Federal state budgetary educational institution of higher education
"Volgograd state medical University" of the Ministry of health of the Russian Federation

Department of clinical laboratory diagnostics

LECTURE №1
Introduction to Clinical Laboratory Diagnostics

The student should know about:

-the basis of the organization of laboratory service;

-purposes, tasks, and the role of laboratory diagnostics in the practical medicine;

- the concept of diagnostic sensitivity and specificity of the test;

-the basic principles of obtaining biological material for biochemical studies.

-the organization of the quality control of laboratory -researches;

-- the concept of screening, prevention and investigation of differential diagnosis, rapid diagnosis;

- the concept of standardization of studies.

Clinical laboratory diagnostics

- is a medical diagnostic specialty, consisting of a combination of in vitro studies of biological material of human body based on the use of :
- hematological, general clinical, parasitic, biochemical, immunological, serological, molecular biological, bacteriological, genetic, cytological, toxicological, virological methods
- for comparing the results of these techniques to clinical data
- and the formulation of laboratory conclusions.

There are following sub-disciplines in accordance with the objects and methods of research in CLD:

-Clinical Biochemistry
- Hematology
- Cytology
- Laboratory Genetics
- General clinical researches
- Immunology
- Isoserology
- Molecular Biology
- Bacteriology
- Parasitology
- Virology
- Toxicology
- Coagulology

Methods of CLD are used in clinical medicine to:

- ✓- confirm the clinical diagnosis or specify it;
- ✓- determine the cause of the disease (at genetic, infectious diseases, poisonings);
- ✓- characterize the form, severity and prognosis of the disease;
- ✓- choose the etiologic and pathogenetic therapy;
- ✓- control over the results of treatment;
- ✓- detect pathology in screening studies of populations that undergo prophylactic medical examination.

Fundamentals of the Theory of Clinical Laboratory Diagnostics:

- Identification of qualitative and quantitative characteristics of the morphological, chemical, and other parameters of biological materials for assessing the functional state of tissues and body systems.
- Identification of physiological stress, early prodromal abnormalities, disturbances in pathological conditions (infectious, inflammatory, necrotic, tumor, immunological, genetic, etc.).

Fundamentals of the Theory of Clinical Laboratory Diagnostics:

- Laboratory tests for the diagnosis and functional diagnosis of diseases, the characteristics of severity, time and duration of illness, prognosis, monitoring treatment and its results.
- Establishing the relationship of structure and function of cells and tissues, and their relationship to clinical symptoms.

- Evaluation of physiological and laboratory parameters of the body of bioliquids, biorhythms (daily, seasonal, zonal), the influence of various factors (social, biological, mechanical, chemical, and physical) on the origin and nature of the pathological process.
- Search laboratory criteria for pathological, compensatory and adaptive reactions and processes aimed at restoring the original condition of the body.
- Development on the basis of clinical and laboratory studies the theoretical basis for the search of diagnostic programs.

- Optimization and development of new methods for studying the chemical and cellular composition of biomaterials, the definition of requirements and indications to conditions of their use;

- Establishment of reference values, limit fluctuations for each parameter of biological fluids and normal modes for individual contingents (by age, sex, occupation, environment);

- Definition of the diagnostic information content of laboratory tests and their fluctuations.

The basis of the clinical laboratory diagnostics are medical technologies, each of which, having undergone scientific approbation and procedure of permission to application, demands specific methodical references, a workplace, health regulations, technical control, training, an economic justification etc.

Fundamentals of the theory of Clinical Laboratory Diagnostics:

1) development of laboratory methods;

2) development of requirements for quality of implementation of analytical methods and means of providing these requirements ;

3) the establishment of normal individual fluctuations of each studied parameter composition and properties of biological fluids and tissues ;

4) the study of natural connections laboratory abnormalities identified with the essence of the pathological process in specific diseases;

5) establishment of diagnostic , differential diagnostic and prognostic value of individual laboratory tests and their combinations;

6) establishment of diagnostic laboratory programs to optimize diagnosis.

Immunoradiometric assay
Enzyme immunoassay– EIA
Fluorescence analysis– FIA
Chemiluminestcence analysis– CLEIA

Spectrophotometry, nephelometry, turbidimetry, fluorometer, flame photometry, polarimetry

clinical chemistry

Antigen

Antibody

10^{-21} 10^{-12} 10^{-9} 10^{-6} 10^{-3} 10^{-0}

pg/ml ng/ml mkg/ml mg/ml g/ml

Immunochemical studies

Progress analytical technologies

Plasma proteins

Medications

Markers of anemia

Thyroid hormones

Infectious diseases

Tumor markers

Markers of diabetes and allergies

Vitamins

PCR

Chromatographic analysis

«Biochemical biopsy» и «Laboratory endoscope»

Conditions of taking biomaterial for clinical laboratory researches:

The most common materials for laboratory tests are blood, urine and other body fluids.

It is necessary to take the material for laboratory tests before eating (fasting). The last meal should be for 8-12 hours (12 hours for studies of lipid profile) before taking material.

The time of taking biomaterial is from 7 to 9 am during the planned studies and at any time for emergency conditions. It is not allowed to take blood sample for routine biochemical analysis the night before.

Obtaining blood for clinical laboratory researches:

1. Native venous blood from large veins (usually from the elbow) without anticoagulants.
2. Venous blood with the addition of anticoagulants.
3. Capillary blood from the finger to determine glucose, clinical analysis of blood and other components;
4. Arterial blood taken from the large arteries (usually the femoral or subclavian) - to determine blood gases.

Venous blood

The use of venous blood for biochemical studies is the most preferable.

Nowadays taking venous blood is done with a thick needle into a glass or plastic tube or industrial vacuum systems, such as Vacutainer.

Depending on the material you need to get (serum or plasma), blood is collected in a clean, dry centrifuge tubes without additives (for serum), with the addition of anticoagulants (for plasma).

Capillary blood

Capillary blood is most often used to determine the glucose or blood count. For capillary blood sampling is used disposable sterile lancet or laser drills.

Clotted and hemolyzed samples are not investigated. Amount of collected blood depends on the number of lab tests and their required amounts of biological material.

For biochemical researches at least 6 ml, for reaerches of system of coagulation - 4.5 ml.

The main chemical additives used for blood analysis:

- Ethylenediaminetetraacetate (EDTA) - an anticoagulant that prevents blood from clotting by binding and effectively removing the calcium ions present in the plasma (calcium is necessary for blood clotting).
- Heparin - an anticoagulant that prevents blood from clotting by inhibiting the conversion of prothrombin to thrombin.It is added to the blood to conduct biochemical studies that require plasma. Anticoagulant properties of heparin are used in therapy.
- Citrate (as sodium salt, ie, sodium citrate) - an anticoagulant that prevents blood from clotting by binding calcium ions (like EDTA). It is added to the blood to examine the processes of coagulation

- **Oxalate (as sodium or ammonium salts, ie, sodium or ammonium oxalate) - an anticoagulant that prevents blood from clotting by binding calcium ions (like EDTA). Used in conjunction with sodium fluoride for the determination of glucose in the blood**

- **Sodium Fluoride - is an enzyme poison which stops metabolizing glucose in the blood after it is collected, ie, retains its concentration. It is used in conjunction with ammonium oxalate specifically for the determination of glucose in the blood**

Obtaining urine for clinical laboratory

Urine collection is carried out after a careful toilet of external genitals to avoid penetration of various substances in the urine.

Urine collected for analysis, can be stored for more than 1.5 - 2 hours (required in the cold). Prolonged standing leads to a change in the physical properties, the growth of bacteria and destruction of elements of urine sediment.

Further collection of urine, depending on the type of study has its own characteristics:

1. For carrying out the general analysis of urine collect only morning urine taken in the middle of urination as it is more concentrated and the pathological elements which have accumulated in kidneys and in urinary ways at night are washed away.

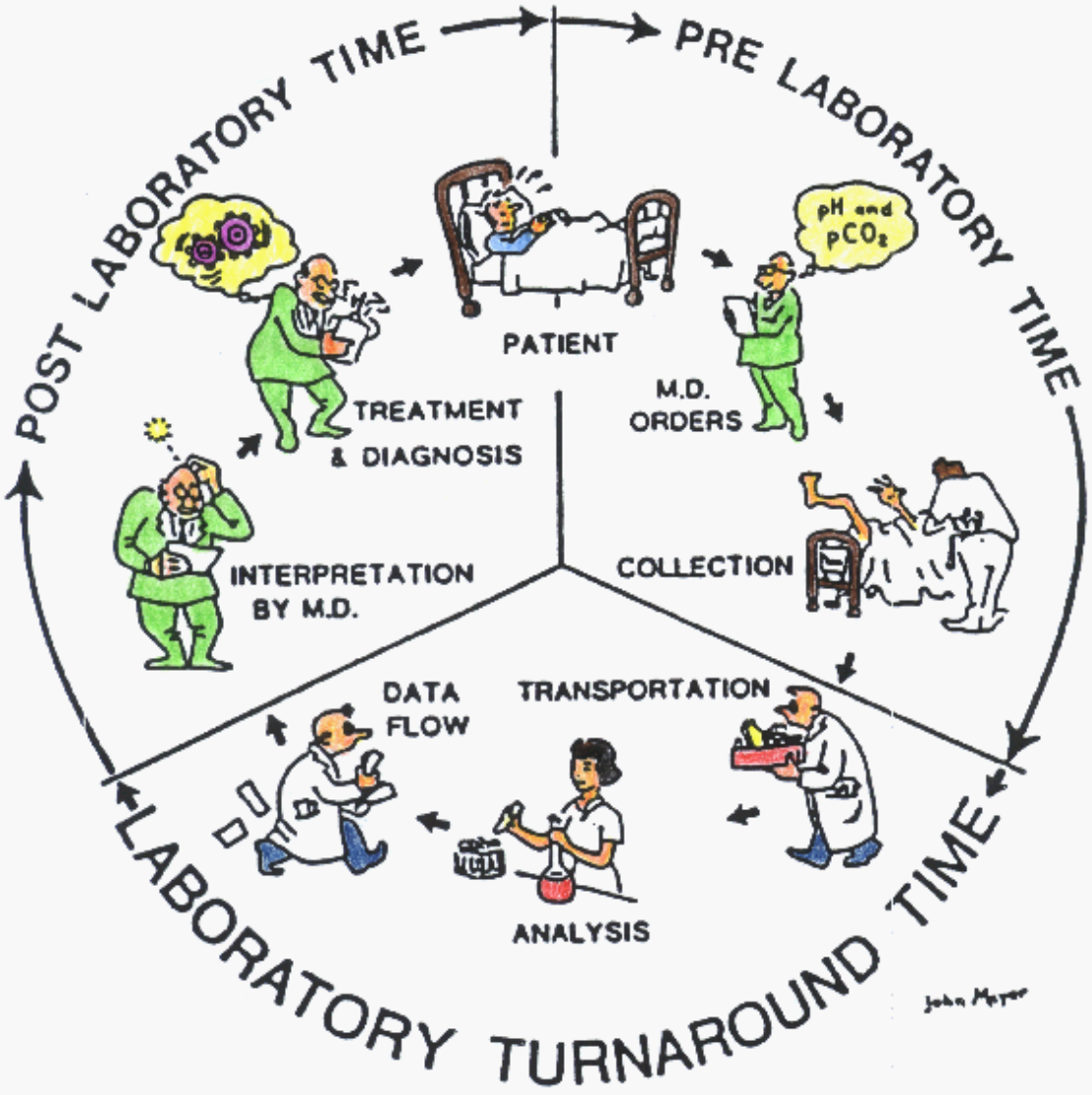
2. For carrying out assay on Zimnitsky (an assessment of concentration ability of kidneys) 8 portions of urine are collected per day

3. To determine the number of formed elements in 1 ml of urine by the method of Nechiporenko (revealing hidden inflammation) middle portion of the first morning urine is collected, no more than 15 - 20 ml.

4. Two glasses test is more often used in urology for women. Urine during urination is divided into two parts. It is important the first portion in this case to be small on volume. Glasses are also prepared in advance and the number of portion is indicated on each container.

5. Collecting of daily urine. The patient collects urine within 24 hours, keeping a usual drinking regimen (1,5-2/ l per day).

The main stages of the laboratory analysis



The reliability of the results depends on the quality of used laboratory methods, instruments, reagents, calibration material, from care of work of the personnel:

- If the deviation is of laboratory parameters is caused by pathology, at repeated researches repeatability and an orientation of deviations are in most cases taped.
- Some forms of pathology are characterized by several changes in laboratory parameters, for example, in acute inflammatory processes at the same time can be changed the number of white blood cells, erythrocyte sedimentation rate, the content of some enzymes, etc.

Some laboratory tests are specific for certain disorders of the body or for a specific type of pathology (for example, organ-specific isoenzymes, paraproteins in multiple myeloma), but most of the tests give results that have only a probable diagnostic character.

Thus, the increase in erythrocyte sedimentation rate is observed at bacterial inflammation, autoimmune process, and the tumor.

Assessing the suitability of a laboratory test for the diagnosis of certain forms of pathology use diagnostic criteria of

-diagnostic specificity,

-diagnostic sensitivity,

-diagnostic efficiency.

- **The diagnostic specificity** of the test for a specific disease - the percentage expression of the frequency of true negative test results in patients not suffering from this disease.

The more specific is method of investigating, the less it gives "false positive" results. False-positive results may lead to incorrect diagnosis and unnecessary diagnostic and therapeutic procedures that may worsen the patient's quality of life.

- **The diagnostic sensitivity of the test for a specific disease is only a percentage expression of the frequency of true positives test results in patients with this disease.**

- ***The more sensitive is this method of investigation, the less it gives "false negative" results. False-negative are such results that, do not reveal the patient's disease*.**

- **Diagnostic significance of positive results** is expressed by percentage of true positive to the total number of positive results, which include also false positive results.
- **The diagnostic significance of negative results** is the percentage of true negative results to the total number of negative results.

The diagnostic efficiency of the test is expressed in percentage of true (both positive and negative) test results to the total number of results.

In the calculations of these characteristics of laboratory tests is introduced amendment to the frequency of the disease among the total number of the surveyed.

Diagnostic efficiency of the method in the diagnosis of phenylketonuria

- PKU meets in 1 case from 10000. Diagnostic sensitivity of test is 100% and specificity is 99.9%.
- Positive predictive value of the analysis is only 10%, that is, nine out of ten positive tests for further study will be false positive.

Quality control assessments

To identify and assess systematic and random errors of measurements made in the laboratory, it is carried out the intra-and inter-laboratory quality control of laboratory researches.

For control measurements are used reference materials: aqueous solutions of standard fused serum, prepared in the laboratory-specific reference tools (brush strokes, microbiological culture, pathogenic fungi, a suspension cyst, etc.).

- **·A number of quality criteria are used:**

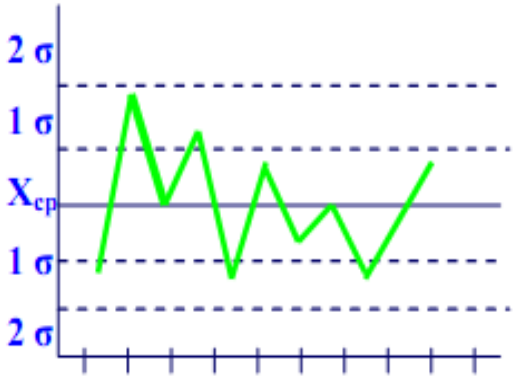
- **Accuracy of measurements - the absence of systematic errors in the results (to control the correctness the material with the studied maintenance of components is used only);**
- **• Measurement error - the deviation of the measurement result from the true value of measured size;**
- **• Systematic measurement error - error that remains constant or varying regularly at repeated measurements of the same size;**
- **• The random measurement error - error that randomly at repeated measurements of the same size;**

- **The convergence of the results of measurements - the lack of significant differences between the results of measurements performed under identical conditions (control of convergence and reproducibility of research results may be achieved by control material with unknown content);**

- **The reproducibility of measurement results - no significant differences between the results of measurements performed in different conditions (at different times, in different places). Reproducibility of the results of studies characterized by their degree of overlap in multiple studies of the same sample of biological material. Reproducibility is expressed as the inverse coefficient of variation of results. The less is the coefficient of variation, the higher is reproducibility;**

- **The correctness of the test results corresponds to the average measurement value of the true value of the measured parameter.**
- **The correct diagnostic information through laboratory studies can be obtained by knowing the normal values of laboratory test, the limits of intra-and Inter-individual fluctuations and the influence of various factors.**

Reliable results



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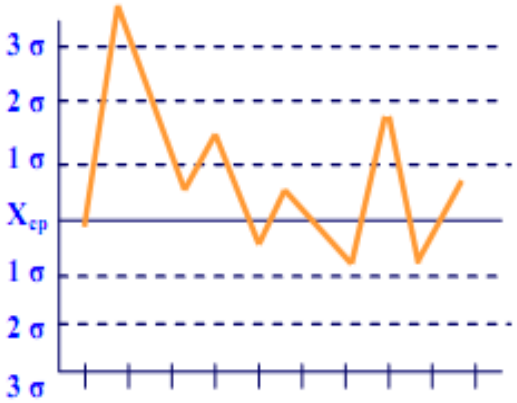
Random errors



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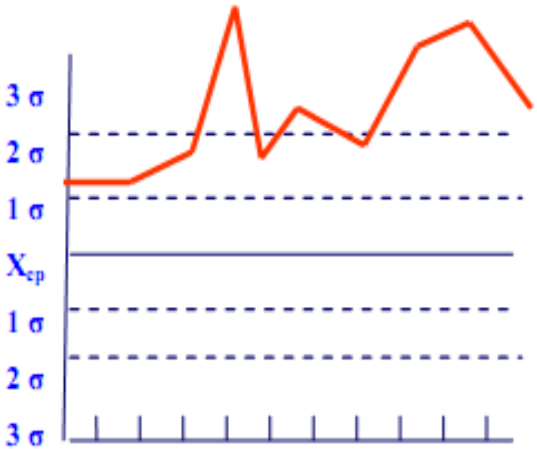
Systematic errors



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Interlaboratory control - a comparative quality control of research results obtained in several laboratories using a common reference material. It includes control of the reproducibility and accuracy, is carried out at least once a quarter under the methodological guidance of the control centers of the national, provincial and regional levels. The control center determines the goals, objectives and procedures for the control experiment, collect and examine the results of control determinations and make recommendations to improve the quality of the laboratory.

Units in Clinical Biochemistry

Unit of quantity of substance is the mol is a quantity of substance in the grams, equal in number to the molecular weight of the substance. 1 mole = 10^3 mmol = 10^6 mkmol = 10^9 nmol = 10^{12} pmol. The content of most substances in the blood is expressed in millimoles per liter (mmol/l).

- **For the indicators which molecular mass is unknown or it can not be measured (total protein, total lipids, etc.), as the unit of use - grams per liter (g/l).**

Units in Clinical Biochemistry

- In those cases where the total weight or number is difficult to determine, measured in international units per milliliter (IU/ml)

- Enzyme activity is expressed in SI is expressed in moles of product formed in 1 second in 1 liter of solution – mol/(s-l), mmol/(s-l), nmol/(s-l).

Types of laboratory researches:

Rapid laboratory diagnostics - quick laboratory testing methods that provide a study in 10-15 minutes after receiving the material. Rapid methods are based on the same or similar chemical reactions as the classical methods of analysis.

Clinical biochemistry - one of the most extensive sections of laboratory medicine, including studies of organic and inorganic substances that are formed during the biochemical reactions and enzyme activity in serum, plasma, blood, urine, cerebrospinal fluid and other biological fluids.

Clinical and laboratory immunology - section of laboratory medicine, which provides the definition of the degree of anti-infective and anti-tumor protect of the body using a set of indicators, and laboratory diagnosis and monitoring the effectiveness of treatment of allergic diseases.

Clinical microbiology (bacteriology, mycology, virology)-microbiological laboratory studies are conducted to identify the causative agents of infectious and inflammatory processes, determine their sensitivity to drugs and monitor the effectiveness of treatment.

Cytology (exfoliative and puncture)

Cytological diagnosis is to examine the structure and identify abnormalities in the structure of cells derived from exudates, synovial and cerebrospinal fluid from the surface of the mucous membranes, as well as tissues and organs in their biopsy.

Cytology (exfoliative and puncture)-cytological diagnosis is to examine the structure and identify abnormalities in the structure of cells derived from exudates, synovial and cerebrospinal fluid from the surface of the mucous membranes, as well as tissues and organs in their biopsy.

Clinical Molecular Biology and Genetics Diagnostic-genetic material is explored - chromosomes, genes, nucleic acid for detection of different types of mutations underlying hereditary diseases and malformations. Modern methods of DNA diagnostics - hybridization analysis, the amplification of genome, polymerase chain reaction, DNA probes, and other irreplaceable in prenatal diagnosis, and is widely used to identify viruses and bacteria.

Clinical Toxicology

Clinical and laboratory parasitology

Laboratory control (monitoring) of drug therapy

Clinical blood (studies of blood coagulation, study of the endocrine system, studies of renal function, liver function tests, tumor markers).