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SPECIFICITY OF MANUFACTURING PROCESS VALIDATION FOR DIAGNOSTIC SEROLOGICAL DEVICES

O. Yu. GALKIN[1,2], *A. G. KOMAR*[3], *M. O. PYS'MENNA*[1]
[1]National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine
[2]Private Limited Company "Profarma Plant", Kyiv, Ukraine
[3]State Company "Ukrainian Medical Certification Center" of the Ministry of Health of Ukraine, Kyiv

E-mail: alexfbt@gmail.com

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The aim of this research was to analyze recent scientific literature, as well as national and international legislature on manifacturing process validation of biopharmaceutical production, in particular devices for serological diagnostics. Technology validation in the field of medical devices for serological diagnostics is most influenced by the Technical Regulation for Medical Devices for *in vitro* Diagnostics State Standards of Ukraine (SSU) — SSU EN ISO 13485:2015 "Medical devices. Quality management system. Requirements for regulation", SSU EN ISO 14971:2015 "Medical devices. Instructions for risk management", Instruction ST-N of the Ministry of Health of Ukraine 42-4.0:2014 "Medications. Suitable industrial practice", State Pharmacopoeia of Ukraine and Instruction ICH Q9 on risk management. Current recommendations for validations of drugs manufacturing process, including biotechnological manufacturing, can not be directly applied to medical devices for *in vitro* diagnostics. It was shown that the specifics of application and raw materials require individual validation parameters and process validations for serological diagnostics devices. Critical parameters to consider in validation plans were provided for every typical stage of production of *in vitro* diagnostics devices on the example of immunoassay kits, such as obtaining protein antigens, including recombinant ones, preparations of mono- and polyclonal antibodies, immunoenzyme conjugates and immunosorbents, chemical reagents etc. The bottlenecks of technologies for *in vitro* diagnostics devices were analyzed from the bioethical and biosafety points of view.

Key words: *in vitro* diagnostics, serological methods, validation, risks, quality management.

William Osler, a prominent Canadian doctor of the XIX century thought medicine to be "a science of uncertainty and an art of probability. One of the chief reasons for this uncertainty is the increasing variability in the manifestations of any one disease." Thus, clinical lab diagnostics developed in the first half of XX century as a field of medico-biological science aimed at narrowing down the "uncertainty" of diagnosis. Today it integrates methods for objective chemical and morphological analysis of biological materials (liquids, tissues, cells) of human organism. The methods of serological diagnostics were developed among the earliest methods for clinical laboratory diagnostics, introduced into practical medicine, and they are still very in demand. Serological methods are used to diagnose both infectious (bacterial, viral, fungal, parasytical) and non-infectious (oncological, endocrine, allergic) diseases [1].

Kits for serological diagnostics are very specific medical devices. Obviously, their purpose is of extraordinary importance, since accurate results of lab diagnostics determine the patient's health and life. That is why special attention is paid to the quality of *in vitro* diagnostics devices (IVDs) that depends on standardization and technical regulation [2].

Venues managing production quality are important in the development of a biopharmaceutical technological process (including making medical devices of biotechnological origin). Efficiently and rationally organized validation of technological processes in accordance to international and national legislature is a mandatory part of production lifecycle for these devices and one of the quality management processes in contemporary biopharmaceutical industry. Validation of the

technological process of biopharmaceutical industry is a mandatory element of providing quality, safety and efficiency [3–6].

Validation of technical processes is done to provide the proof that the process, personnel's actions and manufacturing systems function as intended by their stated goals and result in expected results. Validation is a process (action) that continues during the product's lifecycle, and provides guaranteed quality of the product taking into account all changes it undergoes in time [7]. The tasks of manufacturing process validation for *in vitro* diagnostics devices are as follows. First is confirmation of established parameters of technological processes. Second is providing production quality using the chosen technology. Thirdly, protocols (technological instructions, standard operational procedures — SOP) for the technological process (in accordance with the industrial capacities and equipment's intended purpose) must be confirmed. Fourthly, the equipment must meet all parameters of the technological process and production quality. Fifthly, the personnel must be able to conduct all procedures of the technological process. Lastly, all parameters of the technological process must be accurately reproduced while maintaining established production quality [8].

The aim of our work was to provide the inherent validation specifics for devices of serological diagnostics, using immunoassay kits as an example, based on analysis of contemporary scientific literature and national and international legislature.

General characteristic of in vitro diagnostic devices

Technical regulation concerning in vitro diagnostics devices [9] provides such a definition of IVD (in clinical laboratory diagnostics): medical device, in particular reagent, calibrator, control sample (material), kit, instrument, apparatus, equipment or system, used either separately or in conjunction, and assigned by the manufacturer for *in vitro* application to study samples, in particular samples of blood and tissues, obtained from human organism with the express aim (or mainly) to obtain information: concerning the physiological or pathological state; concerning congenital disorder; to determine the safety and compatibility with potential recipients; to monitor therapeutic measures.

The most technologically difficult and, on the other hand, the most widely employed serological technique is immunoassay, and so we shall now consider the principles of immunoassay kits production validation.

Immunoenzyme analysis (IEA, immunoassay) is an immunochemical technique based on the reaction between antigen and antibody with the application of enzyme-labeled antigens or antibodies. IEA was developed by E. Engvall and A. Pesce and has a significant number of advantages over radioimmunoassay (RIA) [1]. IEA entails practically no threat of radioactive pollution and work with radioactive materials; the results can be determined visually. Reagents for IEA are comparatively cheap and affordable while the method is no less informative, sensitive and trustworthy than RIA [10, 11].

One should note that by today IEA is widely used not only in serological diagnostics of infectious and non-infectious diseases, but in almost all fields of biological and medical science. Such wide implementation arises from its undisputable advantages over other serological methods. IEA has high sensitivity, specificity and reproducibility, is simple, rapid, and adaptable to specific purposes, requires affordable and stable reagents and allows processing multiple samples [1].

From the point of view of localization of the immunochemical reaction, all versions of IEA are either homogeneous or heterogeneous. In the first case, there is no need to view to separate the components into different phases. In the homogeneous assay, the activity of antibody- or antigen-bound enzyme is considerably different from that of free enzyme. In the heterogeneous setting, separation of enzyme-labeled reagents is necessary: the labeled reagent is fixed to the solid phase, and then enzyme activity is measured (after the free ligand with the label is removed from the reaction area). The modification is called solid-phase immunoassay (ELISA), and is the most widely used now [1].

Regardless of the technique modifications it is built around three processes: "antigen-antibody" reaction, fixation of enzymatic label to the formed complex and identification of the latter by physical or physicochemical means. Thus, ELISA includes the solid phase (immunosorbent) — polymer plates with antigens or antibodies adsorbed on the bottom of the wells; studied material (a biofluid) with certain antigens or antibodies; the labeled reagent — antibodies or antigens conjugated to enzyme; substrate-chromogenic mix with chemicals that are substrates for the enzyme and a chromogenic substance whose colour

changes under the influence of the enzyme-driven reaction. If the reaction's results are not identified by sight, a device is used to measure the changed optical density of the studied sample [1].

The polystyrene plates for ELISA are produced with varying sorption parameters. Antigens for IEA can be native, synthetic or recombinant. Antibodies in the immunosorbent or conjugate are usually monoclonal. Due to the limitations of standardization, polyclonal sera are of limited application in IEA. Various bioorganic syntheses are employed to obtain conjugates of antibodies and antigens with enzymes, resulting in covalent bonds; in some cases, it is possible to use high-affinity interactions of different biomolecules. Major enzyme labels in IEA are horseradish peroxidase (most often used), alkaline phosphatase (extraordinarily stable and expensive), β-D-galactosidase, glucose oxidase and several other enzymes [11, 12].

The principles of IVD standardization must follow the recommendations on validation of analytical methods and trials by the State Pharmacopoeia of Ukraine (SPU). The reason for this is SPU methodology is scientifically-based, widely used in medicine and harmonized with international legislature. In the case of pharmaceutical drugs, active substance content is determined in a strictly regulated production recipe (usually it must lie in the range of 95% to 105% of stated content). In IVDs, contents or biological activity of separate components (antigens, antibodies, their conjugates, biologically active substances of chemical or biological origin) of the device do not have such weight. The important thing is the whole system's ability to identify the target substance whose quantity in the studied sample of biological matter was not previously known. An important difference of medical preparations from IVDs is the mandatory inclusion of controls (serving as internal standards of the system) which should be calibrated quantitatively (for quantitative analysis, for example, to determine the immunoglobulin content by class) or semi-quantitatively (for example, to qualitatively identify antibodies to superficial antigens of B hepatitis virus) [2].

The parameters of bioanalytical standardization of IVD and the validation procedure of analytical methods and technologies are interconnected, since validation parameters are, in fact, the parameters of standardization. Applying recommendations of normative legislature on medical devices [9, 13–16], pharmaceutical drugs [17, 18], literature [19, 20] and our own experience in IVD development [21, 22], earlier [2] we worked out an approach to select validation parameters for different kinds of IVDs (Table 1).

General approach to validation of technology for biopharmaceutical production

Validation presupposes collection and analysis of data starting with project development and following industrial production to prove with scientifically-based evidence that the process is able to stably output high-quality production. Validation

Table 1. **Validation parameters for different kinds of IVD [2]**

Parameter	IVD	
	Quantitative	Semi-qualitative (qualitative)
Accuracy	+	–
Precision:		
Convergence	+	+
Intralaboratory precision	+	+
Reproducibility	+	+
Specificity:		
Diagnostic specificity	+	+
Analytical specificity	+	+
Sensitivity:		
Diagnostic sensitivity	+	+
Analytical sensitivity (limit of detection)	+	–
Linearity	+	–
Application range	+	–
Stability	+	+

of the process includes a certain operation sequence, performed during product lifecycle and the production time [23].

Generally speaking, there are three main types of process validation (PV). Prospective validation is done for production lines that are only being founded or were reconstructed. In this case, all qualification steps are required [project documentation (DQ), installation (IQ), operation (OQ) and performance (PQ)], as well as validation of processes and analytical methods. Concurrent validation is done similarly to prospective during serial production if it was not validated before; it requires all qualification steps and validation of processes and analytical methods. Retrospective validation of processes and analytical methods is done during serial production of non-sterile devices (that have not been validated previously) based on previously obtained documented data. Revalidation is done routinely as planned and recorded by the factory in the validation report, and also to re-start the production if documentation and/or production conditions are changed with possible implications for the quality of semi- and final products. The nature of validation is then determined by the factory based on the changes [16].

There are three major steps of validation process (Fig. 1). Firstly, the project design is evaluated. The project profile, scale and possible risks are investigated, revealing critical characteristics of materials and process parameters. The next step is process validation itself, with the object being serial production. The number of validation cycles is determined using the risk management procedure for quality ICH Q9 "Quality risk management" [24]. It is important to draw up an adequate validation protocol which would meet the acceptability criteria and include critical parameters of quality and process characteristics (Fig. 2). The validation of a technological process for variable technological parameters should consider the worst case scenario, setting maximally acceptable error margins within acceptable ranges ("action level" validation) [1]. Distinguishing critical and non-critical criteria is illustrated on Fig. 3, and one possible algorithm of validating production technology (using sterile production as example) is presented on Fig. 4.

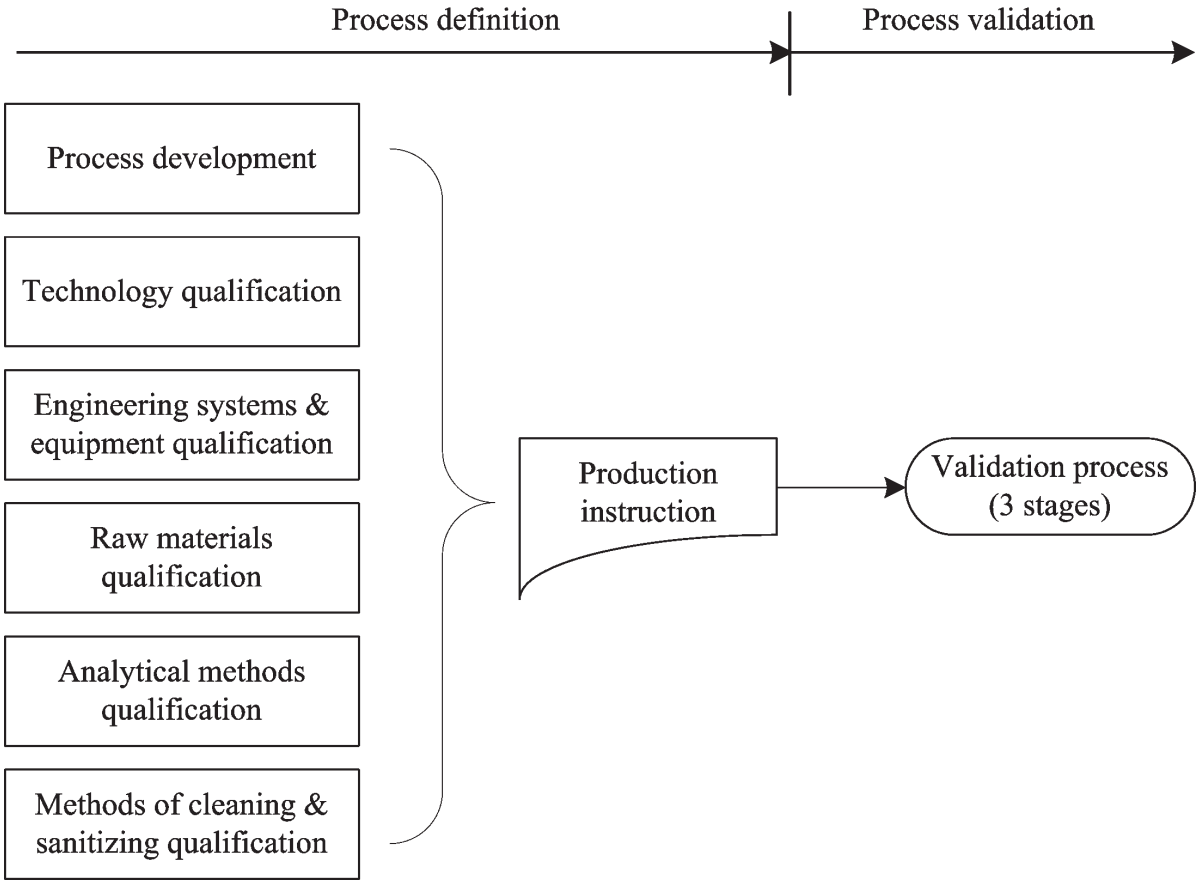

Fig. 1. **Scheme of establishment and execution of the validation processes [26]**

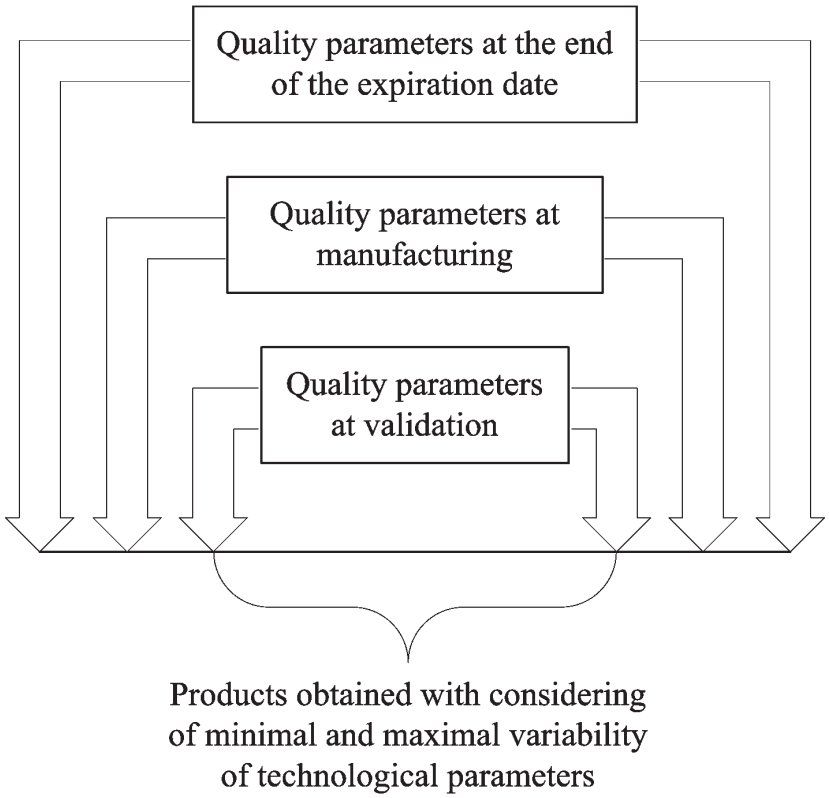

Fig. 2. **The principles of acceptability criteria for quality parameters [7]**

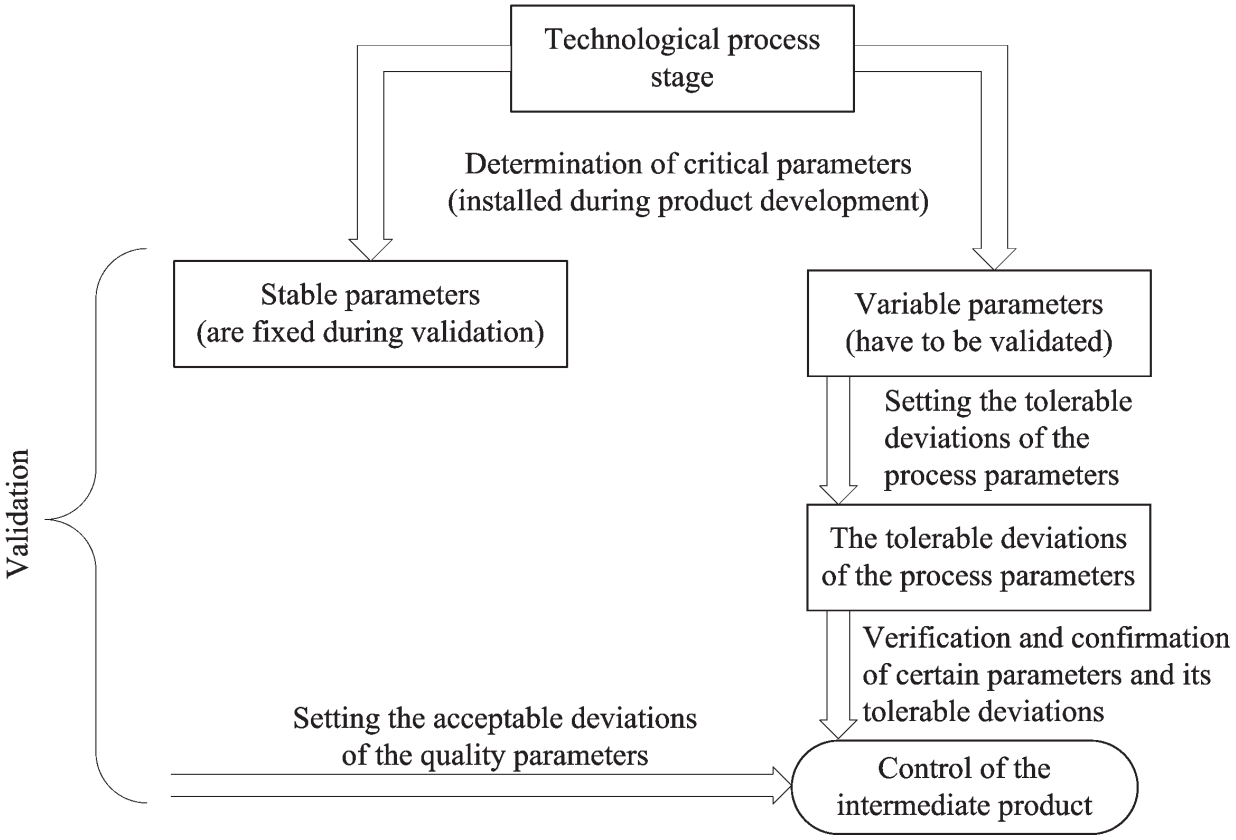

Fig. 3. **Algorithm of sterile production process validation [7]**

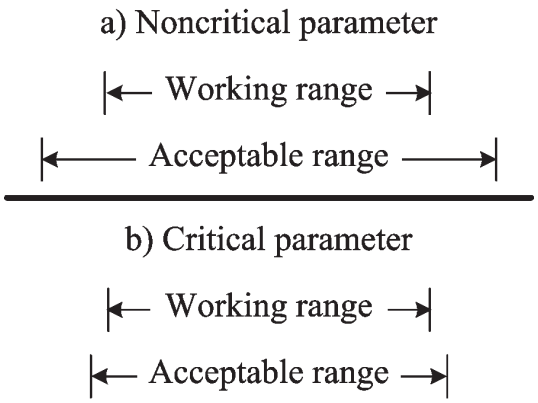

Fig. 4. **The difference between non-critical and critical parameters [26]**

Table 2. **Scheme of validation process for a pharmaceutical production [27]**

Validation object	Preliminary step	Major step
Analytical methods	Qualification of lab equipment at IQ and OQ stages	Validation of pharmacopoeial and non- pharmacopoeial techniques
Technological processes	Qualification at IQ, OQ and PQ stages	Validation of every process (recorded in validation protocols)
Auxiliary processes (purification, sanitation, etc.)	Validation of purification techniques, sanitary treatments, etc.	Validation of every process (recorded in validation protocols)
Engineering systems (providing clean air, water, water vapor, inert gas, compressed air, etc.)	If necessary, qualification of elements of the system (including critical areas, filters) and computer subsystems	Qualification of the whole system (IQ, OQ and PQ)
Industrial and lab rooms (clean rooms and areas, cold storage rooms, etc.)	Qualification at the DQ and IQ steps	Qualification of the outfitted buildings (OQ protocols); during operation (PQ protocols)

Third step is process verification, based on routine analysis of trends, changes, deviations and deficiencies, sampling and analyzing semi- and final products, etc. The general scheme of organizing validation in pharmaceutical factories is presented in Table 2.

The specifications file elements for validation procedures include the following [25]: the concept of construction, construction of the product, testing the construction with the proposed product specification; specifications for raw materials, intermediate materials, blanks, packing materials and final products; standard operational procedures (SOP) for equipment exploitation, including maintenance, production methods and useful and environmentally friendly specifications; validation protocols and reports; procedures for inspections and trials for monitoring during production, specifications of the product and of the acceptability criteria; protocol of sterilization process and report forms (for sterile production); SOP for assembling and handling (including maintenance) of product if necessary.

Validation research on establishing critical stages of non-standard technological processes is an integral part of medical drug development. Irrespectively of validation processes, results of the process validation must be described if the final product's compliance with specifications cannot be guaranteed with an acceptable degree of statistical significance through sample testing before it is allowed for distribution [23].

The design of aseptic and non-aseptic stages of production must allow efficient technological supervision. More strident demands are put on products obtained by fermentation or cell culture with further isolation and purification. For example, usually no cells and viruses should be present during the initial stages of the technological

process and are fully excluded before the isolation stage. This is often ensured by insertion of an additional filtration procedure between isolation and purification of the final product. The quality control is enhanced by the purification stage. For example, culture medium is monitored again at the last stage of chromatographic purification, and such technological processes as fraction collection, sampling, and closing containers can be done under a laminar air flow. Such preliminary validation is documented and outlined in the factory plans that can be used to demonstrate monitoring of its environment, product, personnel, materials and pollutions. The complex list of auxiliary data on production monitoring can be used to submit normative documents and as references for preliminary validation [26].

Validation of biotechnological stages of drug development for in vitro diagnostics equipment

Risk factors. Before a biotechnological process can be validated, it is important to evaluate risk factors associated with the product origin, raw materials, and production procedures. Also, analytical techniques used to characterize and validate the process and the quality of raw materials, intermediate and final products must themselves be validated. For plasmids, recombinant proteins and monoclonal antibodies, the technological process for obtaining the final product usually begins with fermentation or cell culturing accompanied by product recovery followed by multi-stages purification to obtain the pure substance.

Validation begins with thorough engineering of production process for the biotechnological product. This allows reducing risk levels to acceptable. After a detailed description of the technological process it can be validated. The points of reduction of risk factors, and risk levels in case production deviates from the norm must be known. Such information is provided by process validation [28].

The recombinant proteins are synthesized in bacterial and yeast cells and cell cultures of plants, insects, animals and people. Transferring a gene sequence coding the desired protein into an organism requires a transport system with vector DNA. The vector can be a plasmid, a virus, a phage, or an episome. The vector usually contains regulatory elements or sequences to control the expression rate [1]. Cells of vertebrate animals (in particular, mammals) are used to obtain monoclonal antibodies, and every system entails unique risks. Table 3 summarizes some widely known risks of most often used biotechnological production set-ups. The raw materials and semi-products used in culturing also require validation. For example, animal sera, frequently employed as growth factors and cryoprotectants for cell cultures, can transmit the transmissive spongiform encephalopathy virus (TSE) [28].

Endotoxins and nucleic acids are not considered contaminants and risk sources during IVD production, since they do not influence main validation characteristics (specificity, sensitivity, precision) and are safe for the personnel. Meanwhile, viruses and prions can not impact the quality of IVDs but are potentially unsafe for personnel. Proteins (of the non-target, contaminant kind) can be seen as risk factors for qualitative parameters of diagnostics devices, including those with cross-reactivity towards target antigen proteins.

The variability of cell culture can lead to unexpected expression of random products. Protein degradation and aggregation can result in formation of anomalous products that affect activity and/or immunogenicity of the target product. During the isolation and purification procedures the variability of used materials might change the product qualities. Auxiliary materials and/or equipment that come in contact with target biological products can influence the properties and stability of the latter (such as short-term changes in physical and physicochemical parameters of culture)

Table 3. **The most frequent potential risks of using biological objects for biotechnologies [28]**

Potential risk	Bacteria	Yeast	Cell cultures of insects and other animals	Transgene animals
Viruses	–	–	+	+
Prions (TSE)	–	–	+/–	+
Endotoxins	+	–	–	–
Nucleic acids	+	+	+	+
Proteins	+	+	+	+

Table 4. **The most frequently used analytical methods used to validate biotechnological production [28, 29]**

Method	Studied parameters
Peptide mapping	Impurities
Mass-spectrometry	Purity and admixtures, molecular mass, glycosylation
Liquid chromatography	Purity, admixtures, carbohydrates analysis
Electrophoresis	Purity, additives, glycosylated forms
Biological activity	Power of action, tertiary structure
Western-blot	Protein admixtures
Carbohydrate analysis	Glycosylated forms, carbohydrate sequence
Polymerase chain reaction	DNA, viruses, mycoplasmas
Nucleic acid sequencing	Genetic stability
Epitope mapping	Antigen structure

or even contaminate them (for example, the biocomponents of affine or immunoaffine chromatography columns). All these risks can be managed by screening raw materials and establishing criteria of acceptability during the development of reliable processes to incorporate known and potentially unknown risks, provide control specifications for every single procedure and use relevant validated techniques of process analysis [28].

Analytical methods of evaluation of biotechnological processes and products. Validation of analytical methods is an important precondition of process validation in production of biocomponents and diagnostic equipment. As a rule, analyzing biotechnological processes and products is a more complex and cumbersome task compared to classic pharmaceutical chemicals [29]. Table 4 summarizes information on specific analytical methods most often used to analyze biotechnological products.

Peptide mapping is widely employed to demonstrate the difference in a single amino acid between the protein product and its anomalous form. The use of mass-spectrometry has significantly grown in recent years; the technique provides information about the molecular weight of intact product and identifies admixtures by weight. Combined with other methods like peptide mapping, mass-spectrometry can confirm primary structure and posttranslational modifications, such as glycosylation. Ion-exchange high-performance liquid chromatography is used to both analyze purity and to find admixtures during production, and to analyze carbohydrates. Gel filtration allows evaluating aggregation. Determining the stability of the intermediate and final products is also a vital part of biotechnological production [28, 30].

The most important analytical methods are those for determination of bioactivity. In IVDs, biological activity can be seen as immunochemical activity, hence it must be tested with specially modified IEA [31].

Establishing the protein antigen structure (identification and characterization of antigenic determinants by epitope mapping) allows controlling and raising sensitivity and specificity of the techniques and devices for *in vitro* diagnostics [32].

Biological safety and its maintenance. Technologies of IVD component production can put both the personnel and the (semi) product itself in biological danger [33]. Biosafety principles can be maintained with the relevant technical decisions, such as specialized equipment — microbiological boxes (MB). According to international and national classifications (DSTU EN 12469:2017) [34] there are three safety classes for MB. Class 1 MB has an opening through which the worker can conduct his manipulations inside the box; it is built in a way that protects the personnel from emissions of fine contaminated particles from inside the box, by the way of laminar air flow directed through the opening into the box with further filtration and elimination from the box. Class II MB also has an opening to conduct the manipulations; personnel is protected, the risk of the product being polluted/cross-contaminated is minimized, and the contaminations that do occur are eliminated through filtered air flow circulating inside the box and by filtration of used air. In most cases, this is done by unidirectional downward laminar air flow inside the box and air door at the opening. Class III MB has the working area completely isolated, and the personnel separated from the area by a physical barrier (gloves

connected to the box). Filtered air constantly enters the box, and the worked air is filtered to prevent the microorganisms from escaping.

The type of MB must be chosen considering the specifics of used biological objects (bacteriae, microscopic fungi, and viruses) to employ the relevant procedures qualifying and validating the process.

Microbial biosynthesis. Both traditional and recombinant (usually *E. coli*) microbes are used in IVD production. The stage is, as a rule, not too critical from the point of view of the final product, since physical and physicochemical parameters of the process are fully acceptable from the point of recombinant protein stability.

However, the process of microbial biosynthesis must be stable, from the standpoint of technical and economical technology parameters. The stability of critical parameters of culturing process (medium pH, temperature, diluted oxygen concentration, hydromechanical parameters etc) directly influences the realization of potential maximum output of the final product. These parameters should be exacted to validate microbial biosynthesis processes [31, 35, 36].

One guarantee of stable and efficient biosynthesis process is optimal planning in general and selecting equipment in particular. A key problem with using the optimal bioreactor to solve a certain biotechnological task is the so-called "traditional approach" to fermentation equipment. That is, the chosen bioreactor meets quantitative criteria that allow achieving certain technical and economical parameters of the process, yet are not optimized for maximal output. One of the ways to solve this issue is by creating and following an algorithm of calculating the global optimization criterion for choosing bioreactor for periodical microbial synthesis using the program MathCAD [37]. Typical process validation parameters of microbial biosynthesis are collected in Table 5.

Cell and tissue cultures. Cell technologies are widely used in many biotechnological processes, in particular to obtain recombinant proteins and viral antigens. The validation specifics of these technological stages are analogous to those for technologies to synthesize monoclonal antibodies, which will be reviewed separately.

Purification and isolation of recombinant proteins. If the target product is secreted into cultural liquid, its recovery can include a simple procedure of filtration to remove cells and their components. Other methods of purification include centrifuging and adsorption of final product. Such intracellular products as recombinant proteins produced in *E. coli* can be denaturized and localized in inclusion bodies. Bacterial cells, as a rule, are concentrated by centrifuging or filtration, washed, and then disintegrated for homogenization. Afterwards the protein is isolated from IB and refolded [31, 35].

Products and solutions for extraction and recovery have to be validated for proteins localized in IB. There has to be an established degree of refolding of the target protein. The validation should demonstrate the refolding sequence and remove any wrong conformation of the protein product. Special attention should be paid the right conformation of the protein, since this is one of the main parameters of specificity and sensitivity in IVDs [25, 28]. Endotoxin monitoring is not necessary, since, firstly, such proteins are used *in vivo*, and secondly, there are no reports of their possible interaction with human plasma antibodies (as a factor of unspecific interaction of recombinant protein antigens). Typical validation parameters of microbial biosynthesis process are summarized in Table 6.

Chromatographic methods play the main role in isolation and purification of recombinant proteins. Generally, three to five purification procedures are needed to reach the required purity of protein product. That degree of

Table 5. **Typical quality criteria for microbial biosynthesis of recombinant proteins [28]**

Technological process	Quality criteria
Culturing in flasks	Cell density in culture
Culturing in a fermenter for inoculum	Cell density Carbon content
Culturing in industrial fermenter	Cell density Carbon content Level of absorbed O_2 Product titer/expression level Loss of plasmid marker Generation/removal of aerosol

Table 6. **Typical quality parameters for isolation and purification of recombinant proteins [28]**

Technological process stage	Parameters of quality
Microbial cells isolation	Solids output
Cell disintegration Recovery (renaturation)	Cell lysis degree /% of disintegrated product Product output
Chromatography	Product output and concentration Purity (HPLC-chromatography, SDS electrophoresis in polyacrylamide gel, Western-blot) Product degradation/stability Host cell protein content Virus removal Impurities content Resins re-use

Table 7. **Variations in performance and criteria of chromatographic purification [28]**

Variable parameters	Parameters affected by variations in chromatographic purification
Total protein content	Protein purity
Sample volume	Total product output
Conductance	Removal of specific impurities
Throughput rate	pH
Pressure	Resolving power

purity depends on the product's concentration, volume of samples, and risks from impurities present in the raw materials. The parameters in development and validation of the process include characteristics of sorbents and filters that correlate with the characteristics of separation, column packing quality and stability, product purity, impurities profile, and equipment cleaning [28, 31].

Validation of all stages of chromatography and filtration requires orthogonal analytical techniques, some of which could not be included in the manufacturing process. For every step it is important to determine the acceptable range of all parameters and establish the criteria of acceptability for purity and impurities. Clear understanding of what parameters of purity and impurities are for every stage of purification is crucial. Preliminary criteria of purification are determined when the production process is developed, after which they are modified (Table 7). Notably, small changes in work parameters can have a disproportional impact on removal of certain impurities.

Hybridoma technology (obtaining of monoclonal antibodies, McAb). McAb can be obtained either *in vitro* or *in vivo*. The latter is bioethically inadvisable [38]. In any case, it is impossible to omit lab animal use and therefore requirements for the production stage are needed. Standardization of this step can refer to control over the purity of animal lines that should be supported by relevant certificates of the suppliers or competent laboratories. This monitoring is required for high efficiency of production (antibodies synthesis output). In using laboratory equipment or specialized bioreactors to culture hybridomas the important (crucial) parameters are temperature, medium pH, concentration of CO_2 and diluted O_2, mixing speed, culture density, and osmolarity [39, 40]. An important element of intraproduction control is culture medium control since cultures of eukaryotic cells are supersensitive to changes.

Hybridoma cells should also be monitored for mycoplasmic contamination, since it can lead to the loss of antibody production. Another critical step in the work with hybridomas is their cryopreservation and cold storage [1]. Overseeing specific stages of the technology must be included into validation protocols.

Human (animal)-derived materials and their safety. Serological diagnostics equipment has negative and positive control samples.

Table 8. **Factors that influence the quality of biochemical and chemical components**

Factor	Variable parameters
Plate	Material, well shape, adsorptive capacity
Buffer solutions	Content, pH, antigen activity (cross-reactivity of components), storage duration, colloid stability
Antibodies (mono- and polyclonal)	Specificity, titer, affinity, incubation time, temperature, stability, cross-reactivity
Antigens	Stability, epitope parameters, temperature
Immunoenzyme conjugate	Enzyme type, conjugate type, activity, concentration, cross-reactivity
Substrate, chromogenic substance	Concentration, sensitivity, stability, pH
Stop reagent	Concentration, stability

Evaluation of their quality requires various control materials [1]. Undiluted samples of blood plasma, and defibrinated plasma obtained from patients with established clinical diagnoses or from healthy donors are usually used as raw materials in production of control materials (for internal and external supervision) for diagnostics of various pathologies. In this case inactivation of possibly present infectious agents becomes very important. It is conducted with a number of physical and/or physicochemical methods, and their efficiency may also be evaluated during the validation procedures, and the degree of activity of antibodies (or antigens) in control materials must lie within the range determining the diagnostic decision [1].

Control of biochemical and chemical components. Immunoassay kits have such biochemical and chemical components (besides basic antibodies and antigens): immunoenzyme conjugates, immunosorbents (plates), buffer solutions for different purposes (to dilute plasma or emmunoenzyme conjugate, to wash plates), solutions of enzyme substrate, chromogenic and the stop reagent.

We can formulate the following technological criteria that affect the diagnostic characteristics of conjugates. The conjugate quality arises from its components. In the case of antibody conjugates their qualitative parameters depend, on one hand, on specificity, sensitivity, affinity, stability and purity of antibodies, and on the other on activity, stability and purity of the enzyme. The parameters must be controlled at the stages of entry-point and internal control.

The quality of conjugate is directly influenced by manufacturing. In this case one should pay attention to the following "weak places". The reagents used for conjugation must at least influence the activity of antibodies and enzyme. It is also significant to control the presence of unbound molecules of immunoglobulins in synthesized conjugate, since their presence decreases sensitivity of the latter. Unbound enzymes in conjugates might undesirably enhance background noise [11].

Stability of biochemical and chemical components is supported inter alia by preventing microbial growth with antimicrobial preservatives. This is rational since the product is only used *in vitro*, and organization of additional aseptic or "cleaner" industrial conditions is not justified by non-aseptic usage. Efficiency of the relevant preservatives may be ratified during development. General outline of critical factors is given in Table 8.

Recent literature and national and international legislature on validation of biopharmaceutical production including serological diagnostics equipment were analyzed in present study. According to our results, current detailed protocols for validation of drug production processes (including those of biotechnological/biological origin) are not immediately applicable to medical devices for *in vitro* diagnostics. Specifics of the application and raw materials need individually developed validation parameters, and serological diagnostics devices need production validation. For every one of the typical steps of IVD we propose critical parameters that should be incorporated by drawing validation protocols.

In further research we will analyze and evaluate the specific demands of quality monitoring systems for production of serological diagnostics devices.

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ОСОБЛИВОСТІ ВАЛІДУВАННЯ ПРОЦЕСУ ВИРОБНИЦТВА МЕДИЧНИХ ВИРОБІВ ДЛЯ СЕРОЛОГІЧНОЇ ДІАГНОСТИКИ

О. Ю. Галкін[1, 2], *А. Г. Комар*[3], *М. О. Письменна*[1]

[1]Національний технічний університет України «Київський політехнічний інститут імені Ігоря Сікорського»
[2]ТОВ «ПРОФАРМА ПЛАНТ», Київ, Україна
[3]ДП «Український медичний центр сертифікації» МОЗ України, Київ

E-mail: alexfbt@gmail.com

Метою роботи був аналіз сучасної літератури, а також національних та міжнародних нормативних документів щодо валідації процесу виробництва біофармацевтичної продукції, зокрема засобів для серологічної діагностики. До найбільш вагомих керівних документів, які слід використовувати під час організації валідації технології засобів для серологічної діагностики, належать: Технічний регламент щодо медичних виробів для діагностики *in vitro*, ДСТУ EN ISO 13485:2015 «Медичні вироби. Система управління якістю. Вимоги щодо регулювання», ДСТУ EN ISO 14971:2015 «Вироби медичні. Настанови щодо управління ризиком», Настанова СТ-Н МОЗУ 42-4.0:2014 «Лікарські засоби. Належна виробнича практика», Державна фармакопея України та Настанова ICH Q9 щодо управління ризиками. Доведено, що наявні методичні рекомендації щодо валідації процесів виготовлення лікарських засобів, зокрема біотехнологічного походження, неможливо застосовувати безпосередньо для медичних виробів з метою діагностики *in vitro*. Показано, що специфіка сфери застосування та використовуваної сировини потребує індивідуального підходу до встановлення валідаційних показників та організації валідації технології засобів для серологічної діагностики. По кожному з типових етапів технології виготовлення медичних виробів для діагностики *in vitro* (на прикладі імуноензимних наборів, одержання протеїнів-антигенів, у т. ч. рекомбінантних, препаратів моно- та поліклональних антитіл, імуноензимних кон'югатів та імуносорбентів, хімічних реагентів) обґрунтовано критичні показники, які слід враховувати під час складання валідаційних планів. Проаналізовано «вузькі» місця технологій засобів для діагностики *in vitro* з позицій біоетики та біобезпеки.

Ключові слова: *in vitro* діагностика, серологічні методи, валідація, ризики, управління процесом контролю якістю.

ОСОБЕННОСТИ ВАЛИДАЦИИ ПРОЦЕССА ПРОИЗВОДСТВА МЕДИЦИНСКИХ ИЗДЕЛИЙ ДЛЯ СЕРОЛОГИЧЕСКОЙ ДИАГНОСТИКИ

А. Ю. Галкин[1, 2], *А. Г. Комар*[3], *М. А. Пысьменна*[1]

[1]Национальный технический университет Украины «Киевский политехнический институт имени Игоря Сикорского»
[2]ООО «ПРОФАРМА ПЛАНТ», Украина
[3]ДП «Украинский медицинский центр сертификации» МЗ Украины

E-mail: alexfbt@gmail.com

Целью работы был анализ современной литературы, а также национальных и международных нормативных документов по валидации процесса производства биофармацевтической продукции, в частности средств для серологической диагностики. К наиболее весомым руководящим документам, которые должны использоваться при организации валидации технологии средств для серологической диагностики, отнесены: Технический регламент о медицинских изделиях для диагностики *in vitro*, ДСТУ EN ISO 13485:2015 «Медицинские изделия. Система управления качеством. Требования по регулированию», ДСТУ EN ISO 14971:2015 «Изделия медицинские. Руководство по управлению риском», Руководство СТ-Н МЗУ 42-4.0:2014 «Лекарственные средства. Надлежащая производственная практика», Государственная фармакопея Украины и Руководство ICH Q9 по управлению рисками. Доказано, что имеющиеся методические рекомендации по валидации процессов изготовления лекарственных средств, в т. ч. биотехнологического происхождения, невозможно применять непосредственно для медицинских изделий с целью диагностики *in vitro*. Показано, что специфика сферы применения и используемого сырья требует индивидуального подхода к установлению валидационных показателей и организации валидации технологии средств серологической диагностики. По каждому из типовых этапов технологии изготовления медицинских изделий для диагностики *in vitro* (на примере иммуноэнзимных наборов, получения протеинов-антигенов, в т. ч. рекомбинантных, препаратов моно- и поликлональных антител, иммуноэнзимных конъюгатов и иммуносорбентов, химических реагентов) обоснованы критические показатели, которые следует учитывать при составлении валидационных планов. Проанализированы «узкие» места технологий средств диагностики *in vitro* с позиций биоэтики и биобезопасности.

Ключевые слова: *in vitro* диагностика, серологические методы, валидация, риски, управление процессом контроля качества.