Biochemical blood analysis

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In order to correctly assess the state of health, it is necessary to obtain as much information as possible. The results of laboratory tests are a source of valid information that reflects changes in the body's metabolism and can be used in assessing the patient's health.

According to data presented by the World Health Organization WHO, laboratory examination provides around 80% of the information leading to the establishment of a correct diagnosis.

The most extensive and important part of the laboratory examinations is the analysis of blood, blood serum or plasma. Blood is a relatively easily available material, and its composition reflects a number of biochemical processes taking place in various tissues.

Another relatively frequently analyzed material is urine. Analyzes of other body fluids (gastric secretions, duodenal contents, amniotic fluid, cerebrospinal fluid, saliva, sweat, etc.) are required specifically only for a limited number of examinees, their frequency is considerably lower, and they are often only performed in specialized workplaces.

A doctor can use a wide range of laboratory methods at different stages of the diagnostic and therapeutic process. However, it is necessary to indicate them rationally, to use them effectively, then to evaluate and correctly interpret the obtained results.

Laboratory tests performed in clinical-biochemical laboratories are:

- *according to availability* - **basic**, **specialized**, **highly specialized**,
- *in terms of execution speed* - **routine**, **statistical**, **from vital indication**.

Directly in the doctor's office or at the patient's bedside, some examinations can be performed with advantage, providing immediate information without the need for analysis in a laboratory (**so-called bed-side diagnostics, point-of-care testing (POCT), near-patient testing**).

Blood sampling

Blood for collection can be obtained from veins, arteries or capillaries. Venous blood is most often collected (mostly from the elbow), less often capillary blood (e.g. from a finger, earlobe or from a warmed foot in a newborn). Arterial blood is taken only exceptionally, mainly for blood gas analyses.

Venous blood sampling

In terms of procedure and equipment for venous blood collection, we distinguish between two collection methods:

- **open** sampling system – the worker performing the sampling is in direct contact with the biological material

  Sampling is done either with a needle directly into the test tube or into a syringe with a very gentle pull using a plunger

- **closed** collection system – handling of the sample after collection is done directly in the collection syringe/tube.

  The worker is thus protected against contamination by the patient's blood during collection, the biological material itself is protected against possible contamination from the outside and against possible breakage during transport and centrifugation. Individual closed syringes/vacuum tubes are color-coded according to the type of preparation substance present inside (hemocoagulation accelerators, separation gel, heparin, EDTA, ...). he separation gel enables perfect separation of the serum from the blood coagulum after centrifugation (by creating an intermediate layer). It is thus possible to insert the sampling syringe/tube directly into the analyzer. The used material is easily disposed of by burning.

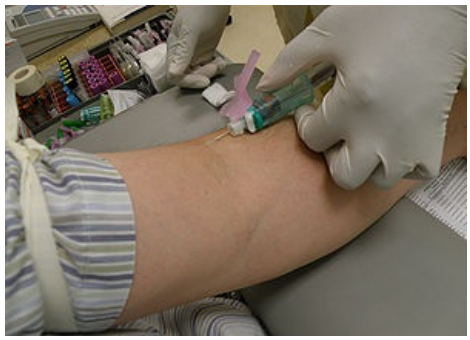
When using a **closed system**, sampling is done in a closed syringe or vacuum tube.

Collection in a closed syringe. The collection kit contains a syringe to which a needle is attached. When blood is drawn into the syringe, it is drawn using a piston. If the patient has sufficiently strong veins, the piston on the syringe can be pulled out and locked immediately before collection, thus creating a vacuum in the test tube through which the blood is drawn. After sampling, the plunger breaks off and the syringe becomes a closed tube. The sampling method is chosen according to the condition of the patient's veins.

Collecting in a vacuum tube. The collection kit includes a needle holder with hemostatic valve, a needle and an appropriate vacuum tube. Before sampling, a suitable needle is inserted into the holder, the position of the vein is stabilized with the thumb at a distance of 2 to 5 cm below the sampling site, a venipuncture is performed, and only

then appropriate tubes are gradually inserted. The vacuum tube must not be placed on the inner needle of the holder before venipuncture, as the vacuum in the tube would be cancelled. The vacuum in the tube ensures both adequate filling of the tube and a sufficient ratio of blood to anticoagulant.

Principles of venous blood collection



collection of venous blood into a vacuum tube, vacutainer system

- find out an allergy to disinfectants or a certain type of patch,
- before taking the arms, the patient does not exercise the hand (distortion of the examination series), the pressure can be increased by opening and closing the palm,
- disinfect the injection site (Jodonal B, Persteril, Jodisol, Ajatin),
- if the veins are clearly visible, take the sample from the unstretched arm,
- if a tourniquet is used (not longer than 1 min), it should be released immediately after the vein is punctured,
- if the tourniquet has been used for a long time or the patient has intensively exercised with the hand, this should be indicated in the request form,
- after sampling, apply a cotton swab moistened with a disinfectant to the injection site,
- when collecting non-coagulable blood, mix the blood immediately after filling by repeatedly rotating the collection tube at least 5 times (do not shake!).

Capillary blood sampling

Capillary blood is used in cases where only a small amount of sample is needed. Main principles when taking:

- by warming the injection site (belly of the finger, earlobe, heel in infants) to ensure good blood circulation (apply a warm, moist compress 3 minutes before the actual sampling)
- disinfect the injection site
- the injection should be directed from the side into the belly of the toe (heel), where there is better blood flow than in the middle
- after being impaled with a lancet, wipe off the first drop (tissue fluid admixture), support the formation of further drops with light pressure (tissue fluid admixture is present in the blood during strong squeezing)
- blood is usually collected in special capillary blood tubes or in small plastic or glass tubes. In strip tests, a drop of blood is applied directly
- after sampling, apply a cotton swab moistened with a disinfectant to the injection site

Blood processing

Blood collected without the use of anticoagulants clots after a shorter time, as a result of the conversion of soluble fibrinogen into a fibrous network of fibrin. **Serum** is obtained by centrifuging the clotted blood . Precipitation time must be sufficient (at room temperature after 15-30 min). However, premature separation of serum from blood elements can lead to additional fibrin formation and serum coagulation. Non-clotting blood must be obtained for some clinical-biochemical examinations. Blood is collected in containers with the addition of anticoagulant (anti-clotting) agents. **Plasma** is obtained by centrifuging non-clotting blood. Blood can be centrifuged immediately after collection, which saves time in acute conditions. Plasma or serum should be separated as soon as possible, but no later than 2 hours after collection (for determination of potassium ions within 1 hour after collection). To separate blood elements from plasma, or blood clots from serum, centrifuges are used. Closed collection syringes/tubes with blood are placed in the so-called rotor, where the sedimentation of substances with a higher density is accelerated by means of centrifugal force. An overload approximately 1000 times greater than Earth's gravity is used to perfectly centrifuge whole or clotted blood. Blood centrifugation is always carried out in closed tubes (preventing the formation of an aerosol, possibly contaminating the sample) for about 10-15 minutes at room temperature or at a temperature of 4 °C.

Preparation of blood plasma

During collection, the collected blood in the test tube is mixed well with an anti-coagulant substance (anticoagulant agent) by careful swirling, which dissolves in the blood and keeps it non-coagulating for a certain time (the volume of the blood does not change). The anticoagulant is put into a test tube in the form of a solution, which is allowed to evaporate. Non-coagulating blood with heparin can also be obtained by sucking the heparin solution from the ampoule into the syringe and creating a fine heparin film on the surface by repeated movements of the piston. We will then take blood into the syringe prepared in this way. Closed sampling systems are already filled with anticoagulants during production. Approximate doses of anticoagulant to prevent clotting of 1 ml of blood:

Approximate doses of anticoagulant to prevent clotting of 1 ml of blood:

Anticoagulant	Dose mg/ml
Sodium oxalate (lithium, potassium)	1−2
Trisodium citrate	3
EDTA.Na_2	1−2
Heparin	4−6 IU (solution), 40−60 IU (dry)

The principle of action of the first three coagulating agents in the table is the binding of calcium ions and thereby reducing their concentration in the blood. Calcium ions are necessary for the activation of several blood clotting factors (factors II, VII, IX and X. Heparin binds to plasma antithrombin III and increases its affinity to thrombin. Some clotting factors are inactivated by the effect of thrombin. When using anticoagulants, it is necessary to take into account by the fact that the composition of the collected blood may change. For example, all anticoagulant agents weigh Ca^{2+} and Mg^{2+}, therefore it is necessary in the case of determining Ca^{2+} use specially prepared heparin. It is also necessary to take into account that during coagulation, not only are coagulation factors activated, but also some components are released from disintegrated platelets.

Task: preparation of blood plasma (link to pdf)

tubes with EDTA, intended for blood count

Factors affecting the result of laboratory examination

The results of laboratory tests are influenced by a number of factors. Their ignorance or underestimation can lead to incorrect interpretation of the result. The laboratory examination includes three phases:

- pre-analytical part – preparation of the patient, collection of biological material and its transport to the laboratory, storage of the sample before analysis, preparation of the sample for processing;
- analytical part – own analysis and calculation of the result;
- post-analytical part – data validation and their transfer and interpretation.

a) Factors of the pre-analytical phase

The most important phase of the examination from the point of view of possible influence on the result is the pre-analytical part, during which the result can be influenced by biological influences (influential and uninfluential), the method of material collection, its transport and storage. If possible, the effort is to eliminate these factors, or minimize, otherwise they must be taken into account when interpreting the result.

Biological influences uncontrollable

Race, ethnic group of the population – different races/ethnic groups have different metabolic pathways, but also the amount of muscle mass, different physiological values of some analytes, or a different frequency of disease occurrence given by a different frequency of certain genes.

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

Age – most parameters in childhood have a lower value of the upper reference limit compared to the adult population. A number of biochemical systems or events are associated with a certain phase of an organism's development. High physiological values during adolescence are mainly due to the development of the skeleton.

Pregnancy – several mechanisms apply that lead to changes in analyte concentrations, enzyme activities and component counts during pregnancy. It can be changes in the production of hormones and their effect on the organism, the influence of the placenta, the transfer of substances from the amniotic fluid, etc.

Biorythms – these are regular linear (age) or cyclic changes in metabolism under the influence of hypothalamic and pituitary hormones (daily, monthly, ...) or changes caused by climatic or seasonal conditions that can be predicted with a certain probability. However, each individual also has non-cyclical, unpredictable biorhythms.

Biological influences controllable

Body weight – can affect analyte concentrations by changing distribution volumes. The concentration of e.g. LDL-cholesterol, triacylglycerols, uric acid, insulin is positively correlated with obesity. Physical activity – affects the change in the composition of body fluids and depends on the duration and intensity of physical activity. Acute strength and exhausting load increases the proportion of anaerobic metabolism, endurance load increases aerobic metabolism. During physical activity, there is an increased utilization of substrates (glycemia initially increases slightly, after longer exercise it decreases, triacylglycerolemia decreases, the concentration of free fatty acids increases), a change in tissue metabolism (increased lactate formation, a decrease in pH), and a movement of fluid from the intravascular space to the interstitial (increase in hematocrit, total protein and substances bound to it), to change the concentration of many hormones.

Diet/starvation – affect the investigated analytes by different mechanisms - depends on the composition and amount of food and fluids taken. Hormones and enzymes are released before and during food intake. When the body is dehydrated, the hematocrit increases, but so does the concentration of a number of substances. A high-protein diet increases phosphates, urea, and uric acid. A diet rich in fats reduces the proportion of nitrogenous substances, e.g. uric acid. A diet rich in carbohydrates increases, for example, lactate dehydrogenase and decreases the concentration of triacylglycerols. A vegetarian diet reduces total and LDL-cholesterol and

triacylglycerols, increases total bilirubin and urine pH. Some foods and drinks can affect some metabolic pathways, e.g. if they contain caffeine, there is an increase in the concentration of catecholamines, glucose and free fatty acids.

Smoking – affects the level of a number of analytes mainly due to the effect of nicotine. Smoking affects glucose metabolism, increases the concentration of cholesterol and triacylglycerols.

Alcohol – alcohol consumption alters biochemical analytes differently depending on whether acute or chronic abuse is involved. In general, it primarily affects the metabolism of glucose, triacylglycerols and increases liver enzymes in the blood. A one-time consumption of alcohol in a moderate and moderate dose minimally affects the examination. Long-term abuse leads to hypoglycemia and keto- and lactic acidosis, the concentration of uric acid increases.

Medicines – they can affect biochemical tests by several mechanisms, e.g. they induce/inhibit liver enzymes, affect binding to transport proteins, act cytotoxicly or interfere with the determination itself. Stress - affects the production of hormones, which subsequently change the metabolism of a number of substances. Stress is often accompanied by more serious illnesses, but in some people even blood sampling itself. The external environment - can have a significant effect on metabolism and subsequently on the concentration of a number of analytes. This is the altitude (from heights above 3000 m), the temperature of the environment, but also the geographical location - rural, urban. Traveling across time zones manifests itself in the change of some analytes, the most common being the retention of sodium ions and fluids with normalization in 2 days after the return. Mechanical influences – e.g. muscle trauma, intramuscular injections increase the activity of ALT, AST, CK and myoglobin concentration, uterine pressure in a high degree of pregnancy increases the ctivity of ALT, digital examination of the prostate increases the activity of prostate specific antigen (PSA).

Material collection

Patient education plays a major role in the entire process of correct laboratory examination.

Collection on an empty stomach − it is recommended that the patient does not eat for about 10-12 hours and is relatively quiet. It is also recommended to drink approx. 2-3 dl of water in the morning. Failure to observe 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).

Sampling time – the concentration of some substances fluctuates considerably during the day (e.g. glucose, triacylglycerols, hormones), others during the month or year. Planned sampling is usually done in the morning. The position during sampling affects the concentration of proteins or substances that bind to proteins (e.g. total proteins, albumin, enzymes, lipids, Ca^{2+}, Fe^{3+} ions). While standing, water escapes from the intravascular environment and thus the concentration of the mentioned substances increases by 5-15%. The standard position of the patient during sampling is the sitting position.

Use of the tourniquet – its application should not be longer than 1 minute, the patient should not "pump" with the arm. With prolonged strangulation of the limb (approx. 5 min) and more pronounced exercise, there is up to a 10% change in the activity or concentration of a number of analytes. This change is most often caused by the transfer of low molecular weight substances from the intravascular space to the interstitium as a result of an increase in the filtration pressure across the capillary wall and metabolic changes at the site of the choke (anaerobic metabolism).

Vein/finger compression affects blood gas concentration, lactate and pH.

Hemolysis (visible at a hemoglobin concentration > 0.2 g/l) – in the hemolytic sample there is an increased concentration of analytes whose concentration inside the erythrocytes is high (e.g. total proteins, lactate dehydrogenase, acid phosphatase, aspartate aminotransferase, from ions mainly K^+, phosphates, Mg^{2+}). auses of hemolysis: use of a too large or small needle, rapid emptying of the syringe, violent shaking of the blood in the tube, moisture in the collection set or in the tube, the presence of detergents in the tube, incorrect ratio of anticoagulant to blood, storage of blood in a refrigerator, leaving it in an environment with increased temperature (in the sun, over a radiator), centrifugation at high speeds.

Transport For transport, serum/plasma is more suitable than whole blood. There is a risk of hemolysis when transporting whole blood. During transport, whole blood is stored at a temperature of 0 °C (melting ice).

Sample stabilization/storage

When the collected blood is left standing for a longer period of time, the energy resources of the erythrocytes are exhausted, which then cannot maintain the basic metabolic processes (there is K^+ leakage from the erythrocytes into the blood, Na^+ transport in the opposite direction). Depending on the analyzed analyte, a low temperature (4 °C, 0 °C (melting ice), −20 °C, −80 °C), protection from light, pH adjustment of the sample, addition of a stabilizer is chosen for stabilization.

b)Factors of the analytical phase

The result of each laboratory examination is characterized by several features that determine to what extent it reflects the real situation and to what extent it is affected by errors. Basic analytical properties of any method include precision and truthfulness. ***Precision*** *expresses* the degree of agreement of the results obtained by repeated analysis of the same sample under predetermined conditions. Accuracy is a general term. The degree of

dispersion of the results (x i), i.e. the numerical value of accuracy, is expressed by the concept of **inaccuracy** . The imprecision is usually reported as the sample *standard deviation s* (it is given in the units of the measured analyte) or as the relative standard deviation (coefficient of variation) CV:

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

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

According to the conditions under which the accuracy determination takes place, we distinguish between repeatability and reproducibility of the method. ***Repeatability*** refers to the accuracy of the method when all analyzes are performed in one series of measurements, on the same day and on the same instrument. The concept of repeatability is identical to the term "accuracy in a series". **Reproducibility** expresses accuracy in time. It is obtained by calculation from determinations that are carried out sequentially over several days on one device. Reproducibility is often referred to as "precision between days".

Accuracy depends only on the distribution of random errors, it has no relation to the actual value of the result.

Random errors arise quite irregularly due to the action of random influences, they can be evaluated statistically. They cause dispersion of the measurement results, which can be characterized by the accuracy of the measurement numerically expressed as the standard deviation s or the coefficient of variation CV. Their influence on the measurement result can be reduced by increasing the number of measurements.

Trueness (formerly *correctness*) expresses the closeness of agreement between the average value obtained from a large number of measurement results () and the agreed reference value (x0). The measure of truth is the deviation (bias), which expresses the difference between the mean value of the results and the agreed reference value:

bias = bias =

The accuracy of the method is determined by the size of the systematic error (bias), which always deviates the result in one direction. Systematic errors can be predicted and calculated and the results corrected. **The agreed reference value** (x_0) represents the true value, which in reality is always unknown. It is obtained using the reference method in a large number of laboratories.

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

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TE = 1,96 • s + bias
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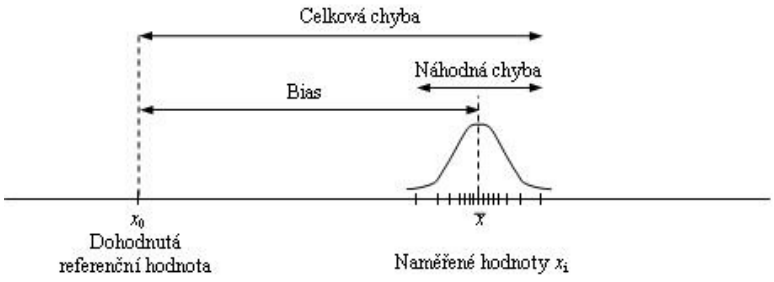
Reliability is determined by the accuracy and truthfulness of measurements. It represents the range that individual values reach during repeated measurement.



Every examination result is always affected to varying degrees by random and systematic errors. The consequence of the existence of errors is the uncertainty of the measurement result. ***Uncertainty*** (*uncertainty, u*) represents the interval of values in which the result of the analysis (x) is with a certain probability. Uncertainty includes many components, while insignificant ones are neglected, significant ones are expressed in the form of standard deviations (or coefficients of variation) as the so-called **standard uncertainty *u*** (ie numerically u = s). The most significant random component of uncertainty is the standard uncertainty of reproducibility, and the most significant systematic component of uncertainty is the standard uncertainty of the calibrator. Therefore, the total (combined) standard uncertainty (uc) can be found from the relation:

The true value is found with some probability in the interval (in the so-called **expanded uncertainty**), which is obtained by multiplying the standard combined uncertainty (uc) by the expansion coefficient (k), which for a 95% confidence level has a value of 1.96 (approximately 2):

In compliance with the principles of good laboratory practice, the result of the laboratory examination should always include information on the extended uncertainty of the determination of the given analyte. The values of the reference limits or limit values should also contain an indication of the uncertainty of the determination

Quality control systems

The correctness of the performed analyzes is checked in clinical-biochemical laboratories at regular intervals using control samples with the declared value of the determined analytes (*internal quality control*). Controls on two levels are usually used; one level is in the range of reference values, the other in the pathological range. The results of control analyzes within ±2 s are considered satisfactory. The control results are displayed graphically in addition to the numerical value.

The laboratory is compulsorily involved in the external quality assessment system. *External quality assessment* is a system of objective evaluation of laboratory results by an independent organization, which is carried out by regular comparison of measurement results evaluated by laboratories with each other and comparison to reference measurement values.

Validation of results *(findings).* Due to the large number of analyzes carried out (thousands) and the large percentage of results within the reference limits, the so-called electronic validation is carried out continuously. Results that are within the specified range, do not have an error message and do not differ in the specified range from the previous examination (finding), are released for release to clinical workplaces automatically. Other findings are retained and submitted to the supervisor for validation. This takes into account the compliance of other performed tests, previous examination, diagnosis, or other clinical data of the patient. If there is any doubt about the correctness of the analyzes carried out, a repeated determination is made to exclude random error.

Interpretation of results

Laboratory examination provides a number of important information for determining/refining the diagnosis, choosing the correct treatment procedure and monitoring its progress, identifying prognostic or risk factors. The results of laboratory examinations are evaluated in relation to physiological values, to the results of other examinations, to the patient's history, or their change over time is monitored. Interpretation of laboratory tests often requires the cooperation of a number of experts (clinical biochemist, hematologist, immunologist, geneticist, microbiologist, toxicologist, pharmacologist, etc.) with the referring physician. In the case of repeating the laboratory examination, knowledge of the characteristics of the analyzed analyte, especially its biological half-life, the rate of stimulation of synthesis or degradation during a pathological process, plays an essential role.

Comparing results to a reference interval

When interpreting the results of biochemical examinations, the most common comparison is made with the so-called reference interval of values. The reference interval includes 95% of the reference values, i.e. 5% of the values are not included (2.5% of the highest and 2.5% of the lowest). Reference values are values obtained from a selected group of individuals (age, sex, race) with a defined state of health (minimum 120 reference individuals); it depends, among other things, on the method of collection, transport and storage of samples (see task 1.5), the method of determination and the method of statistical evaluation. This selected reference population does not include, for example, pregnant and lactating women or a priori "healthy" blood donors, individuals who are sick, with genetic predispositions, obese, alcohol, drug users, etc.

In the statistical evaluation of reference intervals, it is necessary to exclude outliers, to test the values from the point of view of normal distribution, or to choose suitable procedures for transforming data into a normal distribution, to determine reference intervals by non-parametric and parametric methods, to test the effect of age/sex.

Depending on the frequency distribution of reference values, a parametric or non-parametric statistical method can be used to estimate the reference interval. If the reference values have a *normal* frequency distribution (graphically expressed by a Gaussian curve), the so-called *parametric method* using mathematical parameters can be used to calculate the reference limits. First, the arithmetic mean and standard deviation are calculated from the reference values with:

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n}$$

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

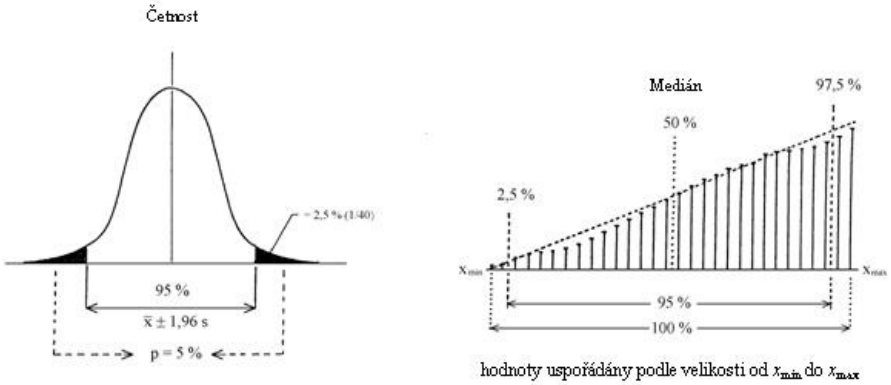
Estimates of the reference limits are then calculated from these parameters:

$$\bar{x} \pm a \cdot s$$

If the reference interval is to contain 95% of the reference values, a value of 1.96 (approximately a value of 2) is substituted for the coefficient - see the following diagram.

If reference values do not follow a normal frequency distribution, they can often be transformed to a normal frequency distribution using an appropriate mathematical function (e.g. logarithm, inverse). Arithmetic mean and standard deviation are determined from the transformed values, estimates of the reference limits are calculated, which are then retransformed back.

A non-parametric method can also be used to determine the reference interval. In this case, the type of value distribution does not matter. However, it is only necessary to examine a larger sample reference group than in the previous case, sort the obtained values in ascending order of size and separate the appropriate percentage of values (usually 2.5%) at both ends - see the following diagram.



The interpretation of laboratory results using the 95% interval of reference values is also an expression of probability: in a healthy individual, there is a 95% probability that his determined value will fall within the given reference interval. The remaining 5% represents the probability that the result will be lower (2.5% probability), or higher (2.5% probability) than lower, or upper reference limit.

The validity of the reference limits taken from the literature must always be verified for the method used in the laboratory and for the given population. As the number of different determinations increases, the probability that all determined parameters will lie within their reference intervals decreases. Therefore, the probability that (in a "healthy" individual) we will get any of the results outside the reference range increases.

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Desicion Analysis

Another interpretation of the results is the so-called *decision analysis* . In this evaluation, we do not work with an interval of reference values ("from - to"), but with a single **borderline** (*critical*) **value** (*decision limit*), which allows us to mark the determined result as a *positive test* (these are usually higher values than the borderline value) or as a *negative test* (usually borderline and lower values). A positive test is associated with a certain risk of existing or future disease. Risk can be expressed verbally (risk increased, high, ...) or numerically (probability in %, etc.) The threshold value is usually calculated in such a way as to achieve the maximum of the so-called test yield, i.e. the highest probability of matching the test with the diagnosis. Concepts and their practical application will be explained in more detail in clinical biochemistry. When monitoring patients during disease progression or medical therapy, laboratory findings are compared with previous results. With this procedure, mindless comparison of data is not possible, but it is necessary to take into account the analytical and biological intraindividual variability of the given parameter.

Critical difference

The result of the laboratory examination is affected not only by the error during the analysis, but also by the intraindividual variability of the measured parameter. To assess two consecutive measurement results in a certain time interval (depending on the monitored parameter) in the same patient, it is necessary to know the so-called **critical difference** . This is a minimal percentage difference in the results, caused by a change in metabolism, not an analytical error or the variability of a given parameter over time in a given individual. Calculation of the critical difference (*critical difference* , CD):

$$CD = 2,77 \cdot \sqrt{CV_a^2 + CV_i^2}$$

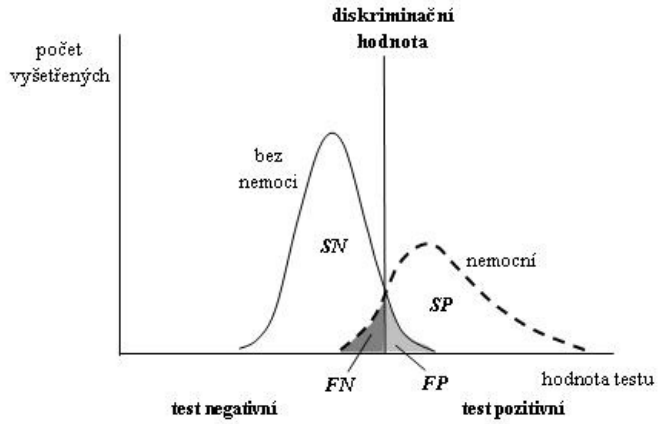
Wheree 2.77 is the coefficient related to the 95% probability interval for 2 subsequent measurements; CVa analytical coefficient of variation (intermediate accuracy of measurement); CVi intraindividual biological variation describing the dispersion of the monitored parameter values over time in an individual (see database: https://www.westgard.com/biodatabase1.htm, CVi values are given here as CVw in percentage).

The CD calculator can be found, for example, on the website of the Czech Society of Clinical Biochemistry http://www.cskb.cz

If the difference between two subsequent measurements is greater than the CD value, then these results differ from each other with a certain probability (mostly 95%), i.e. they are not caused by analytical or intraindividual biological variability, but reflect a change in the patient's clinical condition. If the difference of two subsequent measurements is smaller than the permissible CD value, the arithmetic mean for the given parameter is calculated. If the critical difference is exceeded, a third examination is performed after a certain time.

Diagnostic accuracy of laboratory examination

The task of any laboratory examination/test should be to differentiate individuals with the presence or absence of a particular disease. In most laboratory tests, however, there is a partial overlap between the healthy and the sick, creating an additional group of healthy people with a positive test (false positives) and a group of sick people with a negative test (false negatives). We can therefore divide the investigated persons into four groups (see Fig. 1-4 and the truth table below).



SN... zdraví s negativním testem (**správná negativita**)

FN... nemocní s negativním testem (**falešná negativita**)

SP ... nemocní s pozitivním testem (**správná pozitivita**)

FP ... zdraví s pozitivním testem (**falešná pozitivita**)

Truth table: Classification of investigated persons

Number of persons examined	With illnes	No disease	In total
With a positive test	SP	FP	FP+SP
With a negative test	FN	SN	SN+FN
In total	SP+FN	SN+FP	SN+FP+SP+FN

Diagnostická citlivost (senzitivita) testu vyjadřuje pravděpodobnost, že pozitivní test vyjadřuje skutečně pozitivní diagnózu (jinak řečeno, že u nemocné osoby bude výsledek testu pozitivní neboli tzv. procento správně pozitivních nálezů):

$$diagnostická\ senzitivita = \frac{SP}{SP + FN}(\times 100\%)$$

The diagnostic specificity (specificity) of the test expresses the probability that a negative test expresses a truly negative diagnosis (in other words, that the test result will be negative in a healthy person, or the so-called percentage of correctly negative findings):

$$diagnostiká\ specifita = \frac{SN}{SN + FP}(\times 100\%)$$

The value of both quantities ranges from 0-1 (0-100%), the higher the value, the more diagnostically valuable the test. In practice, methods with a specificity of at least 0.7 are used.

Diagnostic sensitivity expresses the results of the test in relation to sick individuals, diagnostic specificity in relation to healthy individuals. In an ideal case, the laboratory test allows you to clearly separate healthy from sick individuals (sensitivity and specificity are equal to 1). **The limit** (**discriminative value**, *cut-off value*) above which we can consider a change in analyte concentration as a positive finding is of fundamental importance for decision-making . There is an indirect relationship between diagnostic sensitivity and specificity, i.e. the higher the diagnostic sensitivity (lower false negativity) of the laboratory method, the lower the diagnostic specificity (higher false positivity) and vice versa.

Communication of biochemical investigation results

In most cases, a sample of blood or other biological material is sent for examination to a central clinical-biochemical laboratory. For the sake of simplicity, the required examinations are marked in the accompanying forms (requests). The application should contain relevant information about the material collection, storage conditions and the method of its transport. The results of the determination together with their interpretations (numerical, graphic, verbal) are then returned on the results sheet (findings report). Different workplaces use different types of forms and score sheets.

Links

Related Articles

- Blood
- blood plasma
- Blood sampling for examination
- Blood count
- Haemocoagulation ■ Examination of blood coagulation ■ Examination of bleeding ■ Sedimentation of erythrocytes
- Biochemical analysis of blood ■ Laboratory examination of acid-base balance
- Blood culture ■ CRP ■ PCT