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# SEROLOGICAL METHODS IN MODERN BIOTECHNOLOGIES AND THEIR BIOANALYTICAL STANDARDIZATION

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***Context*** – Serological methods have become to use first turn before all methods of clinical laboratory diagnostics, and they stay remain extremely relevant to the present day. Serologic methods are used for diagnostics as infectious (bacterial, viral, fungal, parasitic), and non-infectious (oncological, endocrine, allergic) diseases. A significant proportion of diagnostic examinations carried out by the laboratory service relates precisely to serological tests. Serologic methods remain an indispensable part of ensuring the sanitary and epidemiological well-being of each country. Development of serological diagnostics products is the subject of immunobiotechnology – a section of biotechnology, which aims to create diagnostic and immune preparations for the diagnosis, treatment and prevention of human and animal diseases. Products for serological diagnostics belong to a class of medical devises. Obviously, the problem solved by such medical products is extremely responsible, because the health and life of the patient depends on the correctness of the outcome of the laboratory study. That is why, at the present stage, special attention is paid to the quality of medical devises for *in vitro* diagnostics, which, in turn, is connected with questions of standardization and technical regulation. A review of the medical and biological characteristics of the main common serological methods of diagnosis was made. The modern scientific and regulatory requirements for the analytical quality of products for serological *in vitro* diagnostics were summarized.

***Keywords*** – serological methods, *in vitro* diagnostics, medical devises, analytical quality.

## I. INTRODUCTION

William Osler, a prominent Canadian physician of the 19th century believed that "medicine is a scienceofuncertainty and an art of probability; one of the main causes of this uncertainty is increasing variability of manifestations of any disease" (1). A branch of medical and biological sciences formed in the first half of the 20th century, clinical-laboratory diagnostics, was aimed to minimize the scope of "uncertainty" when establishing the diagnosis. At present, clinical laboratory diagnostics combined laboratory methods of objective chemical and morphological analysis of biological materials (fluids, tissues, cells) of a human body. Such objective testing enables to carry out assessment of the condition of body organs, physiological systems and their spare capacities; to identify deviations from normal state and pathological disorders in activity of organs, human body systems; to establish diagnoses of diseases and perform laboratory control over pathological process dynamics, treatment and rehabilitation results. Meeting the challenges facing modern medical science and practice is impossible without patient's laboratory examination [1-3].

Serological diagnostics methods were among the first ones suggested and implemented into practical medicine from the whole complex of clinical laboratory diagnostics methods; they still remain highly urgent nowadays. Serological methods are used for diagnostics of both infectious (bacterial, viral, fungal, and parasitic) and non-infectious (oncologic, endocrine, allergic) diseases. Considerable portion of diagnostic examinations performed by laboratory service belong to serological tests. Serological methods remain an indispensable component of assurance of sanitary-epidemiological welfare of our country [1, 4-5].

It is worth mentioning that the range of methods and technologies used has greatly expanded from the time elapsed since implementation of laboratory examination of patients for diagnostic purposes. Advance in science and technology in the second half of the 20th century has perceivably increased the capabilities of laboratory diagnostics – almost all chemical and cellular components of biological materials have become accessible for assessment, and available methods, technologies, and equipment allowed characterizing the condition of body organs and systems with great precision. A whole branch of research-and-production activities involved in development and manufacture of devices for clinical laboratory diagnostics has been formed [6-7]. Appropriate scientific developments are the subjects of biotechnology – science and industry, which emerged in the second half of the 20th century, and aims to create useful products, drugs and technologies on the basis of biosynthesis or biotransformation processes. Objects in biotechnology are

biological agents – viruses, prokaryotes, cells and tissues of plants, humans and animals, their components and extracellular substances used in biological processes. The formation of the modern biotechnology industry took place at the junction of fundamental biological sciences (molecular and cellular biology, biochemistry, microbiology, virology, genetics) and engineering and technological disciplines. Development of serological diagnostic tools is the subject of immune biotechnology – a section of biotechnology, which aims to create diagnostic and immune products for the diagnosis, treatment and prevention of human and animal diseases [1, 8-9].

Thus, the purposes of the work were a comparative analysis of serological methods used in immune biotechnology and generalization of various regulatory approaches to their analytical standardization.

## II. COMPARATIVE CHARACTERIZATION OF SEROLOGICAL METHODS: MEDICAL AND BIOTECHNOLOGICAL ASPECTS

Immune reactions are used in diagnostic and immunological tests. The methods applied for this purpose are serological ones, i.e., methods for identification of antibodies and antigens using "antigen-antibody" reactions in blood serum and other body fluids and tissues. Identification of antibodies against certain antigens in patient's blood serum allows performing diagnostics of diseases of both infectious and non-infectious origin. Serological tests are used for identification of microbial antigens, various bioactive substances, blood groups, tissue and tumor antigens, immune complexes, cellular receptors etc.

**Agglutination reaction.** Agglutination reaction is adhesion of corpuscles (bacteria, erythrocytes etc.) by antibodies in the presence of electrolytes (sodium chloride). Agglutination reaction is manifested in the form of flocks or sediment comprised of corpuscles (e.g. bacteria) "agglutinated" by antibodies. Such interaction results in formation of particles – agglomerates, which are precipitated (agglutinate). Agglutination reaction can involve bacteria, protozoa, fungi, yeasts, Rickettsia, erythrocytes and other cells, either living or killed. The reaction occurs in two phases: the first one is specific binding of antigen and antibody, and the second one is non-specific (formation of visible agglutinate). Microorganisms found in agglutinate remain alive, yet they lose their mobility.

At present, AR is used for identification of antibodies in blood serum of patients, e.g. in brucellosis (Wright's test, Hedelson's reaction), typhoid fever and paratyphoid fevers (Widal's test) and other infectious diseases; identification of causative agent isolated from a patient; determination of blood groups using monoclonal antibodies against erythrocyte alloantigens.

AR is widely used for serological diagnostics of infectious diseases and determination of antigenic structure of isolated microorganisms. In order to determine antigenic structure of a causative agent isolated from the body of a patient or a carrier, specific immune serum is used, which is produced via immunization of animals (e.g. rabbit, donkey, or sheep) with certain microorganisms. Microbial identification is performed through agglutination reaction on glass with adsorbed or monoreceptor serums, or in test tubes with species-specific agglutinated serums. Adsorbed serums contain antibodies only against antigens specific for this particular microbe, and monoreceptor ones contain antibodies only against one specific antigen of a causative agent. Species-specific serums contain antibodies against all antigens of a certain microbial species [10-12]. Antibodies can be identified in patient's blood serum using agglutination reaction in such diseases as typhoid fever and paratyphoid fevers (Widal's test), brucellosis (Wright's test), tularemia etc. [13-17].

**Indirect hemagglutination reaction.** Indirect hemagglutination reactions are based on the use of erythrocytes with adsorbed molecules of a known soluble specific substance involved in "antigen-antibody" reaction.

In case of detection of antibodies, the relevant antigens are pre-adsorbed on erythrocytes, and the reaction itself is called indirect (passive) hemagglutination reaction (IHAR). Reverse indirect hemagglutination reaction (RIHAR), on the contrary, is intended for identification of antigens (or microorganisms themselves) and provides for preliminary adsorption of specific antibodies on the erythrocyte surface. Hemagglutination inhibition reaction (HAIR), when a virus isolated from a patient is neutralized with specific immune serum and then combined with erythrocytes is also used in serological tests. The absence of hemagglutination is indicative of the conformity between the virus and the immune serum used.

Co-agglutination reaction (CAR) is close to IHAR. It is used for identification of antigens using antibodies bound with Staphylococcal protein A (so-called antibody diagnostic preparation) [18]. Protein A is known to have affinity for Fc fragment of IgG molecule of humans and certain animals [19, 20]. This peculiarity of the mentioned bacteria allows obtaining antibody-based Staphylococcal diagnostic preparations. They contain standard suspensions of bacterial cells loaded with antibodies which specifically react with antigenic determinants of examined infectious disease agents. CAR reaction results in formation of flocks comprised of Staphylococcal protein A, antibodies of diagnostic serum and the microorganism being identified.

At present, various methods for production of erythrocytic diagnostic preparations have been developed, which differ in erythrocyte sensitization methods (using tannin, glutaraldehyde, chromic chloride, rivanol etc.), their affiliation with a certain species (human, sheep, chicken, turkey etc.), reaction preparation options (in microtitration plates, test tubes, capillaries) and accounting of results (visual, instrumental).

IHAR has rather high sensitivity and specificity parameters, which stipulates for its broad use in diagnostics of infections caused by bacteria, Rickettsia, and protozoa. Erythrocytic Salmonella O-diagnostic preparation, representing a suspension of erythrocytes with adsorbed O-antigens of various Salmonella groups on them, is used for IHAR preparation with patient's serum when the clinical diagnosis of Salmonella infection needs to be specified. Erythrocytic Vi-diagnostic preparation represents erythrocytes sensitized with purified Vi-antigen of *Salmonella typhi*; it is used in IHAR for identification of typhoid fever bacteria carriage [21, 22]. Erythrocytic tests

for diagnostics of diphtheria, tetanus, schistosomiasis, influenza virus, chickenpox virus etc. have been developed and manufactured [23, 24]. CAR-based diagnostic preparations are used for identification of causative agents of genital herpes, Streptococcus infection, dermatoses, cattle pox etc. [18, 25, 26].

**Precipitation reactions.** Precipitation reaction in agar gel, or diffuse precipitation method, allows to study the composition of complex water-soluble antigen mixtures in detail. Gel (semi-liquid or denser agar) is used for the reaction preparation. Each component in antigen composition diffuses towards the relevant antibody at different rates. That is why complexes of different antigens and relevant antibodies are located at different sites of a gel plate, where precipitation lines are formed. Each of the lines conforms to a certain specific "antigen-antibody" complex. Precipitation reaction is typically carried out at room temperature. Besides usual linear immunodiffusion, which has no practical value at present, two most widely used modifications of the method are described: double radial immunodiffusion by O. Ouchterlony[27] and simple radial immunodiffusion by G. Mancini [28].

Precipitation reaction is a very sensitive method, and it is used in studies of various protein and polysaccharide antigens in forensic practice – for determination of species of blood, sperm, serum stains present on underwear and various objects. This reaction can also be used for detection of various admixtures in milk, fish and meat products, identification of the nature of proteins in the composition of paints of the "old masters" [29-30].

**Neutralization reaction.** Immune serum antibodies are capable of neutralizing the effect of microorganisms (in particular, viruses) or their toxins on sensitive cells and tissues, which is due to blocking of causative agent antigens with antibodies, i.e. their neutralization. This reaction is most frequently used in viral diseases either for detection of antibodies in patient's blood or for identification of viruses isolated from patients.

Virus neutralization reaction (NR) is based on the fact that specific antibodies bind antigenic determinants of surface viral proteins and prevent the virus adsorption on the sensitive host cell. As a result, the virus loses its ability to reproduction, which is confirmed by its inability to damage cells in *in vitro* system and cause diseases in sensitive animals.

Cell cultures, chicken embryos, and laboratory animals (newborn or adult mice, hamsters) are used for determination of viral NR.

A known virus-containing material (allantoic fluid of chicken embryos, cultural fluid etc.) is used for serological diagnostics of viral infections, i.e. establishment of the concentration of anti-viral antibodies in patient's blood serum. The virus is titrated preliminarily. Virus titer is interpreted as its dose assuring 50 % biological effect – causing cytopathic effect (CPE) in 50 % of infected cell cultures ($TCD_{50}$), or death or development of a disease in 50 % of infected animals ($LD_{50}$ or $ID_{50}$). For NR preparation, 100 $TCD_{50}$ of virus in equal volumes is added to subsequent dilutions of patient's blood serums. After incubation of reagents at temperature 37 °C for 1-2 hours, their mixtures are added to a sensitive system (a suspension of susceptible cells, chicken embryos, or laboratory animals). The results are calculated after the relevant period.

Delay in viral cytopathic activity in cell culture with addition of the mixture of virus and patient's serum (in the presence of degenerative changes in cells in control test tubes where only virus was added) is indicative of the presence of antiviral antibodies in the patient's body. Absence or decrease in number (versus the control) of diseased and dead animals or chicken embryos is indicative of the same fact.

One NR modification is color test. It is based on the ability of cells cultures growing *in vitro* to decrease the nutrient medium pH due to accumulation of metabolic products and thus change the color of the medium containing an indicator phenol red from pink to yellow. Enteroviruses and some other viruses inhibit metabolism of infected cells and prevent the medium color change [10-12, 30-32].

**Complement fixation test.** Complement fixation text (CFT) is based on the fact that when antigens and antibodies conform to each other, they form an immune complex to which complement is bound via antibody Fc fragment; thus, complement fixation via "antigen-antibody" complex occurs. Complement fixation occurs at the first reaction step upon formation of "antigen-antibody" complex; in this case, hemolysis of erythrocytes sensitized with antibodies will not occur (positive reaction). If an antigen and an antibody do not conform to each other (or antigen or antibodies are absent in the tested sample), the complement remains free and binds to "erythrocyte – anti-erytrocytic complex" at the second step, causing hemolysis (negative reaction) [10-12]. CFT is used for detection of antibodies in patients' blood serum in bacterial, viral, protozoal infections, as well as for identification and typing of viruses isolated from patients [30, 31].

Antigens applicable for CFT are killed bacteria suspensions, extracts prepared from these suspensions, individual chemical microbial fractions. The main requirement to the antigen is the absence of inhibition of complement activity: it must not possess anti-complementary properties. To identify these antigenic properties, the complement is additionally titrated in the presence of antigen used in the reaction. CFT is used in diagnostics of syphilis (Wassermann reaction), gonorrhea (Bordet-Gengou reaction), toxoplasmosis, Rickettsia and viral diseases [30, 31].

**Immunofluorescence reaction.** Immunofluorescence reaction (IFR) is based on "antigen-antibody" reaction, in which the antibody is marked with a certain fluorescent dye (fluorochrome). Upon formation of such complex, it can be identified using luminescent microscopy or other device capable to register the relevant signal [33]. Fluorescein isocyanate and isothiocyanate etc. are used as fluorescent tags.

Immunofluorescence reaction (or fluorescent antibodies method) has two principal variants: direct and indirect. In the direct variant, specific immune serums labeled with fluorochromes raised against each type of test antigen are used for identification of different antigens. In this case, a large set of labeled antibodies is required, which can complicate the task a lot. In indirect IFR variant, only one immune serum is labeled with fluorescent tag – the one

raised against human or animal immunoglobulins, which is a donor of serum specific for the test antigen (in other words, anti-species serum with fluorescent tag). At the first IFR stage, interaction of relevant specific antibodies (immune serum) and antigen (tested causative agent) with formation of "antigen-antibody" complex occurs. At the following stage, anti-species immunoglobulins labeled with fluorochrome are attached to the formed complex [10-12].

IFR allows performing qualitative and quantitative identification of surface and intracellular antigens is samples of cellular suspensions (cell cultures, bacteria, mycoplasms, Rickettsia), samples of blood, bone marrow, alveolar swabs, thin tissue slices. The method allows carrying out detailed evaluation of biological samples for the presence of certain antigenic determinants, typical of certain causative agents or diseases, to perform quantitative assessment of both surface and intracellular proteins and receptors. Examinations and assessment can be performed either manually using fluorescent microscope or in automated mode using fluorescence-based flow cytometry device or microarray cytometers. The use of confocal microscope and robotized fluorescent microscope in combination with image processing software is possible. Currently available automated technologies allow analyzing several dozens of antigens in one sample using a set of various fluorescent markers in the format of high-informative microscopy and cytometry and approximately two times lower maximum set of antigens using up-to-date flow cytometry or confocal microscopy. IFR has found wide use in serological diagnostics of infectious and oncologic diseases, fundamental and applied studies in microbiology, cellular biology, genetics, pharmacology etc. [29, 31, 34, 35].

**Enzyme immunoassay.** Enzyme immunoassay (EIA) is an immunochemical method based on "antigen-antibody" reaction using antigens or antibodies labeled with enzyme. EIA preparation is not associated with potential hazard of radioactive contamination and handling of radioactive materials; the assay results can be determined visually. Reagents for EIA are relatively cheap and accessible. At the same time, EIA is non-inferior compared to RIA in terms of informative value, sensitivity, and reliability [36-38].

It should be mentioned that at present EIA is widely used not only in serological diagnostics of infectious and non-infectious diseases, but almost in all branches of biological and medical science. Such wide EIA prevalence is due to its several doubtless advantages over other serological methods. EIA is characterized by high sensitivity, specificity and reproducibility of results, easiness of execution, availability and stability of reagents, methodological flexibility (possibility of modifications), rapidness, and possibility of automation for large-scale tests.

From the viewpoint of immunochemical reaction location, all enzyme immunoassay variants can be divided into homogeneous and heterogeneous ones. In the first case, there is no need to separate the components into different phases. In homogeneous analysis, the activity of enzyme linked with antibodies or antigen is essentially different from the activity of free enzyme. In heterogeneous methods, separation of reagent linked with enzyme is mandatory: the labeled reagent is fixed on a solid phase, after which measurement of its enzymatic activity is carried out (the free ligand with label is preliminarily removed from the reaction zone). This principal method modification has been called enzyme-linked immunosorbent assay (ELISA). ELISA is the main enzyme immunoassay type used for today.

Irrespective of the assay modification, it provides for occurrence of three processes: immunochemical "antigen-antibody" reaction, fixation of enzymatic tag to the formed complex and identification of such tag with one or another physical or physicochemical method. Based on this, the necessary ELISA elements are as follows: solid phase (immunosorbent) – special plates made of polymer material with absorbed antigens or antibodies on the bottom of the wells; test material (body biological fluids) containing a certain antigen or antibodies to it; reagent with a tag – antibodies or antigens conjugated with an enzyme; substrate-chromogenic mixture containing substances serving as a substrate for the enzyme used, and a chromogen capable to change its color as affected by an enzymatic reaction. If the reaction results are identified by other method than visual, a relevant device is additionally required for measurement of the test sample optical density change.

A range of polystyrene plates for ELISA with different sorption characteristics are manufactured. Antigens used in EIA can be native, synthetic, or recombinant. Antibodies in immunosorbent or conjugate composition are typically monoclonal: due to limited capabilities to standardize polyclonal serums, their use in EIA is minor. Various bioorganic synthesis reactions with covalent bond formation are used to obtain conjugates or antibodies and antigens with enzymes; in some cases, the use of non-covalent binding is possible via the application of high-affinity interactions of various biomolecules. The main enzymatic tags in EIA are horseradish peroxidase (the most frequently used enzyme), alkaline phosphatase (an extremely stable and expensive enzyme), β-D-galactosidase, glucose oxidase, and certain other [38, 39].

Sometimes, signal amplification systems are used in ELISA; a classical example of such system is avidin-biotin system [38, 39]. It has been known for a long time that assurance of potent interaction between biotic and avidin, an egg albumen glycoprotein, requires only tetrahydro-imidazole ring of vitamin. Thus, carboxylic group of valerianic acid residue in biotin can be modified, and thus active biotinylated derivatives can be obtained. In particular, biotin bound to macromolecule retains its ability to bind avidin active center (the binding constant is $10^{15}$ $M^{-1}$) [40]. As avidin molecule is comprised of four identical subunits, biotin residues of two different biotinylated proteins can be concomitantly bound to it.

Practical use of avidin-biotin system is limited by high basic capacity of avidin and presence of carbohydrate residues in its molecule, which, in its turn, stipulates for high non-specific binding level. Another biotin-binding protein, called streptavidin, has been identified in bacteria *Streptomyces avidinii*[41]. Like egg albumen avidin, streptavidin also forms very potent and specific non-covalent complex with biotin, and it is comprised of four identical subunits. Each subunit contains one biotin binding center. Unlike avidin, streptavidin is non-glycosylated and

has neutral pH level [40]. A study [42] describes two options of this type of analysis for identification of specific IgE-antibodies, serum concentration of which is extremely low.

Enzyme-linked immunosorbent spot, ELISPOT assay is a modification of EIA method, and was initially developed for quantification of B-lymphocytes secreting specific antibodies [43]. Over time, ELISPOT was adapted to various tasks, first of all, for identification and quantification of cells producing cytokines. This method allows visualizing the product being secreted on the surface of an individual cell, activated or stimulated to an immune response.

ELISPOT allows performing both qualitative (immune protein type) and quantitative (number of cells wit relevant molecular signal) evaluation. Modern ELISPOT test is carried out using so-called ELISPOT readers equipped with computed test results detection-visualization systems, which allows for maximum automation of the procedure and assurance of higher precision compared to manual examination.

A specific EIA modification, hybridization-enzymatic assay (HEA), is used as one of the options for detection of polymerase chain reaction results.

EIA also served as a basis for development of rapid tests (so-called dot blot ELISA), which provide for immersion of solid phase with the reagent into the test sample. Such test systems become more and more prevalent when rapid results of individual tests are needed in laboratory diagnostics of infectious diseases. For example, they can be used to identify markers of hepatitis B and C, HIV etc. [44, 45].

Enzyme immunoassay has found extremely broad use in serological diagnostics of many infectious diseases (HIV infection/AIDS, viral hepatites, TORCH infections, sexually transmitted infections etc. [37, 46, 47]), oncologic diseases (identification of cancer markers [48]), endocrine disorders [49], allergy conditions [37, 50, 51], evaluation of the level of antibody-mediated and cellular immunity [52].

***Immunochromatographic assay.*** Immunochromatographic assay (ICA) is another immunochemical assay type, in which the test sample is applied on the surface of solid phase – membrane with pre-applied reagent, and the test result has the appearance of colored band.

One of two ICA formats, direct or competitive method, is used depending on the tested analyte nature and peculiarities. In direct ICA (sometimes also called "sandwich" variant), conjugate of antibodies against the searched analyte with a certain tag, applied on the membrane, is used. Anti-species antibodies specific to primary antibodies (e.g. mouse monoclonal antibodies against human immunoglobulins) are immobilized on the membrane control line. Following application of the sample containing target analyte (e.g. viral antigen), the sample contact with the membrane bearing the conjugate results in the analyte binding with the conjugate of labeled antibodies. After that, the immune complex penetrates into the test zone, where it binds specific antibodies, forming the immune complex "antigen – conjugate of antibodies raised against the antigen and tag – secondary anti-species antibodies". The excess of unbound conjugate binds anti-species antibodies on the control line. Thus, identification of two lines on the test strip represents the positive test result. In the absence of analyte in the sample, the conjugate binds anti-species antibodies on the control line only, forming one band on the test strip. The similar principle can be applied for detection of specific antibodies as well. In this case, labeled antigen is immobilized in the test zone, and antibodies specific to this antigen are immobilized in the control zone. This ICA modification is used for identification of high molecular weight compounds, in particular, various serological markers of infectious diseases (antigens and specific antibodies).

Competitive ICA has been used for detection of low molecular weight compounds. This method modification is based on the competition between the analyte and the immobilized analyte conjugate with protein carrier for the limited number of specific antibodies binding centers contained in the antibodies-tag conjugate. Application of analyte-containing sample results in the analyte binding with the tag on the membrane. Further, the immune complexes pass through the test zone, where the conjugate of analyte with protein carrier is immobilized. The immune complexes cannot bind this conjugate due to the steric difficulties: low molecular weight substances usually have one antigenic determinant and, consequently, the antibodies have one binding center with the antigen, which is already occupied with the analyte. Further, the immune complex binds anti-species antibodies located on the control line. As a result, the absence of stained band in the test zone and the presence of staining in the control zone are indicative of the fact that concentration of measured substance in the test sample exceeds its limit value for this test. In the absence of analyte in the sample, conjugate of antibodies with tag binds the conjugate of antigen with protein carrier immobilized in test line zone. Unbound conjugate of antibodies with tag reaches the control line zone and binds snit-species antibodies there. Thus, the presence of two stained lines (the test one and the control one) is used for detection of low molecular weight substances, in particular, metabolites of narcotic compounds [44, 53].

***Immunoblot.*** Immunoblot (western blot, protein immunoblot) is a complex analytical method intended for detection of specific proteins, which provides for consecutive combination of the following methods: protein electrophoresis in polyacrylamide gel under denaturing conditions (usually in the presence of sodium dodecyl sulphate) or in native condition; transfer of proteins after their electrophoresis on a membrane (nitrocellulose, polyvinylidene fluoride) with following detection using specific antibodies (like EIA, IFR or RIA) [54, 55]. The method was suggested by a team headed by G. Stark, and conventionally called western blot as one of the tetrads of molecular biology methods intended for analysis of biomolecules: DNA (Southern blot), RNA (Northern blot) and post-translation protein modifications (Eastern blot) [56].

Immunoblot is used for analysis of proteins of both tissue and cellular origin. Several different methods are used for test sample preparation, which allow homogenizing it to the level of individual proteins. Essential aspect of these procedures is parallel protection of proteins to be examined from proteolytic enzymes which can be found in the initial sample, and from denaturing

factors of chemical and physical origin. Separation of test sample proteins using electrophoresis can be performed by their isoelectric point (pI), molecular weight, or electric charge [57]. If a combination of two separation criteria is used, this is so-called two-dimensional electrophoresis. For further detection, proteins separated after electrophoresis are transferred with gel plate on a membrane using one of the following techniques: 1) membrane is placed on the gel, and a stack of filter paper is placed on the top, and the whole pile is transferred into a special buffer solution (protein transfer occurs as affected by capillary forces); 2) combination of this technique with passage of electric current. After this "blotting", proteins are fixed on a membrane with maintenance of their location. Essential step of assay required for assurance of successful detection is blocking of free membrane areas from non-specific interactions using an inert protein (e.g. bovine serum albumin) in the presence of detergent (e.g. Tween-20 or Triton X-100). This procedure precludes any non-specific interactions of specific antibodies, involved in detection procedure, with the membrane (minimizes the possibility of false positive results). Specific antibodies interact with relevant antigens on membrane; detection is performed using secondary antibodies (e.g. anti-species) labeled with an enzyme, fluorochrome, or radioactive tag; further accounting of results is carried out in a manner similar to EIA, IFR or RIA (qualitative or quantitative). Evaluation of results can be performed using special software (31, 56). It should be mentioned that immunoblot is an extremely sensitive and specific method, which stipulates for its use in serological diagnostics as a confirmatory (verification) test, in particular, for establishment of final diagnosis of HIV infection/AIDS, hepatitis C, Lyme disease, bovine spongiform encephalopathy, feline acquired immunodeficiency syndrome etc. [58, 59].

## III. BIOANALYTICAL STANDARDIZATION

Fulfillment of targets of clinical laboratory diagnostics is performed using the relevant devices – a separate type of medical devices. Medical device for *in vitro* diagnostics (IVD) is interpreted as a medical device, in particular, reagent, calibrator, control material, kit, instrument, apparatus, equipment or system, whether used alone or in combination, intended by the manufacturer for use *in vitro* for examinations of specimens in laboratories, including blood and tissue samples derived exclusively from the human body, solely or principally for the purpose of providing information: concerning a physiological or pathological state; concerning a congenital abnormality; to determine the safety and compatibility of donations with potential recipients; to monitor therapeutic measures [10, 11]. Development and manufacture of human and animal diseases diagnostic agents is one of the most important branches of immune and analytical biotechnology [12, 13].

It should be mentioned that precision of test results obtained using IVD devices is extremely important, as it influences life and health of patients. In view of social value of quality of IVD devices and clinical laboratory diagnostics, an important general issue is scientific justification of bioanalytical standardization parameters of serological *in vitro* diagnostic devices (with following implementation of these requirements into national regulatory framework). Requirements of leading global pharmacopeias can be used for establishment of IVD devices standardization parameters only to a very limited extent. The existing standards specialized in medical devices and their manufacture – Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices (IVDMD), ISO 13485:2016 "Medical devices. Quality management systems. Requirements for regulatory purposes", and ISO/IEC 17025:2017 "General requirements for the competence of testing and calibration laboratories" formulate only general requirements to quality and safety of products. Table 1 generalizes the requirements of IVDMD pertaining to bioanalytical standardization of serological diagnostics devices.

*Table 1*

**Requirements to Technical Regulation Pertaining to Bioanalytical Standardization of IVD Devices**

| Characteristic | Requirements to development and manufacture |
|---|---|
| Safety | Avoidance of risk for clinical condition or safety of consumers, users' health, including due to safe design and construction. Assessment of potential risks versus benefits for patient or consumer.<br>Provision of appropriate safety measures for avoidance of potential risks associated with the use of devices, which cannot be eliminated.<br>Informing of users and consumers on potential risks associated with the use of devices, which cannot be eliminated via safety measures. |
| Serviceability (quality) | Achievement of operating characteristics (in particular, analytical and diagnostic sensitivity, analytical and diagnostic specificity, accuracy, repeatability, reproducibility), including control of possible hindrances, as well as detection limits.<br>Traceability of accepted nominal values of calibrators and/or control materials has to be assured via the use of available reference measurement procedures and/or available reference materials (reference samples) of higher order. |
| Stability | Safety and quality of devices has to remain unaltered during the shelf life established by the manufacturer, and it may not become deteriorated to the level resulting in hazard to user's health and safety when the device is exposed to stress which may occur under normal conditions of use.<br>Characteristics and operating parameters may not become deteriorated during the use in accordance with intended purpose due to storage and transportation conditions. |

For justification of IVD device standardization principles, it is expedient to use the recommendations on validation of analytical procedures and tests stated in SPU as input data. This expediency is due to the fact that these methodological approaches are scientifically justified, they are widely used in pharmaceutical branch, and they are harmonized with international guidance documents. At the same time, several peculiarities and differences of evaluation of pharmaceutical products and control of *in vitro* diagnostic agents should be formulated. In case of a medicinal product, active substance (active pharmaceutical ingredient) content in it is sufficient; this parameter is stipulated by a drug product manufacturing formula and it is strictly regulated (it typically has to be within 95% to 105% of the nominal content). In case of IVD device, content or biological activity of an individual component of the device (antigens, antibodies, their conjugates, bioactive substances of chemical or biological origin) has no principal value – the important factor is the ability of complex of the device ingredients (system) to identify a certain analyte, the content of which in the biological material test sample is not known in advance, in a reliable and precise manner. The essential difference between medicinal products and IVD devices is the mandatory content of controls (actually being internal standards of the system) in the composition of the latter, which have to be calibrated quantitatively (for quantitative test methods, e.g. for measurement of content of various immunoglobulin classes) or semi-quantitatively (for qualitative test methods, e.g. for identification of antibodies against hepatitis B virus surface antigen).

We have to mention again the interrelation between the parameters of IVD devices bioanalytical standardization and analytical techniques validation procedure, as validation characteristics are actually standardization parameters. Thus, we suggest to proceed to justification of IVD devices validation characteristics. Based on recommendations of normative documents pertaining to medical devices [10, 11, 14, 15], medicinal products [16, 17], literature data [19, 20] and our own experience in development of IVD devices [21, 22], we suggest the following approach to selection of validation characteristics for various IVD device types (table 2). Further, we suggest discussing each validation characteristic in a more detailed manner.

*Table 2.*

**Validation characteristics for various IDV device types**

| Characteristic | IDV device type | |
|---|---|---|
| | Quantitative | Semi-quantitative (qualitative) |
| Accuracy | + | – |
| Precision<br>Repeatability<br>Intermediate precision<br>Reproducibility | <br>+<br>+<br>+ | <br>+<br>+<br>+ |
| Specificity<br>Diagnostic specificity<br>Analytical specificity | <br>+<br>+ | <br>+<br>+ |
| Sensitivity<br>Diagnostic sensitivity | | |
| Analytical sensitivity (limit of detection) | +<br>+ | +<br>– |
| Linearity | + | – |
| Range | + | – |

Remarks:

"–" – examination of the characteristic is not expedient;

"+" – examination of the characteristic is expedient.

## IV. REFERENCES


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# СЕРОЛОГІЧНІ МЕТОДИ В СУЧАСНИХ БІОТЕХНОЛОГІЯХ ТА ЇХ БІОАНАЛІТИЧНА СТАНДАРТИЗАЦІЯ

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Серологічні методи використовувались в першу чергу перед усіма методами клінічної лабораторної діагностики, і вони залишаються надзвичайно актуальними і донині. Серологічні методи застосовуються для діагностики як інфекційних (бактеріальних, вірусних, грибкових, паразитарних), так і неінфекційних (онкологічних, ендокринних, алергічних) захворювань. Значна частка діагностичних обстежень, проведених лабораторною службою, стосується саме серологічних тестів. Серологічні методи залишаються невід'ємною частиною забезпечення санітарного та епідеміологічного благополуччя кожної країни. Розробка продуктів серологічної діагностики є предметом імунобіотехнології – розділу біотехнології, метою якого є створення діагностичних та імунних препаратів для діагностики, лікування та профілактики захворювань людини та тварин. Продукти для серологічної діагностики належать до класу медичних апаратів. Очевидно, що проблема, вирішена такими медичними препаратами, є надзвичайно важливою, адже від вірогідності результатів лабораторного дослідження залежить здоров'я та життя пацієнта. Ось чому на сучасному етапі особлива увага приділяється якості медичних пристроїв для діагностики in vitro, що, в свою чергу, пов'язано з питаннями стандартизації та технічного регулювання. У роботі здійснено огляд медикобіологічних характеристик основних поширених серологічних методів діагностики. Узагальнено сучасні наукові та нормативні вимоги до аналітичної якості продукції для серологічної діагностики in vitro.

***Ключові слова –*** серологічні методи, in vitro діагностика, медичні апарати, аналітична якість.