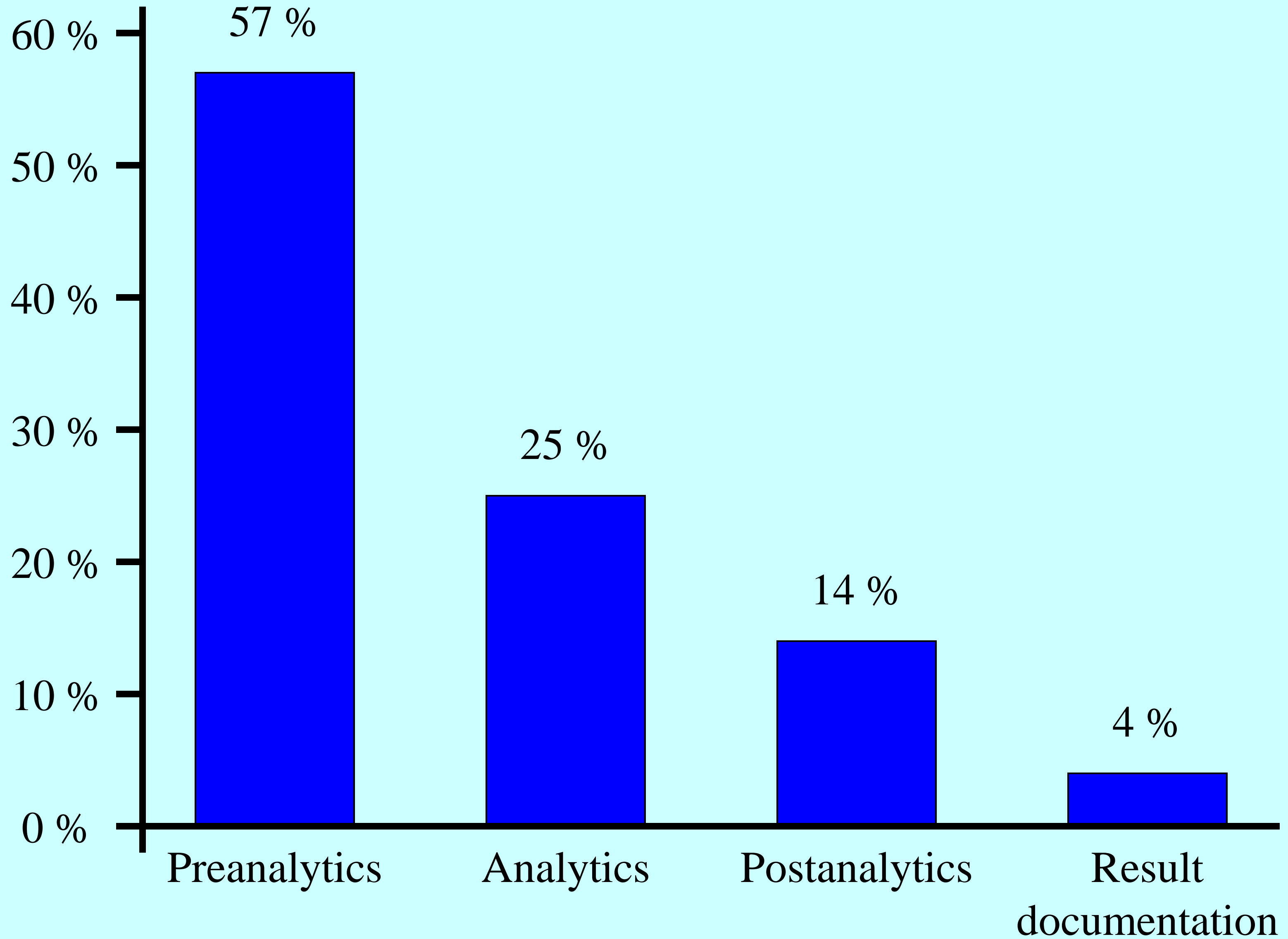
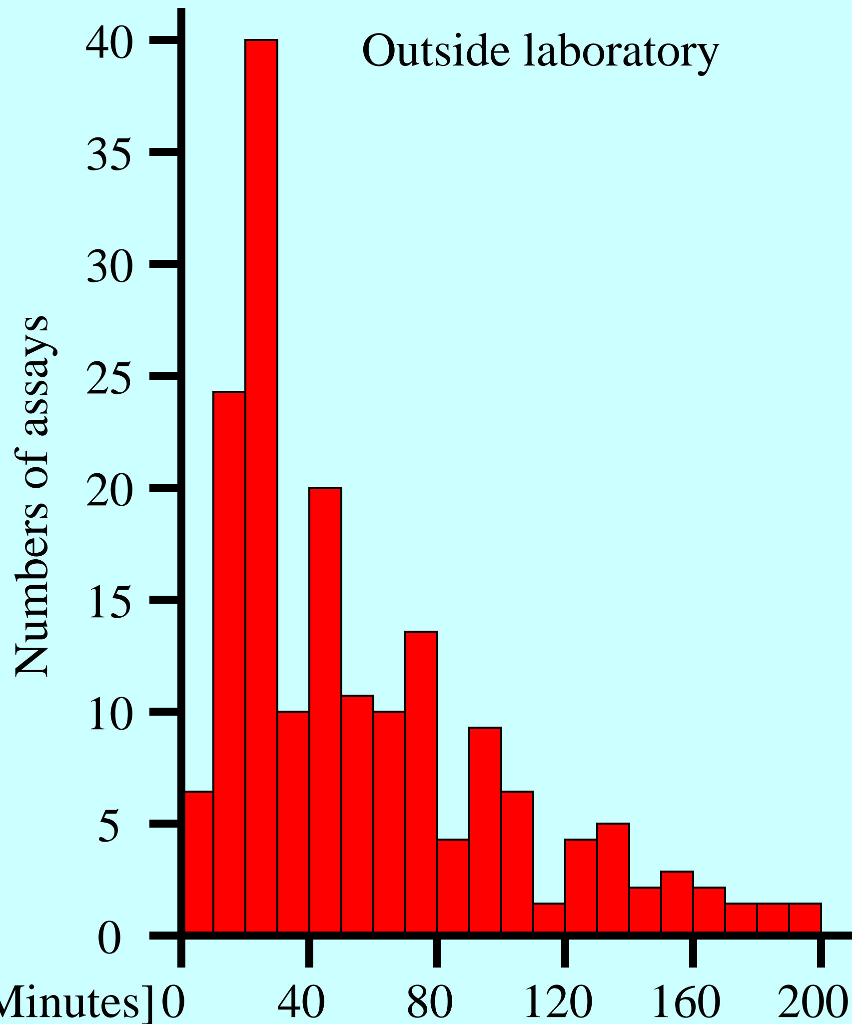
Phases in laboratory analytics

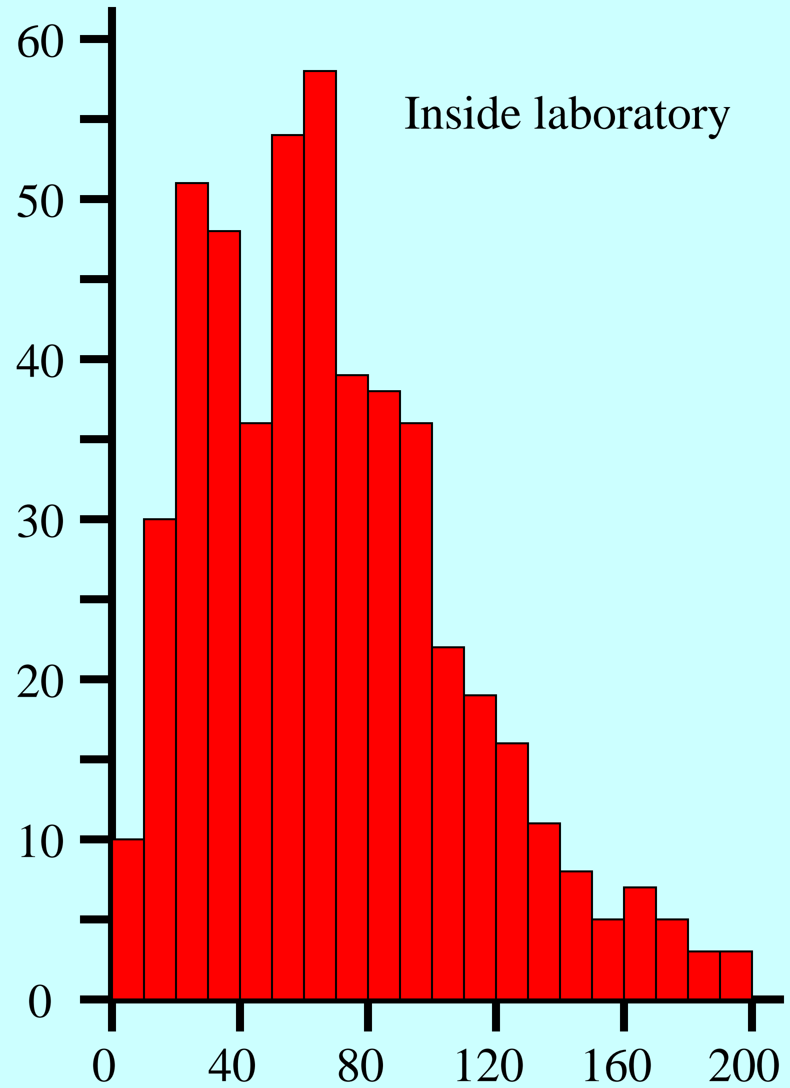


Time required for sample handling

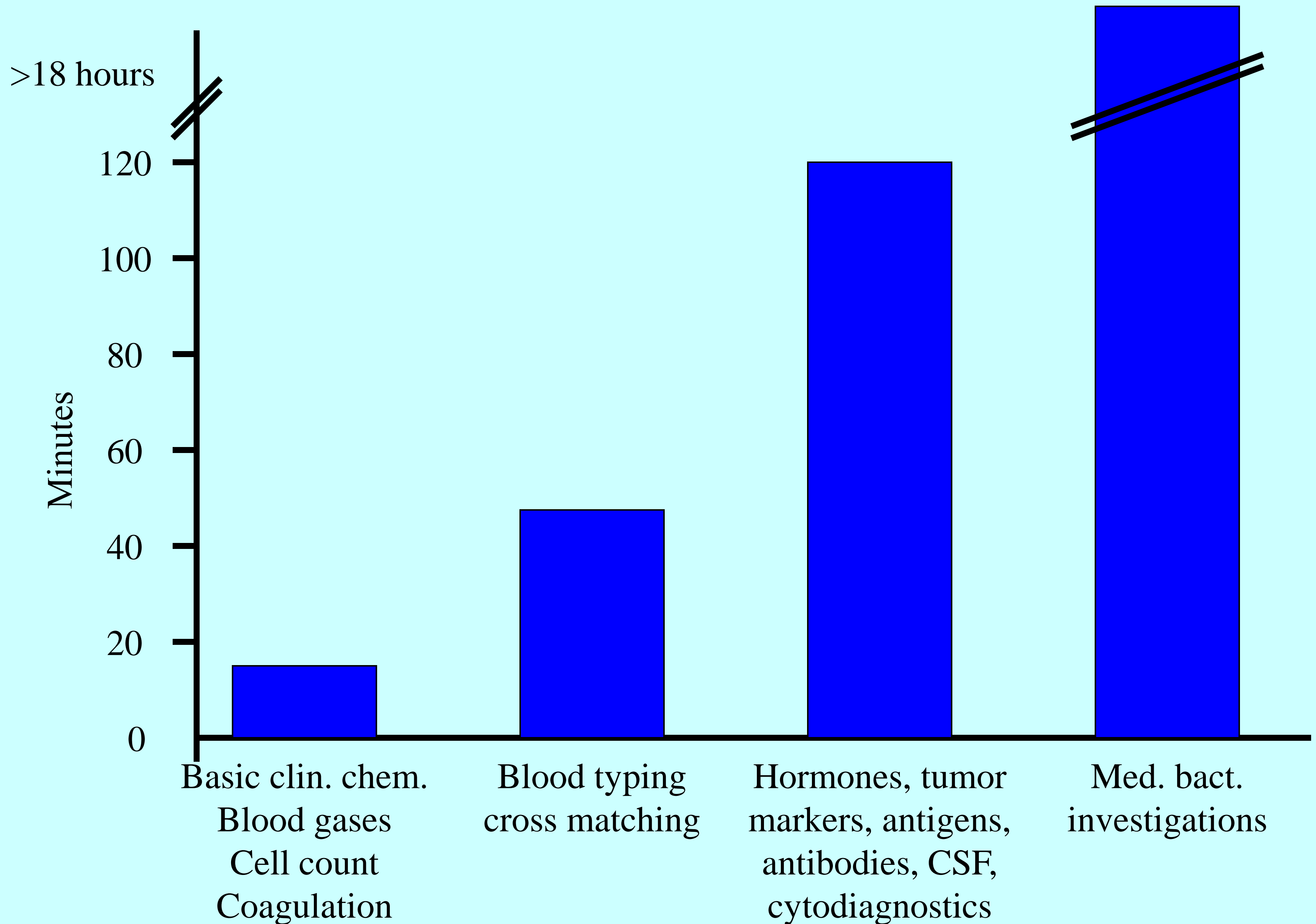


Documentation of preanalytical times during a usual working day





Time required for different analyzes



Distribution of the reported problems by phase of testing

Phase of testing	**Total [%]**
Preanalytic	
Test initiation	21.7
Specimen collection and handling	33.9
Total	55.6
Analytic	13.3
Postanalytic	27.8
Inconsistent result	3.3
Total	**100**

What is praenanalytics?

Praeanalytics includes all factors affecting the sample prior to its analysis.

This includes:

The correct indication for the laboratory investigation

Professional preparation of the patient (e. g. circadian rhythms, starvation, skin desinfection)

Correct choice of the samples and sample handling (blood sampling and transport)

What is analytics?

Analytics includes all steps of sample measurement

It includes the estimation of precision and accuracy of the results (control cards)

It is in the responsibility of the physician or clinical chemist performing the analysis

It is subject of laboratory organisation

It is subject of internal and external quality controls

What is postanalytics?

Postanalytics is the understanding of the laboratory results for clinical use. This requires specific knowledge of:

Reference values

Potential errors and influence factors

Sensitivity and specificity

Positive and negative predictive value

Prevalence of a disease

Evaluation of the critical difference (course of the data in the clinical course of the disease)

Typical errors - I

Sample volume (e. g. „laboratory-induced“ anemia after frequent sampling of large volumes

Circadian rhythm

Clinical course of the disease (e. g. creatin kinase activity 4 - 6 hours after myocardial infarction)

Inadequate adapation of the patient prior to sampling (e. g. water volume shift)

Typical errors - II

Artificial hemolysis (e. g. thin cannula, rapid sampling, too intense mixing, freezing)

Contamination (e. g. infusion therapy (may cause „occult anemia“), alcoholic desinfectants, microbes)

Dilution (e. g. infusion therapy (potassium), squeezing the finger in capillary sampling procedures)

Drugs (e. g. increase of amylase after infusion of hydroxy-ethyl starch for plasma expansion)

Sample transport (e. g. time, heat, cooling, freezing)

Typical errors - III

In-vitro metabolism (e. g. falsely low blood glucose, falsely low alcohol, falsely high lactate)

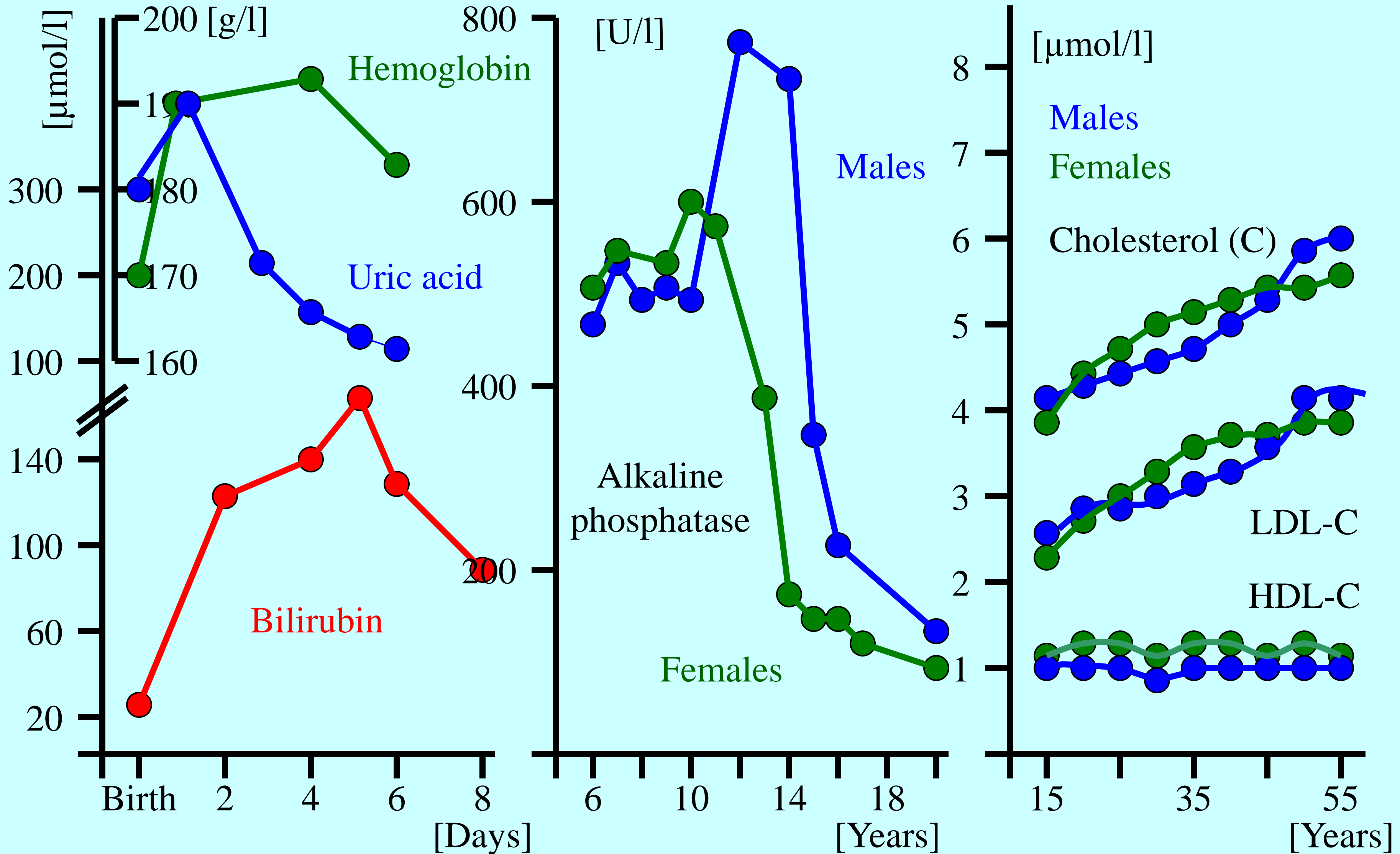
Insufficient mixing (e. g. clots in citrate, EDTA or heparin anticoagulated blood, sedimentation of blood cells (e. g. falsely low or high values of Hb and Hct)

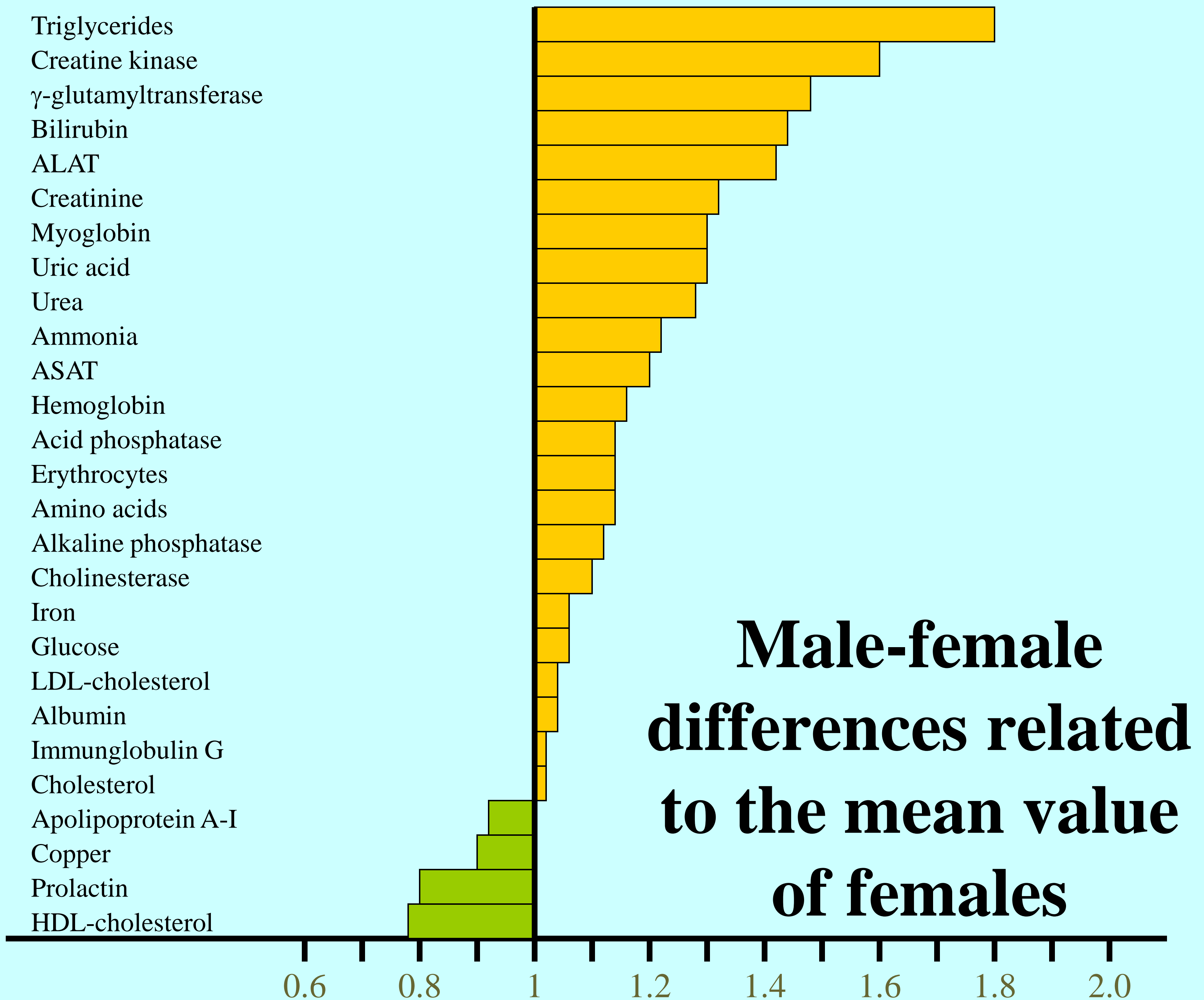
Protein absorption at the tube wall

Light sensitivity (e. g. degradation of bilirubin or vitamins)

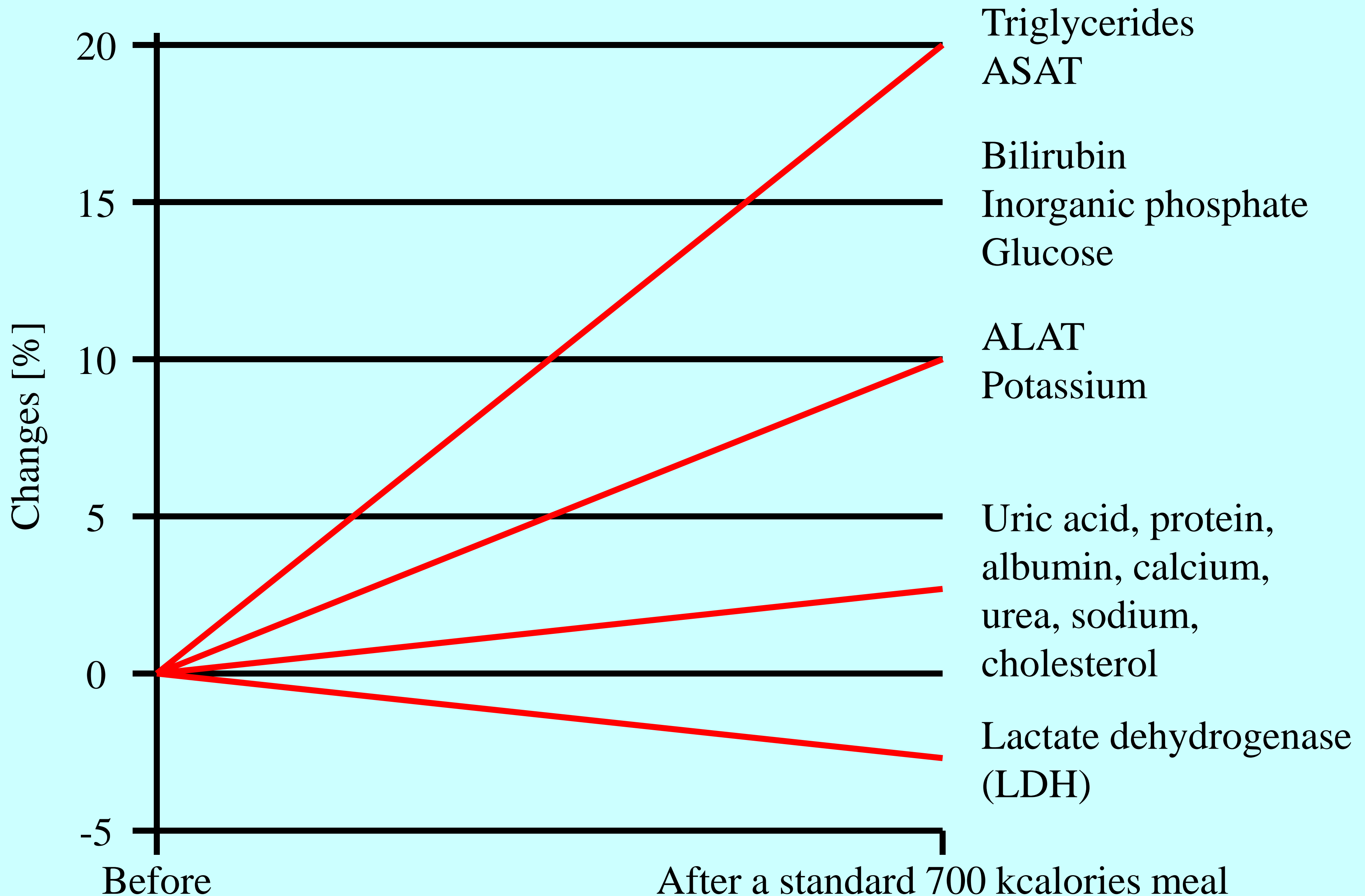
Mix-up of samples

Age dependence of various substrates and enzyme activity

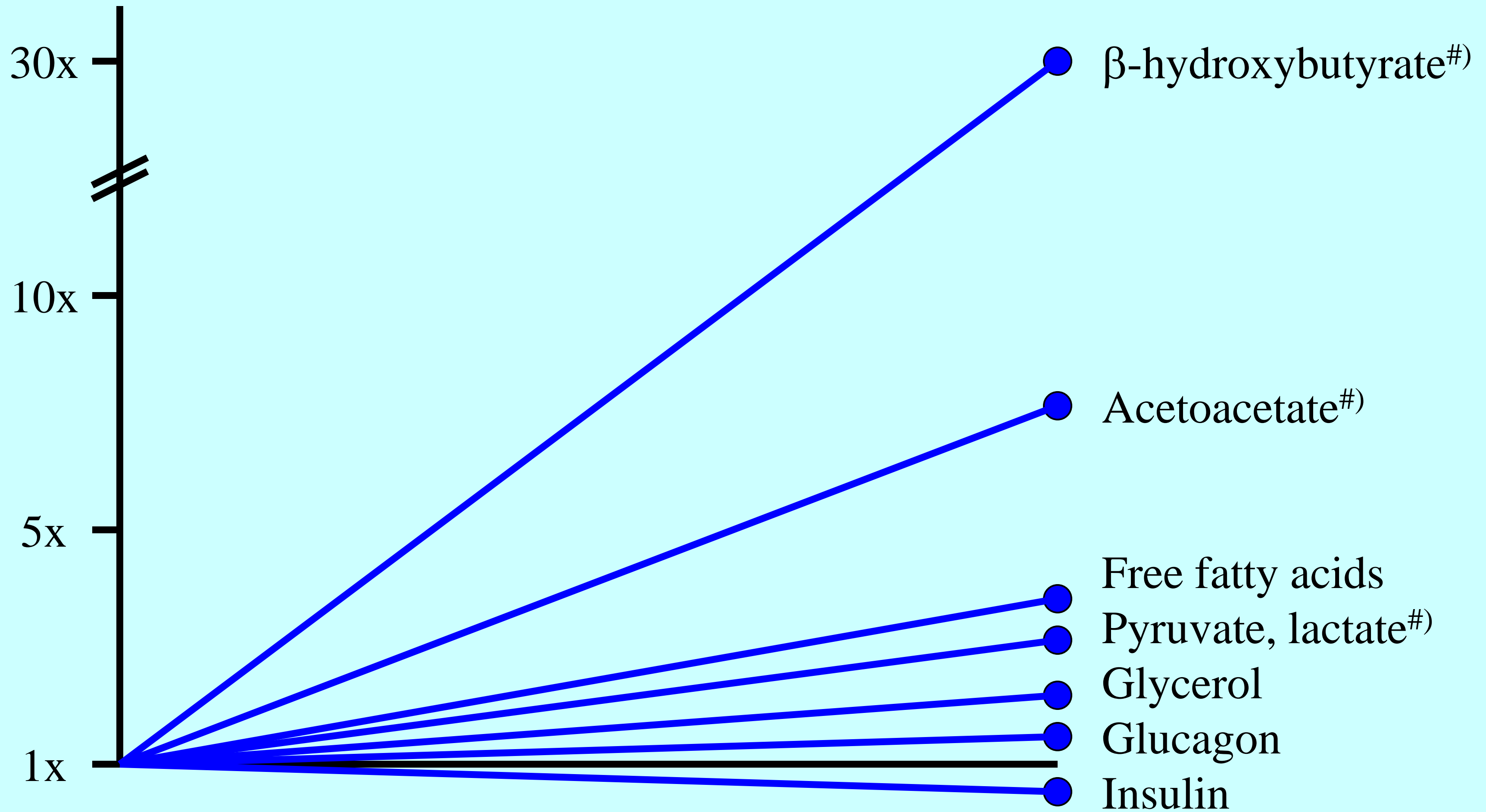



Triglycerides
Creatine kinase
γ-glutamyltransferase
Bilirubin
ALAT
Creatinine
Myoglobin
Uric acid
Urea
Ammonia
ASAT
Hemoglobin
Acid phosphatase
Erythrocytes
Amino acids
Alkaline phosphatase
Cholinesterase
Iron
Glucose
LDL-cholesterol
Albumin
Immunglobulin G
Cholesterol
Apolipoprotein A-I
Copper
Prolactin
HDL-cholesterol
0.6
0.8
1
1.2
1.4
1.6
1.8
2.0
Male-female differences related to the mean value of females

Change of the serum concentration after a standard meal



Effect of starvation on blood analytes

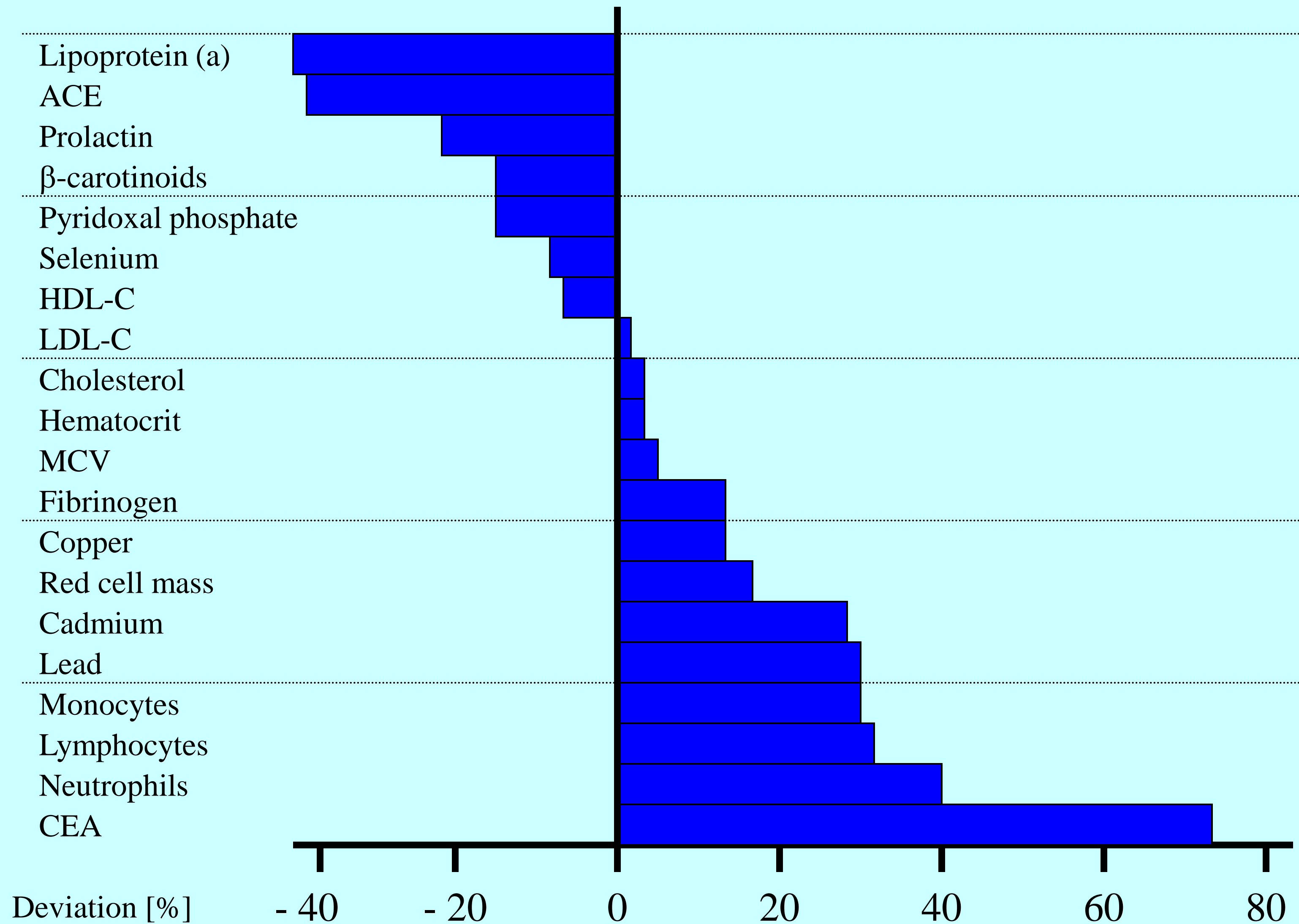


Variation of seeveral analytes after 40 - 48 hours starvation

#) Starting point after 14 hours starvation

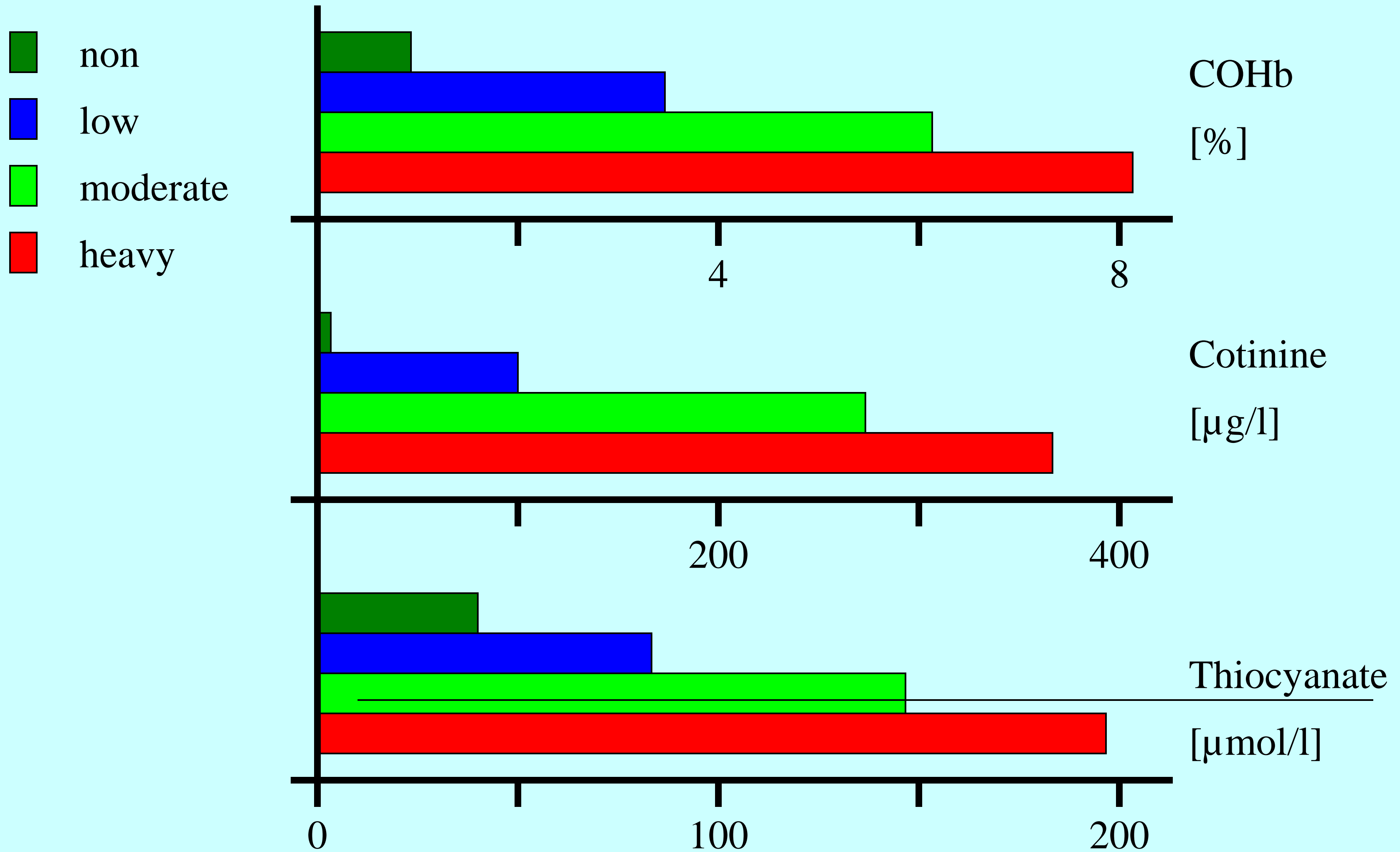
Effect of smoking on analytes - I

Variations caused by chronic cigarette consumption

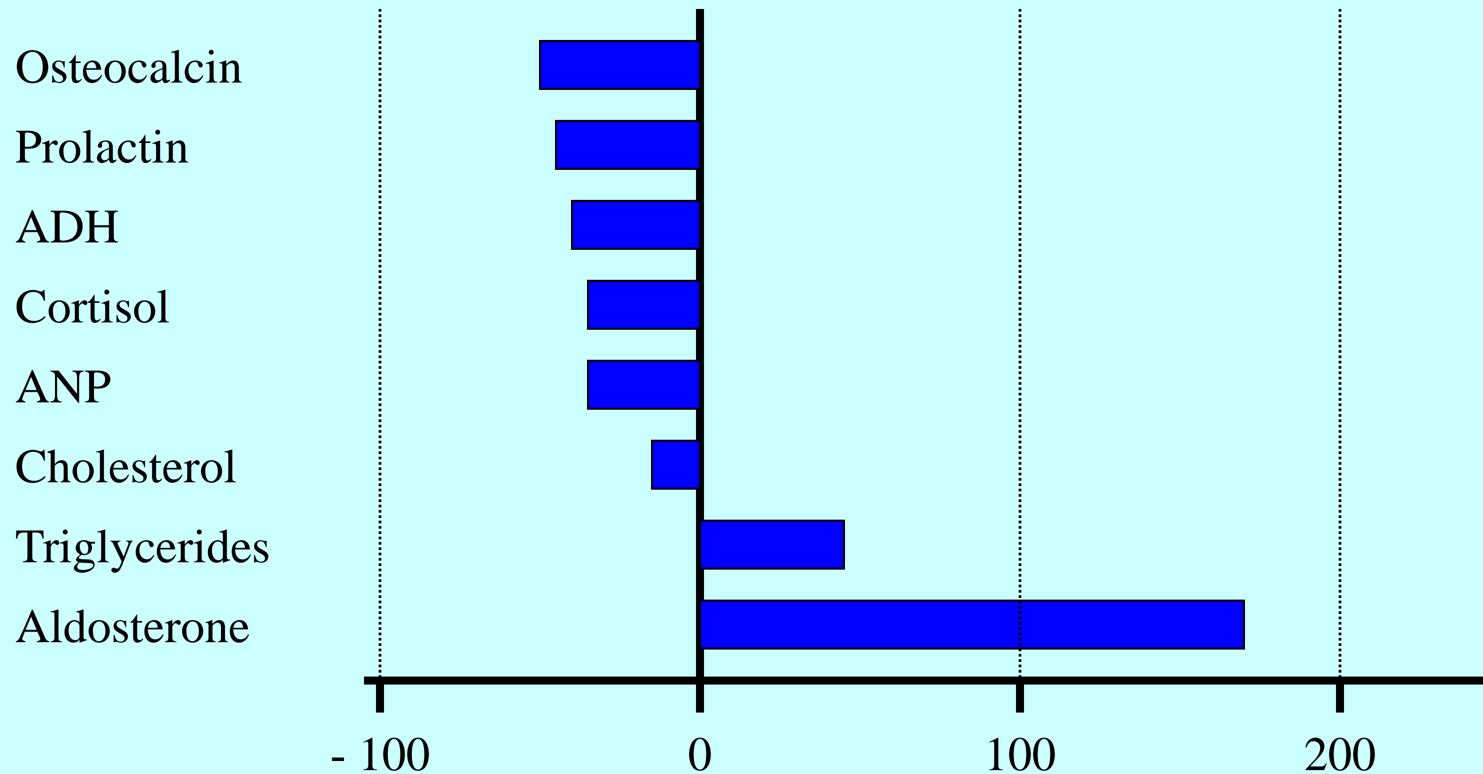


Effect of smoking on analytes - II

Effects caused by cigarette smoke constituents

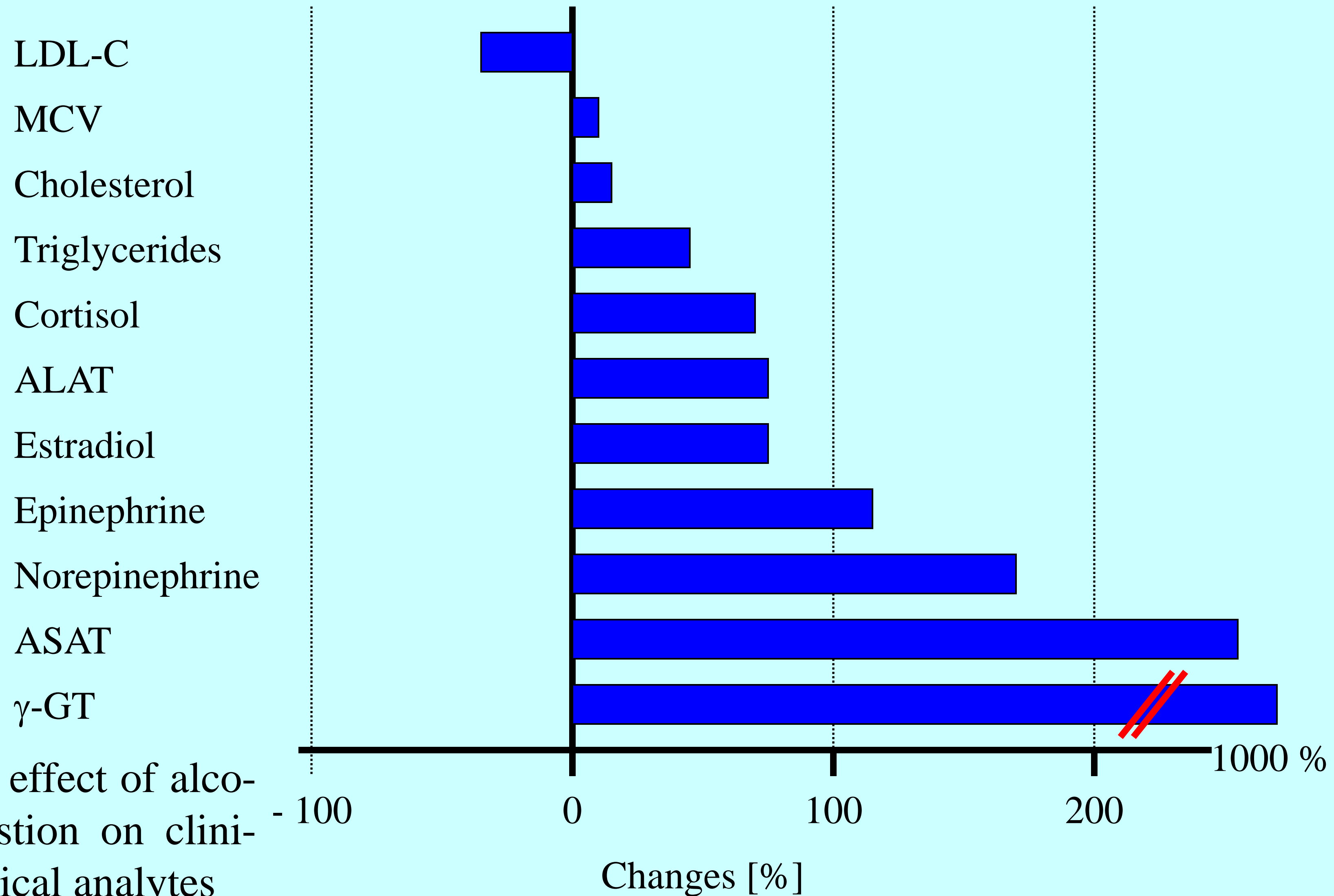


Effect effect of alcohol intake on analytes - I



Acute effect of alcohol ingestion on clinical chemical analytes

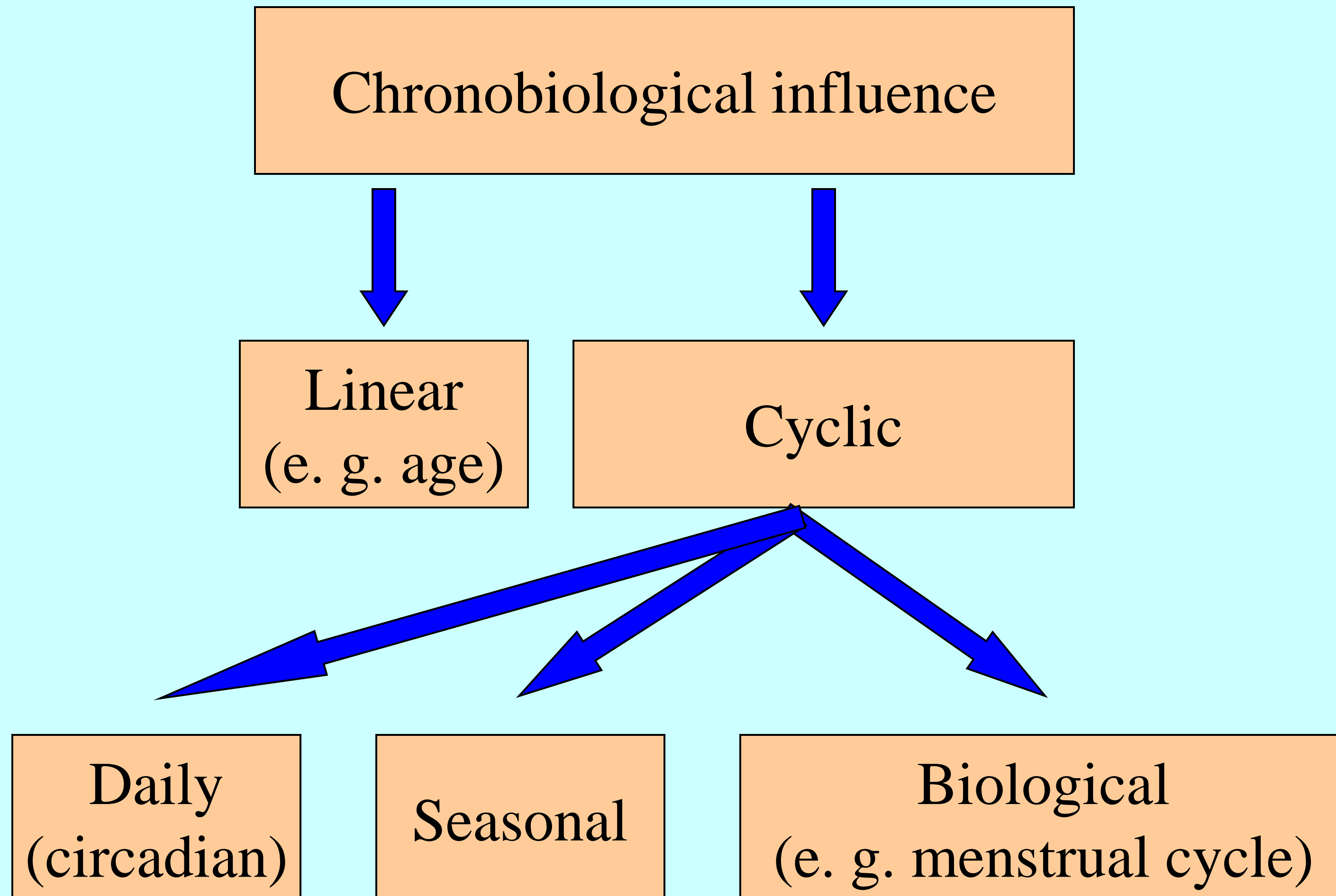
Effect effect of alcohol intake on analytes - II



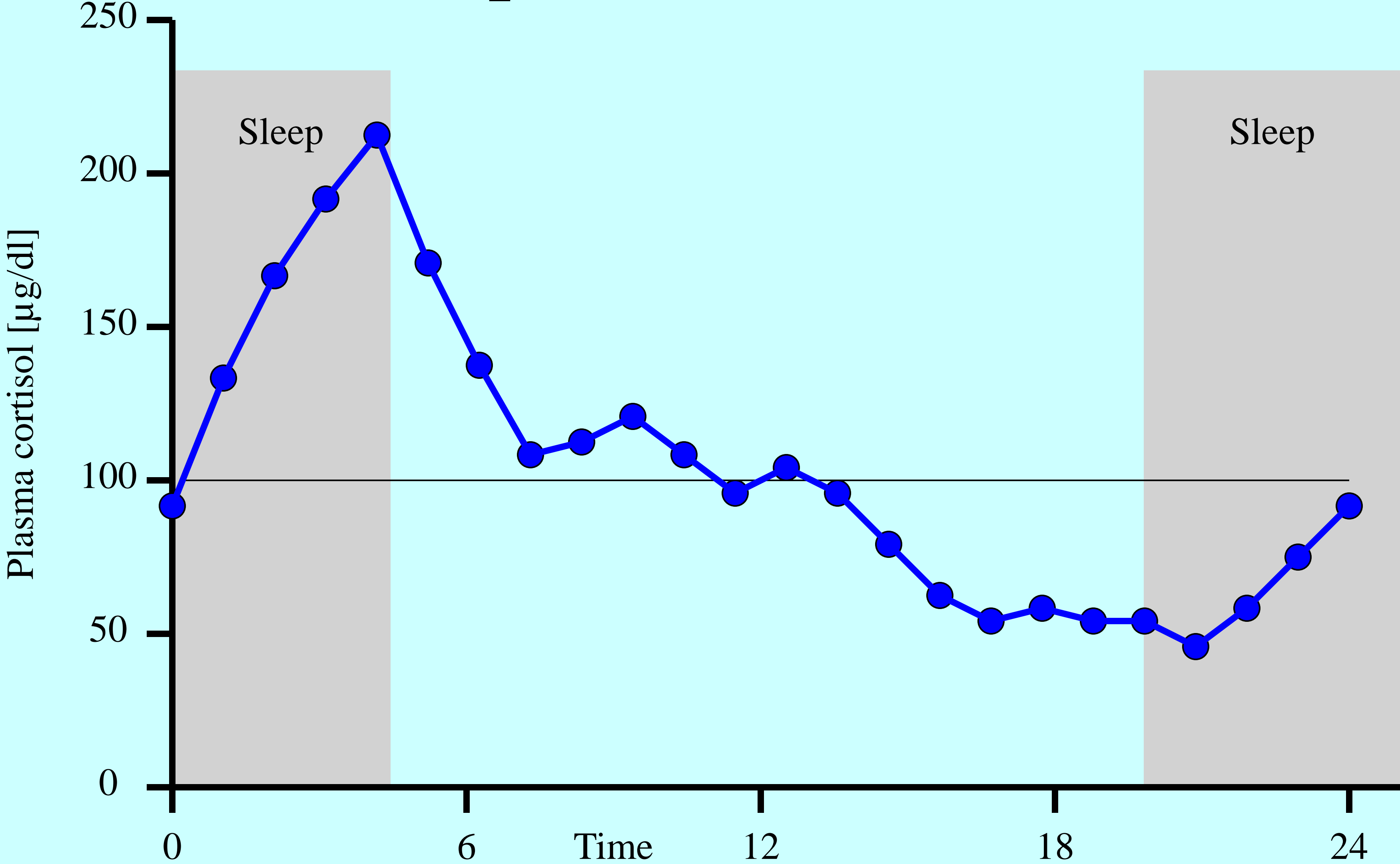
Chronic effect of alcohol ingestion on clinical chemical analytes

Chronobiological influences

Biological parameters are affected by several types of chronobiological influences



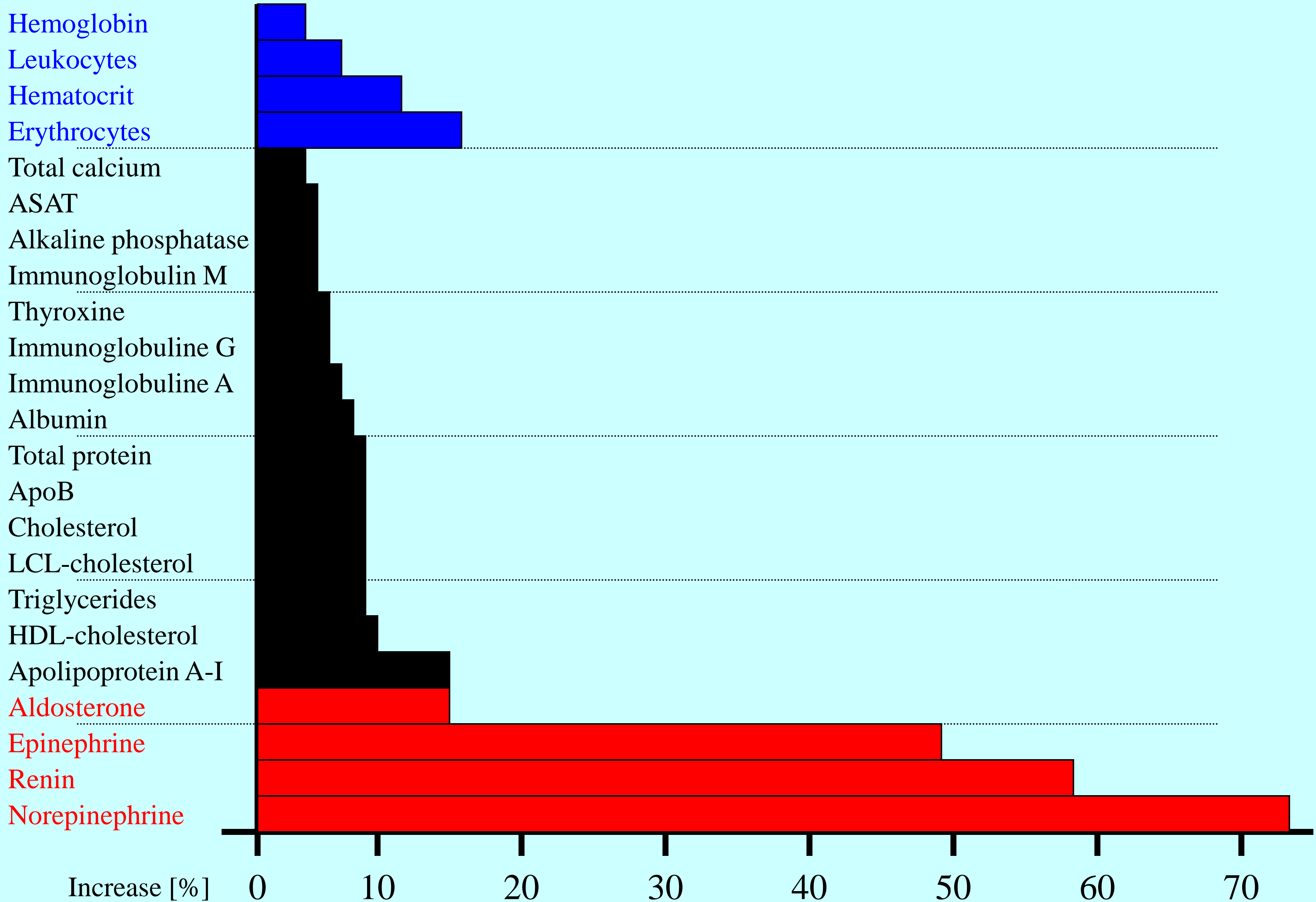
Diurnal variation of cortisol plasma concentration



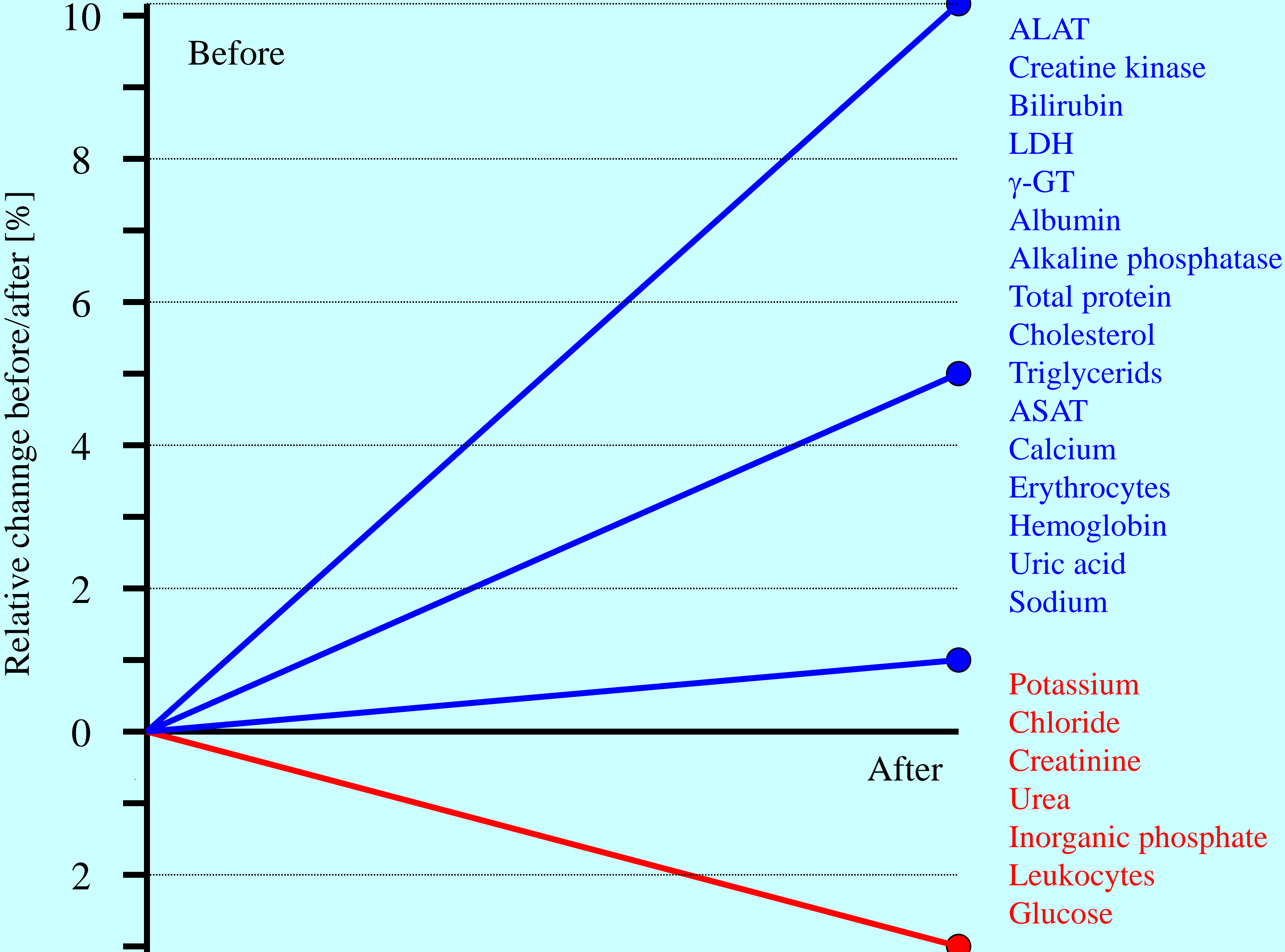
Diurnal variation of selected analytes

Analyte	Maximum (time of day)	Minimum (time of day)	Amplitude (% of daily mean)
ACTH	6-10	0-4	150-200
Cortison (S, U)	5-8	21-3	180-200
Testosterone	2-4	20-24	30-50
TSH	20-2	7-13	5-15
T4	8-12	23-3	10-20
Somatotropin	21-23	1-21	300-400
Prolactin	5-7	10-12	80-100
Aldosterone	2-4	12-14	60-80
Renine	0-6	10-12	120-140
Epinephrine (S)	9-12	2-5	30-50
Norepinephrine (S, U)	9-12	2-5	50-120
Hemoglobin	6-18	22-24	8-15
Eosinophils	4-6	18-20	30-40
Iron (S)	14-18	2-4	50-70
Potassium (S)	14-16	23-1	5-10
Phosphate (S)	2-4	8-12	30-40
Sodium (U)	4-6	12-16	60-80
Phosphate (U)	18-24	4-8	60-80
Volume (U)	2-6	12-16	60-80
Body temperature	18-20	5-7	0.8-1.0°C

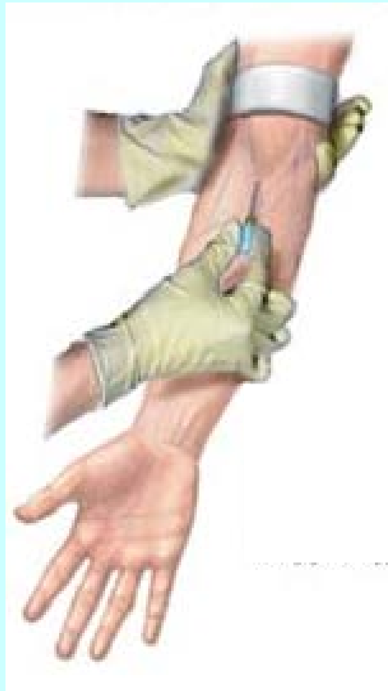
Effect of change from supine to upright position

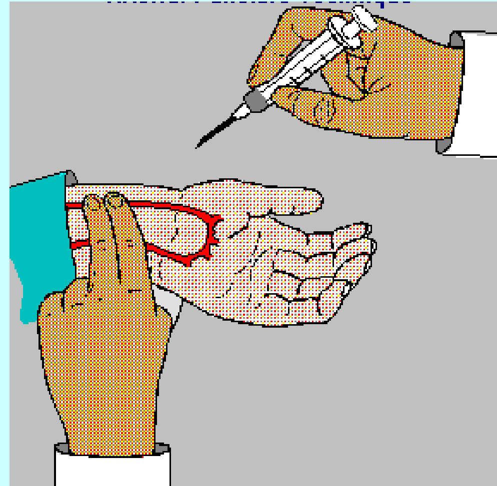


Effect of a 6 minute tourniquet application



Blood sampling





In clinical routine blood samples are taken from:

- # veins
- # capillaries
- # arteries

Type and volume of the blood sample depend on the age of the patient and the analyses for which the blood is required.

Tubes for blood sampling - I

Vials are produced from numerous manufacturers (e. g. Sarstedt, Becton Dickinson).

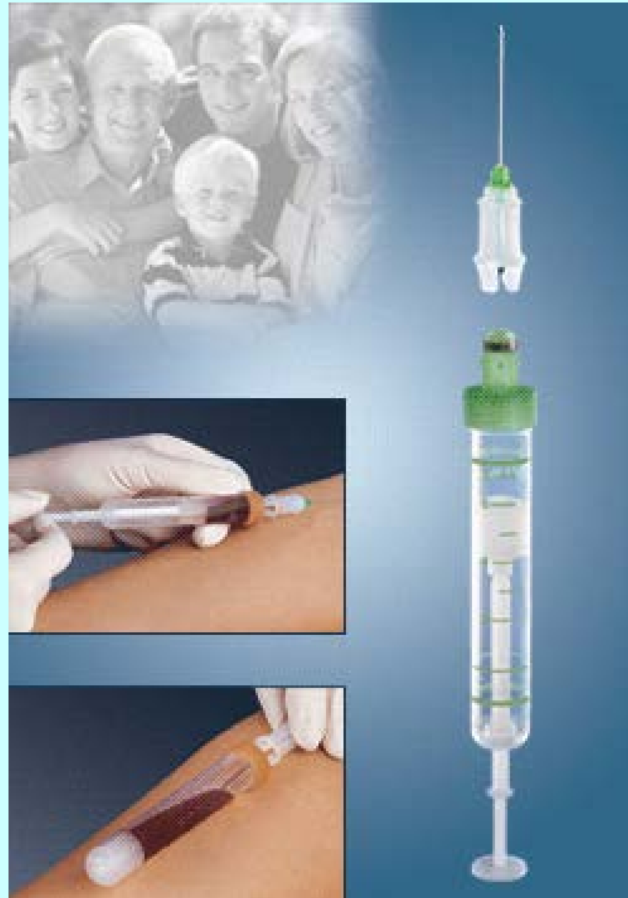
The tubes differ in respect to their sampling technique (e. g. vacutainer, monovettes).

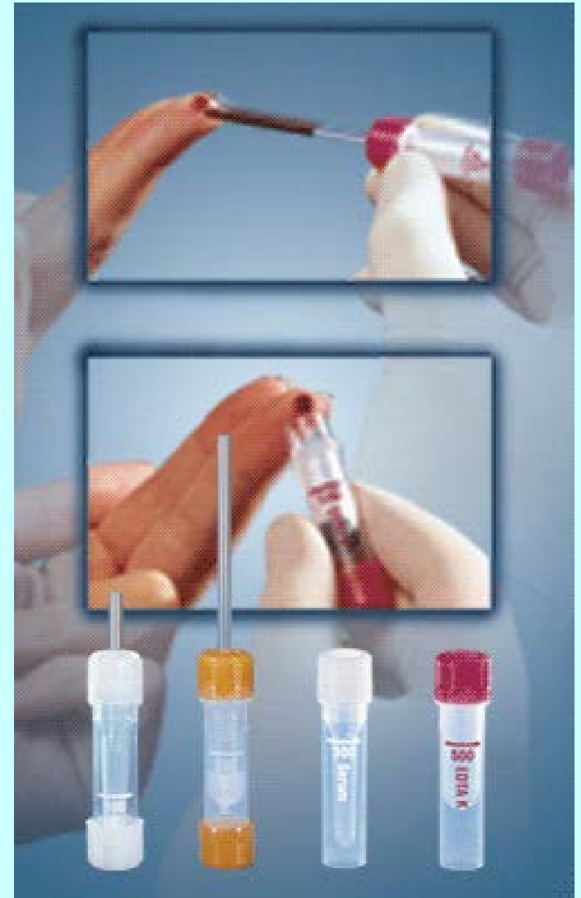
Tubes are of different sizes (vials for venous or capillary sampling).

The tubes differ in respect to their additives (e. g. citrate for clotting analyses, EDTA for hematological analyses, serum tubes).

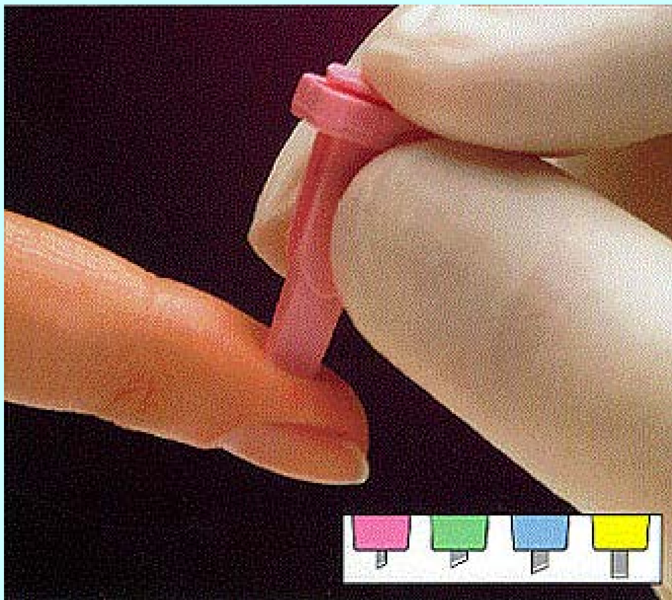
Manufacturers use a colour code (e. g. red for EDTA, green for citrate, white for serum) which is not subject of a standard.

Tubes for blood sampling - II





Capillary blood sampling - I



Some sampling devices offer a choice of needle penetration depths.

Select the penetration which gives you adequate bloodflow with minimum discomfort and avoid pressing the finger when sampling.

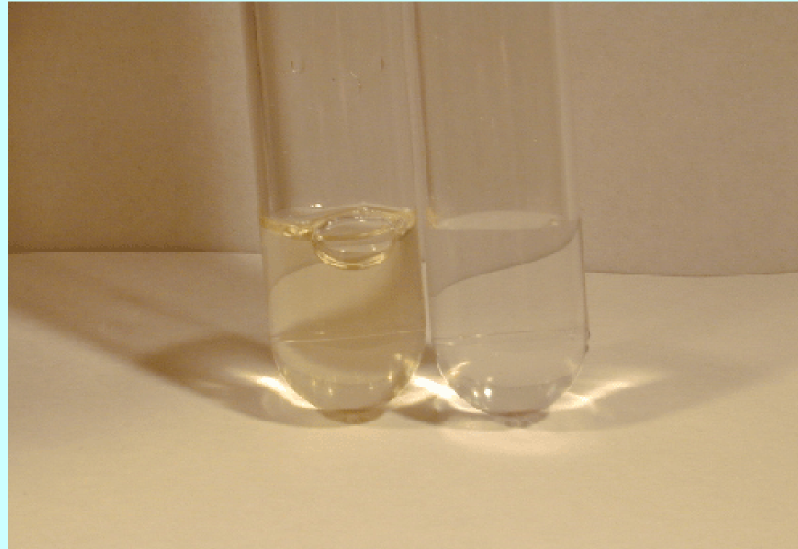
Capillary blood sampling - II

Capillary blood sampling is used for many laboratory parameters (clinical chemistry, hematology, clotting analysis, blood gases).

The determination of blood gases require sampling of „arterialized“ blood after hyperaemization of the skin.

However, especially in patients with circulatory failure (shock) the obtained values of capillary blood gases are not representative.

Cerebrospinal fluid



Normal cerebrospinal fluid has a very low protein concentration and looks like water. If there is a bleeding into the cerebrovascular fluid, red blood cells are rapidly lyzed and the cerebrospinal fluid becomes hemolytic. After a bleeding a longer time ago, cerebrospinal fluid shows xanthochromia.

Top left: Very mild (left) and severe (right) hemolysis of CSF.

Bottom left: Mild hemolysis (left) and xanthochromia (right).

Top right: Xanthochromic (left) and normal (right (CSF).

Urine analysis

Left: Normal urine

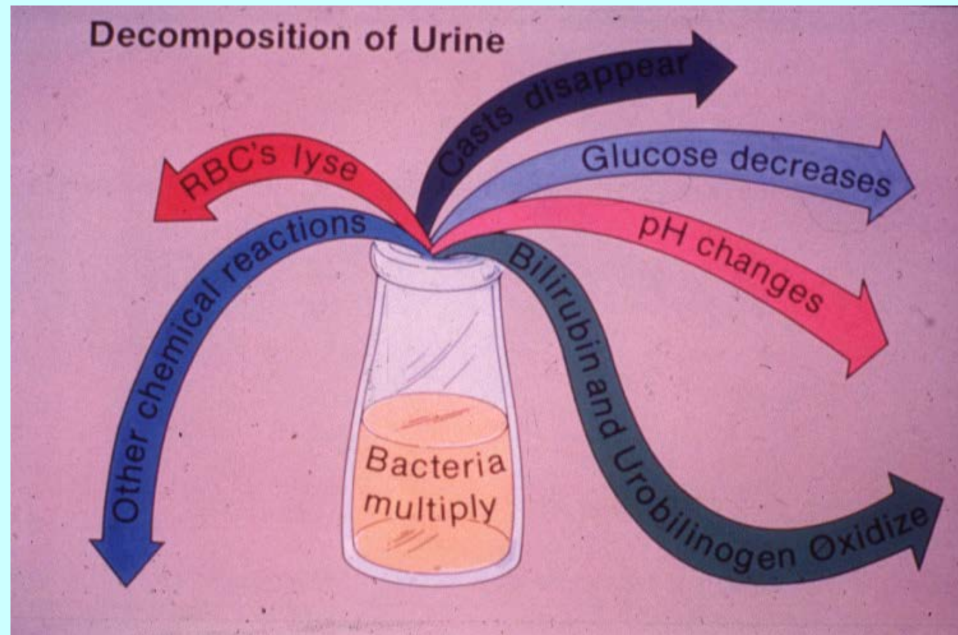
Right: Catheter urine in a severely ill patient

Types of urine specimens

Different types of urine specimens and their use in the laboratory

Random or spot urine	Qualitative and quantitative chemical determinations and/or cellular constituents and casts
First morning urine	Cellular constituents and casts
7-10 a. m. (second morning urine)	Quantitative determination related to creatinine
24 h urine	Quantitative determinations (e. g. protein loss, creatinine clearance)

Decomposition of urine



Decomposition of urine begins within 30 minutes of voiding:

Cells and Casts lyze or dissolve

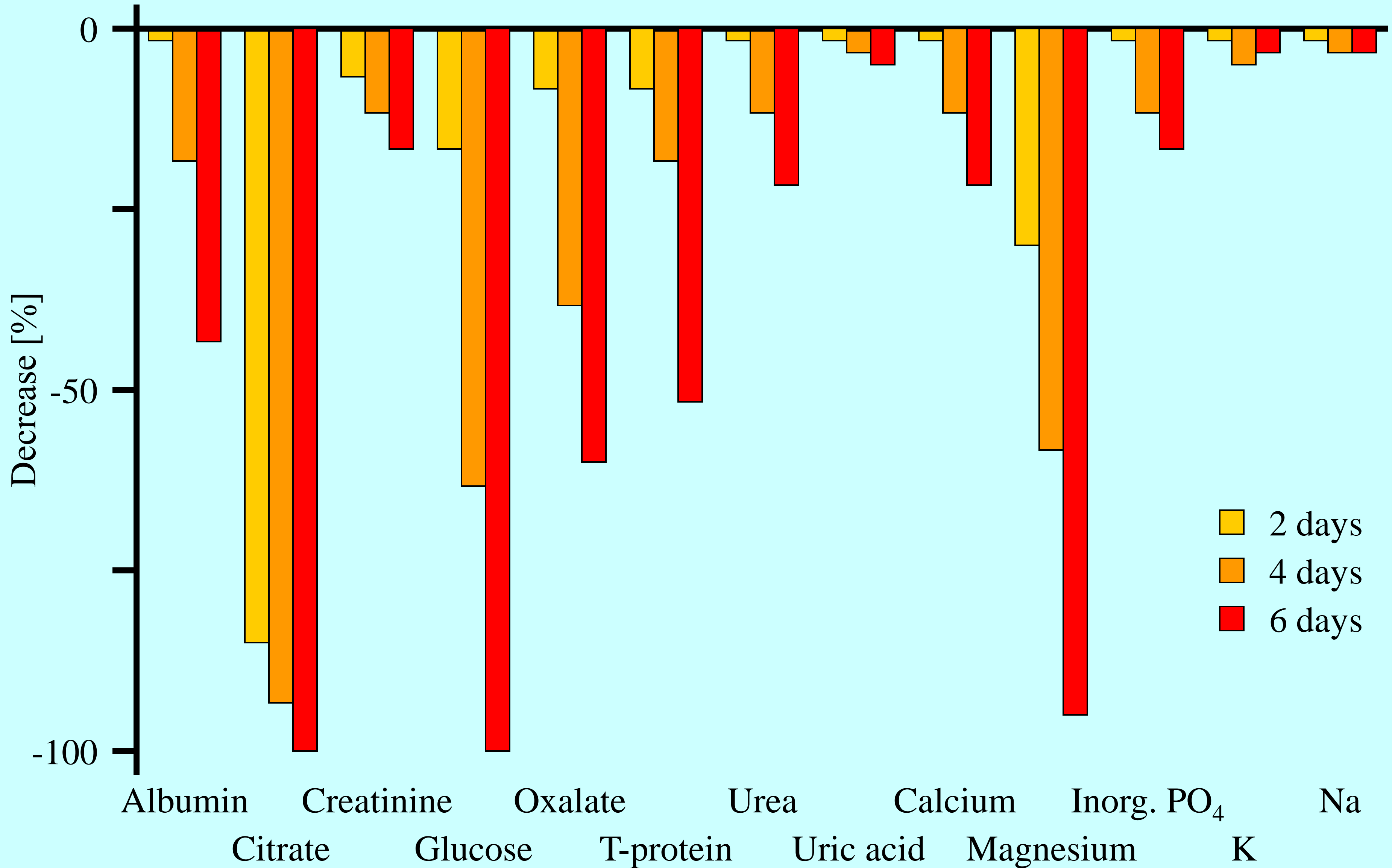
Glucose and other chemicals are lost

Bilirubin and Urobilinogen oxidize and are no longer detected by reagent strips.

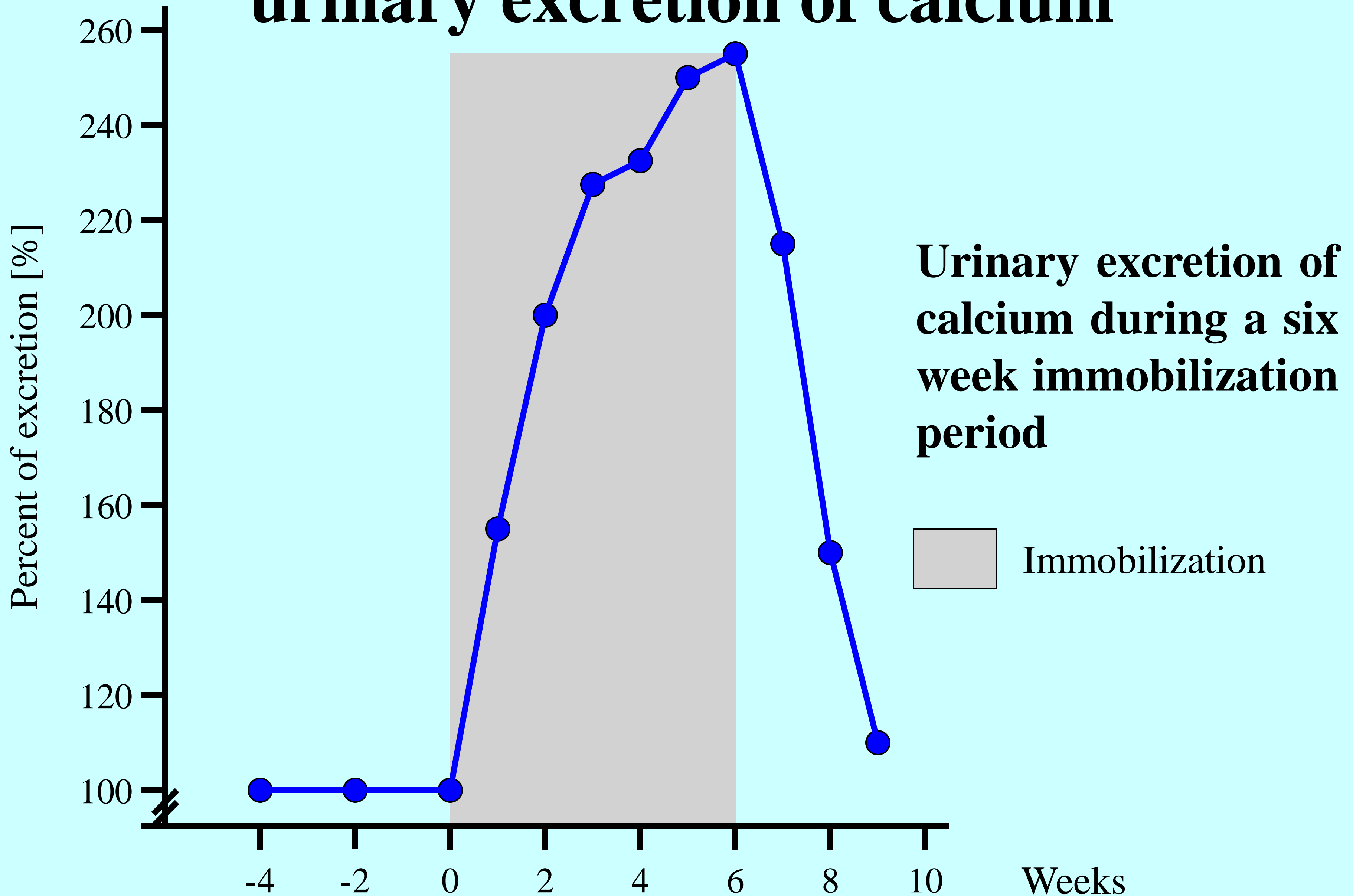
Bacteria multiply, causing an increase in pH as urea is broken down to ammonia and increased turbidity of the specimen is seen.

Influence of storage time on urine analytes

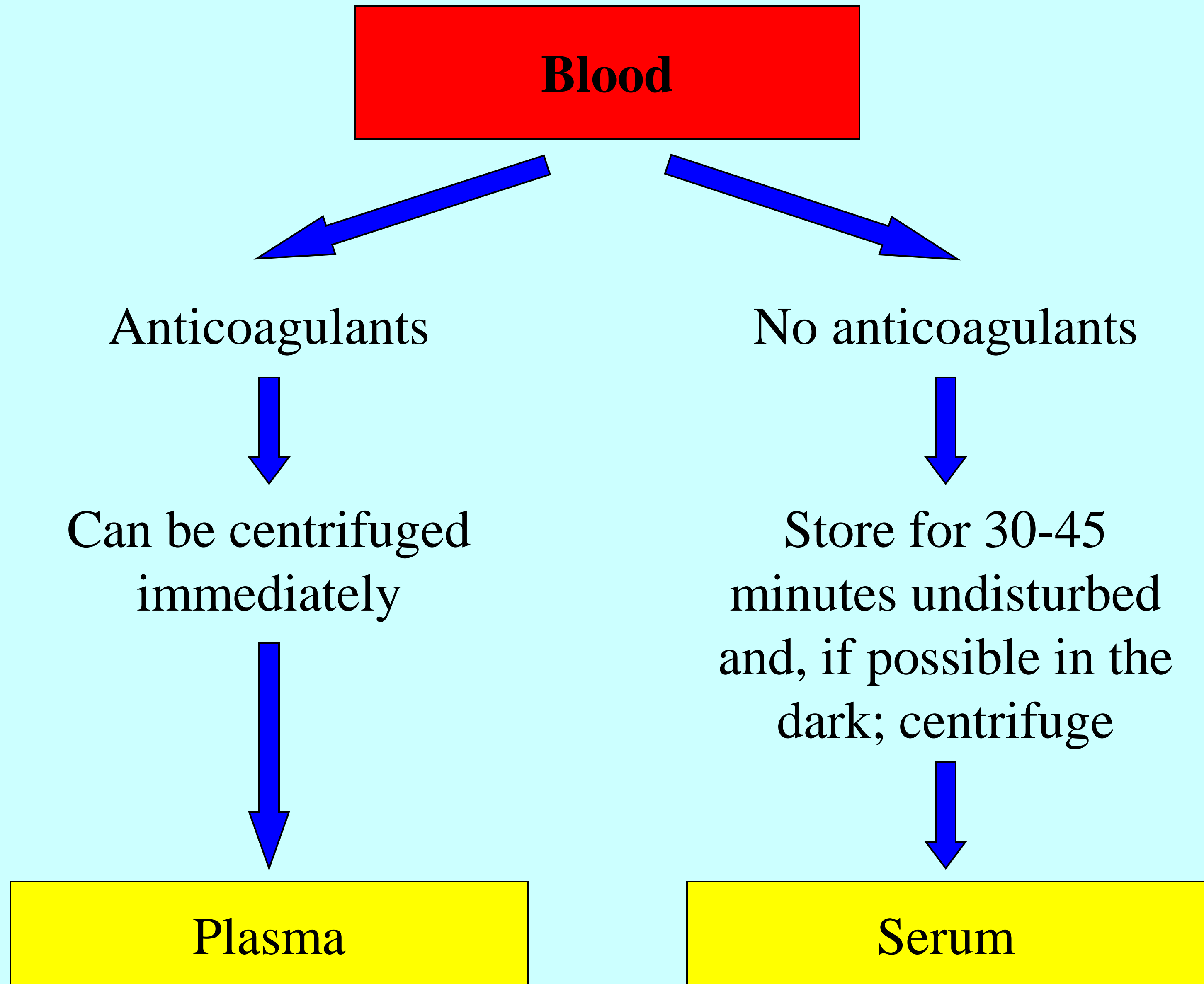
Samples without additives; storage at room temperature



Effect of immobilization on the urinary excretion of calcium



Plasma and serum



Different types of plasma

Different anticoagulants:

EDTA-plasma:	K_2EDTA, K_3EDTA
Citrate-plasma	
Heparin-plasma:	Li-heparin, Na-heparin, NH_4-heparin

Different centrifugation forces:

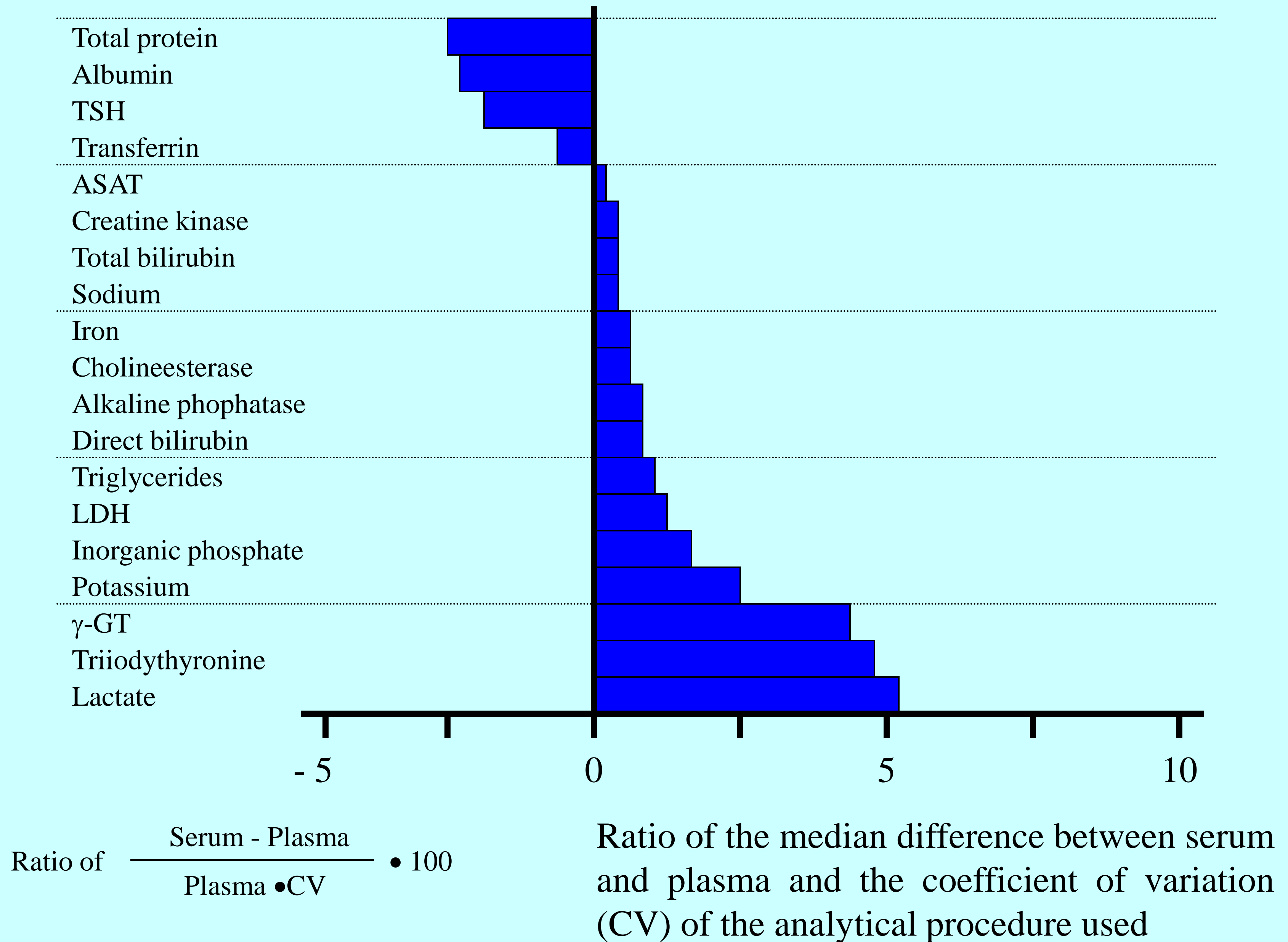
Plasma	**Relative centrifugal force (g)**	**Centrifugation time [min]**
Platelet-rich	150-200	5
Platelet-poor	1000-2000	10
Platelet-free	2000-3000	15-30

Plasma-serum differences of analytes - I

Analytes with diagnostically relevant serum/heparinized plasma concentration differences and their main causes

Analyte	% change in comparison to the mean in plasma	Main cause of the serum/ plasma difference
Potassium	+ 6.2	Lysis of cells, particularly platelets
Inorganic PO_4	+10.7	Release from cellular elements
Total protein	-5.2	Effect of fibrinogen
Ammonia	+38	Thrombocytolysis, hydrolysis of glutamine
Lactate	+22	Release from cellular elements

Plasma-serum differences of analytes - II



$$\text{Ratio of } \frac{\text{Serum - Plasma}}{\text{Plasma} \bullet \text{CV}} \bullet 100$$

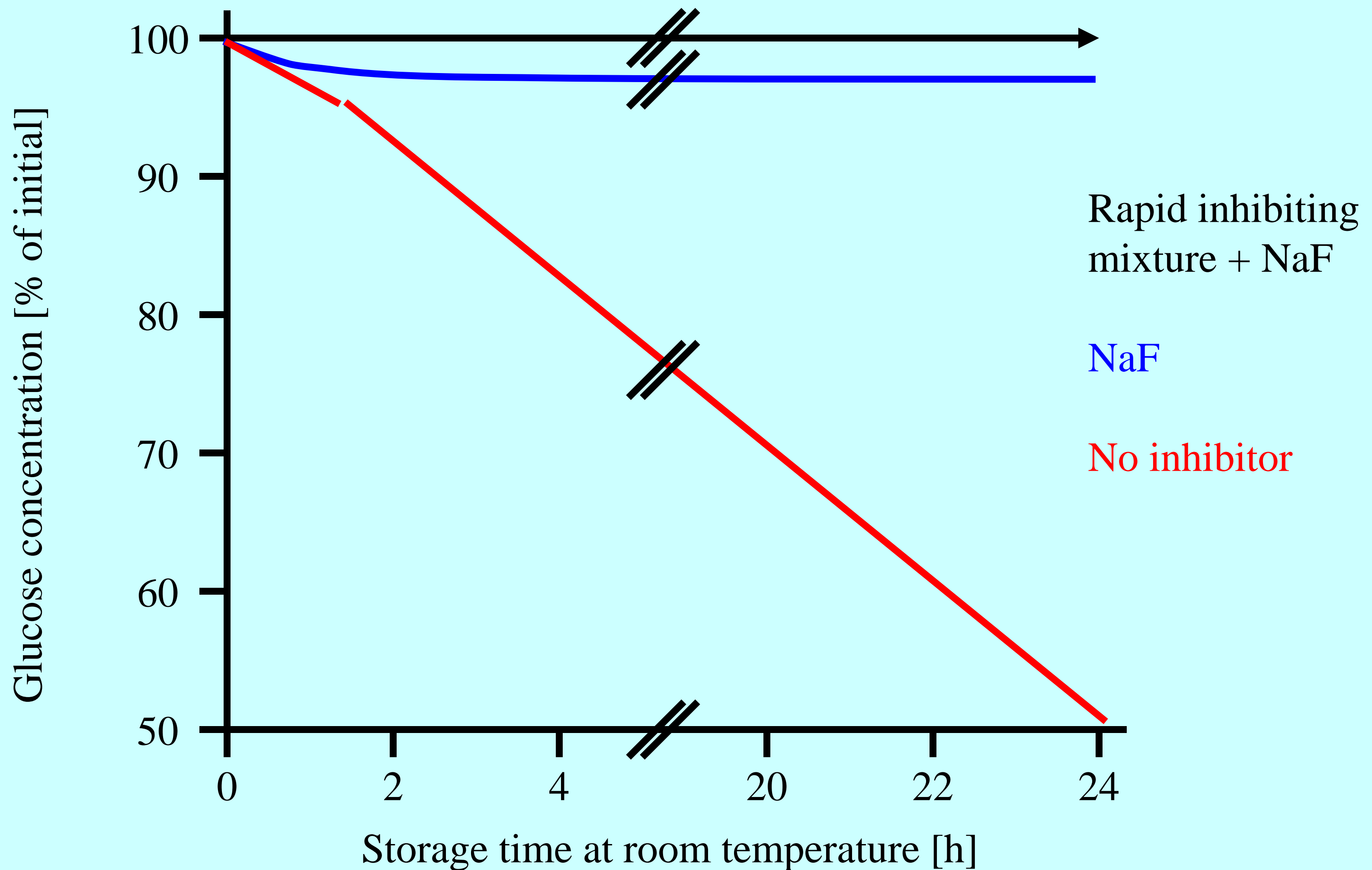
Ratio of the median difference between serum and plasma and the coefficient of variation (CV) of the analytical procedure used

Additives and colour codes of tubes
(Becton-Dickinson)

Tube	Application	Colour
Plain (non-additive)	Clinical chemistry and serology	Red
Heparin (14.3 U/l)	Plasma chemistry	Green
K_2 or K_3 EDTA (1.5 mg/ml)	Hematology and selected chemistry	Lavender
Sodium citrate (0.105 mmol/l)	Coagulation	Blue
Sodium fluoride (2.5 mg/ml) K-oxalate (2.0 mg/ml)	Glucose, lactate	Gray
Sodium iodoacetate (0.5 mg/ml) Heparine (14.3 U/l)	Glucose	Green

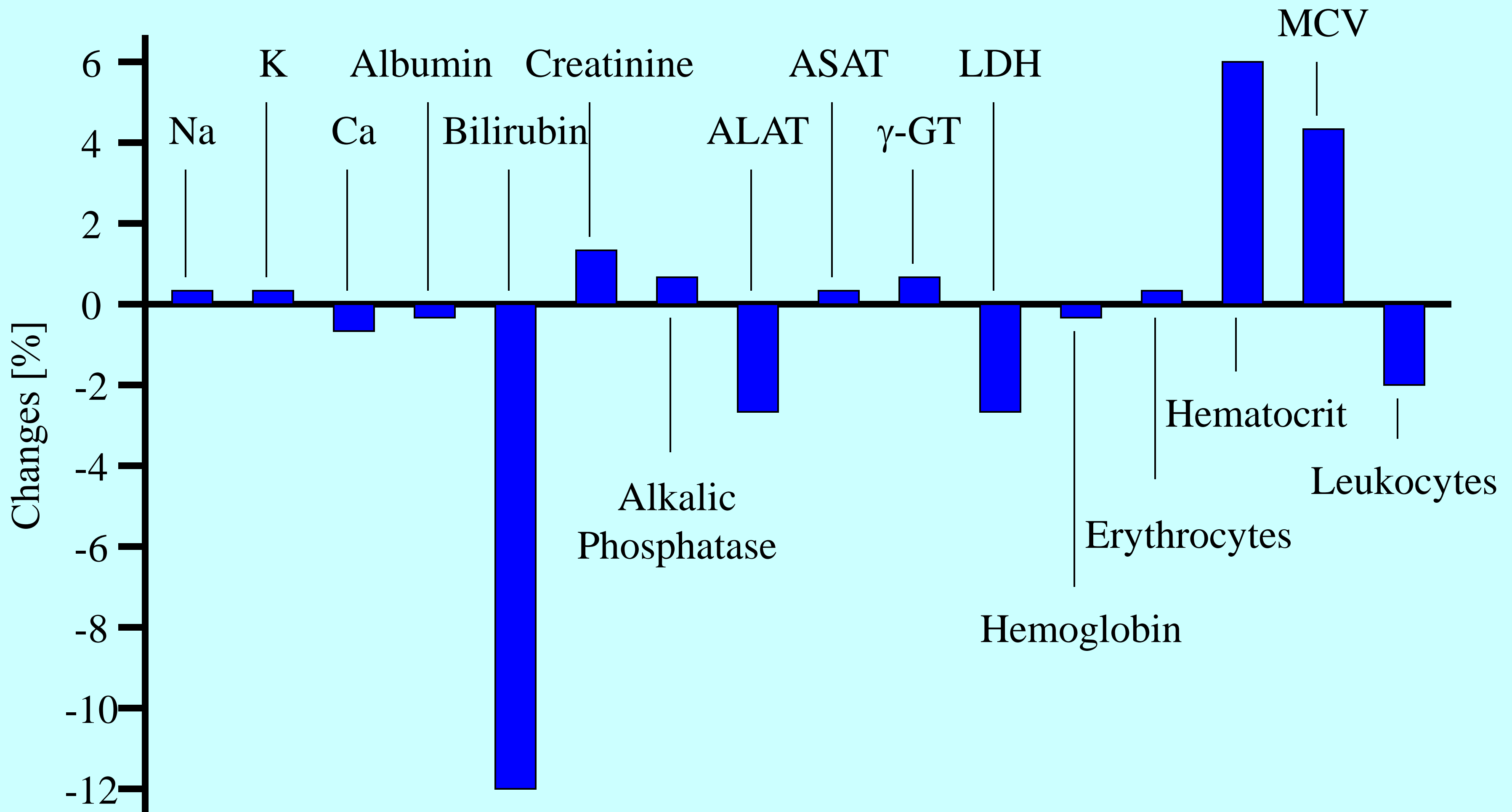
Degradation of glucose

Preservation of glucose for glycolytic inhibitors



Effect of time and temperature during transport

Stability of various analytes during mail transport



Management of samples - I

The procedure is governed by the stability of the constituents of the sample. The most important causes for the alterations to the quality of specimen are:

Metabolism of the blood cells
Evaporation/sublimation
Chemical reactions
Microbiological decomposition
Osmotic processes
Effect of light
Gas diffusion

Rapid transport and short storage times improve the reliability of laboratory results.

Specimens and samples are preserved longer the cooler they are stored (but note exceptions!).

Specimens and samples should always be stored in closed vessels (evaporation).

The danger of evaporation also exists in refrigerators (condensation of moisture on the cooling elements).

Management of samples - II

Storage problems are reduced if disposable sampling systems are used.

Separating agents (e. g. gel separators) improve the serum/plasm yields and enable serum to be left in the original tubes above the blood.

Avoid shaking the sample vessels because of risk for hemolysis

Always store sample vessels containing blood vertically; the clotting procedure is accelerated.

Lable infectious material and handle it with particular care.

Avoid storing of whole blood.

Blood samples should reach the laboratory within 45 minutes of collection in order to ensure that centrifugation and separation of the sample is carried out within 1 hour.

Avoid glycolysis to keep glucose, lactate and pH stable. Glycolysis can be avoided by the addition of an inhibitor in conjugation with an anti-coagulant.

Management of samples - III

Avoid the effect of light otherwise there will be a fall in the values of bilirubin, vitamin C, porphyrins, creatine kinase and folic acid.

Reduce contact with air as far as possible. If this is not done, evaporation/sublimation will result in an apparent increase in the concentration/activity of all non-volatile components. This is particularly the case when the volume of the sample is relatively small and the surface area is relatively large.

Whole blood should not be stored in the refrigerator. When urine is cooled, salts may be precipitate out of the solution (calcium and magnesium phosphate, uric acid).

For certain analytes the specimens/samples should not be deep frozen because of aggregation, precipitation or denaturation.

Management of samples - IV

A very common source of error is the inadequate mixing of deep-frozen samples after they have been thawed. Concentration gradients are prod-uced during thawing as the concentrated solution first melts and then runs down the sides of the vessel.

After thawing, the sample should therefore be inverted several times, avoiding the formation of foam. Look for undissolved material, and if necessary bring into solution by careful warming.

Store samples after analysis in such a way as to permit the confirming of results, checking the identity of samples or performing additional tests for medical or legal reasons.

Sample storage - I

Recommended storage time and conditions for analytical samples

Samples for	Storage time	Temperature
Clinical chemistry	1 week	Refrigerator
Immunology	1 week	Refrigerator
Hematology	2 days	Room temperature
Coagulation	1 day	Refrigerator
Toxicology	6 weeks	Refrigerator
Blood grouping	1 week	Refrigerator

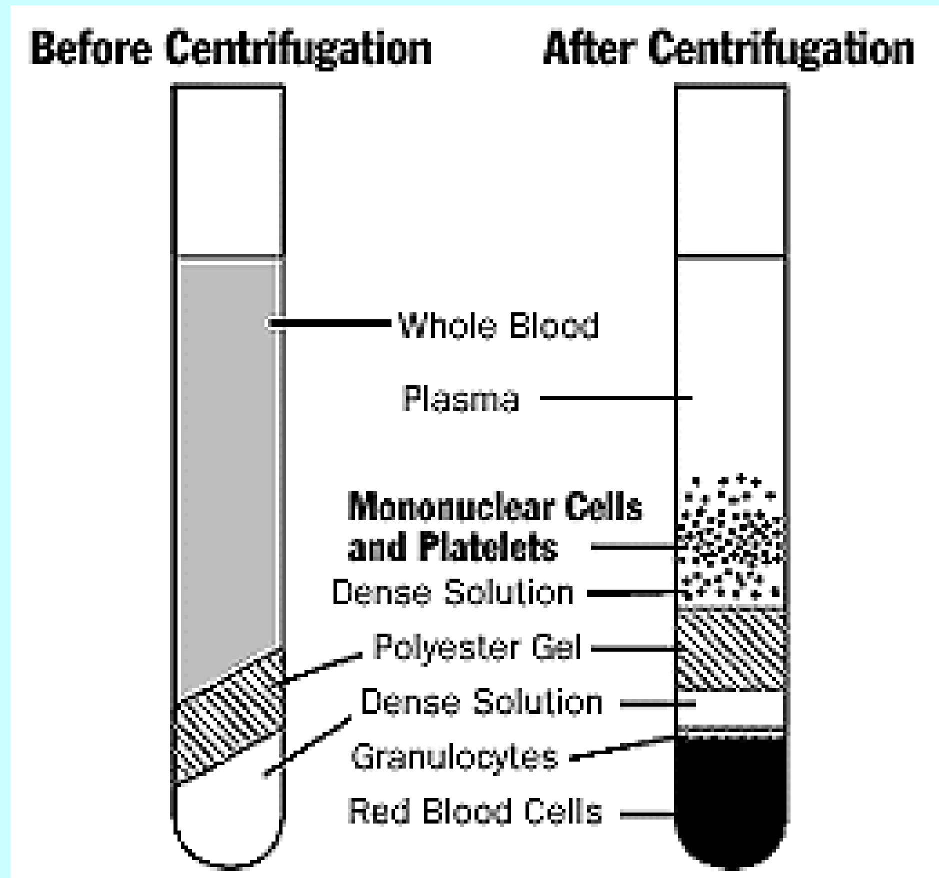
Sample storage - II

Examples of blood and urine constituents which should not be stored frozen

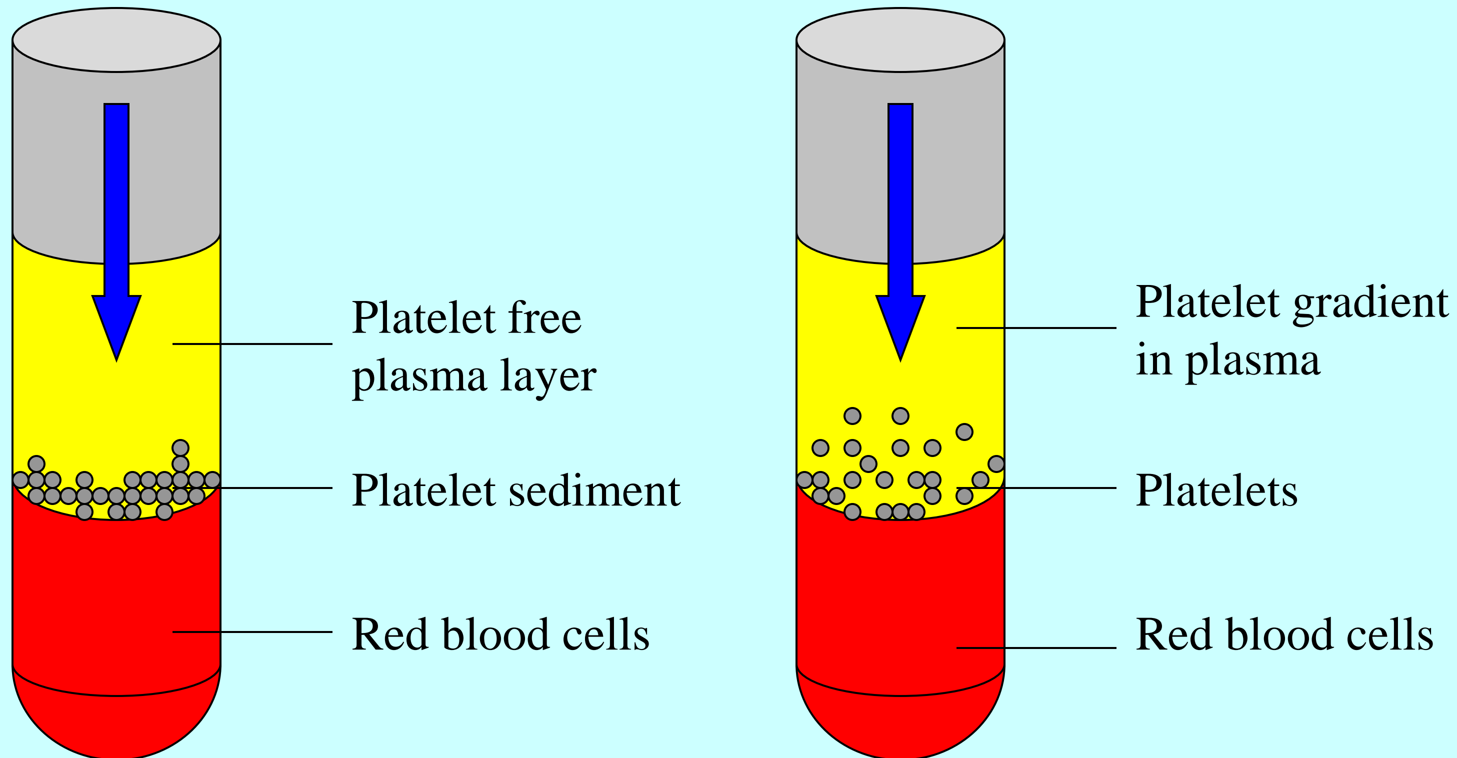
Sample	Analytes
Serum/plasma	Lipoprotein electrophoresis Lipoprotein X Apolipoprotein A-I and B LDL-cholesterol (prevented by addition of glycerol) Fibrin monomer positive plasma#)
EDTA-blood	Hematology
Urine	IgG Sediment Uric acid (precipitations)

#) Negative test result, prolonged PTT, shortened thrombin time, shortened reptilase time

Separation of blood components by means of centrifugation



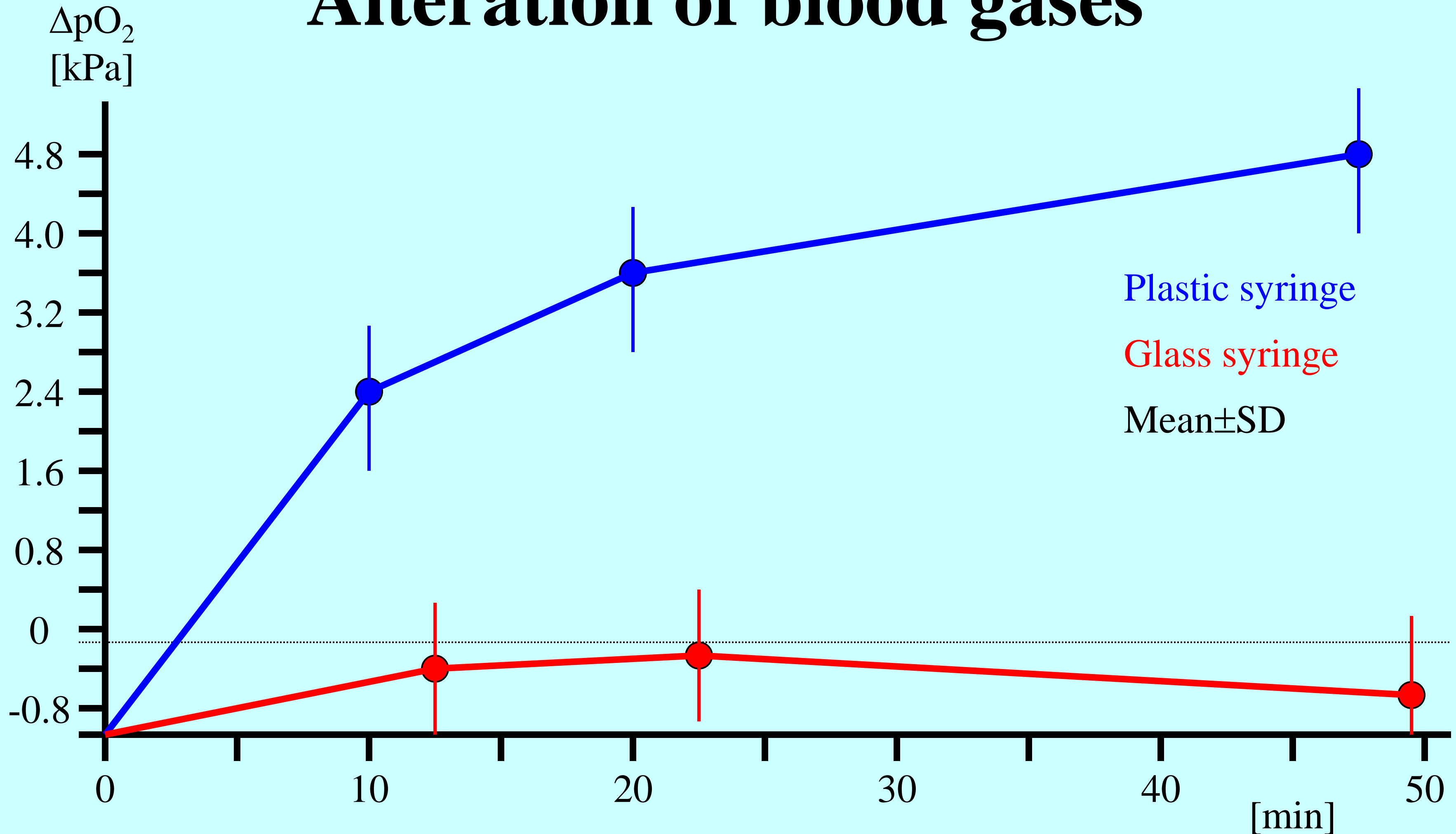
Platelet contamination after insufficient centrifugation



Left: Sufficient time and centrifugation force.

Right: Insufficient time and centrifugation force. Sample probe will pick up platelets present in plasma giving rise to spurious results

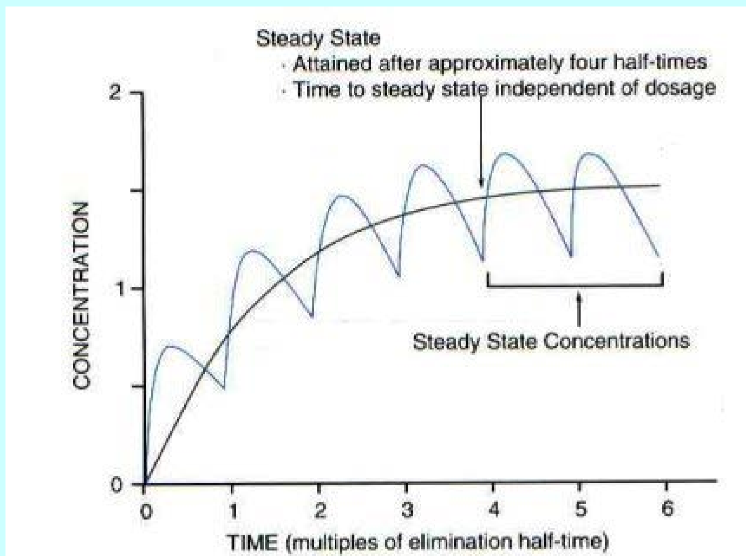
Alteration of blood gases



Alteration of pO_2 in whole blood (pO_2 = 85 mmHg (11.3 kPa)) stored in a plastic or glass sysringe for 45 minutes at room temperature (4.8 kPa = 36 mmHg).

The right time for therapeutic drug monitoring

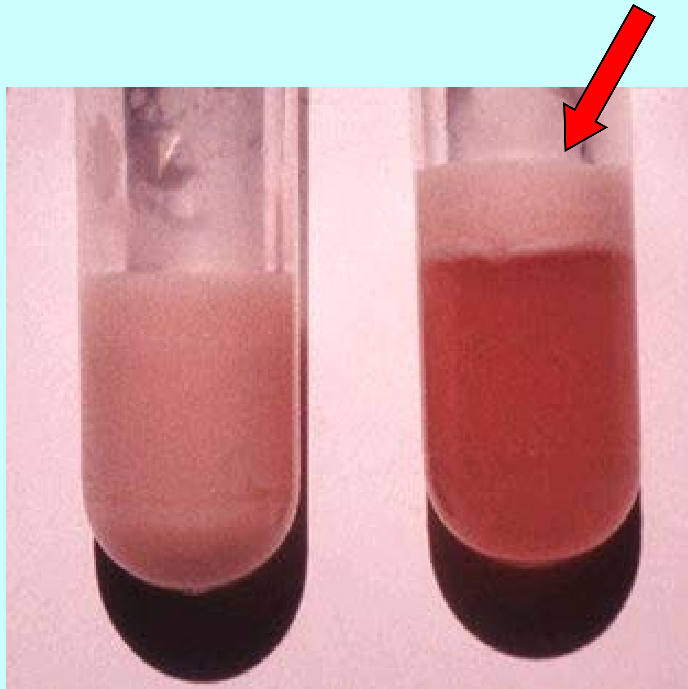
Time at which blood sample should bee taken



Long term therapy:
Basically always in the steady-state (after approximately 5 half-lifes)

Intravenous therapy:
One must wait until the distribution phase is completed (approx. 1-2 hours after completion of the in-fuion

Lipemia



Hyperlipemia is due to an increase in plasma lipids, namely cholesterol and triglycerides. Hypertriglyceridemia produces visible lipemia and indicates an elevation in chylomicrons or VLDL. When hyperlipemia is from increased chylomicrons, e.g. postprandial hyperlipemia, chylomicrons will form a fat layer above a clear infranatant when serum or plasma samples are refrigerated. When hyperlipemia is due to increased VLDL, a fat layer does not form, and the sample is turbid (lipemic). When there is a combination of both chylomicrons and VLDL, a fat layer will form above a turbid infranatant.

Hypercholesterolemia is generally caused by an increase in LDL and HDL and does not produce a visible hyperlipemia. It is important to remember that lipemia will interfere with laboratory tests, especially hematologic and biochemical tests. Therefore, fasting samples should always be collected for testing.

Mechanisms of interference in lipemia

Inhomogeneity:

Triglyceride-rich lipoproteins float during centrifugation and storage of serum/plasma samples. When then analyzed without careful mixing, triglycerides and other constituents may be inhomogenously distributed in the sample. This may cause a disproportionately high concentration of lipids in the upper layer and cause interference in other methods like total protein. On the other hand, lipids may replace water in the upper phase leading to a lower apparent concentration of water-soluble components like electrolytes.

Water displacement:

It is responsible for the higher concentration of sodium and potassium in direct ion-selective electrode measurement compared to flame photometry. In exceptonal cases, lipids can can replace up to 10 % of the water content of a serum/plasma sample.

Interference by turbidity:

Photometric procedures are sensitive to turbidity at nearly all wavelengths.

Interference by physicochemical mechanisms:

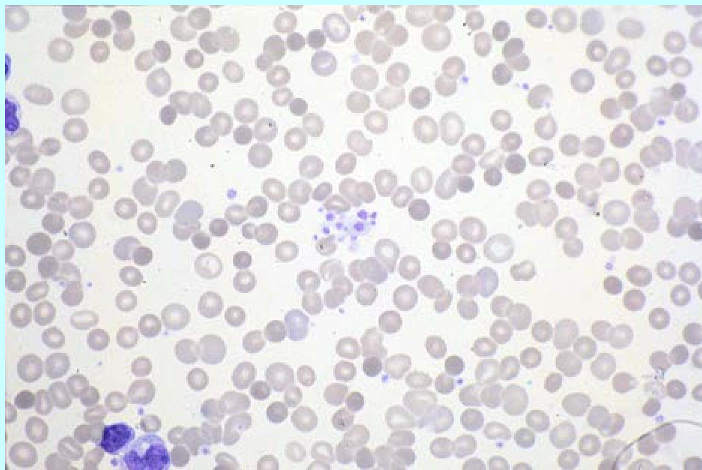
Lipoproteins in the sample may incorporate lipophilic constituents, thereby decreasing their accesibility to antibodies. Likewise, electrophoretic and chromatographic procedures may be disturbed by lipoproteins.

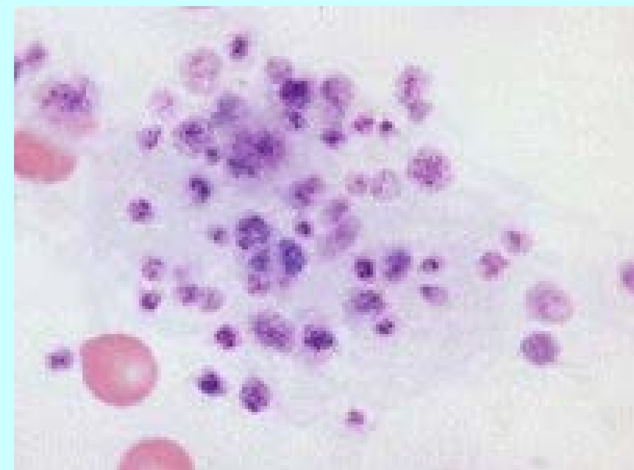
Pitfalls with endogenous antibodies

Endogenous antibodies can significantly affect the results of laboratory analyses. Distinct pitfalls are:

Cold agglutinins,
Cryoglobulins
EDTA-dependent antibodies
Macroenzymes
Autoantibodies
Heterophilic antibodies

Aggregation of platelets





In clinical routine, aggregation of platelets may result in low platelet counts (EDTA induced pseudo-thrombocytopenia)

Macroenzymes - I

The possibility of complexes with immunoglobulins (macro-enzymes) has been demonstrated for all diagnostically relevant enzymes.

A consequence of such phenomena is an increased biological half-life of such enzymes.

The increased half-life may in turn result in enhanced enzyme activity which can provoke further diagnostic measures.

The phenomenon of macroenzymes is primarily observed in elderly patients with chronic diseases.

Macroenzymes - II

Well-described examples are macro creatine kinase (CK) type I and type II.

Macro CK type I is an immunoglobulin CK-BB complex. Type II represents polymers of mitochondrial CK which can be detected by electrophoresis.

Both types of macro CK may affect accurate quantification of CK-MB by means of CK-M-inhibiting antibodies resulting in falsely elevated CK-MB activities.

Another example is macro amylase which is characterized by enhanced activity in serum while urinary amylase excretion is unchanged.

Autoantibodies

Immunoassays can be affected by autoantibodies or heterophilic antibodies.

Well described examples are autoantibodies directed against triiodothyronine and thyroxine. In these cases thyroid hormone concentrations are apparently enhanced since the tracer is bound not only to the receptor antibody added to the sample but also to the autoantibody.

Antiphospholipid antibodies in plasma result in increased aPTT values because the antibody binds phospholipids used as reagent in the assay.

Heterophilic antibodies

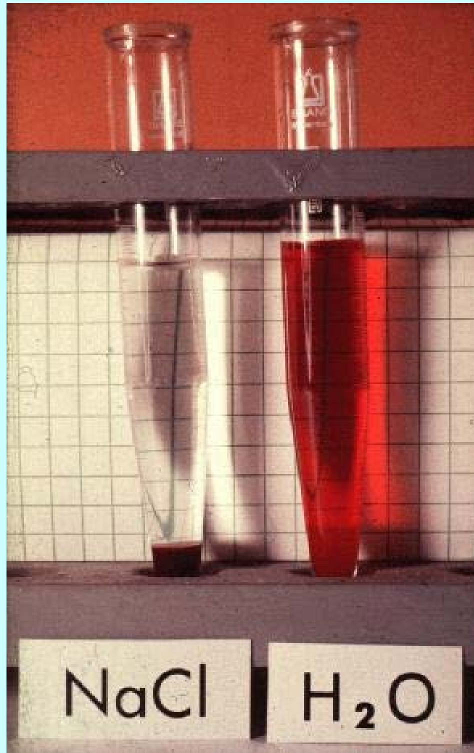
Heterophilic antibodies (e. g. human anti-mouse antibodies (HAMA)) are detected in some human serum samples.

The mechanism underlying generation of these antibodies is unknown.

In some cases, interference by heterophilic antibodies can be of diagnostic significance. If antibodies have anti-mouse specificity and assays employing immunoantibodies from mice are used (murine monoclonal antibodies), interference of these assays is possible.

There are several reports in the literature describing wrong therapeutic measures as a consequence of such antibody induced analytical errors.

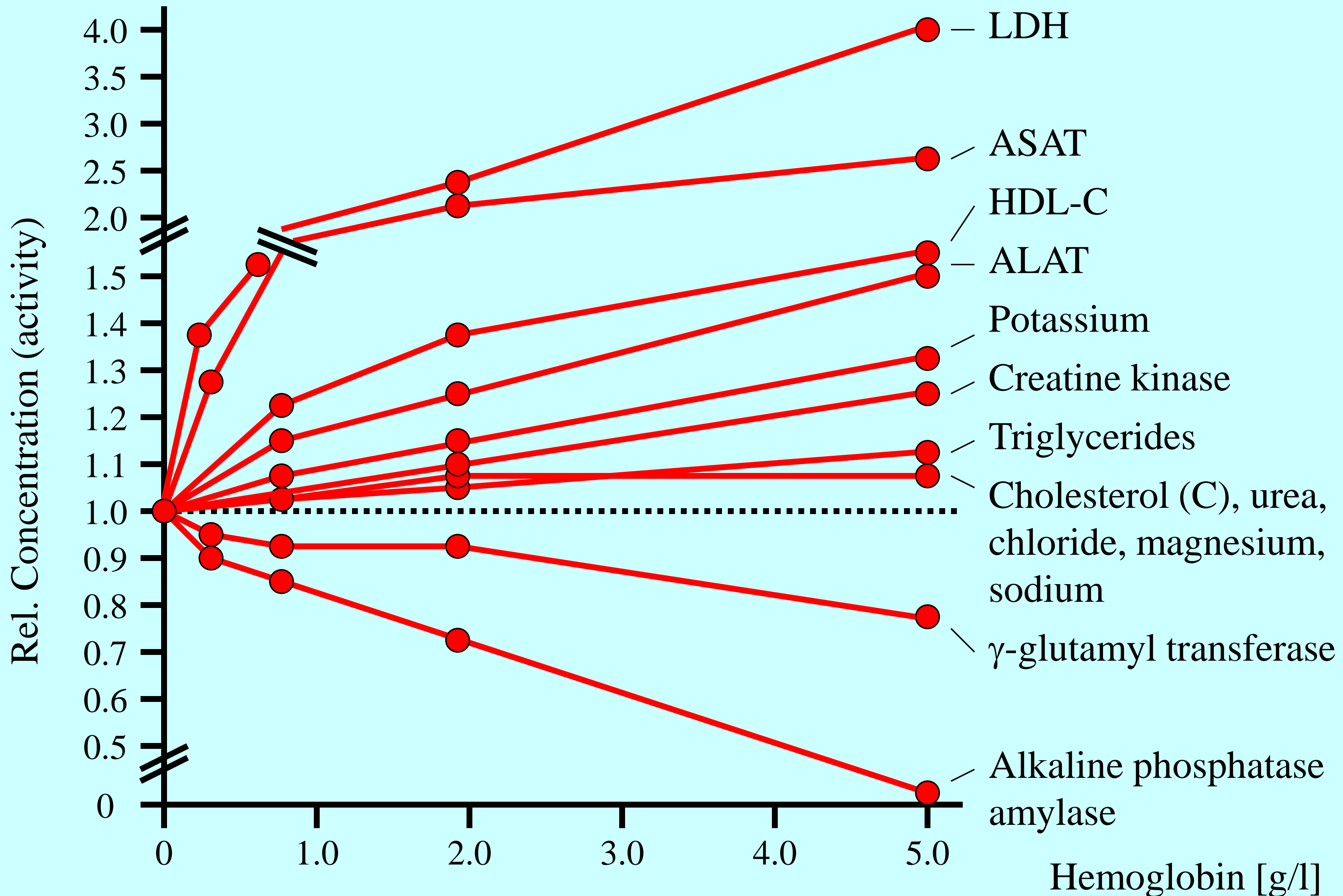
Hemolysis



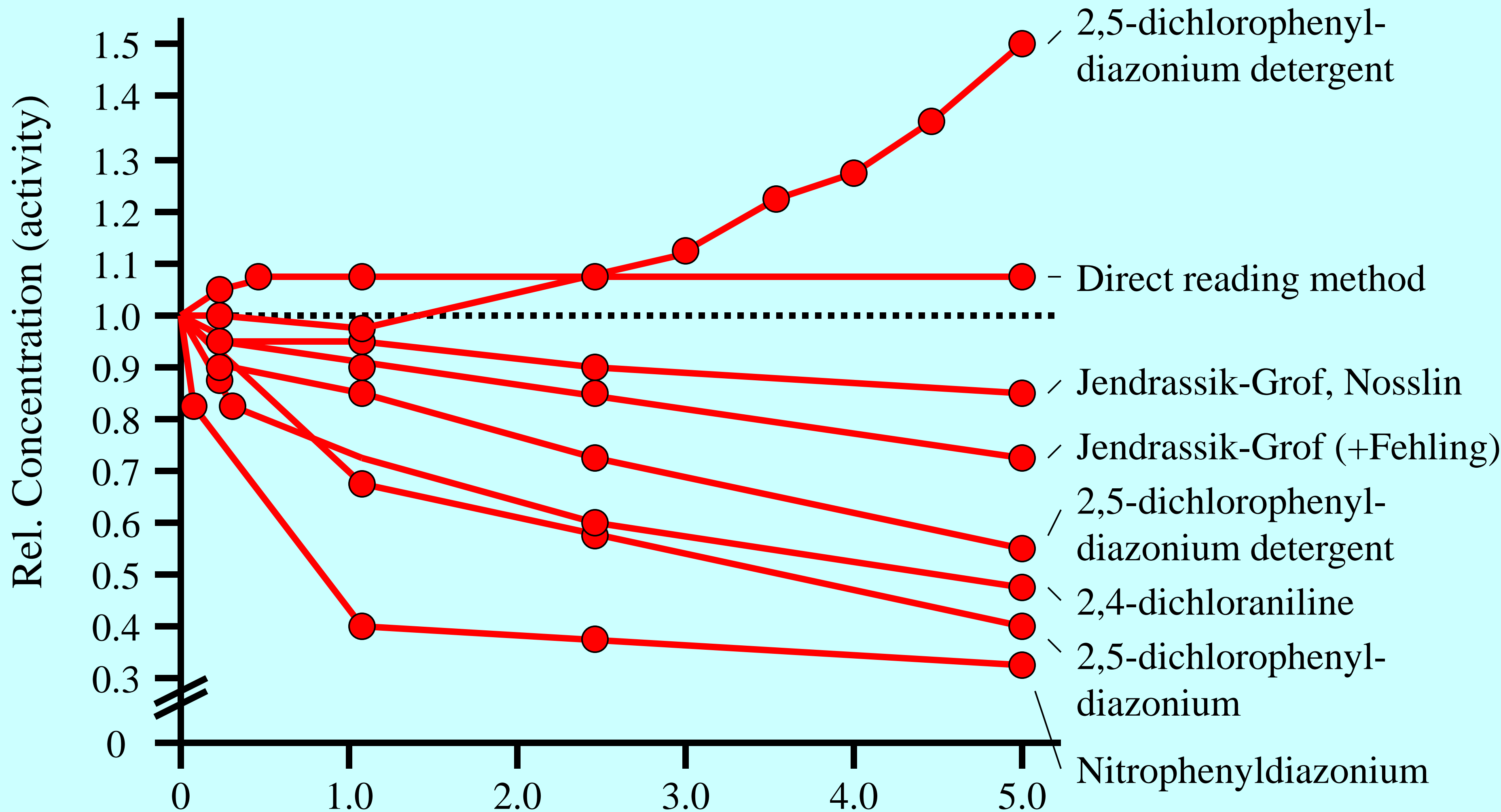
If normal blood is put into a centrifuge and the cells are spun down, the resulting supernatant is yellow plasma or serum. If a hemolytic sample is put in a centrifuge and the cells are spun down, the resulting supernatant is red coloured due to the hemoglobin in solution, and the cell pellet at the bottom of the tube is very pale.

The picture on the left shows a comparison of spinning down a suspension of cells in 0.9 % NaCl and some suspended in water.

Effect of hemolysis



Effect of hemoglobin on total bilirubin determined by different assays



Mechanisms of interference in hemolysis

Increase of intracellular constituents in the extracellular fluid:

The efflux of intracellular constituents may occur in vivo, during sampling and at all stages of the preanalytical phase. Accordingly, hemolysis may be a diagnostically relevant observation, defined as an in-vitro influence factor when occuring during sampling or other steps of the preanalytical phaseas it leads to alteration of the sample composition.

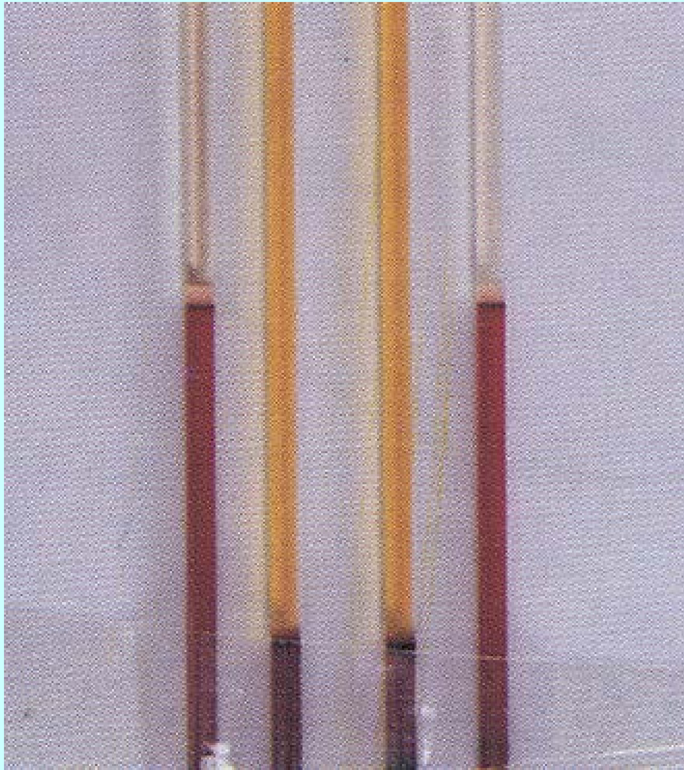
Optical interference:

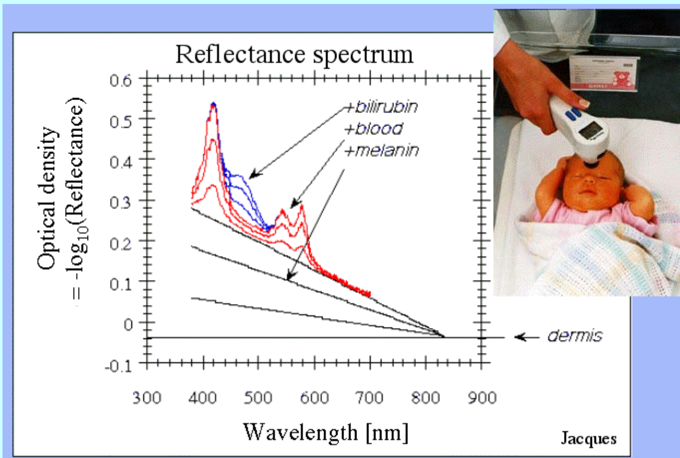
It may be due to the colour of the hemoglobin, which may change during sample storage due to hemoglobin formation. The direction and degree of interference differs not only with the wavelength(s) but also with the type of blank and reagent used.

Interference by intracellular constituents with the reaction mechanism of the assay (chemical, biochemical and immunological interference):

In this case, a method-dependent interference is observed which is not due to optical interference by hemoglobin. Thus, adenylate kinase released from blood interferes with most standard methods for the measurement of creatine kinase activity, the interference being dependent on the concentration of the inhibitors of adenylate kinase.

Hyperbilirubinemia





Biliburin interference arises from its spectral properties and its ability to react chemically with other reagents which are used for the determination of parameters in clinical chemistry.

Top left: Hematocrit capillaries of normal patients (left and right) and a patient with hyperbilirubinemia (middle).

Bottom left: Spectrum of bilirubin determined by non-invasive reflectometry in newborns.