Factors influencing laboratory test results

The results of laboratory tests are influenced by many factors. Their ignorance or underestimation may lead to incorrectly interpretation of the result. Laboratory examination involves three phases:

- **Preanalytical part** – preparation of the patient, collection of biological material and its transport to the laboratory, storage of sample before analysis, preparation of sample for its processing.
- **Analytical part** – own analysis and result calculation.
- **Postanalytical part** – data validation, data transfer and their interpretation.

a) Factors of prenalytical phase

The most important phase of examination from the point of view of possible influencing of the result is the preanalytical part during which the result can be influenced by biological influences (controllable and uncontrollable), the way of material collection, its transport and storage. If it is possible, we try to eliminate (eventaully to minimalize) these factors. Otherwise, they must be taken in account when interpreting results.

Uncontrollable biological influences

Race, ethnic group of population – different race/ethnic groups have different metabolic pathways, but also the amount of muscle mass, different physiological values of some analytes, resp. a different frequency of disease due to a different frequency of certain genes.

Gender – mostly does not affect the physiological values of analytes, if so, in women the values are slightly lower than in men. The differences are mainly due to the proportion of hormones and habit. Before puberty, the differences in values between girls and boys are minimal.

Age – most parameters in childhood have a lower upper reference value compared to the adult population. Many biochemical systems or processes are associated with a certain stage of the organism development. High physiological values during adolescence are mainly due to skeletal development.

Pregnancy – there are several mechanisms that lead to changes in analyte concentrations, enzyme activities, and number of components during pregnancy. We can see changes in the production of hormones and their effect on the organism, the influence of the placenta, transfer of substances from amniotic fluid, etc.

Biorhythms – these are regular linear (age) or cyclic changes in metabolism under the influence of hormones of the hypothalamus and pituitary (daily, monthly…) or changes caused by climatic or seasonal conditions, which can be predicted with some probability. However, noncyclic, unpredictable biorhythms also occur in each individual.

Controllable biological influences

Body weight – can influence analyte concentrations by changing the volume of distribution.

Concentrations of LDL-cholesterol, triglycerides, uric acid, insulin are positively correlated with obesity.

Physical activity – influences the change in body fluid composition and depends on the length and intensity of the physical load. Acute strength and exhaustive exercise increase

es the proportion of anaerobic metabolism, endurance exercise aerobic metabolism. During physical activity there is increased utilization of substrates (glycemia initially increases slightly, after longer exercise decreases, triacylglycerolemia decreases, concentration of free fatty acids increases), metabolism changes in tissues (increased lactate formation, pH decrease), fluid is transferred from intravasal space into the interstitial (increased hematocrit, total protein and substances bound to it), to change the concentration of many hormones.

Diet/starvation – influence of the analysed analytes by various mechanisms – depends on the composition and amount of food and fluids. Hormones and enzymes are washed out before and during meals. During dehydration of the organism the haematocrit increases as well as the concentration of many substances. A protein-rich diet increases phosphates, urea and uric acid. A fat-rich diet reduces the proportion of nitrogenous substances such as uric acid. A carbohydrate-rich diet increases level of lactate dehydrogenase and decreases the concentration of triglycerides. The vegetarian diet decreases total and LDL-cholesterol and triglycerides, increases total bilirubin and pH of urine. Some foods and drinks may influence some metabolic pathways, e.g. if caffeine is contained, catecholamines, glucose and free fatty acids are increased.

Smoking – influences the level of many analytes mainly due to nicotine. Smoking affects glucose metabolism, increasing concentrations of cholesterol and triacylglycerols**.**

Alcohol – consumption of alcohol changes biochemical analytes differently depending on whether it is acute or chronic abuse. In general, it primarily affects the metabolism of glucose, triglycerides and increases the liver enzymes in the blood. Single use of alcohol in moderate and moderate doses minimally affects the examination. Long-term abuse leads to hypoglycaemia, keto – and lactacidosis and increased concentration of uric acid.

Drugs – can influence biochemical examinations by several mechanisms, e.g. they induce/ inhibit liver enzymes, affect binding to transport proteins, act cytotoxically or cancel the biochemical assay.

Stress – influences the production of hormones, which subsequently alters the metabolism of many substances. Stress often accompanies more serious illnesses, but in some people, even blood collection itself.

External environment – can have a significant influence on metabolism and consequently on concentration of many analytes. These include altitude (from altitudes above 3000 m), ambient temperature, but also geographical location – countryside, city. Traveling through time zones is manifested by a change of some analytes, most often it is retention of sodium ions and fluids with normalization 2 days after return.

Mechanical effects – e.g. muscle trauma, intramuscular injections increase ALT, AST, CK activity and myoglobin concentration, uterine pressure in high stage of pregnancy increases ALT activity, digital rectal examination increases prostate specific antigen (PSA) activity.

Collection of material

Instruction of patient plays a major role in the process of proper laboratory testing.

Fasting collection – it is recommended that the patient does not eat for about 10–12 hours and is relatively calm. It is also recommended to drink about 2-3 dl of water in the morning. Non – compliance of fasting results in distorted findings in the parameters of carbohydrate and lipid metabolism. For some special examinations or functional tests, dietary or regimen measures are prescribed (PSA may be positive after cycling).

Collection time – the concentration of some substances varies considerably during the day (e.g. glucose, triglycerides, hormones), others during the month or year. Planned sampling is usually done in the morning. The collection position influences the protein concentration respectively. substances that bind to proteins (e.g. total proteins, albumin, enzymes, lipids, Ca^{2+}, Fe^{3+} ions). In the standing position water leaks from the intravascular environment and thus the concentration of these substances increases by 5–15 %. The standard position of the patient during collection is the sitting position.

Use of tourniquet – its application should not be longer than 1 minute, the patient should not "pump" his arm. Longer constriction of the limb (approx. 5 min) and significant exercise leads to a 10 % change in activity or concentration of a number of analytes. This change is most often due to the transfer of low molecular weight substances from the intravascular space to the interstitium due to an increase in filtration pressure across the capillary wall and metabolic changes at the site of constriction (anaerobic metabolism).

Vein/finger compression – influences concentration of blood gases, lactate and pH.

Hemolysis (visible at hemoglobin concentration> 0,2 g/l) – there is an increased concentration of analytes in the hemolytic sample whose concentration inside erythrocytes is high (e.g. total proteins, lactate dehydrogenase, acid phosphatase, aspartate aminotransferase, ions K^+, phosphates, Mg^{2+}). Causes of hemolysis: use of too big or small needle, rapid emptying of the syringe, rapid shaking of blood in the tube, moisture in the collection kit or in the tube, presence of detergents in the tube, incorrect anti-coagulant/blood ratio, storage of blood samples in fridge or in high temperature surroundings (in the sun, above the radiator), centrifugation at high speed.

Transport – serum/plasma is preferable to whole blood for transport. There is a risk of hemolysis when transporting whole blood. During transport, whole blood is stored at 0 °C (melting ice).

Sample stabilization/storage

Longer standing of the collected blood leads to depletion of erythrocyte energy sources, which cannot maintain the basic metabolic processes (K^+ leakage of erythrocytes into the blood of Na^+ transport in the opposite direction). Depending on the analyte we choose these condition for storage and stabilization of blood samples: low temperature (4 °C, 0 °C (melting ice), −20 °C, −80 °C), light protection, sample pH adjustment, addition of stabilizer.

b) Factors of the analytical phase

The result of each laboratory examination is characterized by several features that determine the extent to which it reflects the real situation and to what extent it is affected by errors. The basic analytical properties of each method include precision and trueness. Precision is the closeness of agreement between independent results of measurements under determined conditions. Precision is a general term. The measure of variance of the results (x_i), i.e. the numerical value of precision, is expressed by the concept of imprecision. The imprecision is reported as the sample standard deviation s (given in units of the analyte to be measured) or as the relative standard deviation (coefficient of variation) CV:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

$$CV = \frac{s}{\bar{x}}(\cdot 100\%)$$

The repeatability and reproducibility of the method are distinguished according to the conditions under which the precision is determined. **Repeatability** refers to the precision of a method where all analyses are performed in one series of measurements, on the same day and on the same instrument. The concept of repeatability is identical to the designation "precision in series". **Reproducibility** expresses precision over time. It is obtained by calculating from determinations carried out gradually over a period of several days on one apparatus. Reproducibility is referred to as "precision between days".

The precision depends only on the distribution of random errors, it is not related to the actual value of the result.

Random errors arise quite irregularly due to random effects, they can be statistically evaluated. They cause a variance in the measurement results, which can be characterized by the measurement precision expressed numerically as the standard deviation *s* or the coefficient of variation CV. Their effect on the measurement result can be reduced by increasing the number of measurements. Random errors arise from imprecision of measurment of the type that is described by a Gaussian distribution (e.g. caused pippeting or signal variability).

Trueness expresses the closeness of agreement between the average (mean) value obtained from a large series of results of measurements and the reference value. The measure of trueness is the bias, which expresses the difference between the mean value of the results and the reference value. Trueness of the method is given by systematic error (bias). **Reference value (x_0):** it represents true value, which in fact is always unknown. It is obtained using a reference method in many laboratories.

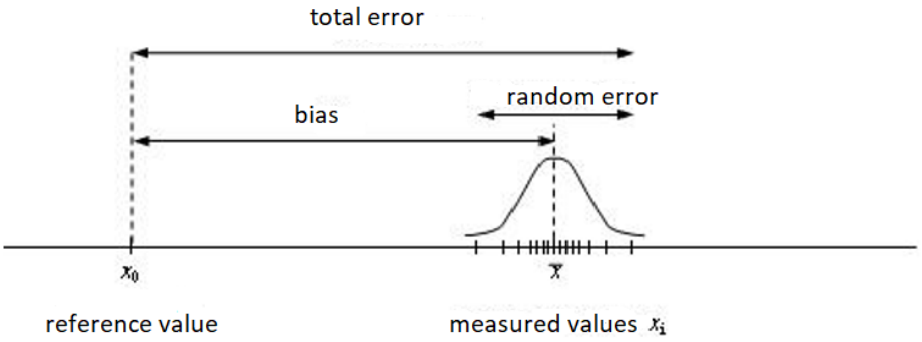
Accuracy expresses the closeness of agreement between the individual (single) measurement result and the reference value. Accuracy combines precision (characterized by standard deviation s) and trueness (characterized by bias), i.e. the effects of random and systematic factors. The measure of accuracy is the total error (TE):

$$TE = 1{,}96 \cdot s + \text{bias}$$

Reliability is determined by the precision and trueness of the measurement. Represents the range that individual measurements reach during repeated measurements.

Repeatibility - the closeness of agreement between results of successive measurements carried out under the same conditions.

Reproducibility - the closeness of agreement between results of successive measurements carried out under changed conditions (e.g. time, operator, reagents …).



Each examination result is always influenced by random and systematic errors. The result of existention of errors is the uncertainty of the measurement result. **Uncertainty** (u) represents the interval of values in which the analysis result (x) is with some probability. Uncertainty involves many components, while insignificant are negligible, significant are expressed as standard deviations (or coefficients of variation) as the so-called standard uncertainty u_s (i.e. numerically u = s). Standard uncertainty expresses individually partial uncertainties of measuring process. The most important random component of uncertainty is the standard uncertainty of reproducibility and the most systematic component of the uncertainty is the standard uncertainty of the calibrator. Combined standard uncertainty (u_c) can be obtained from partial uncertainties of measurement.

The real value is found with some probability in the interval (in the so-called expanded uncertainty), which is obtained by multiplying the standard combined uncertainty (u_c) by the expansion coefficient (k), which has a 95 % confidence level of 1,96 (approximately 2).

If the principles of good laboratory practice are followed, the results of the laboratory examination should always include a detail about the expanded uncertainty of the analyte. Also reference values or limit values should include a detail about uncertainty of determination.

Correlation coefficient expresses measure of association between two variables.

Reference: https://www.wikiskripta.eu/w/Biochemická_analýza_krve