DIRECTIONS FOR LABORATORY WORK

Students are obliged to keep the following rules for safe laboratory work, chemical waste disposal, and the instructions for giving first aid.

The program of practicals is displayed at web pages of Information System of Masaryk University (http://is.muni.cz). Supplementary material will be uploaded at web pages of „VSBC041c Biochemistry II – practice" in Information System. If necessary, instructions that are more detailed will be given at the beginning of lesson.

Students have to be in the laboratory **in time** and be prepared for the lesson. At the beginning of practical lesson, the teacher will check the current knowledge of students. Student not sufficiently prepared will not be allowed to participate in the lesson and will have to do extra practical lesson at the end of semester. All absences have to be supported by the letter of apology at Department of Study Affairs. All absences have to be made up till the deadline given.

Students have to wear laboratory coats. They are also recommended to have protective spectacles. Smoking, eating, drinking, and chewing gums are prohibited in the laboratory.

Work in the laboratory is prohibited for pregnant women and mothers up 9 months after delivery. Female students are obliged to notify the teacher about pregnancy.

Any injury has to be immediately reported to the teacher.

A short record about work is made in an **exercise book**, which is shown to the teacher at the end of practical lesson.

The next week, a **lab report** is presented to the teacher's approval and signature. The lab report is written on free A4 papers and contains the following items: student's name, date, chapter number and title, the name of experiment, brief principle, all results (tables, graphs, equations), all answers to questions from the lab manual.

General guidelines to practical lessons

- *Reagents and other laboratory equipment* are available on laboratory tables. After work, they should be placed back to the original place.
- When *heating* a solution, be sure to point a test tube to open space, away from other people.
- *Pipetting* of solutions is performed by pipettors and bottle-top dispensers.
- *Cleaning* glassware is performed by students during lesson. Aqueous solutions of chemicals are poured down the chemical drain (dark grey sink) together with ample tap water. Rinsed vessels are collected in a marked container.
- *Solid waste* (filter paper, broken glass) is put into a container made for that purpose.
- The workplace must be **kept in order**; all waste must be immediately removed.

Safety guidelines for handling dangerous substances

Corrosives

- Corrosive substances are generally strong acids and strong hydroxides.
- Concentrated acetic acid is also a corrosive.
- Even dilute solutions of these compounds are harmful, especially for eyes.
- When handling corrosives, be sure to wear protective glasses or goggles.
- Corrosives are delivered by pipettors; mouth pipetting is absolutely prohibited.
- Work with corrosives must be performed on the tiled surface in a fume cupboard.
- When heating corrosive liquids, be sure to point a test tube to open space, away from other people.
- Before dilution, acid is always slowly poured into water, while gently stirring.

Toxic compounds

- Examples of toxic substances in practical lessons are methanol and heavy metal salts.
- They are delivered by pipettors; mouth pipetting is absolutely prohibited.
- Work with irritating and fuming substances are performed in a fume cupboard.
- After work with toxic substances, clean the workplace, rinse glassware, and wash your hands thoroughly.

Flammable and explosive substances

- In practical lessons, students may work with natural gas, hexane, alcohols, and concentrated acetic acid. Some of these substances can be explosive when mixed with air.
- All open flames (gas burners) must be extinguished in the presence of flammable liquids.
- The residues of flammable solvents must be collected in labelled containers, never flushed down the drain.
- After the spillage of flammable liquid, extinguish all flames, turn off all electric appliances, and ventilate the room
- If the content of a small vessel flames up, it can be ceased by covering the vessel.
- In case of larger fire, alarm everyone, turn off gas and electricity in the laboratory, remove all flammable substances from the surroundings, and use the carbon dioxide extinguisher.

Biological material

- In practical lessons, you will work with biological material.
- Blood samples are tested, originated from transfusion station.
- Serum or plasma are model samples, prepared by diluting substances in the solution of albumin or prepared from the horse or pig lyophilised sera (e.g. control serum RU or Lyonorm P).
- Urine and gastric juice samples are model aqueous solutions.

- All biological material must be considered as potentially infectious and the precautions given for handling with biological material should be respected:
  - Wear protective gloves.
  - Dispose single-use equipment into labelled containers.
  - Use pipettors with exchangeable tips.
  - All glassware contaminated with biological material must be disinfected (e.g. by 1% Jodonal for 2 h) or sterilized in autoclave.
  - Laboratory table contaminated with biological material must be disinfected (e.g. by 1% Jodonal).
  - Avoid biting your nails or pencil, rubbing the eyes.
  - Always wear a laboratory coat.

First aid

Small injuries (cut)

- Wash the wound with tap water, apply disinfection (Septonex, Ajatin) and cover with sticking plaster.
- If bleeding is more intensive, apply compressive bandage and seek medical assistance.

Electric current injuries

- Turn off electricity, control breathing and heart action, call medical emergency.

Injuries by corrosives

- Wash affected area immediately with running water (do not waste time by taking off clothing)
- If corrosive splashes the eye, immediately rinse the eye (lids kept open) with water from eye-fountain for a few minutes, then seek eye specialist.
- If corrosive gets into mouth (no mouth pipetting!), immediately rinse mouth with plenty of water, and seek medical examination.

Affection by toxic substances

- Follow the instructions of teacher. First aid and elimination of toxic effects depend on the type of substance.

Burning injuries

- Cool immediately the affected area by cold water for several minutes.
- In case of scald (injury from hot vapour) remove the wet clothing and cool with cold water.
- Cover the burned areas with sterile gauze pads.
- In case of more severe burnings, call medical emergency.

Contamination with biological material

- Apply thoroughly disinfection on all contaminated areas and/or wounds including clothing.

1 BLOOD. ANALYZERS. LABORATORY INVESTIGATION. INTERPRETATION

Topics to be reviewed

Blood and its main components. Plasma and serum. Anticoagulants. Haematocrit. Biochemical analyzers. Reference values, critical difference, factors affecting pre-analytical variability.

Pre-lab questions

1. Which factors may induce haemolysis during blood collection?
2. How many revolutions per minute are recommended for the centrifugation of full blood?
3. Explain the term of „dry chemistry“.
4. Haematocrit states the volume ratio of erythrocytes in the whole blood. What volume of plasma can be theoretically obtained after centrifugation of 2 mL of non-coagulable blood, if haematocrit is 0.4?
5. Give examples of disinfectants applied on skin before blood collection.

Introduction

For accurate appraisal of health state many information should be acquired. Results of laboratory examinations are considered as the source of valid information needed for the assessment of health state and the reflection of metabolism changes in body. According to World Health Organisation (WHO) laboratory examinations provides about 80 % information leading to the assessment of correct diagnosis.

The largest and most important part of the clinical-biochemical examinations concerns blood, blood serum and plasma analyses. Blood is easily accessible material and its composition reflects the range of biochemical processes blood is a good indicator of the physiological conditions throughout the body.

Another frequently analysed material is urine. Analyses of the other body fluids (gastric juice, duodenal juice, amniotic fluid, cerebrospinal fluid, saliva, transpiration, etc.) are demanded only in a chosen number of patients, their frequency is much lower, and they are often carried out in the special laboratories. A small number of simple, mostly qualitative tests with direct informative results take place in the doctor’s surgery or at the patient’s bed (*bed-side diagnostics, point-of-care testing, near-patient testing*).

1.1 Blood collection

Blood for analysis may be taken from veins, arteries, or capillaries. Venous blood is usually the specimen of choice, for some analysis and especially in young children is taken capillary blood. The arterial puncture is used only exceptionally, especially for the blood gas analysis.

1.1.1 Venous blood collection

With regard to the method and equipment used for the venipuncture are distinguished two ways of collection:

• **Open collection system** – phlebotomist is in direct contact with the biological material:

– blood is drawn through needle directly into a blood tube;

– blood is drawn into a disposable syringe and by back sticking into a blood tube.

• **Closed collection system** – the sample handling is carried out after the collection directly in the collection syringe or test tube. Thus, the phlebotomist is protected against the contamination by the patient's blood, the biological material is protected against the external contamination and also against breaking during transport and centrifugation. The individual syringes/evacuated test tubes are marked by colour according to the additive used (clotting accelerators, separation gel, heparin, EDTA, ...). The separation gel enables proper separation of serum from the blood coagulum after centrifugation (creation of interlayer). The collection syringe/test tube can be placed directly into the analyzer. The used material is easily burnt down.

The collection is carried out:

- *into the closed syringe* – the blood is drawn out with the help of a piston (Fig. 1-1a) or with immediately before the collection prepared vacuum (pulling the plunger into the base of syringe and locking it into place, Fig. 1-1b). After the collection, the piston is broken off and the syringe is transformed into the closed blood tube. The method of collection is adapted to patients with problematic veins. Vacuum is used in case of strong veins.
- *into the evacuated test tube* (Fig. 1-1c) – the system includes a specific blood-collection needle, a holder into which the needle is assembled before phlebotomy, and evacuated blood collection tubes.

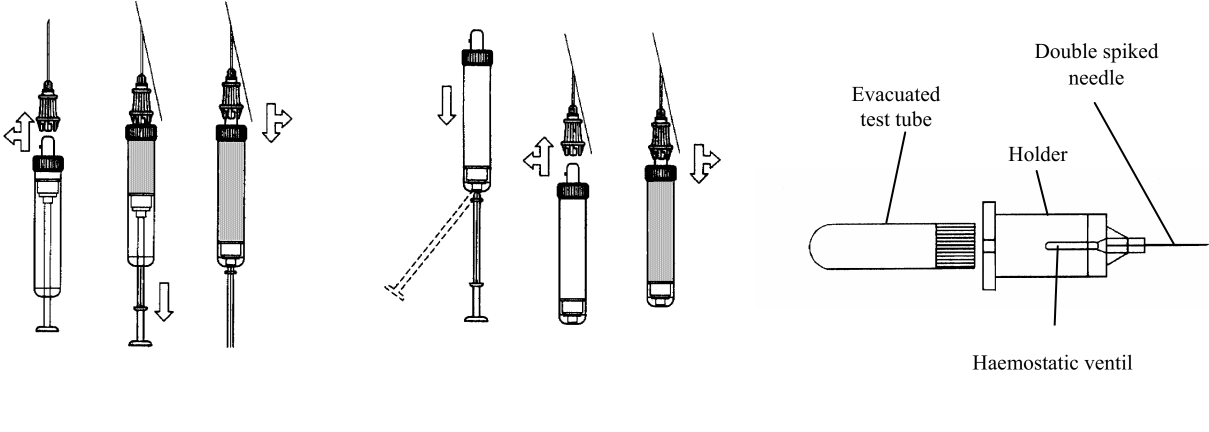


Fig. 1-1a Fig. 1-1b Fig. 1-1c

Specimen collection general instruction

- 10–12 hours before the blood withdrawal patients are not allowed to eat; they have to exclude fat food and alcohol from their diet.
- In the morning before the blood withdrawal, patient can drink about ¼ L of plain water or bland tea.
- Before the blood withdrawal, the patient should be asking for any allergies to antiseptics, adhesives.
- Standard posture of patient at the blood withdrawal is sitting posture.
- Pumping of the fist before venipuncture should be avoided (results of many tests can be affected).
- The site of injection must be disinfected (Jodonal B, Persteril, Jodisol, Ajatin).
- If the veins are visible, the use of a tourniquet should be minimized.
- If the tourniquet is used (not more than 1 min), it should be released before withdrawal of blood begins.
- Press down on the gauze once the needle is out of the arm (to avoid formation of a haematoma).

1.1.2 Capillary blood collection

Capillary blood is used in case only a small amount of the sample is needed. General instructions for specimen collection:

- Warm the site of injection (tip of the finger, an earlobe, the heel or big toe of an infant), ensure good blood supply. Avoid massaging the site.
- The injection site must be disinfected.
- Puncture should be directed onto the outer and upper region of the fingertip, halfway between the centre of the finger pad and the edge of the fingernail.
- After puncture with the lancet, the first drop should be wiped off (dilution of blood with the tissue fluid). Gently apply intermittent pressure to the surrounding tissue. Avoid excessive squeezing or "milking" of the puncture site.
- Blood is usually collected into the special capillary blood test tubes or into the small plastic or glass test tubes. For strip tests, the drop of blood is applied directly on the paper.
- Following collection press clean gauze sponge on the puncture side.

► See video recording and acquaint with the blood withdrawal ways.

► Give main advantages of closed collection system.

1.2 Blood processing

Depending upon the tests that have been ordered, blood sample may be processed before it is analyzed. Most routine laboratory tests are performed on either plasma or serum. When blood is drawn without the addition of an anticoagulant and allowed to stand, it clots after several minutes, because soluble fibrinogen is transformed to the insoluble network of fibrin by the clotting mechanism. **Serum** is excluded from the blood clot after some time and is obtained from clotted blood by centrifugation. Sufficient time is needed for complete blood clotting (at room temperature at least 15–30 min).

To prepare plasma an anticoagulant must be added to the specimen during the collection. Centrifugation of non-clotted blood produces **plasma**. Blood may be centrifuged immediately after the collection.

Plasma or serum should be separated from blood cells or coagulum within 2 hours at the latest (or 1 hour for the determination of potassium ions).

Centrifuges are used to separate components of a mixture on the basis of particle size or density, For separation of blood cells from plasma or serum there is recommended relative centrifugal force 1 000–1 200×*g* (*g* is relative centrifugal force and is measured in multiples of the earth's gravitational field) for at least 10–15 minutes. Centrifugation of blood is always performed in the closed test tubes (to prevent aerosolization of infectious particles) at room temperature or 4 ºC. Prolonged centrifugation or higher centrifugal force cause often partial or complete haemolysis.

Materials: Sodium oxalate, lithium oxalate, potassium oxalate, sodium citrate, disodium salt of EDTA (ethylenediamine-tetraacetic acid) – all as solid compounds, heparin solution in a vial. Blood drawn into a tube with anticoagulant added, centrifuge tubes, centrifuge.

1.2.1 Preparation of blood plasma

Anticoagulants are substances, which prevent blood plasma from clotting when added to blood during the collection procedure. Anticoagulant is placed into the test tube in the form of solution and solvent is evaporated at room temperature. After the collection, blood is mixed gently with the anticoagulant (5 to 10 inversions). Heparin is very often used in such a way that a few drops of heparin solution are drawn into a syringe and spread over the inner walls of the syringe by repeated movements of the piston. The blood sample is subsequently drawn into the syringe. Closed collection systems are filled with anticoagulants during their production.

Approximate amounts of anticoagulants needed to prevent 1 mL of blood from clotting:

Sodium (lithium, potassium) oxalate	1–2 mg
Sodium citrate*	3 mg
EDTA.Na_2 (K_2, K_3)#	1–2 mg
Heparin+	4–6 IU (liquid), 40–60 IU (dry)

* sodium citrate solution is used in haematological tests; that is mixed with blood in the ratio of 1:10 or 1:5;

EDTA.Na_2 … disodium salt of ethylenediaminetetraacetic acid; also dipotassium or tripotassium salts are used

+ heterogeneous mixture of sulphonated mucopolysaccharides, produced by mast cells in the tissues of mammals. It accelerates the action of antithrombin III, which neutralizes thrombin and thus prevents the formation of fibrin from fibrinogen. The amount heparin added depends on material of collection test tube (glass, plastic).

When using anticoagulants we have to take into account potential changes in the composition of collected blood, e.g. all anticoagulants bound Ca^{2+} and Mg^{2+} ions. Therefore, for the determination of Ca^{2+} ions a specially modified heparin should be used. Moreover, during coagulation, the coagulation factors are activated and platelets release the contents of their granules into the plasma.

Procedure

☞ About 5 mL of blood, which has been prevented from clotting by the use of some anticoagulant and collected into centrifugation test tubes, centrifuge for 10 min at 2 500 rpm.

► Draw chemical formulas of common anticoagulants and explain how they act.

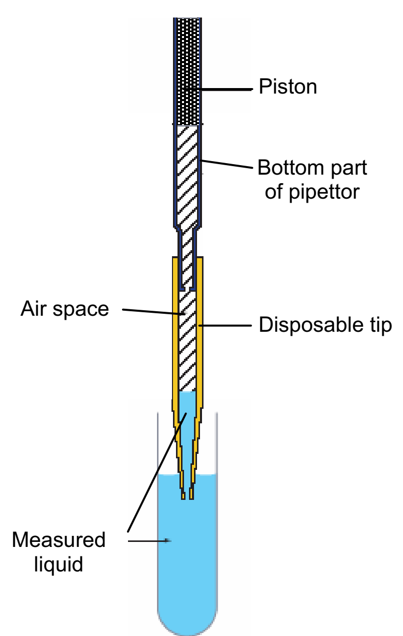
► Consider whether the results of some biochemical determinations can be influenced by the choice of anticoagulant and illustrate with some examples.

- ► Describe the appearance of blood before and after being centrifuged.
- ► Give the main differences in composition of blood plasma and serum.
- ► What does it mean, when we describe plasma/serum as a) haemolytic; b) icteric; c) chylose?
- ► Can be a blood sample intended for the collection of plasma kept at −20 °C?

1.3 Manual methods & pipetting

Manual methods have certainly come a long way. At present, they used various commercial complete kits (in vitro diagnostics kits, IVD kits) containing most of the components needed to perform the determination. However, most manual methods are still labour intensive, time consuming, limited in their reproducibility and are limited in terms of overall throughput. The most significant step in all manual methods is precise and accurate measuring of small volumes of biological sample, which is always potentially infectious and dangerous reagents.

1.3.1 Manual air displacement pipettors



Air displacement pipettors have a piston that moves in a cylinder and always certain volume of air remains between the piston and the sample liquid (volume of all gases significantly depends on external pressure and temperature). Therefore, accuracy of pipetting is affected by atmospheric pressure, temperature, and the nature of the liquid (density, viscosity and homogeneity).

Mechanical pipettors may be **fixed volume** (capable of delivering a single factory-set volume) and **adjustable** (the volume is determined by the operator across a particular range of values). Pipettors must be fitted with the correct disposable tips before use.

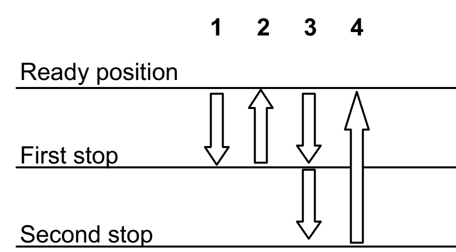
Pipetting results can easily be influenced by user technique. The most common pipetting technique is **forward (direct) pipetting** which discharges all the liquid by one full movement of the piston. The technique employs the blow out function ensuring complete delivery of the liquid (at the end of pipetting the tip must be empty). It is suitable for aqueous solutions, like buffers, diluted acids or alkalis or solutions containing small concentrations of protein or detergent. Pre-rinsing of the tip before the actual pipetting improves the results, but is very often skipped to save time and fatigue.

Reverse pipetting begins and ends with the tip containing liquid. A selected volume plus an excess is aspirated into the tip. The delivery is done without blow out, and thus, the excess volume remains in the tip. It is suitable for heterogeneous samples like blood or serum, viscous, or foaming liquids, or very small volumes of liquid. The advantage of this pipetting technique is reducing error due to film retention.

General Pipetting Operational Considerations

- Select appropriate tip and make sure that the tip is firmly attached to the tip cone. Tips are designed for single use.
- The temperature of the pipetted solution and the pipettor should be the same.
- Hold the pipettor vertically when aspirating the liquid. Once there is liquid in the tip, never lay pipettors on their sides**.**
- When filling volumes ≥ 10 µL**,** pre-rinse each new tip before aspirating the liquid (by filling and emptying the tip for three to five times).
- When aspirating, try to keep the tip at a constant depth approx. 2–5 mm below the surface of the liquid. Avoid air bubbles in the tip during aspiration.
- When pipetting viscous liquids (e.g. serum, blood), leave tip in the liquid for about 1–2 seconds after aspirating before withdrawing it.
- In adjustable pipettors, it is recommended that higher volumes be set first – if going from a lower volume to a higher volume, first surpass the desired volume, and then slowly decrease the volume until the required setting is reached.
- Always control the operating button slowly and smoothly.
- When dispensing, hold tip at an angle, against the inside wall of the vessel/tube if possible.
- Use the same pipetting technique, pipettor and type of tips in serial pipetting procedures.

1.3.2 Forward pipetting procedure



☞ Attach the appropriate tip to the pipettor.

☞ Set the pipettor to the desired volume.

☞ Press the operating button to the first stop (**1**).

☞ Immerse the tip so that 2–3 mm is in the liquid and slowly release the operating button to the ready position (**2**). Avoid the creation of air bubbles in the tip (Fig. 1-2a).

☞ Be sure to allow for a waiting period at the end of the aspiration to ensure completion.

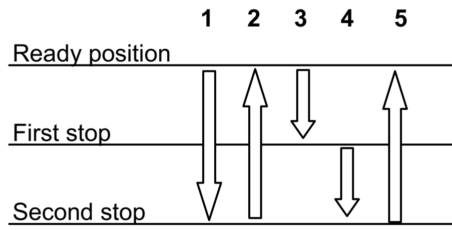
☞ Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid (Fig. 1-2b).

☞ Dispense the liquid into the receiving vessel (Fig. 1-2c) by gently pressing the operating button to the first stop (**3**). After one second, press the operating button to the second stop (blow out) (**3**).

☞ Remove the tip from the vessel, sliding it along the wall of the vessel.

☞ Release the operating button to the ready position (**4**).

1.3.3 Reverse pipetting procedure



☞ Attach the appropriate tip to the pipettor.

☞ Set the pipettor to the desired volume.

☞ Press the operating button to the second stop (**1**).

☞ Dip the tip into the solution to a depth of about 5 mm and slowly release the operating button to the ready position (**2**). This will fill the tip with a volume that is larger than the set volume. Avoid the creation of air bubbles in the tip (Fig. 1-2a).

☞ Wait 1–2 seconds to reach equilibrium in the tip and withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid (Fig. 1-2b).

☞ Dispense the liquid into the receiving vessel (Fig. 1-2c) by depressing the operating button gently and steadily to the first stop (**3**). This volume is equal to the set volume.

☞ Hold the button in this position. Some liquid will remain in the tip and this should not be dispensed.

☞ Remove the tip from the vessel, sliding it along the wall of the vessel.

☞ Remove the tip from the receiving vessel, sliding it along the wall of the vessel, without blowing it out. The liquid remaining in the tip can be delivered into the original vessel by pressing the operating button to the second stop (**4**) or discarded together with the tip.

☞ Release the operating button to the ready position (**5**).

Fig. 1-2a

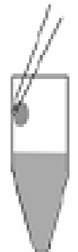


Fig. 1-2b

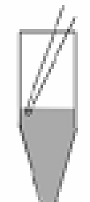


Fig. 1-2c

► State the main differences between forward and reverse pipetting techniques and suggest the technique more suitable for pipetting viscous liquids.

1.4 Portable and desktop biochemical analyzers

Several types of desktop or portable biochemical analyzers will be demonstrated in the student labs during the practical lessons. Some of them (glucometers, Accutrend®, Reflotron®) are based on "*dry chemistry*" principles. The reagents are not used in the form of solution, as you can see in the majority of common analyzers, but all the reagents required for the test are applied to a "dry" carrier material – e.g. a filter paper or carrier film. After the drop of the sample (urine, blood) is placed on the carrier, the reagent is moistened and the reaction proceeds. Coloration is evaluated by the reflex photometry, when the strip is inserted into the apparatus.

Accutrend® analyzers (Roche-Diagnostics) are simple manual analyzers, which are used for quick testing of the chosen parameters from capillary blood by dry chemistry. Accutrend® GC is designed for the determination of glucose and cholesterol. This "pocket" apparatus is for the personal use of e.g. for diabetics, for bedside monitoring or near-patient testing.

Before the examination, the instrument must be calibrated with the help of a coding strip. It is possible to calibrate the both parameters (glucose and cholesterol) at the same time. Successful calibration is indicated by a three-digit code, which appears on the display. The code is loaded and saved.

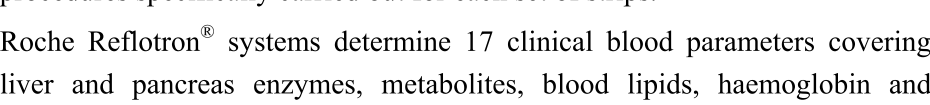
The measurement is carried out with the use of the test strips Accutrend® Glucose and Accutrend® Cholesterol. After the calibration, we can measure with any test strip, whose code is saved in the instrument.

When the test strip is inserted, the apparatus is automatically switched and the code of the required parameter appears.

After that, a drop of blood resulting from the puncture of the finger top is placed on the test zone of the strip. Then the cover is closed and the sample is measured by the reflex photometry method. The level of glycaemia is evaluated after 12 seconds, cholesterolaemia within 180 seconds.

The Accutrend® GC apparatus can be also used as an electronic diary. The obtained values of glycaemia and cholesterolaemia can be saved in the individual directories with the possibility to save also accompanying information describing the conditions of the measurement (e.g. fasting, after the meal, disease etc.). The apparatus is able to save up to 50 values of glycaemia and 20 values of cholesterolaemia.

Reflotron® analyzer (Roche Diagnostics) also belongs to the analyzers working on the principle of dry chemistry. It is used for quantitative determination of a wide range of clinical parameters from blood, serum, plasma, or urine. The system uses reagent Reflotron® strips, different for each determined parameter, and the Reflotron® reflectance photometer controlled by a microprocessor. Very good unity between the analytical results obtained by the analyzer and other routine methods is achieved by the calibration procedures specifically carried out for each set of strips.

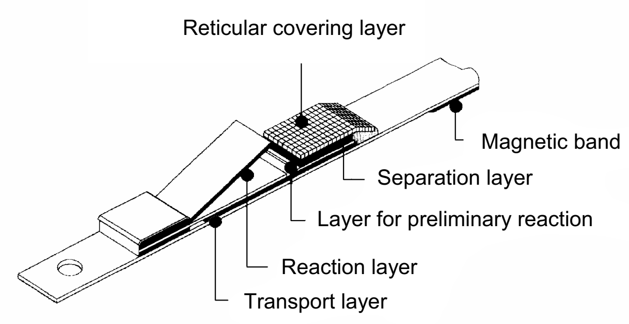


Roche Reflotron® systems determine 17 clinical blood parameters covering liver and pancreas enzymes, metabolites, blood lipids, haemoglobin and

potassium. The installation of a separation system in the strips enables to use samples of the whole blood for the analysis (the consumption of blood for one analysis is 30 μL; it is possible to use capillary blood).

Reaction strips Reflotron® have a magnetic band on the bottom side, which contains specific information about the test: Type of the test, incubation time, wavelength, number of examinations, which should be done, intervals between the individual measurements, mathematical factor for the calculation of the result from the measurement of reflectance, conversion factor to SI units, internal code of the test. After inserting the strip into the instrument, this information controls the following analytical steps.

The detection (upper) area of the strip contains several compartments. The sample, which should be analysed, is applied on the reticular covering layer. It is possible to apply whole blood, serum, plasma. Only accurately measured dose of the sample from the micropipette is always applied. Under the covering layer, we can find the layer of a special glass wool, which traps erythrocytes and the other blood elements.



According to the needs a filtration strip can be placed behind the separation layer for preliminary reaction. This layer can eliminate the interfering substances, may contain the activator or auxiliary reagent (e.g. the strip for the determination of creatinine contains two zones for the endogenous creatinine cleavage and elimination of the endogenous ascorbic acid under the separation zone).

Another zone, so called transport zone, enables the transport of plasma to the actual reaction place. It also contains glass wool, which differs in the composition and orientation of fibres from the previous, so that plasma is sucked to this zone by the capillary force. The transport layer thus serves as a sponge and at the same time as a container for plasma. The reaction layer contains reagent, which is essential for the actual analytical reaction. It is fixed on the special carrier (cellulose film, silica gel, paper). All the above stated hydrophilic layers are fixed on the transparent hydrophobic foil, which protects the optical system against contamination at the same time.

The reaction is started, when the reaction zone is pressed to the reservoir with plasma, which follows the insertion of the strip into the window of apparatus. Thus, the reagent is moistened with plasma and the reaction is activated. The basic principal of the majority of determinations are enzymatic coloured reactions. The resulting coloration is measured by the reflectance photometer directed by the microprocessor.

The advantages of this analyzer include small consumption of blood, non-invasive way of collection without further processing. It is easy to displace the apparatus. Therefore, it is highly recommended for performing of larger screening tests. Disadvantages include much higher costs of the analysis.

Turbox® nephelometer (Orion Diagnostica) is used for specific determination of serum and urine proteins and is based on the principle of immunoprecipitation followed by measurement of the resulting turbidity.

The analyzer consists of a keyboard, reading apparatus for magnetic cards, display, cuvette place with a cover for one cuvette, printer, and measuring element. Besides the analyzer, the producer delivers also complete reagent

sets for the determination of the individual proteins. Each reagent set contains an antibody against the determined protein (antiserum), calibrator (standard of given concentration of the given protein, buffer solution and reagent calibration card. The card has a magnetic print, which contains the calibration curve for the appropriate batch. The information is transferred into the apparatus by slipping the card through the reading part of the analyzer. The instrument analyzes the measurement with respect to the accurate criteria provided on the reagent calibration card.

Samples, calibrators and antiserums are pipetted directly into the special cuvettes (supplied with the instrument), where the immunoprecipitation occurs. After the incubation time, the cuvettes are gradually placed into the cuvette area, where the measurement takes place and the results are printed by the printer. The results are provided in the *Light Scattering Units (LSU)*, which are selected units representing the absolute value of the diffused light, and in the concentration units. Levels, which do not correspond with the criteria given on the magnetic card, are marked with two stars and the explaining reference.

Turbox® system is used for the determination of a large range of the specific proteins (albumin, prealbumin, haptoglobin, transferrin, components of the complement C_3, C_4, orosomucoid etc.).

► See video recording and acquaint with the utilization of automatic and portable biochemical analyzers. What analyzers are based on the principle of dry chemistry?

1.5 Factors influencing results of laboratory examinations

The primary function of clinical laboratories is to provide accurate and timely information, based on in vitro probes examinations, for the diagnosis, monitoring and treatment of the patient. Qualitative and quantitative tests in body fluids provide the ground for medical reasoning. Many factors besides disease affect the composition of body fluids. Ignorance or underestimation of these factors may lead to incorrect interpretation of laboratory results. Laboratory examination includes three phases:

- **Pre-analytical** phase – patient's preparation, collection of the biological material and its transport to the laboratory, preparation of the sample for processing;
- **Analytical** phase – analysis of the sample and evaluation of the result;
- **Post-analytical** phase – validation and the result interpretation.

a) Pre-analytical factors

Probably the most important factors regarding laboratory tests and/or procedures are patient identification and sample identification. It is very important to identify any patient condition or activity that might affect the lab test or diagnostic test being performed. It has been estimated that 1/3–2/3 of errors occur in the pre-analytical phase. The laboratory test value may be affected by physiological variables, sample collection and handling of samples for testing. Physiological factors that affect laboratory test values fall two categories – those that can be controlled and those that cannot.

- **Not controllable biological factors**

Race, ethnicity – All humans today are 99.9% genetically identical and differentiation of the effects of race from those of socioeconomic conditions is often difficult. Nevertheless, various race or ethnic populations differ in the amount of skeleton and skeletal muscles, in physiological levels of some analytes or in distribution of some genes.

Gender – Until puberty, there are few differences in laboratory data between boys and girls. After puberty may be concentration of numerous components or analytes greater in men than in women. Differences in physiological values originate mainly from different distribution of hormones. For example, the higher activity of enzymes originating from skeletal muscle in men is related to their greater muscle mass.

Age – The most important influences, which determine the overall effect of age, are the degree of sexual maturity and the amount of skeletal muscle mass of the individual. Many laboratory parameters in children are lower than adults. Higher physiological values during adolescence reflect e.g. increased osteoblastic activity during bone development.

Pregnancy – Pregnancy causes physiologic changes in many body systems, e.g. changes in production of hormones and their actions, influence of placenta, transfer analytes from amniotic fluid, etc.

Biorhythms – biological rhythms may be linear (age) or cyclic changes of metabolism due to action of hypothalamus and/or pituitary hormones (e.g. circadian rhythms, menstrual cycle) or due climatic or seasonal influences (dietary changes, physical activity, exposure to sunshine, temperature and magnitude of its changes). These cyclical changes may be forecast with some probability.

- **Controllable biological factors**

Body habitus – Body weight may affect concentration of some analytes by changing their distribution volumes. The serum concentration of cholesterol and LDL-cholesterol, triacylglycerols, uric acid, insulin or cortisol positively correlates wit obesity.

Physical activity – The effect of exercise on blood composition depends upon the duration and intensity of the activity and the physical condition of the patient. Muscular activity has both transient and longer lasting effects. In general, acute strenuous and exhausting exercise increased anaerobic catabolism, while prolonged endurance exercise aerobic metabolism. Exercise causes a reduction of cellular ATP, which increases cellular permeability. The increased permeability causes slight increases in the serum activities of enzymes and metabolites originating from skeletal muscle. Levels of these substances return to normal soon after the activity is stopped, with the exception of enzymes, which may remain elevated 24 hours or more. During exercise is increased metabolic activity of skeletal muscle (utilization of glucose and fatty acids, production of lactate).

Diet/fasting/starvation – Diet, food ingestion, fasting and starvation affect investigated analytes by different mechanism. It depends on the composition and amount ingested food and beverages. The hormones and enzymes are released before intake of food and during food intake. The meal rich in protein increases phosphate, urea and uric acid. The food rich in the lipids decreases ratio of nitrogen compounds, for examples uric acid. The vegetarian food decreases both total and LDL-cholesterol and triglycerides, increases total bilirubin and pH of urine. Some food and beverages can affect some metabolic pathways. For example, if the meal contains caffeine, the concentration of catecholamine,

glucose and free fatty acid is increased. Dehydratation may alter test results and make it difficult to draw the patient's blood. Patient may drink water to prevent dehydratation before the collection of blood (coffee is not permitted during the fasting period). Haematocrit and the concentration of many compounds are increased at dehydratation.

Smoking – has undesirable effect on the organism. Smoking may affect the level of many analytes by nicotine. Smoking affects the metabolism of the glucose and increases the concentration of cholesterol and triacylglycerols.

Alcohol – Alcohol consumption changes biochemical analytes in different ways. It depends on the fact whether the abuse is chronic or acute. Generally, it affects mainly the metabolism of glucose, triacylglycerols and increases liver enzymes in blood. Single dose of alcohol (small and medium dose) minimally affects the investigation. Long-term abuse of alcohol results in hypoglycaemia and keto- and lactatacidose, the increasing of serum uric acid concentration.

Drugs – can affect biochemical investigation by a lot of mechanism. For example – drugs induce/inhibit liver enzymes, affect bonding capacity of transport proteins, cause cytotoxicity or may interfere with the determination of analytes.

Stress – Stress affects the production of hormones that consequently change the metabolism of many compounds. Stress is often connected with severe illness and with collection of blood at some persons. The fear accompanying the collection influences concentration of some hormones (e.g. catecholamines). Anxiety that results in hyperventilation may cause acid-base imbalances, and increased lactate.

Environmental factors – These factors can include altitude (above 3 000 m), ambient temperature and geographic localization – countryside or city. Travelling across several time zones affects the circadian rhythm. It results in a change of some analytes, frequently it is retention of sodium ions and water. These values regress within 2 days after return.

Mechanical action – Muscle trauma, intramuscular injection increase the activity of ALT, AST, CK and the concentration of myoglobin. The pressure of uterus in high stage of pregnancy increases activity of ALT, the digital investigation of prostate increases activity of PSA (prostate-specific antigen).

- **Handling of samples**

There are many factors to consider when collecting lab specimens and prior to diagnostic tests. Preparation of the patient prior to the test or diagnostic measure is vitally important to the results of the test.

Sample collection – There are a lot of recommendations. The patients are not allowed to eat approximately 10–12 hours before blood collection. They have to exclude fat food and alcohol from their diet. Patients can drink about ¼ L of plain water or bland tea in the morning before the blood collection. Distorted findings in parameters can occur if patients did not keep fasting. Precautions (e.g. dietary and others) are recommended for some special investigations or functional tests (for example-PSA can be positive after riding bicycle). Haemodilution may occur due to drawing the blood sample from the same arm with an intravenous infusion of fluids running.

The type of blood sample needed is very important. The type of blood depends on the test ordered. Be sure to collect the specimen in the correct blood tube. Certain blood specimens must be collected in tubes with no anticoagulant. Some specimens must be collected in a tube with anticoagulants. Be sure to handle the specimens correctly. Some blood specimens must be gently mixed with the anticoagulant in the tube.

Time of the collection – Concentration of some substances substantially varies during the day (e.g. glucose, triacylglycerols, hormones), whereas the concentration of the others throughout the month or a year. Blood collection is performed usually in the morning hours.

Posture – Postural changes (supine to sitting etc.) are known to vary lab results of some analytes. Going from a supine (lying down on the back) position to standing cause the water to filter from the intravascular environment into the tissues, resulting in a decrease in plasma volume and an increase in non-filterable elements, proteins and substances, which are bound to proteins (e.g. total protein, albumin, enzymes, lipids, Ca^{2+}, Fe^{3+} ions) by 5–15 %. It takes about 20–30 minutes to equalize fluid shifts due to changes in position. Standard posture of patient at the blood withdrawal is sitting posture.

Tourniquet Application – If the tourniquet is used (not more than 1 min), it should be released before withdrawal of blood begins. Pumping of the fist before venipuncture should be avoided (results of many tests can be affected). Prolonged tourniquet application may cause haemoconcentration of non-filterable elements (i.e. proteins). The hydrostatic pressure causes some water and filterable elements to leave the extracellular space. Haemoconcentration may occur due to prolonged tourniquet constriction.

Posture – Postural changes (supine to sitting etc.) are known to vary lab results of some analytes. Going from a supine (lying down on the back) position to standing cause the water to filter from the intravascular environment into the tissues, resulting in a decrease in plasma volume and an increase in non-filterable elements, proteins and substances, which are bound to proteins (e.g. total protein, albumin, enzymes, lipids, Ca^{2+}, Fe^{3+} ions) by 5–15 %. It takes about 20–30 minutes to equalize fluid shifts due to changes in position.

Compression of the vein/finger – influences the concentration of blood gases, lactate, and pH.

Haemolysis – (visible, if the concentration of haemoglobin > 0.2 g/L) – haemolytic sample has increased concentration of the analytes, whose concentration is high in the erythrocytes and vice versa. Haemolysis may occur due to rough handling of the sample or from drawing the blood through a small-gauge needle. *The causes of haemolysis*: the use of extremely big or small needle, rapid evacuation of the syringe, heavy shaking of blood in the test tube, moisture in the collection set or in the test tube, detergents in the test tube, wrong proportion of anticoagulant, maintaining blood in the fridge, on the sun or above the radiator, centrifugation at high speed of rotation.

- **Transport of samples**

The serum/plasma is preferred for the transport than whole blood samples. Haemolysis can occur during the transport of whole blood. During transport, blood samples should be kept at 0 ºC (temperature of thawing ice).

- **Stabilization and storage of sample**

It depends on the determined analyte. There should be low temperature (4 °C, −20 °C, −80 °C), collection into thawing ice, protection against light, correction of the pH of the sample, the addition of the stabilisator.

b) Analytical factors

Results of all laboratory tests are affected by many analytical factors/errors, which determine closeness of measured value to true value. Because "true" value never has been exactly known, it is replaced by an estimate of true value called *traceable* value (sometimes called "conventional true value", "assigned value", "best estimate" of the value, "conventional value", "reference value" or "expected value"). Quantitative analytical results are expressed as "estimated value ± uncertainty", where "estimated value" is the estimate of the traceable value obtained when the analytical method is applied to the test sample. Among basic analytical characteristics are related quantities precision and trueness.

Precision is the ability of analytical method to produce the same value for replicate measurements of the same sample (i.e. closeness of agreement between independent test results x_i obtained under <u>stipulated</u> conditions). By other words, precision refers to how large the uncertainty in an experimental quantity is. Precision <u>is not</u> typically represented as a numerical value (it is a qualitative concept) but is expressed quantitatively in terms of **imprecision**, which is computed as an experimental **standard deviation *SD*** (has the same unit as measured quantity) or a relative standard deviation *RSD* (**coefficient of variation *CV***) of the measurement results:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad CV = \frac{SD}{\bar{x}}(\cdot 100\,\%)$$

where x_i … value of measurement, n … number of measurements and $\bar{x}$ … arithmetic mean:

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

Imprecision depends critically on the specified conditions. These conditions depend on the different factors that may be changed between each test result. Depending on the factors changed, two types of precision can be obtained: the repeatability and the reproducibility. **Repeatability** is simply the precision determined under the same conditions of measurement (the same procedure, the same observer, the same measuring instrument, used under the same conditions, the same location, and repetition over a short period of time). Term of repeatability is identical with the term of precision within-day. **Reproducibility** is simply the precision determined under conditions where the same methods, but different equipments, are used by different operator to make measurements on identical specimens. In general, the reproducibility gives the largest expected precision because it is obtained by varying all the factors that may affect to the results.

Precision depends only on the distribution of random errors and does not relate to the true value. **Random errors** are statistical fluctuations (in either direction) in the measured data due to the precision limitations of the measurement device. Random errors usually result from the experimenter's inability to take the same measurement in exactly the same way to get exact the same number. Fortunately, almost all the random errors follow a Gaussian-shape "bell" curve, and therefore, can be quantified by the standard deviation or the coefficient of variation. The effects of random errors can be minimized by taking many measurements and averaging the results.

Trueness is the closeness of agreement between the average value obtained from a large series of test results $\bar{x}$ and an accepted reference value x_0. Trueness is a qualitative concept. Its quantitative counterpart is *systematic error*, which is called „bias“. **Bias** is the difference between the expectation of

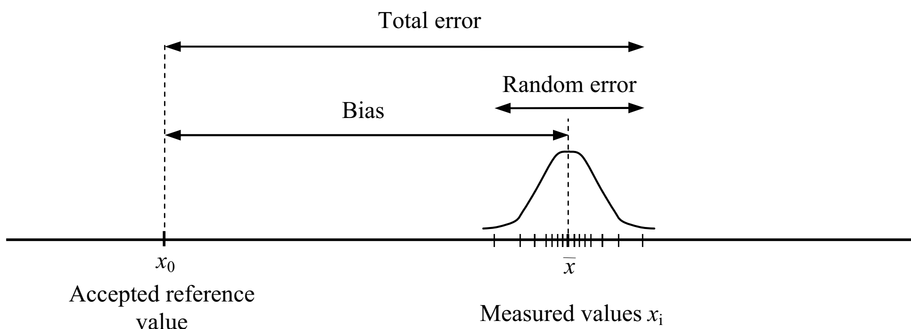
the test results (the average value of the large series of measurements $\bar{x}$) and an accepted reference value x_0:

$$\text{bias} = (\bar{x} - x_0) \qquad \text{bias} = \frac{(\bar{x} - x_0)}{x_0}(\cdot 100\%)$$

The causes of bias may be known or unknown but should always be corrected for when present. The **accepted reference value** x_0 is a value that is accepted as "truth" for a measurable quantity. An accepted reference value usually is obtained as the mean of a large number of measurements by the reference method in the reference laboratories.

Accuracy is closeness of the agreement between the result of a measurement and an accepted reference value. Accuracy is a qualitative concept. Its quantitative counterpart is **total error** (TE), which is a combination of both systematic error and random error:

$$\text{TE} = 1.96 \cdot SD + \text{bias}$$



Systematic errors in some instances can be identified and corrected but depending on its nature may be difficult or impossible to identify or quantify. That means that in many cases one can doubt about the reliability of a particular result of measurement. Unfortunately, not only systematic error can introduce a doubt about a result of measurement: random errors also may move away a result of measurement from the true value. Random and systematic errors act together on a measurement result producing an error of measurement and generating doubt „uncertainty" about the true value.

Uncertainty u characterizes the dispersion of the values that could reasonably be attributed to the measured quantity. Uncertainty is the stated range of the best estimated of any given value within which that value x may be expected to lie with some expressed degree of confidence (i.e. probability).

The uncertainty of a measurement is found by repeating the measurement enough times to get a good estimate of the standard deviation SD of the values. Then, any single value has a **standard uncertainty** equals to the standard deviation (i.e. numerically $u = SD$).

However, if the values are averaged, then the mean measurement value has a much smaller uncertainty, equal to the **standard error of the mean**, which is the standard deviation divided by the square root of the number of measurements.

Uncertainty of measurement comprises, in general, many components. Some of these components may be evaluated from the statistical distribution of the results of a series of measurements and can be characterised by experimental standard deviations. The other components, which can also be characterised by standard deviations, are evaluated from assumed probability distributions based on experience or other information. The most important random and systematic components of the

uncertainty are standard uncertainties of reproducibility and used calibrator, respectively. Therefore, the total (combined) standard uncertainty (u_c) is estimated by the equation:

$$u_c = \sqrt{SD^2_{\text{reproducibility}} + SD^2_{\text{calibrator}}}$$

Then the true value lies with a certain probability in an interval (called an **expanded uncertainty**) that is calculated from a standard uncertainty (u_c) and a coverage factor k, which is equal to 2 (exactly 1.96) at confidence interval of 95%:

$$x \pm 2 \cdot u_c$$

Using good laboratory practice, the measured laboratory data should contain the information about expanded uncertainty. In addition, the reference or cut-off values should contain the information about expanded uncertainty.

1.6 Interpretation of results

Clinical-biochemical examinations provide the clinician with a number of important information to derive the diagnosis, select the correct treatment, and monitor the course of disease to evaluate prognostic or risk factors.

1.6.1 Comparison of results with reference interval

Interpretation of the results of the biochemical examinations is most frequently carried out by the **comparison** with the reference interval. To follow this decision process, these reference values are required for all tests performed in the clinical laboratory, not only from healthy individuals but also from patients with relevant diseases. Epidemiological studies and preventive medicine programs may require reference values from completely healthy individuals, whereas a clinical decision to diagnose a specific disease would preferably require sets of reference values, which discriminate between specific disorders. **The reference values** are values obtained from the chosen group of individuals – reference individuals (age, sex, and race) with defined state of health.

The term *normal* value has been used frequently in the past. Confusion arose because the word normal has several very different connotations, therefore, it is recommended use the term *reference* values.

Reference individual is an individual selected as basis for comparison with individuals under clinical investigation, using defined criteria. A set of selection criteria determines which individuals should be included in the group of reference individuals. Such selection criteria include statements describing the source population, specification of criteria for health or the disease of interest. Ideally, the group of reference individuals should be a random sample of all individuals in the parent population who fulfil the selection criteria. Examples of exclusion criteria for health-associated reference values: pregnancy, excessive exercise, stress, intake of pharmacologically active agents (alcohol, tobacco, oral contraceptives, drug treatment for disease), genetically determined risk, obesity, hypertension. Often separate reference values for sex, age group and other criteria are necessary.

The test results (reference values) from this sample group (the reference group) are analyzed statistically to determine an interval of values that includes a specified percentage of all the values (reference interval) from the sample group.

Reference interval includes 95% of results of reference group; it means that 5% of the results are not included (2.5% of the highest values and 2.5 % of the lowest values). The reference interval can be estimated using parametric or non-parametric statistical method. Using of these methods depends on the distribution of the reference values.

To estimate the reference intervals, it is necessary to exclude outlying values, to test values (Gaussian or non-Gaussian distribution), to estimate reference interval using parametric or nonparametric method, to test influence of age/sex.

The parametric method is used for data having Gaussian distribution (normal distribution). The mean ($\bar{x}$) and standard deviation (*SD*) are calculated from reference values.

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Then the reference interval is calculated

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

where $\boldsymbol{a}$ is coefficient 2 (exactly 1.96) for 95% interval of reference values, $\bar{x}$ mean, SD standard deviation.

The parametric method is based on the determination of ± 2 SD limits (exactly 1.96) from the mean of the reference distribution. In a Gaussian distribution, these limits correspond to the 0.025 and 0.975 fractiles (see scheme below).

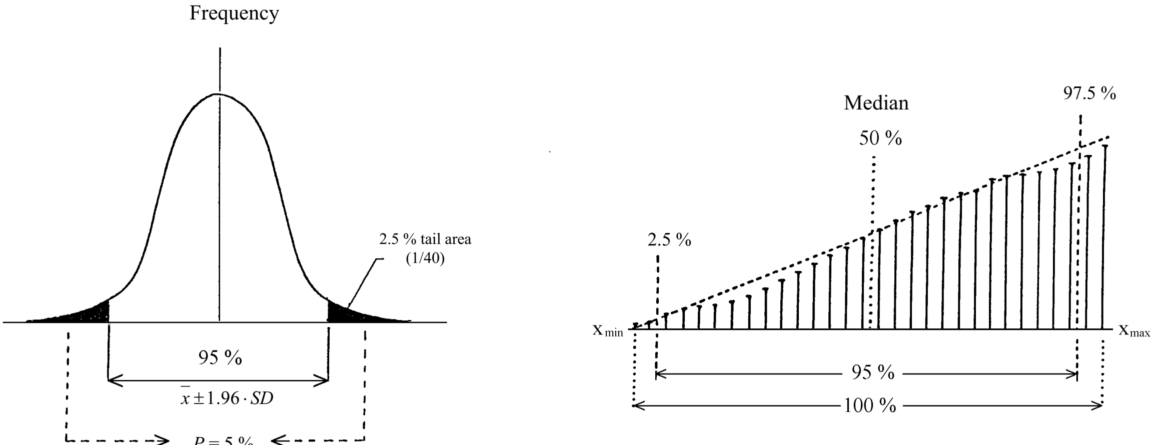
Many analytes have skewed distribution – most frequently with tail towards higher values. In this case, it may be possible to apply a mathematical transformation (for example, taking logarithm) to reduce the data to Gaussian distribution, but such transformation may only approximate the ideal. A reference interval, mean and standard deviation will be calculated after log transforming the observations of the method. The calculated parameters are shown back-transformed into units of the method.

Nonparametric method can be also used for estimation of reference interval. The basic assumption is that the values do not fit a Gaussian distribution. The non-parametric method is based on sorting the reference values in ascending order of magnitude. The reference range can be calculated by ranking the values and deleting the lowest and the highest 2.5 % (see schema below). Such a procedure has the considerable advantage that it requires no assumptions to be made about the characteristics of the distribution and does not require any transformation of the data to be made. The major disadvantage of the non-parametric method is that it cannot be applied to small sets of reference values. The recommended minimum number of reference values is 120.

The interpretation of the laboratory results with the help of the 95% interval of the reference values is the expression of the probability at the same time. Healthy person has 95% probability that his test result will lie in the given reference interval. The remaining 5 % represent the probability, that the result will be lower (2.5 %) or higher (2.5 %) than the reference interval.

It is necessary to verify the validity of the reference intervals taken from the literature (for given method in laboratory and for given population).

Parametric and non-parametric method used for estimation of reference interval:



Values are ordered according to their size from x_{min} to x_{max}

The probability, that the test results of all reference individuals will lie within the reference intervals, decreases with the increasing number of test results. Thus, the probability that the result of healthy individual will lie out of reference range increases.

1.6.2 Decision-making analysis

Another interpretation of the results is so called **decision-making analysis**. In this type of evaluation, we do not use the interval of reference values ("from-to"), but only one **cut-off value** *(decision limit).* This value allows us to describe the determined result as the *positive test* (the values are higher usually than *cut-off value*) or as the *negative test* (usually cut-off value or lower values). The positivity of the test is connected with a certain risk of existing or future disease. The risk could be described verbally (increased, high, etc.) or numerically (probability in percentage, etc.).

The cut-off value is usually calculated in such way, that the maximum of the so-called *substance of the test* is achieved, i.e. the highest probability of the unity of the test with the diagnosis. The ideas and their practical application will be explained in the clinical biochemistry more closely.

In case the patients are monitored during the progression of the disease or the medical therapy, the laboratory findings are compared with the previous results. This procedure does not enable the mechanical comparison of results, but the analytical and biological variability must be taken into account.

1.6.3 Critical difference

Most clinical laboratory tests are not used to aid in the diagnostic process but in monitoring. Data on biological variation are required for the interpretation of changes in serial results. A change may be due not only to the patient improving or deteriorating but also to analytical imprecision and intra-individual biological variation. Therefore, for a change to be significant, it must exceed the so-called critical difference.

Critical difference (CD) or reference change value (RCV) is expressed as statistically significant difference between the two results of the given laboratory test measured in an individual between the given time interval. If data on analytical imprecision, i.e. analytical variation CV_a (repeatability between days), and intra-individual (within-subject) biological variation CV_i are known, then the critical difference can be calculated as:

$$CD = 2.77 \times \sqrt{CV_a^2 + CV_i^2}$$

where the factor 2.77 is derived from 1.96 × √2, where 1.96 is determined by the 95% confidence interval values and √2 arises as we are comparing two results obtained by the same method for given analyte (i.e. results with the same *CV*).

For current database of biologic variations, see http://www.westgard.com/biodatabase1.htm. Calculator of CD is possible to find on the internet – see web pages of Czech Society of Clinical Biochemistry http://www.cskb.cz.

If the difference of two results of the given laboratory test in an individual measured between some definite time interval is higher then calculated CD, it can be expected that the results are different. These results differ with a certain probability (usually 95 %), i.e. they are not caused by the analytical or biological variability, but they reflect the change of the clinical state of the patient.

If the difference of the two measurements is lower than the acceptable CD value, the arithmetic mean is calculated for the given parameter. If the critical difference is crossed, the third examination is carried out after some time.

1.6.4 Diagnostic accuracy

The basic idea of diagnostic test interpretation is to calculate the probability a patient has a disease under consideration given a certain test result. We test some people for the presence of a disease. Some of these people have the disease, and our test says they are positive. They are called true positives (TP). Some have the disease, but the test claims they do not. They are called false negatives (FN). Some do not have the disease, and the test says they do not – true negatives (TN). Finally, we might have healthy people who have a positive test result false positives (FP). Thus, the number of true positives, false negatives, true negatives, and false positives add up to 100 % of the set. We can assume that there are four possible groups of patients – see Fig. 1-3 and the truth table.

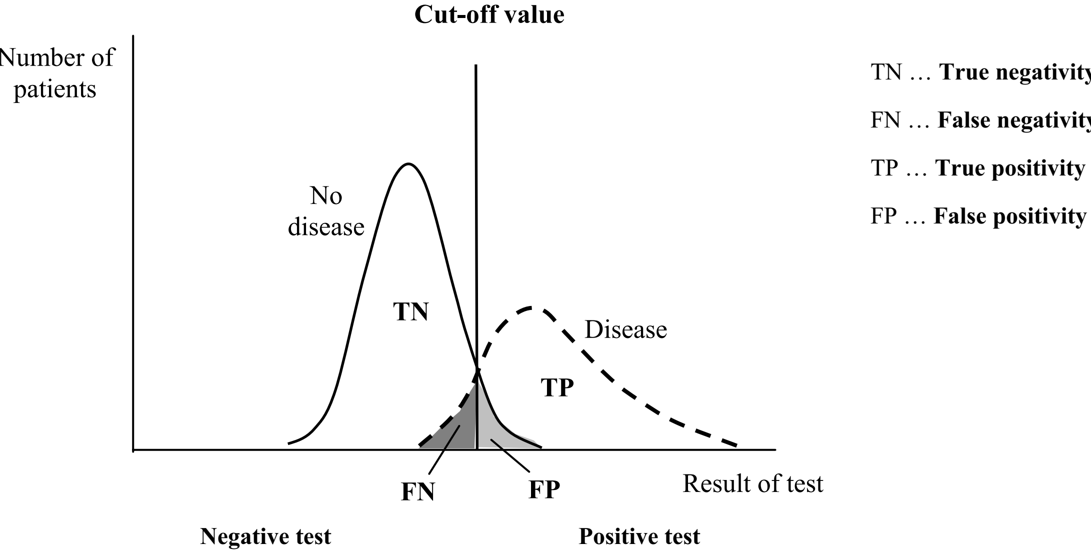


Fig. 1-3: Distribution of individuals with regard to presence of the disease and results of laboratory test

TN is a negative test for patients who do not have the disease, **FN** is a negative test for patients who have the disease, **TP** is a positive test for patients who have the disease, **FP** is a positive for patients test who do not have the disease.

Truth table (2 x 2 table): Classification of patients

Test result	Number of subjects tested		
	Diseased	**No Diseased**	**Totals**
Positive test	TP	FP	TP + FP
Negative test	FN	TN	TN + FN
Totals	TP + FN	TN + FP	TP + TN + FP + FN

Diagnostic accuracy is the ability of the test to discriminate between two states, i.e. disease and no disease, and is described by two key parameters: diagnostic (clinical) sensitivity and specificity.

Sensitivity refers to the proportion of people with disease who have a positive test result**.** Sensitivity is probability that diagnostic test will indicate presence of disease when disease is actually present. This term essentially tells the clinician how good the test is at correctly identifying patients with disease.

$$\text{Diagnostic sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \ (\times 100\ \%)$$

Sensitivity is the percentage of diseased individuals who have a positive test. Sensitivity is also the true-positive rate, e.g. if the sensitivity is 95 %, 5 out of 100 individuals with the disease will test negative. If the sensitivity is 100 % the test recognizes all sick people as such (100 % true positives, 0 % false positives).

Specificity refers to the proportion of people without disease who have a negative test result. Specificity is probability that diagnostic test will indicate absence of disease when disease is actually absent. This term essentially tells the clinician how good the test is at correctly identifying the absence of disease.

$$\text{Diagnostic specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \ (\times 100\ \%)$$

Specificity is the percentage of healthy individuals who have a negative test. Specificity is also the true-negative rate, and the inverse, 1 − specificity, is the false positive rate. If the specificity is 95 =, 5 out of 100 individuals without disease will test positive. If the specificity is 100 % test recognizes all healthy people as healthy (100 % true negatives, 0 % false negatives).

The values of both parameters are changed in the range 0–1 (0–100 %). The closer the sensitivity or specificity is to 100 %, the more sensitive or specific the test.

Sensitivity is the ability of a test to classify correctly an individual as “diseased”. Specificity is the ability of the test to classify correctly an individual as “disease-free”. Both sensitivity and specificity describe how well the test discriminates between patients with and without disease. An ideal test could distinguish absolutely between patients who do and who do not have disease (sensitivity and specificity equals 1). The clinical usefulness of a test is determined by how much it deviates from this ideal. A test where both sensitivity and specificity are close to 1 has good diagnostic ability.

Sensitivity and specificity of given test vary depending on the analyte cut-off level selected to distinguish positive from negative results. When the cut-off value is lowered (i.e. the cut-off line is moved to the left), more diseased individuals are classified as diseased and sensitivity is increased. On the other hand,

if the cut-off value is raise i.e. the cut-off line is moved to the right), more non-diseased individuals are classified as healthy. Sensitivity and specificity are inversely proportional, meaning that as the sensitivity increases (less false negative results, lower FN), the specificity decreases (more false positive results, higher FP) and vice versa.

High sensitivity is required when early diagnosis and treatment is beneficial, and when the disease is infectious. High specificity is important when the treatment or diagnosis is harmful to the patient mentally and/or physically.

1.7 Test requisition forms

The sample of blood or other biological material is sent for examination to the central clinical-biochemical laboratory in the majority of cases. The required examinations are marked into the laboratory order – the test requisition form. The *requisition form* should contain appropriate information about patient (patient name, birth identification number, diagnosis code), ordering physician (ID of health institution, name and qualification number of physician), billing (insurance identification), specimen (source, date of collection) and selected tests (all tests being ordered). Laboratory orders should be entered (electronically or on paper) directly by the physician. In some hospitals and outreach laboratories, laboratory tests are requested on paper requisitions, which are physically sent to the laboratory for entry.

The results together with their interpretation (numerical, graphic, and verbal) are then sent back (electronically or on paper) in the *test results document.* Different workplaces use different design of formularies.

► Familiarize yourselves with the requisition forms used in several hospitals and how to fill out them.

► Which sections are included in all formularies?

► Note the results of the performed examinations into the results document too in course of the semester.

Related problems

► 1. Mr. Z. has is going to come to venipuncture on the next day. Describe, what rules should he follow 24 hours before the collection.

► 2. Mr. S is a race swimmer. He came to the morning venipuncture after two hours training in the swimming pool. What analytes could be affected by the physical strain?

► 3. Miss M. (22 years, league basketball player) came to the regular check-up connected with the venipuncture. She had to stand in the overcrowded tram for one hour before she came to the doctor and she was not allowed to sit in the waiting room either. Analysis of her plasma showed, that the concentration of the total protein was at the upper limit and the level of albumin was slightly raised. Both values were found normal during the second control after two weeks. What can explain the previous results?