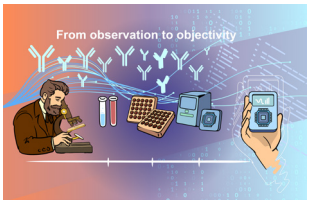
Review, Communication

MAIN STAGES OF IMMUNOASSAY METHODS EVOLUTIONARY DEVELOPMENT

M. B. Rayev*



Laboratory of Cellular Immunology
and Nanobiotechnology, «Institute of Ecology
and Genetics of Microorganisms
of the Russian Academy of Sciences» — branch
of the Perm Federal Research Center of the Ural Branch
of the Russian Academy of Sciences, Ural Branch
of the Russian Academy of Sciences,
614081, Perm, Russia

* Correspondence: mraev@iegm.ru

To cite this article:

Rayev M. B. Main stages of immunoassay methods evolutionary development. *Advanced targets in Biomedicine*. 2025;1(2):7—30. https://doi.org/10.5922/ATB-2025-1-2-1

Received
29.04.2025
Revised
12.06.2025
Accepted
01.09.2025
Published
08.12.2025

Abstract: The evolutionary pathway of diagnostic testing methods can be traced through the historical development and refinement of analytical tools. These tools are designed to identify sources, decipher mechanisms, and control processes inseparable from functional and morphological organization of living organisms under both normal and pathological conditions. In essence, this pathway represents a shift from subjective observation to objective formalization. The importance of this transition has been long recognized and is difficult to overestimate, as illustrated by the consequences of reckless human interference with nature. Furthermore, analysis of the global diagnostic systems market indicates significant growth: valued at USD 14.51 billion in 2021, it is forecast to reach USD 25.88 billion by 2029 (Data Bridge Market Research). The diversity of approaches to developing immunodiagnostic methods, as well as to biomedical analytics in general, is vast. Researchers continue to generate new methodologies. Accordingly, this review analyzes the evolutionary pathways of immunoassay techniques and tools that are actively applied in both laboratory and point-of-care testing.

Keywords: antibody, antigen, serological test, agglutination, immunoassay, affinity, enzyme-linked immunosorbent assay

Обзор

ЭВОЛЮЦИОННЫЙ ПУТЬ ИММУНОДИАГНОСТИЧЕСКИХ СИСТЕМ

М. Б. Раев*

Лаборатория клеточной иммунологии и нанобиотехнологии, «Институт экологии и генетики микроорганизмов, Уральское отделение Российской академии наук» — филиал Пермского федерального исследовательского центра, Уральское отделение Российской академии наук, 614081, Россия, Пермь

* Автор-корреспондент: mraev@iegm.ru

Резюме: Эволюционный путь развития методов диагностического тестирования по существу сводится к истории, связанной с разработкой и совершенствованием инструментов, позволяющих выявить источники, расшифровать механизмы, проконтролировать течение процессов, неотделимых от функционально-морфологической организации и/или состояния живых организмов, причем не только в патологии, но и в норме. По сути это путь от субъективного наблюдения к объективной формализации. Пути и значимость совершенствования последней долго перечислять и трудно переоценить. Достаточно обратить внимание на последствия недальновидно реализованного вмешательства человека в природу и на все, что с этим связано. Кроме того, о многом говорит анализ роста мирового рынка диагностических систем, который фиксирует 14,51 млрд долл. США в 2021 г. и его прогнозируемый рост к 2029 г. — 25,88 млрд долл. США (Data Bridge Market Research). В силу значительного количества методов диагностичекого тестирования в представленном обзоре при описании эволюционного пути способов и инструментов иммуноанализа обращают на себя внимание лишь те, которые задействованы в реальной практике лабораторного и внелабораторного тестирования.

Кючевые слова: антитела, антигены, серодиагностика, иммуноанализа, гглютинацияиммуно-ферментный анализ, аффинность

Для цитирования:

Раев М. Б. Эволюционный путь иммунодиагностических систем. *Современные направления в биомедицине.* 2025;1(2):7—30. https://doi.org/10.5922/ATB-2025-1-2-1

Поступила
29.04.2025 г.
Прошла рецензирование
12.06.2025 г.
Принята к печати
01.09.2025 г.
Опубликована
08.12.2025 г.

Applications of analytical tools range from the diagnosis of plant and animal diseases and the monitoring of human health (including both pathological processes and somatic conditions), to the testing of biological specimens in field and in laboratory settings, to the study of natural disasters and the consequences of active human interference with the environment (wars, ecological disasters, etc.). These tools underpin the methodologies used to objectively evaluate phenomena associated with living organisms.

Immune-analytical systems currently dominate global diagnostic sales. At the same time, growing attention is being paid to nucleic acid fragment detection tests, flow cytometry (which enables simultaneous assessment of a broad range of indicator factors), and various systems suitable for in situ analysis. As diagnostic equipment develops and becomes increasingly complex, there is a need for more research into improving the stability and shelf life of analysis reagents. This is particularly important under diverse environmental conditions, including variations in temperature and humidity. Equally important is minimizing reagent consumption per test, a requirement that extends beyond instrumentally arranged systems [1].

Every story has its beginning

When, in 1890, von Behring and Kitasato obtained antiserum against diphtheria and tetanus toxins and demonstrated its neutralizing properties, few could have anticipated that this would mark the dawn of the serological research era. Their work paved the way to systematic studies of antibody properties and their role in bacterial infections. The diagnostic value of antibody detection was soon confirmed for diseases such as typhoid fever [2] and syphilis [3]. Yet the use of antibodies—originally identified as a key defense mechanism against infection—extended beyond infectious disease diagnosis.

Specificity is a defining feature of antigen-antibody interaction. This became the foundation not only for studying microorganism cell antigens but also for detecting a wide range of molecules, including those with a molecular weight above 5000 Da. It has been demonstrated that smaller molecules (haptens) cannot, on their own, induce the production of specific antibodies. However, when conjugated to a larger carrier (e. g., bovine serum albumin), they can elicit antibody production with the required specificity [4].

In 1930, Karl Landsteiner received the Nobel Prize for his discovery of human blood groups identification, work that began in 1900 when he first described the agglutination of red blood cells in one person caused by the serum of another individual. Immunoassay, as an empirical method for antigen study, rested on two fundamental insights: the specificity of antibodies, and the quantitative character of antibody-antigen interactions. It was demonstrated [5] that the ability of antiserum to precipitate soluble antigens represents a quantitative reaction of antibody-antigen interaction. This was demonstrated using Vibrio cholerae culture medium, described in the late 19th century [6].

The basis of analysis is specificity! But what is the basis of specificity?

In his pioneering paper on the quantitative evaluation of diphtheria antiserum, Paul Ehrlich formulated fundamental principles of antibody formation and specificity [7]. His work laid the foundation for immunochemistry development, and outlined approaches to studying quantitative antigen-antibody interactions. Later, Heidelberger introduced methods for quantitively evaluating precipitation. He also became the first to obtain a purified antibody product.

The polyvalent specificity of antisera results in antigen precipitation, which became the foundation for modern immunoassay methods. Antibodies in antiserum bind to multiple antigenic determinants on complex antigens, forming a three-dimensional precipitate lattice that sediments when optimal antigen-antibody concentration ratios are reached. Although this principle was established in theory [8], it could not be applied in practice even in simple immunoassay methods with single-antigen systems. This limitation was overcome when researchers showed that diffusion of antibodies and antigens in the liquid phase across the surface of adjoining agar gels could be used to evaluate their relative concentrations by the location of precipitation bands [9]. This breakthrough led to the development of the double-immunodiffusion assay in gel, which, with minor modifications, remains widely used today [10; 11]. For the first time, this method enabled the characterization of antigenic interaction between molecules and the assessment of antiserum specificity.

Subsequent innovations led to the development of immunoelectrophoresis technology [12], followed by quantitative radial immunodiffusion assays [13]. The discovery of antigen electrophoretic migration in gels in complex with antibody precipitation formed the basis for the development of rocket immunoelectrophoresis method [14], which allowed rapid quantification of individual antigens in mixtures using monospecific antibodies incorporated into gels. A further modification, two-dimensional (crossed) immunoelectrophoresis, made it possible to perform both qualitative and quantitative analysis of complex antigen mixtures using polyspecific antisera.

Gel precipitation methods played a significant role in antigen analysis and quantification, and were widely used to assess antibody specificity and determine precipitating antibody titers. However, their sensitivity rarely exceeded 5 μg/ml, and they could not be applied to quantify low molecular weight antigens that formed only soluble complexes. These limitations spurred the development of alternative approaches to antigen quantification.

In 1945, Coombs and colleagues introduced an assay [15] first described by C. Moreschi in 1908, in which antihuman immunoglobulin antiserum induced the agglutination of erythrocytes (EA) coated with the corresponding immunoglobulins. This reaction, later known as the Coombs antiglobulin test, became an important clinical tool. Additionally, Coombs was the first to use erythrocytes as an indicator system in immunoassays, paving the way for the creation of numerous erythrocyte-based diagnostic methods [16; 17].

Erythrocytes can bind not only antibodies specific to their membrane antigens (direct tests) but also antibodies targeting antigen-coated erythrocytes (indirect or passive tests). This principle gave rise to additional methods, such as the antigen-coated erythrocyte agglutination assay [18—23]. In both direct and indirect quantitative hemagglutination tests, an erythrocytes suspension is added to serial dilutions of antiserum. If a given dilution contains enough agglutinating antibodies to cross-link surface antigens on neighboring erythrocytes, the cells clump together, or agglutinate; otherwise, they settle to the bottom of the wells in a microtiter plate, forming a compact plaque. The titration endpoint, or serum titer, is defined as the highest dilution that still causes erythrocyte agglutination. For their time, hemagglutination tests represented a major breakthrough in immunodiagnostics, offering high sensitivity, specificity, and technical simplicity.

Further progress came in the late 1970s and early 1980s with the development of the erythroimmunoadsorption method (EIA) [24—26]. This approach relied on a simple detection system where erythrocytes served as a visual indicator, functioning as optically dense corpuscular labels. The key event in EIA was the affinity binding of marker erythrocytes on the walls of U- or V-shaped wells in the immunosorbent carrier, to specifically bound target ligands (antigens or antibodies). While the method offered a convenient one-step detection scheme, assay's simplicity, cost-effectiveness

and accessibility of the corpuscular label, these strengths were offset by low sensitivity, lack of responsiveness and the instability of erythrocyte markers. These shortcomings were largely overcome by the works of Plaksin, who developed a technology for producing stable erythrocyte-based diagnostics and designed an original ultramicro EIA method with superior analytical and procedural performance. This method was successfully applied in the diagnosis of a wide range of diseases.

Tools: replace or supplement?

The development of immune-analytical tests proceeded in two main directions. The first involved the integration of detection instrumentation into immunoassays. While this made diagnostic efficiency and outcomes dependent on advancements in optics, physics, mechanics, electronics, and related fields, it also allowed results to be quantitively formalized. The latter greatly improved accuracy, reproducibility and sensitivity by reducing much of the subjectivity in result interpretation.

The second direction focused on enhancement methods based on the principles and best practices of hemagglutination and erythroimmunoadsorption. Specifically, it involved developing and optimizing instrument-free analytical systems with direct visualization of stereospecific interactions.

The pioneering work of Berson and Yalow was the beginning of the widespread use of radioisotopic labeling in immunoassays. They demonstrated the extraordinary sensitivity of detecting trace amounts of radioiodine-labeled insulin through its binding to specific antibodies. This achievement led to radioimmunoassay (RIA) being used to measure very low concentrations of serum insulin, which quantitatively competed with a radioactive hormone for corresponding antibody binding [27]. The principle of competitive inhibition with standardized radioactive antigens soon became a universal strategy for liquid-phase RIA, widely used to quantify hormones, pharmaceutical drugs, and other low-molecular-weight ligands.

RIA methods evolved along two main lines. On the one hand, there were improvements in labeling techniques and expansion of anti-ligand types that could be modified with radioactive isotopes. On the other, significant effort was directed towards the enhancement of the signal registration during detection processes, which gave rise to two distinct approaches: instrumentally arranged immunoradiometric assays (IRMA) and photodetection in radioautography [27—31]. In recognition of her work in developing RIA, Rosalyn Yalow was awarded the Nobel Prize in Physiology and Medicine in 1977.

Another important milestone in immunoassay development was the introduction of the non-competitive radioallergosorbent test (RAST), first described in 1967 [32]. A simple and visually interpretable heterogeneous assay was used, later adopted by numerous researchers, where anti-IgE antibodies were immobilized on a solid phase. The immune complex that formed after the contact with test samples containing IgE was detected using iodine-131-labeled secondary antibodies.

At the same time, several factors limited the widespread diagnostic use of RIA: the need for special work conditions with radioactive isotopes; the reliance on expensive equipment and qualified personnel; the limited lifetime of radioactive labels; as well as associated health risks.

«Veni, vidi, vici»

The inherent drawbacks of RIA, combined with advances in enzymology, stimulated the development of novel immunochemical methods based on the use of various enzymes as labels. In the early 1970s, three research groups (in Sweden [33—36], Netherlands [37], and the United States [38]) independently proposed

the enzyme-linked immunosorbent assay (ELISA) in nearly identical forms. This development was foreshadowed by earlier studies demonstrating the possibility of conjugating enzymes with antibodies and antigens [39; 40].

Engvall and Perlmann described an assay that used an alkaline phosphatase-antibody conjugate. In this system, soluble IgG and the conjugate-bound IgG competed for binding to antibodies immobilized on a solid phase. Quantification of the ligand was achieved by measuring the enzymatic conversion of colorless p-nitrophenyl phosphate into yellow p-nitrophenol, detected by absorbance at 400 nm. Shortly thereafter, Van Weemen and Schuurs used horseradish peroxidase as the enzyme label in a conjugate system for quantifying urinary chorionic gonadotropin [37]. These two enzymes — alkaline phosphatase and horseradish peroxidase — became the primary tools for subsequent ELISA development.

The fact that specific reaction components interact on a solid-phase surface, and that their sequential replacement is possible, became the basis for designating such assays as solid-phase or heterogeneous. In contrast, homogeneous assays occur within the reaction mixture volume and involve similar steps but without dependence on a solid phase. Indeed, the advent of ELISA marked the beginning of an entire era of immune-analytical methods, the development of which continues today [41—46].

Since the early 1970s, ELISA methods have become widely used in medical diagnostics, biotechnology, and laboratory practice, largely replacing alternative techniques due to their distinctive advantages. Enzyme-linked immunosorbent assay is highly sensitive and allows simultaneous processing of multiple samples in fully or partially automated systems, without requiring extensive sample purification and preparation.

Nevertheless, ELISA has some drawbacks. It requires demanding storage conditions, and both both substrates and enzymatic reaction products are unstable. These issues reduce the reliability of the enzyme-linked immunoassay test systems. ELISA efficacy is determined by the quality of the solid phase (its capacity to bind and stably preserve anti-ligands), the quality of immunospecific reagents, enzyme activity (as the main component of the reporter system), the properties of the substrate system, and the accuracy of the recording equipment.

Much of what has been stated about ELISA equally applies to immunofluorescence, another important immunoassay variant. In 1950, it was shown that fluorescent dyes could covalently bind antibodies without compromising their specific binding ability [47]. Subsequent development of fluorescence-based methods progressed along two parallel paths. Methodologically, immunofluorescence closely resembles enzyme-linked immunosorbent assay. The only difference is that the enzyme label requires a substrate for visualization, where the conversion product serves as a quantitative measure of the target ligand, whereas a fluorescent label 'shows' it directly through its own emission. In both assays, reliable result detection equipment is essential: an ELISA reader for enzyme-based assays and a fluorimeter for fluorescence-based assays. In each case, the results are formalized by a device that compares either the colorimetric difference between reference and test samples or their relative fluorescence intensities [48—51].

Chemiluminescent immunoassay combines features of both ELISA and immunofluorescence. Its defining feature is the detection of specific interactions through the registration of luminescent energy released by a chemical reaction [52]. This method has become widely used and is well established, primarily due to advanced detection equipment, which provides excellent analytical performance with particularly high sensitivity. The synthesis of affinity compound-luminophore conjugates is generally similar to that of fluorescent reagents [53—55].

There is no limit to perfection!

The development and improvement of immunoanalytical researches equipment, advances in the synthesis of fluorochrome conjugates and affinity compounds, and most importantly, researchers' desire to obtain quantitative data rather than solely visualizing cells and tissues in microscopy, led to the emergence of flow cytometry. First described in 1976 [56; 57], flow cytometry provided a means to detect the distribution of cellular antigens that were difficult or impossible to observe with conventional microscopy, while also offering faster and more accurate analysis.

Flow cytofluorimetry has developed more rapidly than any other quantitative immunoassay technique. It allows the evaluation of antigen expression at the level of cell population. Individual cells labeled with fluorescently tagged antibodies exhibit varying luminous intensity depending on their antigen expression. The flow cytometer automatically detects and records these fluorescence signals for each cell. This method can reliably detect even small populations of cells expressing target antigens at very low frequencies.

The high-speed analytical capacity of flow cytometry enables precise quantification of fluorescent labels in individual cells. By using two or more antibody preparations with different specificities, each labeled with a distinct fluorochrome, it is possible to assess the expression of molecules reacting with these antibodies across the entire investigated cell population. Experimental results, along with controls, can be stored and used for comparison in subsequent experiments [58—61].

In essence, a flow cytometer integrates the operational principles of both fluorescence microscopy and hematology analyzers. It combines three core components to implement the method: a light source with a flow system, an optical system, and an electronic data processing module [62—69].

The evolutionary development of immunological diagnostics detailed in this paper can be seen as a continuous path of technological improvement in detection systems. It originated from researchers' desire to quantitatively formalize immune complexes formation using instrumental techniques. This ongoing process of advancement, and the increasing complexity of instrument-based methods, began with the simplest photoelectric colorimeters, which measured changes in luminous flux intensity passing through turbid solutions. These devices led to the the turbidimetric assay (from Latin turbidus — murky) [70—74].

Although the development of immunoassay techniques has been closely associated with advancements in instrumental result registration, they do not cover the full range of possible methodological approaches. This article focuses only on analytical methods that are widely used in global diagnostic and scientific research practice. Literature describes numerous alternative techniques developed in specific research laboratories [52; 75—85]. These methods illustrate the broader potential of analytical approaches rather than representing a strategic focus in diagnostic science.

The great Darwin proved that simplification is also a mechanism of evolutionary development

An analysis of the evolution of immunological research methods would be incomplete without considering an alternative trajectory of analytical development: namely, instrumental-independent techniques. Numerous agglutination tests using erythrocytes as indicator systems form the foundation of a distinct branch of analytical immunochemistry. These methods focus on the detection of stereospecific interactions through diagnostics with indicator element either generated through colorimetric reactions or inherently colored and visible to the human eye. This feature

makes the tests suitable for "in situ" and "at-home" testing, now more commonly referred to as "point-of-care" testing, which is currently a major development priority in immune-analytical assays.

All these methods share a key feature: they are either entirely or almost entirely instrumental-independent. They are applied across a range of disciplines, including biology, medicine, agriculture, and criminology; and are suitable not only for specialized medical facilities, but also for small clinical laboratories, doctors' offices, for home and field use.

The evolution of these methods is influenced by two main factors. First, medical staff require rapid diagnostic assessment during patient encounters. Second, there is a growing public demand for personal health monitoring. Interest is especially high for lifestyle-related conditions such as obesity, drug addiction, alcoholism and atherosclerosis. The current economic climate also favors the development of self-diagnosis analytical systems. For example, the increasing trend among working women to delay childbearing has driven demand for ovulation prediction and early pregnancy detection tools [86—88].

Finally, rapid, instrument-free diagnostic systems are particularly valuable in clinical and emergency medical settings, where they can facilitate urgent blood transfusions during acute emergencies or large-scale epidemic events.

Immunoanalytical testing methods have recently gained considerable importance in veterinary medicine and food industry. They are applied not only in specialized animal clinics and diagnostic laboratories, but also in field conditions and on individual farms. Technological innovations originally developed for human disease diagnostics have proven equally effective in this context [89; 90]. Three main areas of immunodiagnostic application in veterinary medicine and the food industry are as follows:

1) fertility assessment, including measurement of progesterone and estrone sulfate levels in bovine milk [91—95]);

2) diagnostics and detection of infectious diseases, such as parvovirus infection, leukemia and enteritis pathogens [96—98]);

3) food safety monitoring, including detection of toxins and harmful substances in produce [99—102].

The development of simplified, instrument-free immunoassay methods essentially follows the improvement patterns of instrument-based immunoanalytical techniques. One direction focuses on modifying the solid-phase reagent (immunosorbent) to increase the amount of sorbed anti-ligand or to incorporate anti-ligand compound with superior binding characteristics. The other direction concerns improving the detection method, which can be achieved either by optimizing the characteristics of the affinity compound bound to the label, enhancing its reporter characteristics, or increasing the amount of label bound during detection.

Enzyme-linked immunosorbent systems are increasingly used in non-laboratory settings. Particular emphasis is placed on the substrate systems that generate insoluble colored products. Horseradish peroxidase, alkaline phosphatase, and glucose oxidase are the primary enzymes used as labels in such systems.

This approach yields clear visualization of varying colors and staining intensities, as the enzymatic reaction product accumulates precisely at the sites of enzyme label localization. Yet this method has significant limitations: enzyme conjugates retain their analytical properties only for a short period, and the test kits require strict storage and transportation conditions. Other disadvantages include the instability of chromogenic substrates and their products, poor solubility of chromogens in water, insufficient color intensity of certain reaction products, and most critically, toxic, carcinogenic and mutagenic properties of most of the chromogenic compounds.

What if it's even simpler? One step and you're done!

A particularly attractive approach involves the use of non-enzymatic labels, which enables detection through simple visual readout without the need for multi-step addition of manifesting components. This method has two main advantages: first, it ensures the safety of the analysis procedure; second, it reduces labor and processing time. Fundamentally, the aim was to build upon and refine the original advances in hemagglutination and erythroimmunoadsorption systems. This involves enhancing the visualization capabilities of conjugate reporter groups and developing stable, reproducible methods for diagnostics synthesis.

Researchers have now turned their attention to optically contrastive microparticles of both natural and synthetic origin that have the potential to serve as labels in diagnostic reagents. Earlier work focused on attempts to use of anti-ligands conjugated with colloidal gold particles [103—111], likely building on prior experience of using these particles as electron-dense markers in microscopy [112—115]. This method allowed direct visual detection of stereospecific interactions without additional visualization steps. By synthesizing particles ranging in size from 5 nm to 1 μm, researchers produced conjugates suitable for nearly all known assay configurations, as will be discussed below.

Although technological methods used to obtain colloidal gold particles [116—120] will not be detailed here, it is important to note that almost all authors reported instability of the resulting sols. Particle stabilization was accomplished through the conjugate synthesis by mixing metastable colloidal sols with antibodies [121]. The antibody binding capacity was controlled by monitoring red color retention; with excess anti-ligand causing the colloid color to shift from red to blue. Further conjugate particle stabilization was achieved through polyethylene glycol supplementation [118], while more refined size-based fractionation was carried out via density gradient centrifugation with glycerol or sucrose [121].

Researchers successfully developed a stable, optically contrastive label and a detection reagent that maintained stability for several months [122]. However, technical challenges in producing stable reagents, combined with insufficient sensitivity due to the label's low chromophoricity, have limited their widespread use. The large-scale introduction of colloidal gold particle-based detection systems into the diagnostic market has not been realized. Presumably, these systems could not compete with more contrast-rich and stable diagnostic reagents, although they did find application where high sensitivity is not required [179].

Colored liposomes have been proposed as particle labels in approaches that enable simple visual result interpretation. One illustrative liposome preparation method involves first mixing a egg lecithin in a chloroform/methanol system with cholesterol in chloroform, followed by replacing the organic solvent with water. Such particles can carry detectable marker under defined conditions; for example, phosphatidylcholine-based liposomes are saturated with sulforhodamine B, and antibodies specific to human chorionic gonadotropin beta-subunit are covalently attached to their surface. Antibodies targeting the hormone's alpha-subunit are immobilized on a nitrocellulose filter. After sample interaction with the membrane carrier and subsequent incubation with liposome-conjugated antibodies, matrices with distinct spot patterns are formed. A similar approach was successfully implemented in digoxin immunoassay, achieving detection for substance concentrations of 1—2.5 ng/ml in serum [123—125]. In this system, liposomes bound to immune complexes were lysed, and ligand concentration was determined either from the quantity of enzymes released during liposome lysis (horseradish peroxidase or alkaline phosphatase), or from the quantity of dyes saturating the colloidal particles. Despite these successes, liposomal diagnostics

methods have not been widely used in analytical practice, likely either due to the instability of liposomes under changing conditions (pH, ionic strength) resulting in colloid degradation, or to the insufficient label contrast for reliable results.

Latex particles offer substantially greater potential and can be manufactured in a wide size range, similar to colloidal gold particles. Efforts in latex particle synthesis have focused on obtaining functionally active particles with surface groups that are capable of covalent binding to proteins. The glutaraldehyde method represents a straightforward approach to crosslinking affinity compounds to particle surfaces. It established covalent bonds between amino groups on latex particles and target peptides [123]. The use of latex diagnostics in various arrangement formats enables the development of a broad spectrum of analytical systems: agglutination tests (for e. g., streptolysin antibody detection, leptospirosis diagnosis [126—129]), group A streptococcus detection [130—132]; immunochromatographic (lateral flow) assays that provide results within 3—5 minutes (for e. g., pregnancy testing via urinary human chorionic gonadotropin detection) [133; 134]; group A streptococcus detection [135; 136]; influenza diagnostic [137; 138]; and fertility monitoring [139; 140]. Limitations include insufficient sensitivity due to chromophore properties, strict technological requirements for synthesis which increase the risk of non-specific interactions, insufficient stability of latex microspheres during storage (anti-ligand leakage), particularly under non-ideal conditions (e. g., temperature fluctuations), and the hook-effect, which is discussed further below.

Magnetic particles, whose early development was linked to latex technology, are noteworthy for immunoassays, although they require special equipment to realize their potential. Norwegian researchers were among the first to study magnetic particle applications [141; 142], using 4.5 μm magnetic particles to isolate T- and B-lymphocytes from blood. For this purpose, latex particles were loaded with magnetic compounds, and monoclonal antibodies were crosslinked to the polystyrene surface. Following incubation with whole blood, the particles were separated with cobalt-samarium magnets and subsequently analyzed for quantitative evaluation, essentially establishing a magnetic separation technique for sample preparation. Recent years have seen significant advances in applying magnetic iron-carbon particles to immunoassays based on nuclear magnetic resonance. In these constructions, the reporter moment is the change in the fixed proton relaxation time (T2) in the system containing an affinity compound initially adsorbed on a solid phase, the target ligand, magnetic iron-carbon particles functionalized with ligand-specific molecules like antibodies. The proton relaxation time in such a system is inversely proportional to the quantity of magnetic particles bound by the immune complex, which in turn is proportional to the quantity of bound ligand [143; 144]. This method illustrates a hybrid immunodetection approach because it complements the spectrum of both instrumental and non-instrumental immunoassay systems, as both principles are present in the test procedure [145; 146].

Several attempts have been made to develop original detection systems based on any optically dense particles, including spherical particles of silicon dioxide [147—149], polyacrylamide microspheres with a diameter of 3—10 μm [150], iron (III) oxide particles [151], colored polymers [152; 153], and elemental particles (sulfur, selenium, tellurium, and phosphorus [154—158]). While all these attempts produced functional analytical systems, they were not adopted in global diagnostic practice. Based on our experience in the field, this can be attributed to limitations in sensitivity, specificity, and stability of reagents, which in turn affect overall reliability of such systems.

Of particular interest there are studies using dyes as labels. Unlike dyed latex particles, where the loading of chromophores is constrained by the physicochemical parameters of the heterogeneous organic phase / dye system, these approaches use the dye particles themselves as immobilisation matrices for affinity compounds [159—163]. This configuration achieves complete chromophore saturation. Immunoreagents

are produced from water-soluble dyes, which can be converted into fine suspensoid particles in the aqueous phase. Under optimized conditions, the particle surface sorbs well due to hydrophobic interactions and electrostatic forces of various immunoreactive compounds, forming convenient reagents for immunodiagnostics. However, commercial application is greatly limited by instability, particularly under variable storage conditions, including temperature, pH, and ionic strength of the solution.

The issues discussed above underscore major trends in the evolution of detection methods used in immunoassay systems. It is evident that, to satisfy the demands of the developing test systems, detection components themselves must combine at least two elements optimally: a signal-generating element with reporter properties that require no additional steps or efforts for visualization or amplification, and an anti-ligand with sufficient affinity and specificity. A critical factor in test kits development is the stability of these components, particularly the bonding strength between the label and the immunoreactive compound, which is typically achieved through covalent bonding. Equally important is the stability of the label itself, which is determined by its origin. Beyond these component requirements, the development of new high-efficiency detection also hinges on the establishment of robust production technologies, ensuring the generation of durable and stable reagents. Consequently, the main challenges in immunoanalitical systems development include both the careful selection and optimization of component preparation methods, on the one hand, and the design of novel high-yield synthesis technologies for detection reagents, on the other.

As noted above, erythrocytes, dyed latex particles, colloidal gold, and other materials have been used as labels in detection reagents development. This article will primarily focus on analyzing the current state of suspended dye particles as labeling agents, which will be discussed in the sections below.

For several years, Adamov pursued the development of suspension antigens and antibodies as immunodiagnostic reagents [164]. By the late 1960s, Adamov's research stopped for unclear reasons. His approach involved using suspension antigens and antibodies as diagnostic reagents. The antigens and antibodies were immobilized onto particle surfaces via adsorption. To achieve this, various stable in saline solution compounds—including rosolic acid, phenol red, phenolphthalein, alizarin, lead hydroxide hydrate, carmine, methyl orange, thionine, bismuth citrate, α-aminoanthraquinone, dermatol, infusorial earth, polyvinyl chloride F, thymol blue, anthragallol, animal charcoal, and tribasic calcium phosphate—were mixed with solutions of either antigens or immune serum. The resulting reagents were used to detect relevant antibodies and antigens through agglomeration reactions, essentially functioning as agglutination tests. The cessation of this research may have been caused by the following factors: 1) difficulty in producing stable suspension in solutions; 2) instability of the reagents during storage; 3) a high degree of nonspecific interactions.

Nearly 15 years later, an article [165] presented a practical approach to the same concept of developing immunodiagnostic reagents for direct visualization with the usage of hydrophobic dyes as labels. The dyes included four Palanil colors, two Terasil colors, Cubacet, Foron, Resolin, Procinyl, and four Samaron colors. The researchers obtained conjugates of suspension particles of dyes with sheep, rabbit and mouse monoclonal immunoglobulins against hCG. The same method of direct sorption of immunospecific compounds on the particles surface was used. The reagents enabled the hCG determination in the agglutination reaction, but the stability of these reagents did not exceed 2.5 months. Later, the same author reported improvements in assay sensitivity using the previously described reagents, achieving detection limits of

2—13 IU/L for hCG and 10—1000 ng/mL for alpha-fetoprotein in a sandwich assay format. Despite these enhancements, the problem of reagent storage stability persisted [159].

These challenges likely account for the absence of commercial diagnostic kits with instrument-free test systems with direct ligand visualization via colorimetric dyes in the global biotechnological market. Nevertheless, the attractive prospect of highly sensitive direct visualization continues to drive research efforts toward new diagnostic reagents.

In their search for high-contrast labels, researchers explored the use of tetrazolium salts and their reduced forms—formazans—for staining latex microspheres [166—168]. The intense blue and violet colors of reagents based on tetrazolium salts and formazans offer distinct advantages over derivatives of metallic and non-metallic compounds. However, these conjugates still face the same stability and sensitivity limitations. Nevertheless, in the current instrument-free tests diagnostic market, latex-based systems (primarily those configured for immunochromatography) remain the dominant technology.

Researchers eventually turned their attention to colloidal carbon particles, whose intense black color suggested superior potential for reagent development. Early investigations were advanced by two pivotal studies that systematically evaluated the possible use of colloid carbon particles in immunodiagnostics [169; 170]. Both articles reported detailed methodologies for preparing conjugates based on carbon black particles (namely, products from Cabot Corporation, Vulcan & Monarch, USA), and outlined application methods of the obtained reagents in immunoanalytical systems of different formats. Importantly, the method for producing these diagnostic reagents still relied on non-covalent conjugation of immunoreactive compounds to the carbon particles.

The main practical challenges lie not only in enhancing detection system sensitivity through optimal reagent selection, but also in ensuring reagent stability in commercial test kits. This requires developing conjugation methods that facilitate strong covalent bonds between affinity compounds and detection labels. Successful resolution of these challenges would enable the introduction of fundamentally new instrument-free analytical systems to the diagnostic market.

Research initiated in the early 1990s deserves particular attention. For the first time, a technology was developed and validated that addressed both critical requirements for advancing instrument-free diagnostics: strong covalent binding of affinity compounds and the use of the most optically contrasting label (a nanosized colloidal carbon particle) [180]. This achievement led to the creation of a functional diagnostic system and a wide array of test-system models. In terms of both analytical and procedural characteristics, they successfully competed with instrument-based enzymatic test systems [171—175]. By employing a highly visible non-instrumental contrast label and a covalent binding mechanism for the affinity compound, the researchers ensured reliable diagnostics. The size and properties of the carbon label facilitated the development of functional instrument-free assay systems across all express-test formats, including dot-blot assays on both non-porous and porous solid phases (including multiplex versions, [171; 173]), immunochromatography (IC), and immunofiltration (IF) [175; 179].

Recent research has introduced a new approach to enhancing the sensitivity of simplified testing systems with nanozymes—nanoparticles of various compositions that exhibit horseradish peroxidase-like activity. A representative example is protein nanoparticles impregnated with hemin molecules. These diagnostic reagents demonstrate horseradish peroxidase-like activity in the presence of hydrogen peroxide, catalyzing the conversion of the substrate (TMB) with a visible color change [176].

Prussian blue nanoparticles also exhibit horseradish peroxidase-like activity that exceeds the activity of the natural enzyme [177; 178]. The synthesis of Prussian blue nanoparticles involves the controlled mixing of two iron salts. These promising peroxidase alternatives have the potential to replace horseradish peroxidase (HRP), thereby alleviating significant challenges and costs associated with isolating HRP from natural sources. This approach offers several advantages: eliminating labor-intensive purification processes required to achieve reagent purity, and using substantially cheaper and more readily available starting materials for nanoparticle synthesis.

As for the arrangement formats of non-instrumental test systems, the field demonstrates low diversity. There is little transition from agglutination tests in wells of immunoassay plates or Terasaki plates to dot-analysis, immunochromatography, and immunofiltration. Currently, IC systems dominate the market, which presents a curious paradox. The IC method has one major drawback, namely, the hook effect. In certain conditions, it can significantly distort measurement results, particularly at high concentrations of the target ligand. IF systems are free from this problem. However, the number of IF systems on the diagnostic market is notably lower. This discrepancy may be explained by both the technological complexities of IF diagnostic systems production and by manufacturer conservatism.

Conclusions

There is no doubt about the constant demand for relevant tools in diagnostic practice. At the same time, it is clear that significant improvements in testing metrics remain essential—particularly in sensitivity, specificity, accuracy, reliability, reproducibility, accessibility, speed, procedural simplicity, and affordability. It is evident that uniting all these features, along with their required enhancement, into a single analytical system is impossible. Therefore, every type of diagnostic testing used in real clinical practice requires continuous improvement. Fundamental science, in turn, is capable of designing the tools and materials that practical medicine demands.

Without high-quality diagnostic tools we will not be able to achieve early, accurate, and accessible detection of disease symptoms (especially before clinical manifestations appear) together with near real-time patient monitoring and rapid assessment of the body's response to therapeutic interventions. At present, this remains an unfulfilled aspiration for both doctors and patients. However, the analysis of the evolutionary pathways of testing methods detailed in this article suggests that this goal may become attainable in the near future.

Funding: R&D theme 124020500027-7 (Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences).

Conflict of interest. The author declares no conflict of interest.

Финансирование: Тема НИР 124020500027-7 (Институт экологии и генетики микроорганизмов УрО РАН).

Конфликт интересов. Автор заявляет об отсутствии конфликта интересов.

References

1. Harma H. Particle technologies in diagnostics. National Technology Agency. Technolgy Review. Helsinki. 2002, 126, 1—30.

2. Widal F. On the sero-diagnosis of Thiphoid fever. The Lancet. 1896, 148(3820), 1371—1372, DOI: 10.1016/s0140-6736(01)76589-0.

3. Wassermann A., Neisser A., Bruck C. Eine serodiagnostische Reaktion bei Syphilis. Deutsche medicinische Wochenschrift: Berlin. 1906, 32, 745—746.

4. Landsteiner K., Thomas C. C. The Specificity of Serological Reactions. 1936.

5. Heidelberger M. Quantitative absolute methods in the study of antigen-antibody reactions. Bacteriol. Rev. 1939, 3(1), 49—95, DOI: 10.1128/br.3.1.49-95.1939.

6. Kraus R. Ueber spezifische Reaktionen in keimfreien Filtraten aus Cholera-Typhus-Pestbouillonculturen erzeugt durch homologes Serum. [On specific reactions in germ-free filtrates from cholera- typhoid- plague- broth cultures produced by homologous serum]. Published in Wiener klinische Wochenschrift. 1897, 10, 32, 760—763, DOI: 10.3390/toxins16010033.

7. Ehrlich P. The Croonian lecture: On immunity. Proc. R. Soc. Lond. 1900, 66, 424.

8. Marrack J. R. The Chemistry of Antigens and Antibodies. Medical Research Council, Special Report Series. 1938, 230, 194.

9. Oudin J. L'analyse immunochimique du sérum de cheval par précipitation spécifique en milieu gélifié; premiers résultats [Immunochemical analysis of horse serum by specific precipitation in gelified medium; first results]. Bull. Soc. Chim. Biol. (Paris). 1947, 29(1—3), 140—149.

10. Elek S. D. Rapid identification of Proteus. J. Pathol. Bacteriol. 1948, 60(2), 183—192, DOI: 10.1002/path.1700600204.

11. Ouchterlony O. Air-borne infections; infection and spreading of infection in diphtheria with special reference to isolation wards. Acta Med. Scand. 1949, 134(4), 296—309.

12. Grabar P., Williams C. A. Method permitting the combined study of the electrophoretic and the immunochemical properties of protein mixtures; application to blood serum. Biochim. Biophys. Acta. 1953, 10(1), 93—94, DOI: 10.1016/0006-3002(53)90233-9.

13. Mancini G., Carbonara A. O., Heremans J. F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry. 1965, 2(3), 235—254, DOI: 10.1016/0019-2791(65)90004-2.

14. Laurell C. B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 1966, 15(1), 45—52, DOI: 10.1016/0003-2697(66)90246-6.

15. Coombs R. R. A., Mourant A. E., Race R. R. A new test for the detection of weak and incomplete Rh agglutinins. Br. J. Exp. Pathol. 1945, 26(4), 255—266.

16. Gupta S. K., Talwar G. P. Monoclonal antibodies based sandwich erythro-immunoassay and 'dot' enzyme immunoassay for human chorionic gonadotropin in urine. Scand. J. Clin. Lab. Invest. 1986, 46(8), 751—759, DOI: 10.3109/00365518609084047.

17. Gupta S. K., Guesdon J. L., Avrameas S., Talwar G. P. Sandwich enzyme immunoassay of human chorionic gonadotropin using polystyrene beads as solid support. Ann. Inst. Pasteur Immunol. 1985, 136D(1), 47—55, DOI: 10.1016/s0769-2625(85)80074-x.

18. Bravo Oliva J. Rapid agglutination test on microscope slides in routine serological diagnosis. Med. Cir. Guerra. 1959, 21(3—4), 159—170.

19. Crosignant P. G., Polvani F., Surace M. Immunological assay of human chorionic gonadotrophin using HCG-coupled red cells agglutination. Folia Endocrinol. 1963, 16, 650—663.

20. Maurice Y., Provost A. Hemagglutination and hemagglutination inhibition tests with horse sickness virus. Limits of their interpretation. Rev. Elev. Med. Vet. Pays Trop. 1966, 19(4), 439—450.

21. Adler F. L., Liu C. T. Detection of morphine by gemagglutination-inhibition. J. Immunol. 1971, 106, 1684—1685.

22. Dierks R. E., Gough P. M. Passive haemagglutination test for rabies antibodies. Monogr. Ser. World Health Organ. 1973, 23, 147—150.

23. Van Doorn H. R., Hofwegen H., Koelewijn R., Gilis H., Wentink-Bonnema E., Pinelli E., et al. Reliable serodiagnosis of imported cystic echinococcosis with a commercial indirect hemagglutination assay. Diagn. Microbiol. Infect. Dis. 2007, 57(4), 409—412, DOI: 10.1016/j.diagmicrobio.2006.10.002.

24. Coombs R. R. A. Assay utilizing red cells as markers. In: Immunoassay for 80's. Ed. A. Voller. MTP Press, Lancaster. 1981, 17—34.

25. Coombs R. R. A., Munro A. J. Storage-stable antibody-linked eritrocytes. Pat. 2138132. 1984.

26. Guesdon J. L., Avrameas S. Sensitive titration of antibodies and antigen using eritro-immunoassay. Ann. Immunol. 1980, 131, 3, 389—396.

27. Berson S. A., Yallow R. S. Insulin antagonists, insulin antibodies and insulin resistance. J. Med. 1958, 25(2), 155—159, DOI: 10.1016/0002-9343(58)90022-6.

28. Ekins R. P. Radioimmunoassay and saturation analysis. Basic principles and theory. Br. Med. Bull. 1974, 30(1), 3—11, DOI: 10.1093/oxfordjournals.bmb.a071163.

29. Teale J. D. Radioimmunoassay. 2 Scientific Foundations of Clinical Biochemistry. Chapter 19. Heinemann Medical. 1978, 279.

30. Erlanger B. F., Borek F., Beiser S. M., Lieberman S. Steroid-protein conjugates. I. Preparation and characterization of conjugates of bovine serum albumin with testosterone and with cortisone. J. Biol. Chem. 1957, 228(2), 713—727.

31. Byrnes D. J., Henderson L., Borody T., Rehfeld J. F. Radioimmunoassay of cholecystokinin in human plasma. Clin. Chim. Acta. 1981, 19, 111(1), 81—89, DOI: 10.1016/0009-8981(81)90424-1.

32. Wide L., Bennich H., Johansson S. G. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet. 1967, 2, 1105—1107, DOI: 10.1016/s0140-6736(67)90615-0.

33. Engvall E., Perlmann P. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry. 1971, 8, 9, 871—874, DOI: 10.1016/0019-2791(71)90454-x.

34. Engvall E., Jansson K., Perlmann P. ELISA. Quantitative assay of protein antigen, IgG by means of enzyme — labelled antigen and antibody coated tubes. Biochem. Biophys. Acta. 1971, 251, 427—434, DOI: 10.1016/0005-2795(71)90132-2.

35. Engvall E., Perlmann P. Enzyme — linked immunosorbent assay (ELISA). Quantitation of specific antibodies by enzyme-labelled anti-immunoglobuline in antigen coated tubes. J. Immunol. 1972, 109, 129—135.

36. Engvall E. Enzyme-linked immunosorbent assay (ELISA). Biomedical applications of immobilized enzymes and proteins. Ed.: T. M. S. Chang. Plenum Press: N. Y. 1977, 2, 87—96.

37. Van Weemen B. K., Schuurs A. H.W.M. Immunoassay using antigen-enzyme conjugate. FEBS Lett. 1971, 15, 232—236, DOI: 10.1016/0014-5793(71)80319-8.

38. Rubinstein K. E., Schneider R. S., Ullman E. F. "Homogeneous" enzyme immunoassay. A new immunochemical technique. Biochem. Biophys. Res. Commun. 1972, 47, 846—851, DOI: 10.1016/0006-291X(72)90570-0.

39. Avrameas S., Uriel J. Méthode de marquage d'antigènes et d'anticorps avec des enzymes et son application en immunodiffusion [Method of antigen and antibody labelling with enzymes and its immunodiffusion application]. C. R. Acad. Hebd. Seances Acad. Sci. D. 1966, 262(24), 2543—2545.

40. Nakane P. K., Pierce G. B. Enzyme-Labelled antibodies preparation and application for the localization of antigen. J. Histochem. Cytochem. 1966, 14, 929, DOI: 10.1083/jcb.33.2.307.

41. Lam S. K., Devine P. L. Evaluation of capture ELISA and rapid immunochromatographic test for the determination of IgM and IgG antibodies produced during dengue infection. Clin. Diagn. Virol. 1998, 10(1), 75—81, DOI: 10.1016/s0928-0197(98)00002-6.

42. Dutaud D., Aubry L., Henry L., Levieux D., Hendil K. B., Kuehn L., et al. Ouali Development and evaluation of a sandwich ELISA for quantification of the 20S proteasome in human plasma. J. Immunol. Methods. 2002, 260(1—2), 183—193, DOI: 10.1016/s0022-1759(01)00555-5.

43. Pare J., Simard C. Comparison of commercial enzyme-linked immunosorbent assays and agar gel immunodiffusion tests for the serodiagnosis of equine infectious anemia. Can. J. Vet. Res. 2004, 68(4), 254—258.

44. Desai N., Filipovits J., Goldfarb J. Secretion of soluble HLA-G by day 3 human embryos associated with higher pregnancy and implantation rates: assay of culture media using a new ELISA kit. Reprod. Biomed. Online. 2006, 13(2), 272—277, DOI: 10.1016/s1472-6483(10)60626-8.

45. Ebinuma H., Miida T., Yamauchi T., Hada Y., Hara K., Kubota N., et al. Improved ELISA for Selective Measurement of Adiponectin Multimers and Identification of Adiponectin in Human Cerebrospinal Fluid. Clin. Chem. 2007, 53(8), 1541—1544, DOI: 10.1373/clinchem.2007.085654.

46. Tsao Z. J., Liao Y. C., Liu B. H., Su C. C., Yu F. Y. Development of a Monoclonal Antibody against Domoic Acid and Its Application in Enzyme-Linked Immunosorbent Assay and Colloidal Gold Immunostrip. J. Agric. Food Chem. 2007, 55(13), 4921—4927, DOI: 10.1021/jf0708140.

47. Coons A. H., Kaplan M. H. Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody. J. Exp. Med. 1950, 91(1), 1—13, DOI: 10.1084/jem.91.1.1.

48. Frank D. S., Sundberg M. W. Fluorescent labels comprising rare earth chelates. US Patent 4283382A. 1978.

49. Lovgren T., Heinonen P., Lehtinen P., Hakala H., Heinola J., Harju R., et al. Sensitive bioaffinity assays with individual microparticles and time-resolved fluorometry. Clin. Chem. 1997, 10, 1937—1943.

50. Chan W. C. W., Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science. 1998, 281, 2016—2018, DOI: 10.1126/science.281.5385.2016.

51. Arai R., Ueda H., Tsumoto K., Mahoney W. C., Kumagai L., Nagamune T. Fluorolabeling of antibody variable domains with green fluorescent protein variants: application to an energy transfer-based homogeneous immunoassay. Protein Eng. 2000, 13, 369—376, DOI: 10.1093/protein/13.5.369.

52. Thorpe G. H., Williams L. A., Kricka L. J., Whitehead T. P., Evans H., Stanworth D. R. A rapid luminescently monitored enzyme immunoassay for Ig E. J. Immunol. Methods. 1985, 79(1), 57—63, DOI: 10.1016/0022-1759(85)90391-6.

53. Khadzhiolov A. I. Analysis of luminescence of tissue and fluids in the human and animal organism. Izv. Meditsinskite Inst. Bulg. Akad. Naukite Sofia Otd. Biol. Meditsinski Nauki. 1951, 1, 33—64.

54. Akimoto K., Shinmen Y., Sumida M., Asami S., Amachi T., Yoshizumi H., et al. Luminol chemiluminescence reaction catalyzed by a microbial peroxidase. Anal. Biochem. 1990, 189(2), 182—185, DOI: 10.1016/0003-2697(90)90104-h.

55. Terashima T., Okochi N., Kanno M., Satomi S., Taguchi Y., Mori S., et al. Measurement of the chemiluminescence from rat hepatocytes on reperfusion injury: preliminary report. Nippon Geka Gakkai Zasshi. 1992, 93(8), 869.

56. Fulwyler M. J. Optical chamber with spherical reflective portion and apparatus employing same. UK patent 1561042. 1976.

57. Herzenberg L. A., Sweet R. G. Fluorescence-activated cell sorting. Sci. Am. 1976, 234(3), 108—117.

58. Cook L., Irving D. Microsphere-based flow cytometric assays. J. Clin. Immunoassay. 1989, 12, 36—39.

59. Frengen J., Schmid R., Kierulf B., Nustad K., Paus E., Berge A., et al. Homogenous immunofluorometric assays of α-fetoprotein with macroporous, monosized particles and flow cytometry. Clin. Chem. 1993, 39, 2174—2181.

60. McHugh T. M. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. Methods Cell Biol. 1994, 42, 575—595, DOI: 10.1016/s0091-679x(08)61096-1.

61. Soukka T., Paukkunen J., Härmä H., Lönnberg S., Lindroos H., Lövgren T. Supersensitive time-resolved immunofluorometric assay of free prostate-specific antigen with nanoparticle label technology. Clin. Chem. 2001, 47, 1269—1278.

62. Kruth H. S. Flow cytometry: rapid biochemical analysis of single cells. Anal. Biochem. 1982, 125(2), 225—242, DOI: 10.1016/0003-2697(82)90001-x.

63. Wan Dilla M. A., Dean P. H., Waerum O. D., Melamed M. R. Flow Cytometry: Instrumentation and Data Analysis. Academic Press: New York. 1985.

64. Jensen B. D., Horan P. K. Flow cytometry: rapid isolation and analysis of single cells. Meth. Enzymol. 1989, 171, 549—581, DOI: 10.1016/S0076-6879(89)71030-2.

65. Nolan J. P., Sklar L. A. The emergence of flow cytometry for sensitive, real-time measurements of molecular interactions. Nat. Biotechnol. 1998, 16(7), 633—638, DOI: 10.1038/nbt0798-633.

66. Edwards B. S., Oprea T., Prossnitz E. R., Sklar L. A. Flow cytometry for high-throughput, high-content screening. Curr. Opin. Chem. Biol. 2004, 8(4), 392—398, DOI: 10.1016/j.cbpa.2004.06.007.

67. Simons P. C., Shi M., Foutz T., Cimino D. F., Lewis J., Buranda T., et al. Ligand-receptor-G-protein molecular assemblies on beads for mechanistic studies and screening by flow cytometry. Mol. Pharmacol. 2003, 64(5), 1227—1238, DOI: 10.1124/mol.64.5.1227.

68. Waller A., Simons P., Prossnitz E. R., Edwards B. S., Sklar L. A. High throughput screening of G-protein coupled receptors via flow cytometry. Comb. Chem. High Throughput Screen. 2003, 6(4), 389—397, DOI: 10.2174/138620703106298482.

69. Sklar L. A., Edwards B. S., Graves S. W., Nolan J. P., Prossnitz E. R. Flow cytometric analysis of ligand-receptor interactions and molecular assemblies. Annu. Rev. Biophys. Biomol. Struct. 2002, 31, 97—119, DOI: 10.1146/annurev.biophys.31.082901.134406.

70. Engler R., Judon C., Langlade J. P., Jayle M. F. An immunonephelometric determination of rabbit haptoglobin. Article in French. C. R. Acad. Hebd. Seances Acad. Sci. D. Sci Nat. 1975, 280(18), 2157—2159.

71. Van Munster P. J., Hoelen G. E., Samwel-Mantingh M., Holtman-Van Meurs M. A turbidimetric immuno assay (TIA) with automated individual blank compensation. Clin. Chim. Acta. 1977, 76(3), 377—388, DOI: 10.1016/0009-8981(77)90165-6.

72. Evans S. E. Rapid determination of alpha-fetoprotein in amniotic fluid using an automated immunoturbidimetric technique. Ann. Clin. Biochem. 1980, 17(3), 130—133, DOI: 10.1177/000456328001700305.

73. Kleine T. O., Merten B. Rapid manual immunoturbidimetric and immunonephelometric assays of prealbumin, albumin, IgG, IgA and IgM in cerebrospinal fluid. J. Clin. Chem. Clin. Biochem. 1980, 18(4), 245—254, DOI: 10.1515/cclm.1980.18.4.245.

74. Spencer K., Price C. P., Anthony F., Wood P. J. Kinetic immunoturbidimetry: the measurement of pregnancy specific beta 1glycoprotein. Clin. Chim. Acta. 1979, 99(2), 177—187, DOI: 10.1016/0009-8981(79)90041-x.

75. Boguslavski R. C., Li T. M. Homogeneous immunoassay. Appl. Biochem. Biotechnol. 1982, 7(5), 401—414.

76. Oellerich M. Enzyme-immunoassay: a review. J. Clin. Chem. Clin. Biochem. 1984, 22(12), 895—904.

77. Van Weemen B. K. ELISA: highlights of the present state of the art. J. Virol. Methods. 1985, 10(4), 371—378, DOI: 10.1016/0166-0934(85)90055-2.

78. Ekins R. P. Merits and disadvantages of different labels and methods of immunoassay. In: Immunoassay for the 80's. MTP Press: Lancaster. 1981, 5—16.

79. Ekins R. P., Dacubu S. The development of high sensitivity pulsed light, time-resolved fluoroimmunoassay. Pure Appl. Chem. 1985, 57, 3, 473—482.

80. Shall R. F., Tenoso H. J. Alternatives to radioimmunoassay: labels and methods. Clin. Chem. 1981, 27, 7, 1157—1164.

81. Olsson T., Thore A. Chemiluminescence and its use in immunoassay. In: Immunoassay for the 80's. MTP Press: Lancaster. 1981, 113—125.

82. Woodhead J. S., Weeks I. Chemiluminescence immunoassay. Pure and Appl. Chem. 1985, 57, 3, 523—529.

83. Fukada H., Haga A., Fujita T., Hiramatsu N., Sullivan C. V., Hara A. Development and validation of chemiluminescent immunoassay for vitellogenin in five salmonid species. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2001, 130(1), 163—170, DOI: 10.1016/s1095-6433(01)00381-6.

84. Lin J., Ju H. Electrochemical and chemiluminescent immunosensors for tumor markers. Biosens Bioelectron. 2005, 20(8), 1461—1470, DOI: 10.1016/j.bios.2004.05.008.

85. Liu Y. M., Mu H. B., Zheng Y. L., Wang C. Q., Chen Y. H., Li F. R., et al. Capillary electrophoretic immunoassay for alpha-fetoprotein with chemiluminescence detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2007, 855, 2, 280—285, DOI: 10.1016/j.jchromb.2007.04.033.

86. Lindstedt G., Himmelmann C. E., Salsmans R., Valentin K. Home testing for pregnancy-can it be recommended? Scand. J. Clin. Lab. Invest. 1982, 42(4), 371—376.

87. Wang C. F., Gemzell C. Neocept: a simple, sensitive urine test of early pregnancy in women undergoing ovulation induction. J. Reprod. Med. 1982, 27(4), 193—195.

88. Nielsen M. S., Barton S. D., Hatasaka H. H., Stanford J. B. Comparison of several one-step home urinary luteinizing hormone detection test kits to OvuQuick. Fertil. Steril. 2001, 76(2), 384—387, DOI: 10.1016/s0015-0282(01)01881-7.

89. Anderton D. J., Gronow M., Kungdon C. F.M., Ghesman J. Tools for decentralized clinical biochemistry testing. Optimal use of the clinical laboratory. Karger: Basel. 1986, 129—136.

90. Jeffers J. G., Shanley K. J., Meyer E. K. Diagnostic testing of dogs for food hypersensitivity. J. Am. Vet. Med. Assoc. 1991, 198(2), 245—250.

91. Laing J. A., Heap R. B. The concentration of progesterone in milk of cows during the reproductive cycle. Br. Vet. J. 1971, 127(8), 19—22, DOI: 10.1016/s0007-1935(17)37432-8.

92. Sauer M. J., Foulkes J. A., Cookson A. D. Direct enzyme immunoassay of progesterone in bovine milk. Steroids, 1981, 38, 45—53, DOI: 10.1016/0039-128X(81)90020-9.

93. Davies J., Fletcher N. A. Evaluation of an enzyme immunoassay kit for the quantitative assessment of progesterone in buvine milk samples. Vet. Record. 1987, 1209110, 257—258, DOI: 10.1136/vr.120.11.257.

94. Saba N. J., Hatterslay J. P. Direct estimation of estrone sulphate in cow serum for a rapid pregnancy diagnosis test. J. Reprod. Fertil. 1981, 62, 87—92, DOI: 10.1530/jrf.0.0620087.

95. Hamon M., Fleet I. R., Holdsworth R. J., Heap R. B. The time of detection of estrone sulphate in milk and the diagnosis of pregnancy in cows. Br. Vet J. 1981, 137(1), 71—77, DOI: 10.1016/s0007-1935(17)31790-6.

96. Shen J. T. Gamma stick. US Patent 4135884A. 1979.

97. Al-Yousif Y., Anderson J., Chard-Bergstrom C., Bustamante A., Muenzenberger M., Austin K, et al. Evaluation of a latex agglutination kit (Virogen Rotatest) for detection of bovine rotavirus in fecal samples. Clin. Diagn. Lab. Immunol. 2001, 8(3), 496—498, DOI: 10.1128/CDLI.8.3.496-498.2001.

98. Al-Yousif Y., Anderson J., Chard-Bergstrom C., Kapil S. Development, evaluation, and application of lateral-flow immunoassay (immunochromatography) for detection of rotavirus in bovine fecal samples. Clin. Diagn. Lab. Immunol. 2002, 9(3), 723—724, DOI: 10.1128/CDLI.9.3.723-724.2002.

99. Heathcote J. G., Hillert J. R. Aflotoxins: Chemical and biological aspects. Development's in Food science. Elsevier: Amsterdam. 1978, 1, 97—108.

100. Watanabe H., Satake A., Kido Y., Tsuji A. Monoclonal-based enzyme-linked immunosorbent assay and immunochromatographic assay for enrofloxacin in biological matrices. Analyst. 2002, 127(1), 98—103, DOI: 10.1039/B109427K.

101. Zhou P., Lu Y., Zhu J., Hong J., Li B., Zhou J., et al. Nanocolloidal gold-based immunoassay for the detection of the N-methylcarbamate pesticide carbofuran. J. Agric. Food Chem. 2004, 52(14), 4355—4359, DOI: 10.1021/jf0499121.

102. Zhang H., Meyerhoff M. Gold-coated Magnetic particles for solid-phase immunoassay: enhancing immobilized antibody binding efficiency and analytical performance. Anal. Chem. 2006, 78, 609—616, DOI: 10.1021/ac051720x.

103. Egger D., Bienz R. Colloidal gold staining and immunoprobing of proteins on the same nitrocellulose blot. Anal. Biochem. 1987, 166, 413—417, DOI: 10.1016/0003-2697(87)90592-6.

104. Kramarcy N. R., Sealock R. Commercial preparations of colloidal gold-antibody complex frequently contain free active antibody. J. Histochem. Cytochem. 1991, 39(1), 37—39, DOI: 10.1177/39.1.1983872.

105. Chakraborty U. R., Black N., Brooks H. G., Campbell C., Gluck K., Harmon F., et al. An immunogold assay system for the detection of antigen or antibody. Ann. Biol. Clin. 1990, 8, 403—408.

106. Martin J. M., Paques M., van der Velden-de Groot T. A., Beuvery E. C. Characterization of antibody labelled colloidal gold particles and their applicability in a sol particle immunoassay (SPIA). J. Immunoassay. 1990, 11(1), 31—47, DOI: 10.1080/01971529008053256.

107. Moeremans M., Daneels G., Van Dijck A., Langanger G., De Mey J. Sensitive visualization of antigen-antibody reactions in dot and blot immune overlay assay with immunogold and immunogold silver staining. J. Immunol. Meth. 1984, 74920, 353—360, DOI: 10.1016/0022-1759(84)90303-X.

108. Leuvering J. H., Thal P. J., van der Waart M., Schuurs A. H. Sol particle immunoassay (SPIA). J. Immunoassay. 1980, 1(1), 77—91, DOI: 10.1080/01971528008055777.

109. Leuvering J. H., Goverde B. C., Thal P. J., Schnurs A. H. A gomogeneous sol particle immunoassay for human chorionic gonadotrophin using monoclonal antibodies. J. Immunol. Meth. 1983, 60, 9—23, DOI: 10.1016/0022-1759(83)90330-7.

110. Zhang C., Zhang Y., S. Wang S. Development of multianalyte flow-through and lateral-flow assays using gold particles and horseradish peroxidase as tracers for the rapid determination of carbaryl and endosulfan in agricultural products. J. Agric. Food Chem. 2006, 54(7), 2502—2507, DOI: 10.1021/jf0531407.

111. Lee E. J., Kim Y., Lim J., Kim M., Kang C. S., Lee J. H., et al. Simple immunohistochemical staining method using large sized gold colloid conjugated secondary antibody. Ann. Clin. Lab. Sci. 2007, 37(2), 152—157.

112. Gamliel H., Polliack A. Scanning immunoelectron microscopy markers. Isr. J. Medl Sci. 1979, 15(8), 639—646.

113. Mannweiler K., Hohenberg H., Bohn W., Rutter G. Protein-A gold particles as markers in replica immunocytochemistry: high resolution electron microscope investigations of plasma membrane surfaces. J. Microsc. 1982, 126(Pt 2), 145—149, DOI: 10.1111/j.1365-2818.1982.tb00363.x.

114. De Waele M., De Mey J., Moeremans M., Broodtaerts L., Smet L., Van Camp B. Colloidal gold as a marker for the light microscope detection of leukocyte cell surface antigens with monoclonal antibodies. J. Clin. Immunol. 1982, 2(3 Suppl), 24S—31S.

115. Hutchison N. J., Langer-Safer P. R., Ward D. C., Hamkalo B. A. In situ hybridization at the electron microscope level: hybrid detection by autoradiography and colloidal gold. J. Cell Biol. 1982, 95(2 Pt 1), 609—618, DOI: 10.1083/jcb.95.2.609.

116. Romano E. L., Stolinsky C., Hughes-Jones C. An antiglobulin reagent labeled with colloidal gold for use in electron micriscipy. Immunochem. 1974, 11, 521—522, DOI: 10.1016/0019-2791(74)90162-1.

117. Roth J. The preparation of protein A-gold complexes with 3 nm and 15nm gold particles and their use in labelling multiple antigens on ultra-thin sections. Histochem J. 1982, 14(5), 791—801, DOI: 10.1007/BF01033628.

118. Horrisberger M., Rosset J. Colloidal gold, a useful marker for transmission and scanning electron microscopy. J. Histochem. Citochem. 1977, 25, 295—305, DOI: 10.1177/25.4.323352.

119. Horrisberger M., Vonlanthen M. Simultaneous localization of hepatic binding protein specific for galactose and of galactose-containing receptors on rat hepatocytes. J. Histochem. Cytochem. 1978, 26, 960—966, DOI: 10.1177/26.11.722052.

120. Frens J. A device for quantative measuring of shivering in goats. Lab. Anim. 1973, 7(3), 287—288, DOI: 10.1258/002367773780944003.

121. Slot J. W., Geuze H. J. Sizing of protein A-colloidal gold probes for imunoelectron microscopy. J. Cell Biology. 1981, 90(2), 533—536, DOI: 10.1083/jcb.90.2.533.

122. Van den Pol A. N. Colloidal gold and biotin-avedin conjugates as ultrasructural markers for neural antigens. Quartery J. of Experimental Physiology. 1984, 69, 1—33, DOI: 10.1113/expphysiol.1984.sp002771.

123. Litchfield W. J., Freytag J. W., Adamich M. Highly sensitive immunoassays based on use of liposomes without complement. Clin. Chem. 1984, 30(9), 1441—1445.

124. Rongen H. A., Bult A., van Bennekom W. P. Liposomes and immunoassays. J. Immunol. Methods. 1997, 204(2), 105—133, DOI: 10.1016/s0022-1759(97)00041-0.

125. Compbeli R. L., Wagner D. B., O'Connel J. P. Solid phase assay with visual readout. US Patent 4703017A. 1987.

126. Remington J. S., Eimstad W. M., Aranjo F. G. Detection of immunoglobulin M antibodies with antigen-tagged latex particles in an immunosorbent assay. J. Clin. Microbiol. 1983, 17(5), 939—941, DOI: 10.1128/jcm.17.5.939-941.1983.

127. Sakai Y., Hibino M. Distribution-Analyzing latex immunoassay (DALIA): methods for determination of antigen and for elimination of non-specific reaction induced by rheumatoid factor. Chem. Pharm. Bull. 1989, 37, 11, 3010—3014, DOI: 10.1248/cpb.37.3010.

128. Liu Y. C., Dong G. J., Zhao Y. C. Preparation of diazotzed polystyrene latex and its use in agglutination assays. J. Immunol. Meth. 1989, 124, 159—163, DOI: 10.1016/0022-1759(89)90348-7.

129. Gerber M. A., Caparas L. S., Randolph M. F. Evaluation of a new latex agglutination test for detection of streptolysin O antibodies. J. Clin. Microbiol. 1990, 28(3), 413—415, DOI: 10.1128/jcm.28.3.413-415.1990.

130. Reichwein B., Jungkind D., Guardiani M., Gilbert R., Prosswimmer G., Amadio P. Comparison of two rapid latex agglutination methods for detection of group A streptococcal pharyngitis. Am. J. Clin. Pathol. 1986, 86(4), 529—532, DOI: 10.1093/ajcp/86.4.529.

131. Fujikawa S., Ohkuni M. A new latex agglutination test for rapid diagnosis of group A streptococci. Jpn. Circ. J. 1986, 50(12), 1251—1252, DOI: 10.1253/jcj.50.1251.

132. Chang M. J., Mohla C. Ten-minute detection of group A streptococci in pediatric throat swabs. J. Clin. Microbiol. 1985, 21(2), 258—259, DOI: 10.1128/jcm.21.2.258-259.1985.

133. Hanlon J. T., Caiola S. M., Muhlbaier L. H., Dennis B. H., Edelman D. A., Dingfelder J. R. An evaluation of the sensitivity of five home pregnancy tests to known concentrations of human chorionic gonadotropin. Am. J. Obstet. Gynecol. 1982, 144(7), 778—782, DOI: 10.1016/0002-9378(82)90351-9.

134. Imai S., Kouda Y., Nishihara T., Kinoshita M. Immunochromatographic assay with improved colored latex. US Patent 5266497A. 1991.

135. Kojima T., Arai M., Sadamoto S., Ikedo M., Yui I. Evaluation of the diagnostic reagents which detect group A Streptococcus with the immunochromatographical method. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi. 2002, 12(2), 91—95.

136. Ehrlich T. P., Schwartz R. H., Wientzen R., Thorne M. M. Comparison of an immunochromatographic method for rapid identification of group A streptococcal antigen with culture method. Arch. Fam. Med. 1993, 2(8), 866—869, DOI: 10.1001/archfami.2.8.866.

137. Yatsyshina S. B., Kulichenko T. V., Artemova I. V., Rybalka O. V., Elkina M. N. Use of immunochromatographic tests in a laboratory diagnostic algoritm for influenza. Epidemiology and infectious diseases. Current issues. 2018, 1, 48.

138. Mitamura K., Yamazaki M., Ichikawa M, Kimura K., Kawakami C., Shimizu H., et al. Evaluation of an immunochromatography test using enzyme immunoassay for rapid detection of influenza A and B viruses. Kansenshogaku Zasshi. 2004, 78(7), 597—603, DOI: 10.11150/kansenshogakuzasshi1970.78.597.

139. May K. Home tests to monitor fertility. Am. J. Obstet. Gynecol. 1991, 165(6Pt2), 2000—2002, DOI: 10.1016/s0002-9378(11)90566-3.

140. Nielsen M. S., Barton S. D., Hatasaka H. H., Stanford J. B. Comparison of several one-step home urinary luteinizing hormone detection test kits to OvuQuick. Fertil. Steril. 2001, 76(2), 384—387, DOI: 10.1016/s0015-0282(01)01881-7.

141. Vartdal F., Gaudernack G., Funderud S., Bratlie A., Lea T., Ugelstad J., et al. HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation--a fast and reliable technique. Tissue Antigens. 1986, 28(5), 301—312, DOI: 10.1111/j.1399-0039.1986.tb00500.x.

142. Lie O., Vartdal F., Funderud S., Gaudernack G., Olsaker I., Freysadal E., et al. Immunomagnetic isolation of cells for serological BoLA typing. Anim. Genet. 1988, 19(2), 75—86.

143. Khramtsov P., Kropaneva M, Byzov I., Minin A., Mysik A., Timganova V., et al. Conjugation of carbon coated-iron nanoparticles with biomolecules for NMR-based assay. Colloids and Surfaces B: Biointerfaces. 2019, 176, 256—264, DOI: 10.1016/j.colsurfb.2019.01.009.

144. Khramtsov P., Barkina I., Kropaneva M., Bochkova M., Timganova V., Nechaev A., et al. Magnetic Nanoclasters Coated with Albumin, Casein and Gelatin: Size Tuning, Relaxivity, Stability, Protein Corona, and Application in Nuclear Magnetic Resonance Immunoassay. Nanomaterials. 2019, 9, 1345, DOI: 10.3390/nano9091345.

145. Khramtsov P., Kropaneva M., Bochkova M., Timganova V., Zamorina S., Rayev M. Solid-phase nuclear magnetic resonance immunoassay for the prostate-specific antigen by using protein-coated magnetic nanoparticles. Microchim. Acta. 2019, 186(12), 768, DOI: 10.1007/s00604-019-3925-4.

146. Khramtsov P., Kropaneva M., Bochkova M., Kiselkov D., Timganova V., Zamorina S., et al. Nuclear magnetic resonance immunoassay of tetanus antibodies based on the displacement of magnetic nanoparticles. Anal. Bioanal. Chem. 2021, 413(5), 1461—1471, DOI: 10.1007/s00216-020-03112-7.

147. Mitani K., Une H. Dyed inorganic composite particles bound with immunoreactive substances for use in microtiter tests. Europ. Pat. 250700A2. 07.01.88.

148. Nilsson K. G. I. Preparation of nanoparticles conjugated with enzyme and antibody and their use in heterogeneous enzyme immunoassay. J. Immunol. Meth. 1989, 122, 273—277, DOI: 10.1016/0022-1759(89)90274-3.

149. Wechers J. H., Van Es R. M., Keiser G. D., Van Doorn A. W. J., Van Gelder W. M. J. Stained sols of non-metallic elements or compounds, their preparaition and use. Europ. Pat. 89202883.8. 30.05.90.

150. Haun M., Wasi S. Biotinylated antibodies bound to streptavidin beads: a versatile solid matrix for immunoassays. Analyt. Biochem. 1990, 191, 337—342, DOI: 10.1016/0003-2697(90)90228-2.

151. Zelikman I., Hjerten S. Protein immunoassay based on agglutination of antibody-coated ferric oxide particles. J. Immunol. Meth. 1988, 114, 267—273, DOI: 10.1016/0022-1759(88)90183-4.

152. Hirschfeld T. Dye target reagent. US Patent 4166105. 28.08.1979.

153. Tarcha P. J., Wong M., Donovan J. J. Indicator reagents, diagnostic assays and kits employing organic polymer latex particles. Europ. Patent 89116594.6. 28.05.1990.

154. Russel J. C., Yang H., Yost D. A. Method for determinig sample analyte amount-involves reacting sample with colloidal non-metal particles before optical measurement of analyte-particle complexes. Europ. Patent 298368A. 11.01.89.

155. Spallholz J. E. Stable isotopic immunoassay method employing nonradioactive selenium label. US Patent 4341757A. 27.07.82.

156. Van Es R. M., Keiser G. D., Van Doorn A. W.J. Method for an increased visualisation of the reacthion product of a specifically binding substance and a corresponding bindable substance, and kit therefore. Europ. Patent 89202884.6. 23.05.90.

157. Wechers J. H., Van Es R. M., Keiser G. D., Van Doorn A. W. J., Van Gelder W. M. J. Stained sols of non-metallic elements or compounds, their preparaition and use. Europ. Patent 89202883.8. 30.05.90.

158. Yost D. A., Russel J. C., H. Yang H. Non-metal colloidal particle immunoassay. Europ. Patent 0298368A2. 11.01.89.

159. Gribnau Th. C. J., Roeles F., Leuvering J. H. W. The application of water-dispersible hydrophobic dyes as labels in immunoassays. Europ. Patent 80201241.9. 02.05.85.

160. Snowden K., Hommel M. Antigen detection immunoassay using dipsticks and colloidal dyes. J. Immunol. Meth. 1991, 140, 57—65, DOI: 10.1016/0022-1759(91)90126-Z.

161. Xie Y., Xue C., W. Lou W. Establishment and application of dipstick sandwich colloidal dye immunoassay for circulating antigen detection in schistosomiasis patients. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 1999, 17(3), 129—131.

162. Xiang X., Tianping W., Zhigang T. Development of a rapid, sensitive, dye immunoassay for schistosomiasis diagnosis: a colloidal dye immunofiltration assay. J. Immunol. Methods. 2003, 280(1—2), 49—57, DOI: 10.1016/s0022-1759(03)00196-0.

163. Xiao X., Wang T., Ye H., Qiang G., Wei H., Tian Z. Field evaluation of a rapid, visually-read colloidal dye immunofiltration assay for Schistosoma japonicum for screening in areas of low transmission. Bull. World Health Organ. 2005, 83(7), 526—533.

164. Adamov A., Agafonov V. Suspension antigens, antibodies and immunosorbents. Medicine: Moscow. 1969.

165. Gribnau Th. C. J., Van Sommeren A., Van Dinther F. DIA-disperse dye immunoassay. Affinity Chromatogr. Biol. Recognit. Proc. Int. Symp. 1983, 5, 375—380.

166. Anderson R. R. A solid phase system incorporating tetrazolium salts for use in ligand-receptor assays. Europ. Patent 253578A2. 20.01.88.

167. Rounds D. E. Immunoassay utilizing formazan-prelabeled reactants. US Patent 4786589A. 22.11.88.

168. Szewczuk A., Kuropatwa M., Rapak A. The use of antibodies labelled with dyes for fast and simple assay of some human proteins by immunofiltration technique. Arch. Immunol. Ther. Exp. 1992, 40, 325—329.

169. Kang J., Youn B., Oh Y. H. Carbon black immunochemical label. US Patent 5252496A. 12.10.1993.

170. Van Doorn A. W. J. Immunodetermination using non-metallic labels. Europ. Patent 88202597.6. 21.06.89.

171. Rayev M. B., Ambrosov I. V., Briko N. I. A Novel Method for the Serodiagnosis of Group A Streptococcal Antibodies. Streptococci and the Host. Ad. Thea Horaud et al. Advances in Experimental medicine and biology. Plenum Press: New York and London. 1997, 418, 327—329.

172. Rayev M., Shmagel K. Carbon-protein covalent conjugates in noninstrumental immunodiagnostic systems. Journal of Immunological Methods. 2008, 336, 1, 9—15, DOI: 10.1016/j.jim.2008.03.005.

173. Khramtsov P., Bochkova M., Timganova V., Zamorina S., Rayev M. Dot immunoassay for the simultaneous determination of postvaccination immunity against pertussis, diphtheria, and tetanus. Anal. Bioanal. Chem. 2017, 409(15), 3831—3842, DOI:10.1007/s00216-017-0327-5.

174. Khramtsov P., Kropaneva M., Kalashnikova T., Bochkova M., Timganova V., Zamorina S., Rayev M. Highly stable conjugates of carbon nanoparticles with DNA aptamers. Langmuir. 2018, 34(35), 10321—10332, DOI: 10.1021/acs.langmuir.8b01255.

175. Kropaneva M., Khramtsov P., Bochkova M., Lazarev S., Kiselkov D., Rayev M. Vertical Flow Immunoassay Based on Carbon Black Nanoparticles for the Detection of IgG against SARS-CoV-2 Spike Protein in Human Serum: Proof-of-Concept. Biosensors. 2023, 13, 857, DOI: 10.3390/bios13090857.

176. Khramtsov P., Bochkova M., Timganova V., Zamorina S., Rayev M., Kiselkov D. Albumin nanoparticles loaded with hemin as peroxidase mimics for immunoassay. Chemistry Select. 2022, 7, 3, e202103892, DOI: 10.1002/slct.202103892.

177. Khramtsov P., Kropaneva M., Bochkova M., Zamorina S., Rayev M., Timganova V., et al. Prussian blue nanozymes with enhanced catalytic activity: size tuning and application in elisa-like immunoassay. Nanomaterials. 2022, 12, 10, 1630, DOI: 10.3390/nano12101630.

178. Khramtsov P, Kropaneva M, Bochkova M, Timganova V, Kiselkov D, Zamorina S, et al. Synthesis and Application of Albumin Nanoparticles Loaded with Prussian Blue Nanozymes. Colloids and Interfaces. 2022, 6(2), 29, DOI: 10.3390/colloids6020029.

179. Oh Y. K., Joung H. A., Kim S., Kim M. G. Vertical flow immunoassay (VFA) biosensor for a rapid one—step immunoassay. Electronic Supplementary Material (ESI) The Royal Society of Chemistry. Lab Chip. 2013, 13, 768—772, DOI: 10.1039/C2LC41016H.

180. Plaksin D., Rayev M., Gromakovskaya E. Method of stereospecific analysis and method of obtaining a conjugate for stereospecific analysis. Patent of the Russian Federation No.2089912 of 10.09.1997. Bulletin “Inventions”. 1997, 25, 336.

The author

Mikhail B. Rayev, Associate Professor, PhD, Doctor of Biological Sciences, Head of Laboratory of Cellular Immunology and Nanobiotechnology of the Institute of Ecology and Genetics of Microorganisms of the Russian Academy of Sciences — branch of the Perm Federal Research Center of the Ural Branch of the Russian Academy of Sciences, Russia.

ORCID: 0000-0001-6882-4928

Об авторе

Михаил Борисович Раев, доцент, доктор биологических наук, заведующий лабораторией клеточной иммунологии и нанобиотехнологии Института экологии и генетики микроорганизмов Уральского отделения Российской академии наук» — филиала Пермского федерального исследовательского центра Уральского отделения Российской академии наук, Россия.

ORCID: 0000-0001-6882-4928