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MODERN TECHNIQUES OF IMMUNOCHEMICAL ANALYSIS: INTEGRATION OF SENSITIVITY AND RAPIDITY

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The review covers history and development prospects of immunochemical analysis. Advantages and prospects of antibodies as detecting agent, modern requirements to immune-analytical methods and preconditions for two clusters formation (homogeneous relatively insensitive rapid assays and heterogeneous high sensitive and long duration assays), as well as the ways of improvement of analytical characteristics of these immunoassays are considered in detail. Forecast regarding most promising directions of immunochemical analysis, in particular, multiparametric analytical systems is made. Possibilities to develop universal immune-analytical systems, comprising high sensitivity of heterogeneous assays and detection rapidness of homogeneous assays (for example, immunoassays using polyelectrolytes or magnetic colloidal particles) are considered.

Potential and advantages of antibodies as bioanalytical receptors

In course of accumulation of knowledge about antigen-antibody interaction as highly affine and highly specific reaction an interest to antibodies as the means for the detection of antigens of various chemical nature was growing. The history of immunoassay started from immune precipitating methods, in which after extended incubations visually detectable insoluble antigen-antibody aggregates were formed. Important is the possibility to carry out the immunoprecipitation with the use of unfractionated antiserums, containing antibodies to a defining compound, and various samples without their pre-processing (except for very turbid mediums). Such procedural simplicity, the initial reason of which was the absence of tools for more sensitive detection of immune complex, provided high viability of this approach, applied till the moment for the assay of many proteins and determination of blood-group specificity [1]. Maximal reduction of the determination duration was not demanded from immune precipitation; obtaining of the results of clinical test in 1−2 days after sampling was perceived as a norm several decades ago. More continuing incubation was not observed as a disadvantage of the procedure, but as a definite guarantee of greater reliability and reproducibility of the results due to achievement of final (equilibrium) stage by the immune-precipitating processes.

An important, revolutionary progress within the development of immunoassay was the occurrence of analytical systems, in which one of implemented immune reagents was the complex with a marker, detected in extremely low concentrations. In the beginning of 1950-s such procedure was fulfilled for radio-active isotopic tags by Yalow and Berson [2], who were awarded for this development in 1977 with the Nobel Prize. A bit later the methods started developing with the use as the tags of enzymes, fluorophors, other compounds. New systems of detection were actively described, allowing to detect various compounds in the concentrations up to $10^{-10}-10^{-12}$ M in several hours. Transfer to non-isotopic tags excluded the need in special equipment and safety measures and thus called even more wide spread of immune-diagnostics. The main area of its implementation was and remains to be medicine, which greatly exceeds in the scopes of commercialization the ecological monitoring, control of quality and safety of food products and agricultural raw materials, other areas, in which immunoassay is also actively used.

In recent years many researchers has suggested to use in the analysis other receptor molecules as an alternative to antibodies. On the one hand, these are natural agents with similar recombination of structural elements of a molecule and possibility of obtaining of millions of its variants with different specificity [3−5]. On the other hand — synthetic compounds, in which a mark of an analyte is created artificially, providing the possibility of its selective detection [6] [7−10]. Nevertheless, antibodies remain to be and in the near future, probably shall remain to be the receptor, the most widely implemented for specific detection and quantification of content of the compounds of various nature [11]. To a large extent it is conditioned with a significant «allowance», which was obtained by antibodies as a result of their active use for decades. At the moment there are commercially available antibodies to dozens of thousands compounds of practical importance. The approaches were developed, which allow obtaining antibodies to the agents, which traditionally were not observed as inducers of immune response — ions of heavy metals [12−14], carbon nanoparticles [15, 16], vitamins [17], highly toxic compounds [18]. The technologies of obtaining of hybrids and display libraries allow to perform rapid screening and selection of antibodies with necessary specificity, and then producing the given antibodies in unlimited quantities [19, 20]. Affinity and specificity of antibodies can be purposefully changed with the use of methods of gene engineering, which lately have been intensively implemented for molecular design of antigen-binding sites of antibodies [21, 22]. The procedures of conjugation of antibodies with various markers were developed, combining high product output and high level of preservation of its functional properties [23].

Modern requirements to immune-analytical methods

However, although we may give a unique reply on the question of choosing of bio-receptor element, the situation with the choice of an analytical method is much more complicated. Now, as the labels in immunoassay (in commercial tests and in the developments recommended to the introduction into the practice) are used enzymes, fluorophores, liposomes, co-factors, various nanoparticles, etc. The analysis is performed in the volume of a solution, at the surface of polystyrene plates, electrodes, in flow-through cells, membrane pores, etc. Formation of immune complexes is registered by photometric, fluorometric, amperometric, potentiometric, gravimetric, magnetic detectors, etc. By this each of these «etc.» — are the dozens of more rare variants, representing nevertheless, the interest for the researchers.

What is the ground for such variety and is it needed? Should we choose one, the best at the moment method, and use exactly it for the determination of various antigens and for the solution of various practical tasks? As such solution shall simplify demands to equipment and reagents, skills of specialists, interpretation of obtained outcomes. Unfortunately or luckily, but such unification appears to be possible. Immune-chemical methods are used for solution of various tasks, differing in practical requirements. On the one hand, these are tests, performed in specialized, stationary laboratories, from which it is expected the maximum sensitivity and reliability, but is pretty acceptable the range of several hours between sampling and obtaining of the assay results. On the other hand, in a significant number of cases it is required rapid, for minutes, obtaining of the information directly at the site of sampling (at the site of the primary screening medical examination, by attending of a doctor of the patient in house, in case of self-control of the patient, at examination of natural and industrial objects). These two niches of immunoassay at the moment are successfully filled with various analytical methods.

The task of reliable highly sensitive diagnostics is solved by enzyme-linked immunosorbent assay, immune-fluorescent assay, radio-immune assay — heterogeneous methods, based on continuing (dozens of minutes or hours) incubations of reagents till achievement by immunochemical reaction of equilibrium condition, separation of bound and nonbound components and high-sensitive quantitative registration of corresponding markers in the set of immune complexes.

An alternative approach is performance of homogenous immunochemical reactions, achieving equilibrium in minutes, and direct detection on this or that parameters of content in a reaction medium of created immune complexes. To this group we should assign immune-analytical systems, based on modulations of activities of an enzyme marker [24], changing of polarization of a fluorescent marker [25], registration of immunochemical complexes on changing of optical properties of the medium (nephelometry) [26], etc. Due to the absence of the stage of reagents separation

and corresponding influence on registered signal of components of samples (so named matrix effect) and non-reacted immunoreagents, and as well as due to relatively low sensitivity (in comparison with the registration of markers on heterogeneous immunoassays) the given analytical methods are generally characterized by sufficiently higher limits of detection. Initially they were also developed as the methods for stationary laboratories, but the development of technologies and miniaturization of the blocks of data processing allowed suggesting the number of portable mobile systems, suitable for field tests (first of all — for the registration of polarization of fluorescence) [27].

To the test-systems for rapid screening with low sensitivity it is worth to assign also membrane immunochromatographic test-systems (test-strips) [28–30], in which rapidness of detection is assured by the use of homogenous (in the volume of flowing on test-strip fluid) and rapid heterogeneous (in micro-volumes of membrane pores) interactions, and immunoreagents are conjugated with coloured ultradisperse markers (colloidal gold, latexes, etc.) for rapid direct detection [31, 32].

So, the modern situation in immune diagnostics is characterized by some established «division of duties» between relatively long (hours) analytical methods with low limit of detection and rapid (minutes) methods, defining sufficiently higher concentrations of target compound, and in the number of cases — representing only qualitative information that its content in the sample is higher than a definite limit. Each of these classes of methods is well adapted for its special tasks, and its practical use is supported by developed technologies on production of corresponding test-systems, presence of serial equipment for assaying and final measurements, informing and professional training of specialists — users of the developed test-systems.

Nevertheless, the established situation should not be considered as a final optimal solution. The wish to combine the benefits of the given approaches and to suggest immune-analytical systems, comprising high sensitivity and rapidness of detection is natural. In the present review we shall observe the developments held in this area in recent years. The figure 1 summarizes the existing variety of approaches, targeted at improvement of the characteristics of immunoanalytical systems.

Methods of reduction of detection limit in immunoassays

Achievement of a low limit of detection of immunoassay demands the combination of high-affine interaction of antibodies with corresponding antigens and the potential of marker detection (or a parameter, accompa-
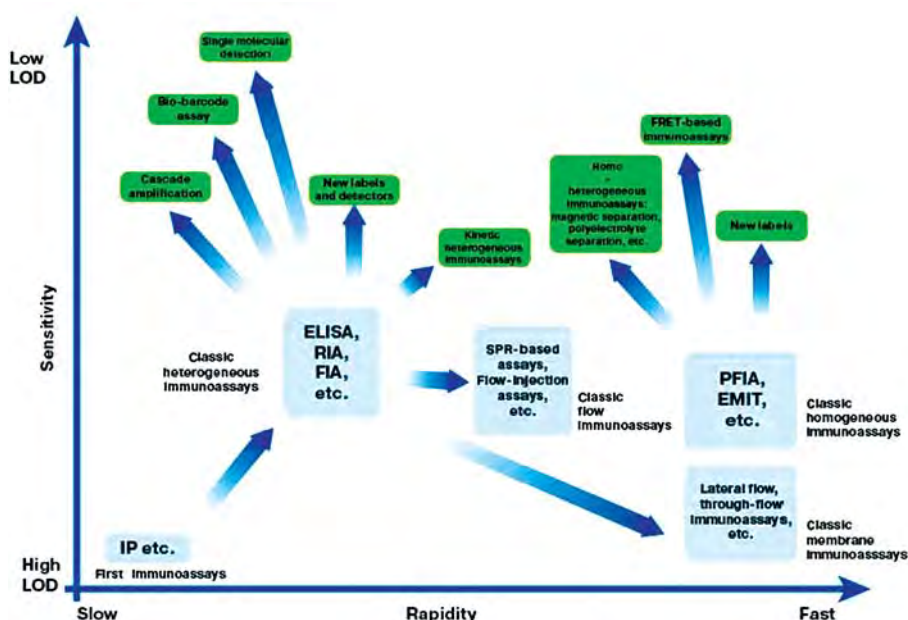

Fig. 1. **Variety and evolution of immunoassays.**
Abbreviations: IP — immunoprecipitation,
ELISA — enzyme linked immunosorbent assay, RIA — radioimmunoassay, FIA — fluorescent immunoassay,
PFIA — polarization fluorescence immunoassay, EMIT — enzyme multiplied immunotechnique,
SPR — surface Plasmon resonance

nying formation of the immune complex) in extremely low concentrations.

In 1986 Jackson and Ekins [33] held theoretic comparison of non-competitive and competitive immunoassays in the notions of sensitivity, precision, kinetics and working range of analyte. They defined theoretic limits of detection of these methods, their connection with the characteristics of antibodies.

It's worth to note, that the peculiarity of dependence between the binding constant of immunochemical reaction and the limit of detection of the target antigen significantly depends on the accomplished format of the immunoassay — non-competitive or competitive. In the first case the antigen is detected directly in the process of immune complex formation, the way it is happening, for example, in a sandwich format of the analysis with formation of complexes antibody — antigen — labelled antigen. In the second case a competition between the antigen in a sample and the second antigenic agent for binding with antibodies is registered. For non-competitive analysis it is potentially possible to detect extremely small concentrations of antigen, if it is allowed by the sensitivity of marker detection or direct detection of immune complex, as well as low background signal. There is a number of works, describing the possibility to detect a single antigen molecule with these sources of signal strengthening [34, 35].

A significant role is played by the optimization of the ratio of reagents used. It should assure, on the one hand, a minimal limit of detection, and on the other hand — maximum reliability and (for quantitative methods) precision of analysis. These requirements cannot be performed simultaneously and demand definite compromise solutions. For the formats of analyses with direct dependence between the concentration of antigen and the number of detected markers (such as a sandwich scheme) increase of concentration of immune reagents enhances the number of detected complexes. However this increase may be accompanied by the growth of non-specific (background) binding of markers. For competitive analysis with reverse dependence between the concentration of analyte and the number of detected markers the reduction of the limit of detection is assured by low concentrations of immunoreagents [36], but it is accompanied by low amplitude of signal and low precision of determination [37].

Despite doubtless importance of affinity of immune interaction for high sensitive assay, it should not be suggested that its growth to infinity shall assure unlimited lowering the detection limit. In this case the issue about minimal detected concentration of a marker becomes critical. Nevertheless, for modern practice the choice of the most affine antibodies from the variety of available ones allows reducing significantly the detection limit, achieving in the number of cases subnanogram levels [38].

Traditionally immunologists note the existence of natural limits on affinity of complex-formation of antibodies with antigens, defined by the nature of induction of immune response. This is connected with the fact that for antibodies with the kinetic constant of dissociation of the order 10^{-4} sec^{-1} and less the time of half-life of the complex of antigen with B-cell receptor becomes greater, than the time of endocytosis of this complex. Respectfully, further increase of the time of half-life of the complex already does not assist B-cell proliferation [39]. Maximum value of the kinetic constant of association is defined by the speed of diffusion of immunoreagents in the solution. In this respect for the antibodies of IgG class, specific to protein antigens, the equilibrium constant of association usually do not exceed 10^{10} M^{-1} [40]. However, these limitations do not exclude the possibility of that, a significantly higher level of complementarity with antigen-binding site of antibodies, and correspondently, a higher constant value of binding is achieved for some antigens. There is also a number of works describing non-dissociating complexes of antigen-antibody with infinite affinity [9, 10]. Another way to increase the affinity of immune interaction, successfully fulfilled in the number of recent developments, is the targeted design of antigen-binding parts of recombinant antibodies [41−43].

So, in a non-competitive analysis the use of high sensitive ways of registration of immune complexes or bound markers can potentially lead to significant lowering the detection limit (fig. 2). For example, the systems of cascade amplification of signal in immunoassay are developed actively, in which a marker bound with immune complex marker after separation of the reaction mixture components acts as the inducer of formation of a big number of molecules, which are detected at the final stage of the test. (The simple variant of such cascade is a traditional ELISA, which detects not molecule of enzyme bound with the antigen-antibody complex as it is, but the products of catalyzed reaction). Although theoretically such cascade or several cascades can significantly reduce the detection limit, the restrictor for
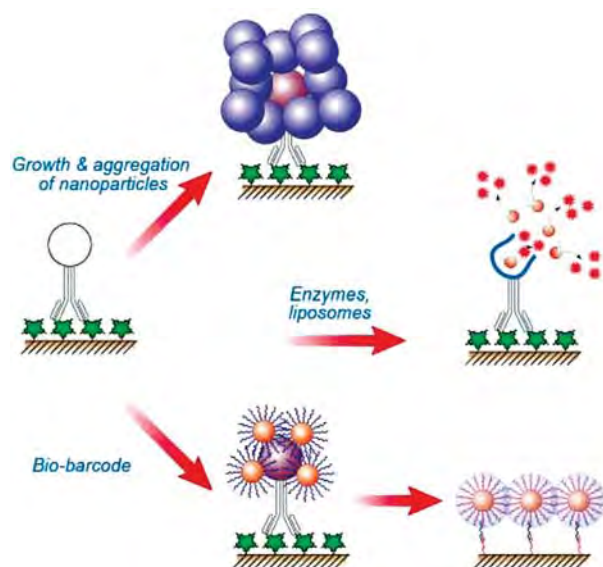
Fig. 2. **Appoaches for signal amplification in immunoassays**

this approach is non-specific binding, resulting in increase of background signal. Nevertheless, there is a number of developments in which strengthening of the signal assures detection of single molecules of antigen. For example, to reach this target can be used the formation of micro-dispersive colloidal complexes on the ground of a single molecule of a marker [44].

Highly sensitive assays may be realized also by PCR amplification of target nucleotide sequences [45−47]. The given nucleic acid lateral flow immunoassay allow to detect a few bacterial cells in grams of tested samples [45].

Evidently, that the limit of detection shall always be worse than the theoretically possible one, as not only registration of some signal is necessary, but also confirmation of difference of its level from non-specific interaction in the analytical system.

The ways of improvement of analytical characteristics of heterogeneous immunoassays

First of all, the sensitive heterogeneous immunoassay does not necessarily demand incubation of reagents till achievement of chemical equilibrium [48]. The simplest solution, which does not demand the changes of reagent bases and the means of detection is the reduction of duration of the assay stages. Traditionally recommended prolonged stages of ELISA (an hour and more for each immune stage) improve first of all reproducibility and precision of the assay. In the number of cases

it is demonstrated, that incubation duration can be reduced to 10−15 minutes with not very big reduction of amplitude of detected signal and almost without alteration of the assay operating range [49, 50]. In accomplishment of kinetic assay its reproducibility starts to play important role, demanding, for example, strict match of the times of incubation for all samples of tested series. However, modern means of automation [51], used in the number of immune enzyme analyzers, allow fulfilling this task. Regarding heterogeneous immunoassays it should be considered that the reason for their extended duration is not heterogeneity of interaction (between reagents presenting in the solution and immobilized at the surface of carrier), but slow diffusion-controlled processes of establishing of equilibrium between the layers of fluid, locating at different distances from a carrier. Considering this, promising are the developments, in which this diffusion exchange is significantly accelerated and reagent medium is structured in the way that time losses due to diffusion become insignificant.

For mixing of pre-surface layers and the total volume of fluid ultrasound processing recommended itself well for the number of test-systems demonstrating 2−5-fold reduction of the analysis duration [52].

An effective solution of the problem of diffusion limitations is transfer of interactions in microvolumes, which is possible with the use of highly sensitive systems of detections of markers (for example, modern fluorescent detectors) [53−55]. Such miniaturization is used in many developed immunochips. Immunoassay at a chip on sequence of stages matches classic solid-phase immunoassay. The principle difference is that antibodies monolayer is immobilized at the surface of silicon, quartz or polymeric materials (Teflon, polycarbonate) [56] of about 100 μm^2 in area. To compare — the area of only a bottom of the well of a standard 96-well plate for ELISA comprises $3 \cdot 10^7$ μm^2, i.e. 300 thousand times greater ([36]. Currently immunochips are usually used for multiparametric tests, in the frameworks of which small areas of binding are incubated till achievement of equilibrium with the total sample volume. In this case achieved immunochemical reaction remains to be diffusion-controlled, and performance of the test requires several hours [57]. Incubation of reagents directly in the areas of binding by the formation of thin layers of fluid allows sufficient reduction of time for formation of detected complexes. As a result

the duration of the assay can comprise 10–15 minutes, corresponding modern demands to express analysis [58, 59].

Significant limitation for the introduction into practice of immunochip technology yet 5–10 years has been the involvement of complicated and expensive optical equipment — confocal microscope, CCD-camera, etc — for highly sensitive detection of a tagged compound in the quantities, which can bind at a small area of a chip. At the moment to solve these tasks relatively cheap portable devices have been invented, which shall assist more rapid introduction of immunochips into clinical practice [58, 60].

Reduction of duration of heterogeneous immunoassay is also reached by its transfer into the flow mode with the use of cells of small diameter. Flow-injection analysis, suggested by Ruzicka and Hansen [61], is grounded on automatic injection of fixed volume of a sample into continuous flow of a buffer solution. Carriers (sorbents) with immobilized antibodies are used to separate detected specific complexes in flow-injection immunoassay, and a detector registers product of enzymatic reaction after binding of enzyme-marker with the sorbent. The use of flow-injection systems for immunoassay incites the transfer of immunochemical and enzymatic reactions into kinetic mode, reducing the contact time to several minutes and respectively reducing the duration of the assay. The necessary conditions for obtaining of reproducible data in kinetic mode is stability of such parameters as the contact time of reagents, temperature and the volume of analyzed sample. In this respect for flow-injection immunoassay principal significance has the availability of serially produced devices for automatic performance of all injection manipulations.

Membrane immunochromatographic assay, the brief specification of which was presented in the section 2, can also be qualified as a variant of flow heterogeneous immunoassay. In a classical variant the main limitation of immunochromatography is low sensitivity due to the use of colloidal dyed particles as directly detected markers. In recent years a number of developments have been suggested, which overcome this limitation and accomplish amplification of detected signal (see section 3), or transfer to alternative markers, revealed in lower concentrations [32, 62]. Thus the number of works depicts the option of more sensitive detection in the membrane assay of colloidal semiconducting fluorescent markers [63, 64]. Significant reduction of detection limit can be achieved due to transfer from optical detection of coloured markers to the registration of their other physical properties, for example, electrical and magnetic. Thus, highly-sensitive immunochromatographic determination of troponin with the use of magnetic nanoparticles is described in [65]. Several immunochromatographic systems with electrochemical registration of markers are suggested [66–68], confirming the potential of this approach. The Table summarize several example of the application of different labels in rapid tests.

Priority labels for rapid immunoassays

Label	Example(s) of application
Spheric gold nanoparticles	[97, 98]
Other gold nanoparticles	[99, 100]
Liposomes	[101, 102]
Gold nanoparticles + marnetic particles	[103]
Different nanoparticles	[104–106]
Fluorescent dyes	[107, 108]
Marnatic particles	[109, 110]
Quantum dots	[64, 111, 112]
Lanthanides	[113, 114]
Latex particles	[115]
Upconverting phosphor	[116, 117]
Eu(III) chelate microparticles	[118, 119]

Homo+heterogeneous immunoanalytical systems

Considering benefits and disadvantages of homogenous and heterogeneous immunoanalytical methods it is considered advisable to combine in one scheme rapid formation of immune complexes in the solution and the efficacy of their detection in heterogeneous systems. A promising approach, assuring achievement of this target is implementation of polyelectrolytes in immunoassay. Polyelectrolyte separation in immunoassay can be performed on the ground of:
• Interaction of counterions (polycation-polyanion) pair [69, 70];
• Interaction of polyelectrolyte-ion of metal pair [71];
• Monomer polymerization [72–76].

The first variant of separation was performed with the use as counterions of linear water soluble polyelectrolytes: polyanion — polymethacrylic acid, polycation — poly-N-ethyl-4-vinylpyridinia or poly-N-N′-diethyl dyallialammonium. These polymers interact

with each other in a wide range of conditions almost immediately forming an insoluble complex. Due to this, having obtained a conjugate of one of polyelectrolytes with an immunoreagent, we may perform detection of analyzed antigen in solution as in case of usual rapid homogenous methods, as then, with the addition of counterion into the system, to separate rapidly formed immune complexes (Fig. 3).
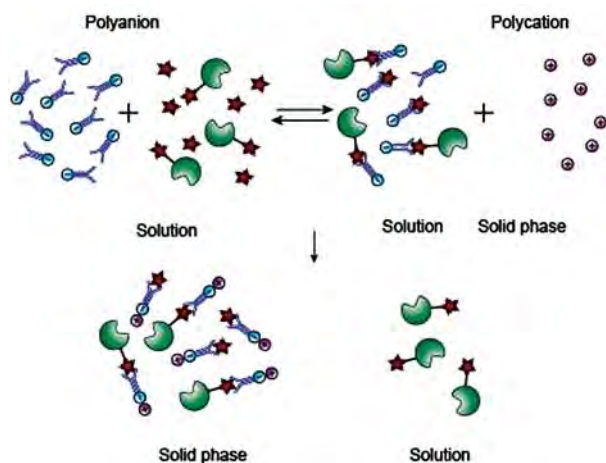

Fig. 3. **Principle of polyelectrolyte separation in immunoassay**

Performed comparison of traditional solid-phase ELISA and ELISA on the basis of poly-electrolytes for detection of such compounds as insulin, testosterone, immunoglobulins, hepatitis B surface antigen demonstrated that the use of polymeric carriers allows reducing the time of analysis from 2−3 hours to 15−30 minutes without losses in sensitivity and specificity [77].

The second variant of separation implements the polyelectrolyte-ion of metal pair. The procedure, suggested by Auditore-Hargreaves K., is based on the use of polymeric carriers, solubility of which depends from the presence of cations [72]. Water-soluble polymers can be precipitated, for example, with the reduction of pH or adding of such ions as Ca^{2+}, and repeatedly transferred into the solution — by the increase of pH and adding of chelating agents (ion-citrate, ethylendiaminetetraacetic acid, etc). Developed is homogeneous immunoassay on the basis of algic acid, which assures 30 minutes identification of antigens, molecular weight of which is within the range from 100 to 50000 Da [72].

The third variant, suggested by Hoffman et al, is grounded on the generation of a solid polymeric phase from soluble monomer. On the basis of this principle systems of immunoglobulins detection with free-radical and temperature initiation of polymerization are developed. The first system (polymerization *de novo*) is grounded on the use of two types of antibodies conjugates: antibodies(1)-fluorophor and antibodies(2)-organic monomer [73−75, 78]. After completion of immunochemical reaction the reaction of polymerization is initiated by free radicals. In the results of polymerization insoluble polymeric particles are formed, content of the marker in which is proportionate to the quantity of antigen in the sample. The second system is based on implementation of temperature-dependent polymers [73, 79]. Here, the same as by polymerization *de novo*, two types of conjugated antibodies are used. Formation of specific immune complexes happens at the temperature lower the critical one for the given polymer, and increase of temperature allows separating the complex from the solution, by this the number of marked antibodies, included into precipitate, is proportionate to the content of antigen in the sample. The most promising for this immunoassay are polymers and co-polymers of acrylamide with N-alkyalacrylamide.

Poly-N-isopropylacrylamide and its co-polymers, except for thermal precipitation, can be precipitated by 14−20% ammonium sulfate. Free IgG at do not precipitate such concentrations of salt. So this method allows separating free immunoglobulins from conjugated with polymer ones.

On the ground of this principle there immunoanalytical methods with the use of enzymatic and fluorescent markers were developed. They demonstrated efficiency for the detection of immunoglobulin G, hepatitis B surface antigen, *Chlamidia trachromatis* etc. [73, 80].

Together with interpolyelectrolyte interaction, an efficient solution, combining all benefits of homogeneous and heterogeneous immunoassays, is the implementation of magnetic immune sorbents [81−83]. The use of these particles as a solid phase in immunoassay gives the possibility to increase significantly the area of surface to immobilize reagents, distribute them equally along the total volume of the reaction medium, hence accelerating heterogeneous interactions. By this after completion of the first stage of the assay implementation of outside magnetic field assures simple and rapid separation of reagents. The use of magnetic colloidal particles (MNP) as a solid phase in ELISA allows improving its analytical characteristics. There is a number of works on MNP implementation

in ELISA for the detection of compounds of various nature — pesticides, hormones, mycotoxins, allergens, proteins, viruses, bacteria [84–87]. By this the duration of specific interactions can be reduced to 5–10 minutes, and the analysis in general up to 20–30 minutes. It is important also that magnetic separation allows performing pre-concentration of target compound from a big sample volume. This concentration allows additional reduction of the detection limit of the target compound 1–2 orders [87].

Ordinary centrifuging can be also used to separate immunosorbent from the reaction medium. This approach is accomplished in the number of test-systems with the use of antibodies, immobilized at the particles of high-disperse latex, usually — polystyrene (which allows using standard protocols of absorption immobilization being developed for ELISA) [88].

Homogeneous immunoanalytical systems

Implementation of homogeneous methods of immunoassays is significantly limited with the influence of sample components on registered signal, due to which it is complicated to distinguish this influence from specific complex formation. Pretty solution of this problem is the use of differential measurements in the assay, when results of measuring with specific immune reagents and with antibodies with the compound, knowingly not present in the test sample, are compared [89].

Nevertheless, solutions with minimal influence of matrix on the result of the assay are preferable — despite the absence of the separation of reaction mixture components before measurements at homogeneous assay. In this respect significant interest is drawn by new markers and new ways of immune complexes registration. Thus, the application of lanthanides complexes as markers allow to reduce impact of background signal due to possibility of prolonged registration and integration of fluorescence [90, 91]. An alternate approach is the use of long wavelength (600–1000 nm) fluorescence that also reduces background impact from sample constituents [92–94].

One more promising approach is the use of the signal, generated by close spatial approach of interacting immune reagents. For example, a number of immunoassay systems was described, grounded on the effect of fluores-
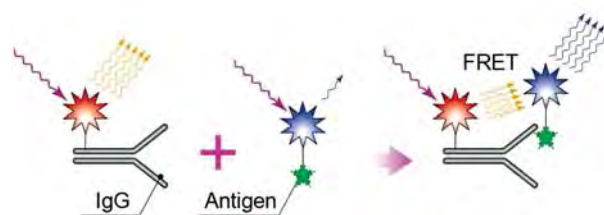

Fig. 4. **Principle of FRET-based immunoassay**

cence resonance energy transfer (FRET) [95, 96], occurring by the approach of two markers, conjugated with antibody and antigen (Fig. 4).

Conclusion and Prospects

As the presented brief analysis shows, the developments, targeted at increase of analytical characteristics of immunochemical test-systems are extremely diverse. The efforts of the researchers are not concentrated on acceleration of sensitivity or increase of sensitivity of rapid tests, but suggest various solutions within the field of options, depicted at the fig.1. In this respect it is complicated to make exact forecast regarding more promising directions of immunoassay. However, it should be expected that the principle of multilevel diagnostics, including preliminary screening and further confirming test shall preserve in near future. Due it can be also expected the preservation of two clusters — more rapid and more sensitive analytical methods. However, the introduction into practice of developments, related with analytical markers, new methods of assay performance and registration of immune complexes shall lead to substitution of conventional methods with new ones. Modern means of registration of ultra-small signals and data processing allows effective control over the content of compounds in samples in extreme low concentrations. By this, diagnostic decisions shall be made considering information about content of a big number of diagnostically significant compounds, which shall results into development of multiparametric analytical systems, as well as information about peculiarities of patient's metabolism, considered in diagnostic data bases within the frameworks of the shift to personified medicine. Simplification and increase of sensitivity of test-systems for field assay shall results into their active implementation in non-medical spheres.

101

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СУЧАСНІ МЕТОДИ ІМУНОХІМІЧНОГО АНАЛІЗУ: ПОЄДНАННЯ ЧУТЛИВОСТІ ТА ШВИДКОСТІ

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Огляд присвячено історії та перспективам розвитку методів імунохімічного аналізу. Докладно висвітлено переваги і перспективи використання антитіл як детектуючого агента, сучасні вимоги до методів імуноаналізу та передумови для формування двох груп методів (гомогенних експрес-методів з відносно невисокою чутливістю і гетерогенних високочутливих з більшою тривалістю поставлення), а також можливості поліпшення аналітичних характеристик цих методів. Обговорено прогнози найбільш перспективних напрямів подальшого розвитку методів імунохімічного аналізу, зокрема мультипараметричних аналітичних систем. Розглянуто можливість створення універсальних підходів імуноаналізу, що поєднують високу чутливість гетерогенних і швидкість поставлення гомогенних методів (наприклад, на основі поліелектролітів або магнітних колоїдних частинок).

Ключові слова: імунохімічний аналіз, гомогенні та гетерогенні методи.

СОВРЕМЕННЫЕ МЕТОДЫ ИММУНОХИМИЧЕСКОГО АНАЛИЗА: СОЧЕТАНИЕ ЧУВСТВИТЕЛЬНОСТИ И СКОРОСТИ

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Обзор посвящен истории и перспективам развития методов иммунохимического анализа. Детально рассмотрены преимущества и перспективы использования антител в качестве детектирующего агента, современные требования к методам иммуноанализа и предпосылки для формирования двух групп методов (гомогенных экспресс-методов с относительно невысокой чувствительностью и гетерогенных высокочувствительных с большей длительностью постановки), а также возможности улучшения аналитических характеристик этих методов. Обсуждены прогнозы наиболее перспективных направлений дальнейшего развития методов иммунохимического анализа, в частности мультипараметрических аналитических систем. Рассматривается возможность создания универсальных подходов иммуноанализа, сочетающих высокую чувствительность гетерогенных и быстроту постановки гомогенных методов (например, на основе полиэлектролитов или магнитных коллоидных частиц).

Ключевые слова: иммунохимический анализ, гомогенные и гетерогенные методы.