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IMMUNOCHROMATOGRAPHIC FOOD CONTROL TOOLS: NEW DEVELOPMENTS AND PRACTICAL PROSPECTS

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Abstract
In the modern food production technologies, the tools and means of simple and rapid testing raw materials, intermediate products and the final ready-to-consume food products are in high demand. This monitoring allows determining the content of toxic and pathogenic contaminants and confirms the compliance of the objects being tested with the established regulatory requirements. Mobile tests tools and means (so called test systems) provide the opportunity of wide range monitoring without involving the specialized laboratories and highly qualified specialists. Thus, test systems for detection of toxic and pathogenic contaminants serve as the useful addition to confirming instrumental analytical methods. An actively developing approach for this field testing is the using of immunochromatographic test strips, in which strips all the necessary reagents are applied to the membrane components of the analytical system. Contact of the test strip with the sample being tested, initiates all further interactions and generates the recordable or visually assessable optical signal. The market of test systems based on immunochromatographic analysis is constantly growing, thus offering the permanently widening choice of solutions. However, in recent years there has been a real boom of new developments in immunochromatography field, thus offering various options for highly sensitive and information capacitive analytical systems. This study systematizes these developments and provides their comparative assessment in terms of prospects for their technological implementation and practical application in the coming years. The opportunities of designing the antibodies and alternative receptor molecules for controlling the affinity and the selectivity of recognition of the compounds being monitored are considered. The advantages and limitations of the new nanodispersed markers and non-optical methods for their registration in immunochromatography are discussed. The methods for quantitative assessment of the contaminants content via immunochromatography are characterized. The developed design options of the test systems for multiplex control — simultaneous detection of several compounds — are presented. Examples of integration of immunochromatographic tests with the systems of automatic registration, processing, transfer, storage and analysis of results of numerous tests are represented.

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Introduction

New data on the factors, which affect the quality and safety of food products, expand the range of contaminants that require control — natural and man-made compounds, as well as the microorganisms. Timely detection of these substances in food products allows for the effective prevention of negative consequences for the consumers' health, like acute poisoning, various diseases and long-term physiological disorders.

The public health protection is ensured by a set of analytical monitoring methods [1,2] made of two levels with different methodologies and instruments. Thus, when characterizing contamination of food products with toxic compounds, methods of primary screening testing and confirmatory identification characteristics are used [1,3]. At the first level, immunochemical and other bioreceptor methods based on the specific binding of controlled compounds by complementary biomolecules — antibodies, proteins of other classes, oligonucleotide receptors (aptamers), etc. — are widely represented [4,5]. The second level of the control involves the substantiated identification of the molecular structures of the detected contaminants. For this purpose, mass spectrometry or other means of detailed characterization of fragments (components) of

Table 1. Comparative evaluation of chromatography and immunoassay

	Chromatographic methods for monitoring toxic contaminants	Immunochemical methods for monitoring toxic contaminants
Instrumental implementation	Stationary equipment for specialized laboratories	Small devices for mass testing
Characteristics of the results obtained	Sample components are separated and identified individually	Target compounds bind selectively to the receptor. The resulting complex is detected
Advantages	➢ High sensitivity ➢ Unified procedures ➢ Grounded identification ➢ Supporting conclusions	➢ Quick testing ➢ Simple procedures ➢ Low cost ➢ Wide screening

target molecules and reconstruction of their structure are usually used [6,7].

These two groups of analytical approaches differ in their principles and, as a consequence, in the measurement results. Therefore, they cannot be considered as competitors or potential replacements for each other. Table 1 summarizes the main differences between chromatographic and immunochemical analytical methods.

It is extremely important that the methods of each control level ensure high efficiency, productivity and a minimum number of false positive and false negative results. This review presents the modern capabilities of immunochromatographic analytical systems (lateral flow test strips). These test systems are promising and actively developing means of preliminary screening control of toxic and pathogenic contaminants of food products. In addition, immunochromatographic test systems successfully detect various biomarkers that are significant for other areas — medicine, veterinary, agriculture, monitoring the state of environmental objects and biosafety [8].

Objects and methods

The object of the study was domestic and foreign developments in the field of immunochromatographic control of toxic and pathogenic contaminants of food products, described in articles and patents. The area of research included modern developments of immunochromatographic systems and methods for their use. The search was carried out in the ScienceDirect, PubMed, Google Scholar, eLibrary databases and other open electronic sources. The following keywords were used: immunochromatography, lateral flow assay, membrane test systems, nanodispersed markers, antibody–nanoparticle complexes, registration of labeled immune complexes. The keywords were used in English and Russian versions in a general search of databases and in groups of works related to the control of major food contaminants. In addition, thematically related articles were searched for by citation chains. Non-peer-reviewed, uninformative and duplicate sources, as well as sources that are indirectly related to the research topic, were excluded from the search results. For general assessments, we mainly used recent (last 5 years) review publications, as well as descriptions of experimental developments containing unambiguous quantitative comparative assessments of traditional and proposed new immunochromatographic systems.

General principles of immunochromatographic test systems arrangement and operation

In monitoring food technologies and final products, two groups of test systems based on antibody (immune) recognition of target objects are actively used — microplate enzyme immunoassay (EIA) kits and immunochromatographic tests. To date, immunochromatography is the most successful from the known analytical methods, effectively adapted to field testing without the involvement of equipment and other additional resources [9,10].

Figure 1 shows the processes occurring during immunochromatography. All reagents required for selective detection of controlled toxic or pathogenic objects are pre-applied and dried in certain areas of membrane components of the test strip. As a result, contact of the test strip with the liquid sample initiates:

1) movement of the sample along the membranes of the test strip;
2) interactions of an analyte potentially present in the sample with specific immunoreagents;
3) as a result, the formation of labeled specific immune complexes in certain areas of the test strip.

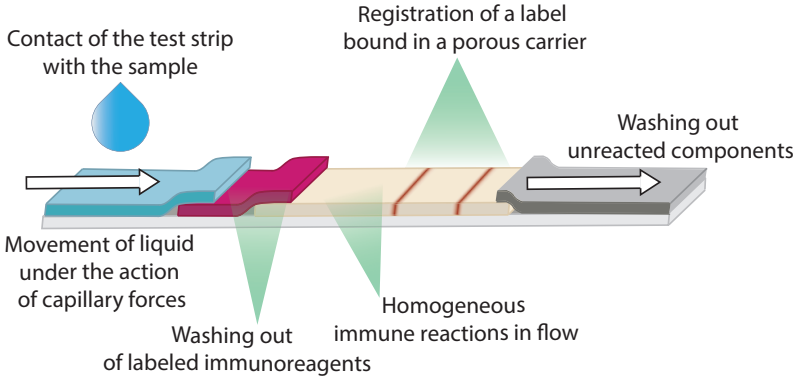

Figure 1. Scheme of the immunochromatographic test system and the processes occurring in it

The presence or absence of these complexes in the simplest version is controlled visually — by a colored label included in their composition. The corresponding coloring allows, based on the testing results, to make a prompt conclusion about the presence or absence of the controlled compound in the sample.

The most well-known problems solved using immunochromatography are pregnancy detection by changes in the concentration of chorionic gonadotropin, for which the very first tests were proposed [11], and monitoring antibodies to the causative agent of COVID-19, for which immunochromatography was successfully applied only recently [12]. This method is in high demand in medical diagnostic practice, which determines priority analytes and forms the basis for commercial demand for immunochromatographic test systems. In addition, immunochromatography is increasingly used in veterinary medicine, environmental monitoring, and food control [8]. However, an assessment of the prospects for technical re-equipment of this method should, first of all, focus on the current tasks of the largest consumers of test systems — medical diagnostics.

Despite the successful technological implementation of the immunochromatography principle, increase in publications describing its new variants occurs in recent years (Figure 2). These articles are focused, as a rule, not on extensive expansion (the application of known methods to new compounds), but on the description of the proposed changes in the testing format. This situation is not typical. As a rule, after the transition of new technical solutions to mass production, their improvement is fixed in the form of know-how or other documents protecting intellectual property. In the case of immunochromatography, there is a boom in new scientific is observed. It is clear that the significance of emerging scientific publications is not the same. Most of the author's proposals remain at the level of laboratory prototypes. However, research activity in the field of immunochromatography deserves attention primarily for assessing what immunochromatographic tests users can expect in the near future, which of the new ideas have the greatest chances of being transformed into reproducible and universal technological solutions.

The proposed review is based on a comparative analysis of the practical prospects of the developments according to uniform criteria, rather than on subjective assessments. What issues will need to be addressed to move from laboratory prototypes to mass production and use of test systems? We analyze which ideas are easily integrated with existing technologies, and which require more complex adaptation. Without pretending to make unambiguous forecasts (the success of implementation depends on many factors), we believe that this critical review of new developments will help users prepare for the analytical solutions of the future.

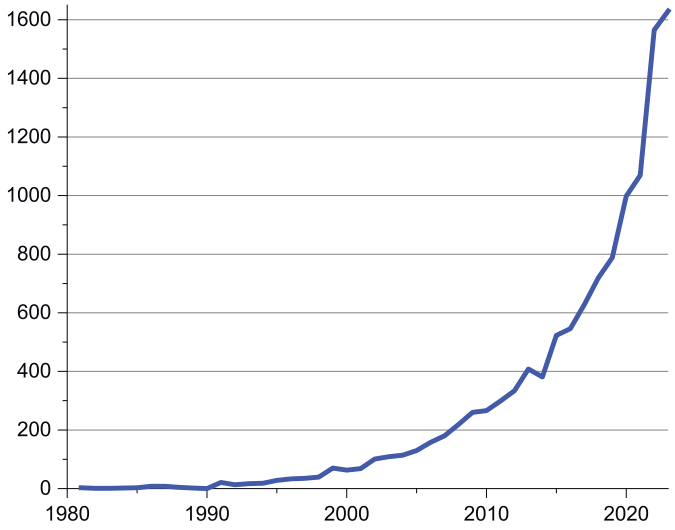

Figure 2. Dynamics (1980–2022) of Scopus indexed publications that meet the Theme criterion: (immunochromatogr* or (lateral and flow and immun*)). The information by state as of 20.10.2024

Main groups of developments for improving immunochromatographic analytical systems

It should be recognized that the same methodological solutions that provided the basic advantages of immunochromatography are currently becoming limitations in the progress of its capabilities. Table 2 summarizes the relationships between the advantages and disadvantages of immunochromatography.

It is clear from Table 2 that proposals for the progress of immunochromatographic test systems cannot be limited to one of their components, which limits the effectiveness of the test systems. Depending on the specific tasks to be solved, needs to change one or another design features of the test systems arise. In this regard, it seems convenient to divide new developments in the field of immunochromatography into five groups, shown in Figure 3. These groups simultaneously reflect both the successive stages of the testing procedure and the directions of changes in

Table 2. The main properties of immunochromatographic test systems, considered as their advantages and limitations

Property	Positive assessment	Negative assessment
All reagents are applied to the membranes of the test strip	Simple completion	Limited number of reagent combinations
Contact of the test strip with the sample initiates all subsequent processes	Easy-to-use	The reactants interact in a strictly fixed order
The duration of the analysis is determined by the movement of liquid along the pores of the membranes	Quick testing	The interactions of the reactants may not reach equilibrium
The colored label in the composition of specific complexes can be registered visually	Using outside the laboratory, without instrumentation support	Subjectivity of results assessment
Specific complexes can be detected immediately after their formation	Quick obtaining of results	No provision for signal development/ amplification

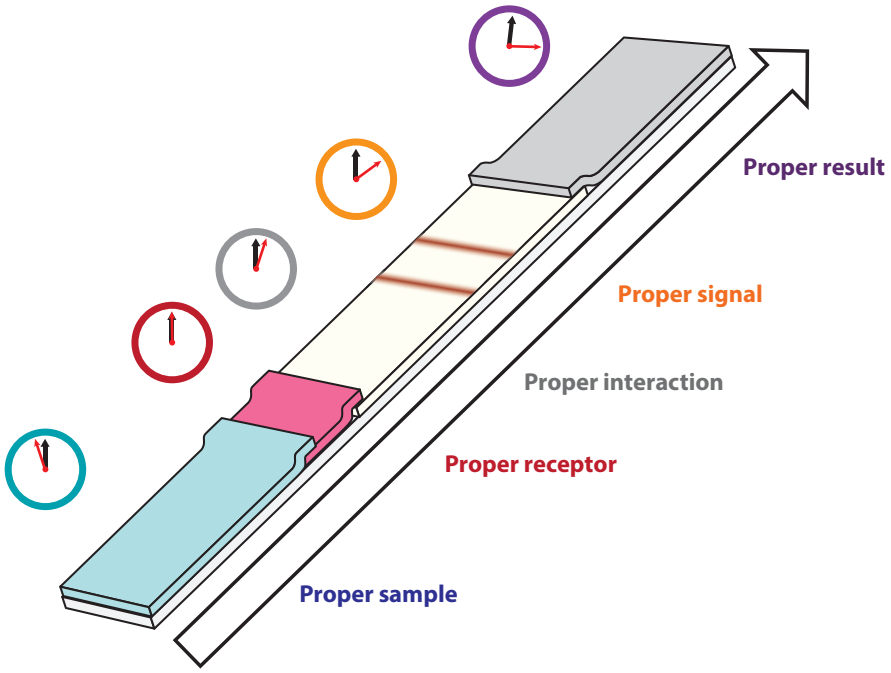

Figure 3. Five groups of methods of impact on the parameters of immunochromatography, localized in time and space

the components of the test systems or processes occurring during immunochromatography.

The analytical characteristics of test systems can be improved by changing the sample preparation, the receptor reagent used, the conditions of immunochromatographic interactions, signal generation, and the processing and interpretation of results. In general, approaches belonging to different groups can be effectively combined within a single test system. However, if we are talking about changes in the same stage of immunochromatography, then, as a rule, it is necessary to choose one of the proposed changes or, based on several ideas, to construct a new approach with possible additional features.

Most of the proposed developments are aimed at lowering detection limits. It should be noted that even with established and controlled maximum permissible levels of contaminants contents, test systems that detect the contaminants in significantly lower concentrations are in demand. They allow for simplified handling with samples and increased reliability of the results obtained. In addition, a lowering in the detection limit is usually accompanied by increased accuracy in measuring analyte concentrations.

However, quite often the improvements declared in publications are disproportionately greater than could be expected from the changes made. For example, with a small increase in the intensity of label staining, a decrease in the detection limit by tens to hundreds of times is described. It is important to take into account that the detection limits of immunoassay systems have theoretical limitations described in the previous century by Jackson and Ekins [13]. Therefore, achieving record levels should be associated with the use of either extremely high-affinity interactions (high binding constants in the antigen-antibody reaction) or methods for generating a detectable signal in the analysis that combine a high response per unit complex formed with extremely high reproducibility of measurements [14]. Confirmed super-record sensitivities [15,16] should be accompanied by fundamental changes in testing methods that ensure the possibility of overcoming the calculated chemical-kinetic thresholds.

Effective sampling and sample preparation techniques

It should be recognized that immunochromatographic tests, initially proposed for work with biological fluids (urine, blood), are most effectively compatible with liquid samples. Target compounds are uniformly distributed in the volume of such samples, and matrix components that can potentially affect the movement of liquid along the test strip and the implementation of specific interactions during this movement are usually successfully separated. This separation is ensured either by means of selected membrane elements of the test strip or by simple and rapid actions directed to the original sample, such as filtration or chemical precipitation of some of its components. Dilution of liquid samples is often proposed as a sample preparation. This idea is useful as a way to introduce components into the reaction mixture that promote washing out and uniform movement of immunoreagents on the membrane, as well as preventing non-specific interactions. However, significant dilution of samples reduces the sensitivity of testing (in terms of the unit volume of the original sample) and therefore cannot be considered a good solution [17].

When moving to solid samples, we should take into account the uneven distribution of contaminants in them and the regulatory requirements for sampling procedures that should compensate for this unevenness. Therefore, testing is forced to lose the compactness characteristic of

traditional immunochromatography variants. It is important that the complication of sampling and sample preparation does not lead to the loss of the advantages provided by subsequent testing. The requirements for simple and rapid analytical procedures should still be met [18]. For this purpose, sample preparation methods with rapid destruction of solid components and effective release of target compounds are being developed. The transfer of traditional extraction protocols with evaporation recommended for chromatographic analytical methods can hardly be considered acceptable, in contrast to protocols with rapid separation of the extract and solid components. However, when proposing new sample preparation methods, it is necessary to additionally confirm that they ensure complete extraction of target compounds.

It should be noted that in some cases there is no need for a strict quantitative assessment of the degree of contamination. In this case, simple methods that extract most (but not necessarily 100%) of the contaminant from samples are acceptable. In this regard, the idea of the participants of the recent European MycoKey project, who developed a compact kit for testing grain contamination with mycotoxins, seems beautiful and promising. It uses a compact vacuum cleaner to collect microparticles from the surface of grains. These particles contain toxigenic mold fungi that produce mycotoxins. The obtained test results are close to the data of generally accepted extraction protocols [19].

From the point of view of practical prospects, new immunochromatographic systems for monitoring food toxicants with a modified sample preparation stage should be assessed primarily based on whether they retain rapidity and ease of use. These requirements are taken into account when monitoring the quality of liquid food products, including commercially available immunoanalytical systems. Analysis of solid food products will require the additional instrumental support. At the same time, the question of effective and universal solutions acceptable for technological implementation remains open today.

Recognition of structurally related analytes (selectivity management)

Immunochromatographic testing involves the use of antibodies as bioreceptor molecules that selectively bind target compounds. Natural combinatorial mechanisms allow the creation of active sites of antibody capable of binding to various compounds with high affinity and specificity. However, the complexity of food contaminant control is due to the potential presence of structurally similar compounds in the tested samples — the original techno- or biogenic contaminants, the products of their transformation in living organisms and in the environment. Designing antibodies that recognize each such substance is difficult and often impossible. In addition, the need to work with a wide range of immunoreagents of different selectivity during testing increases the cost of analysis and makes it more complex.

As a rule, one of the requirements is set for immunoassay of structurally related compounds: either individual or group-specific testing. In the first case, antibodies are selected that recognize one substance — the most common, the most dangerous, etc., while binding to other substances of this family is minimized. In the second case, antibodies should bind the maximum number of toxic representatives of the controlled family of substances with comparable efficiency.

Initially, various approaches to the design of derivatives (haptens) used in the synthesis of immunogens were applied to manage the selectivity of antibodies [20,21]. In some cases, combinations of different haptens during immunization and analysis make it possible to significantly change the selectivity of testing and bring it closer to practical requirements [22,23]. Moreover, the selectivity of test systems is not a quantitative parameter that strictly corresponds to differences in the affinity of interaction of antibodies with different haptens. It has been shown that by varying the concentrations and ratios of reagents in a competitive immunoassay, the assay can be made either more highly specific or broadly specific [24]. This approach is most effective for nonequilibrium analytical methods, which include immunochromatography.

Another promising resource is immunodetection of small molecules using non-competitive interaction schemes. When selecting schemes for conducting immunoassay of low-molecular compounds, it is taken into account that binding of two antibody molecules to these substances is impossible due to steric hindrances. Therefore, so-called sandwich schemes with the formation of antibody-antigen-antibody complexes are excluded for them and competition between the antigen in the sample and the antigen-protein conjugate introduced into the system for binding to antibodies is realized. Unfortunately, for competitive immunochromatography schemes, detection of low analyte concentrations is pretty impeded. This leads to a slight decrease in the degree of binding of labeled antibodies to the antigen-protein conjugate, which within one test strip cannot be compared with binding in case of the analyte absence [14]. To overcome these limitations, variants of the so-called open sandwich and other immunoassay formats have been proposed [25,26]. Thus, the formation of an antibody-antigen complex and the recognition of this complex (but not its components) by the second antibody makes it possible to obtain direct dependences of the recorded signal on the concentration of the analyte and thereby eliminate the difficulties of competition described above.

In recent years, based on the established approaches to obtaining recombinant antibodies, computational methods for molecular design of their antigen-binding sites have been developed. As a result of these calculations and subsequent genetic substitutions, it is possible to increase affinity and eliminate undesirable cross-interactions [27–29]. Such improved antibody preparations may be a promising tool for immunochromatography.

From the point of view of practical application, the approaches described in this section do not require fundamentally new technological solutions and can be applied quite quickly to improve existing test systems. The only significant point that should be noted is the difference in the immobilization conditions on marker nanoparticles for recombinant and natural full-length antibodies.

Replacement of antibodies with alternative receptor molecules

Of interest are the developments of membrane test systems — analogues of immunochromatographic ones, in which other bioreceptors are used instead of antibodies. The most actively considered in this regard are oligonucleotide receptors — aptamers, which have already been selected for thousands of practically in-demand compounds and are successfully used in various analysis formats [30–33]. Researchers note a number of indisputable advantages of aptamers in comparison with antibodies:

- low cost of production;
- high degree of reproducibility of the properties of different drugs;
- ease of chemical modification with fixed stoichiometry of products;
- stability over a wide range of conditions, including thermal stability.

These advantages certainly deserve to be applied in new analytical systems. Despite the fact that a large number of prototype aptachromatographic test systems have already been described, they have not become the subject of wide manufacture. To a large extent, this is due to the difference in the structures of the analyte binding sites: conformationally stabilized components of the protein globule in the case of antibodies and very labile short nucleotide chains in the case of aptamers. This lability limits the efficiency of aptamer-analyte interactions when they are transferred to the kinetic regime and carried out in the near-surface flow in membrane pores. To realize the analytical potential of aptamers in membrane tests, it is apparently necessary:

- more detailed characterization of their structure and reactivity under different conditions;
- adaptation and modification of existing solutions for immobilization and stabilization of test system components in relation to aptamers.

It is also necessary to mention molecular reagents as promising analytical reagents. Molecularly imprinted polymers (MIPs) are the preparations obtained by polymerization of a mixture of monomers in the presence of target molecules for detection and subsequent leaching of the analytes. The polymer structure forms niches for analyte binding, which are characterized by geometric complementarity and affinity interaction of individual groups of the polymer and analyte. Traditionally, MIPs were used in columns for preliminary enrichment of tested samples. However, recent developments in the field of surface imprinting and the demonstrated capabilities of highly selective detection of molecules of different structures presenting in complex matrices indicate the prospects of MIPs as components of various bioanalytical systems [34,35]. Although the appearance of such commercial products is still a long way off, the extreme cheapness and stability of MIPs determine the advisability of continuing developments in this direction.

Variation of interaction conditions during immunochromatography

Traditional immunochromatography is based on the use of a standard set of reagents — a nanoparticles — antibodies conjugate and binding reagents in the test and control zones. In this case, the production of test systems includes the same actions for applying reagents to membranes, and in immunochromatography, the same order of processes is carried out, determined by the washing out and movement of specific reagents along the membranes.

However, such a standard order may not be optimal for a number of analytical tasks. Often users are ready to partially sacrifice rapidity and, by extending the testing by several minutes, obtain a significant (up to one or two orders of magnitude) gain in the detection limit. In this regard, the typical order of interaction in a number of new developments varies:

- the reaction of the analyte with the antibody-label conjugate is implemented outside the test strip and is carried out in a separate test tube, where, under homogeneous conditions, the formation of the immune complex is significantly accelerated [36,37];
- instead of directly conjugating specific antibodies with a label, a combination of native specific and conjugated anti-species antibodies is used, which allows for independent variation of the content of specific antibodies (for effective competition) and label (for an intense signal) in the system [38,39];
- the dynamics of release of reagents applied to membranes is modulated by the addition of various slowly soluble compounds [40,41];
- the reagents are applied to the working membrane, performing a barrier function and modulating the speed of movement of reagents or focusing them in certain areas of the membrane [42,43];
- to form immune complexes, several successive stages of movement of different immunoreagents along the test strip are used [44,45];
- to control the movement of reagents and their effective removal from the pores of the working membrane, test strips are fixed on microrotor systems [46,47];
- traditional test strip geometry is integrated with the additional means for concentrating sample components from a large volume [48–50].

The most critical issue in the scalable implementation of these ideas is the reproducibility of analytical procedures. In addition, a number of developments involve complicating the procedure for manufacturing test systems with

strict orientation of the application of reagents within a separate test strip.

Markers for immunochromatography

Nano- and submicron-sized particles are used in immunochromatography as carriers for immunoreagents and detectable markers. This choice simplifies analytical methods, allowing the formation of immune complexes on membranes to be recorded in real time — by an optical or other signal from the bound marker. Larger total area of the particle preparations accelerates interactions on their surface and ensures rapid testing.

The markers in mass-produced test systems are essentially limited to gold nanoparticles and colored latex particles. Although significant lowering in the detection limit have been shown for a number of alternative markers, the relationships between particle parameters and test system characteristics using them are considered in scattered studies and are not presented as universal conclusions. The choices of particle sizes, the composition of their conjugates with antibodies, and the conjugation method are based on a limited number of recommendations, the scope of which remains a subject of debate. Therefore, when considering ultrafine particles as reagents for immunochromatography, the characterization of new preparations is in demand. A useful tool for achieving this goal is the passportization of the properties of candidate nanoparticles, presented in Table 3. It allows one to focus on a number of a priori known properties of the materials used. In addition, it allows excluding unwarranted hopes for preparations if their analytically significant characteristics do not undergo significant changes.

From a variety of candidates nanoparticles — immunochromatographic carriers and markers proposed in developments in recent years and characterized in review publications [51–54], we will highlight two groups that appear to be the most promising.

The first group — quantum dots — are semiconductor metal particles of the core-shell structure, characterized by stable fluorescence, the peak of which is determined by the particle size. The advantages of quantum dots in comparison with traditional colorimetric markers:

- proportional increase in signal with increasing excitation light at an extremely low background signal;
- stability of glow (no fading);
- common excitation light for simultaneously used particles of different sizes;
- possibility of multi-color multi-assay;
- the possibility to select the composition of quantum dots and their spectral characteristics when working with different matrices, excluding autofluorescence of sample components.

The combination of these advantages leads to a reduction in the detection limits of immunochromatography by several dozen times [55,56]. Note that the need for radiation generating fluorescence is not a critical complication. Such radiation can be provided by a variety of simple devices, including hand-held detectors of currency authenticity operating in the same ranges of excitation and emission light. To date, a number of portable systems for working with immunochromatographic tests based on quantum dots have been described. In them, background radiation is excluded to increase the accuracy of measurements (the test strip is placed in a closed chamber), and the signal is recorded by a portable detector or a smartphone camera.

Recent developments have led to commercially available nanoparticle preparations with hydrophilic coatings and reactive surface groups for conjugation with antibodies. These capabilities are extremely valuable for the technological implementation of new highly sensitive immunochromatographic systems.

The second group of promising carriers and markers are magnetic ultradisperse particles. As follows from Figure 4, their use in immunochromatography leads to the integration of a number of advantages:

- elimination of diffusion limitations for immune interactions;
- increasing the area of contact of analyte molecules with antibodies;
- collection of analyte molecules from a large sample volume;
- simple and rapid separation of immune complexes by applying an external magnetic field;
- easy removal of sample matrix components;
- concentration of the re-solubilized drug in a small volume before running the immunochromatography.

As a result, specific immune complexes can be registered on the test strip membrane both by the intrinsic optical properties of magnetite particles and by using complexes with chromogens — organic dyes or other functionalized nanoparticles. The gains achieved in the detection limits, determined primarily by the concentration factor, amount to about two orders of magnitude [57,58].

The most critical issue in creating such serial tests is the choice of conditions for the effective movement of magnetic particles along immunochromatographic membranes. Potentially, the magnetic particles can be modified

Table 3. Key characteristics for the evaluation of new marker particles for immunochromatography

Quantitative characteristics	Qualitative characteristics
Limit of detection of the marker from the unit volume	Price and availability
Limit of detection of the marker per unit area	Ease of conjugation
Number of markers attached to one immunoreagent	Stability under storage
Changes in marker signal during conjugation	No influence of samples' matrix on the marker signal

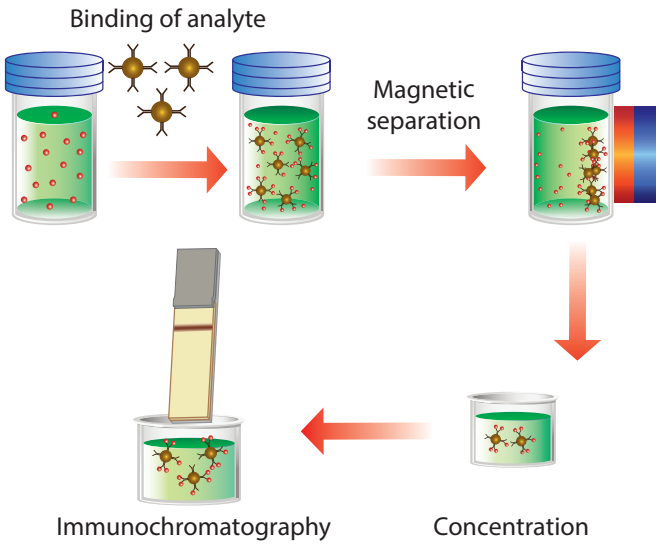

Figure 4. Integration of analytes' concentrating with the help of magnetic ultradisperse immunosorbents and immunochromatography

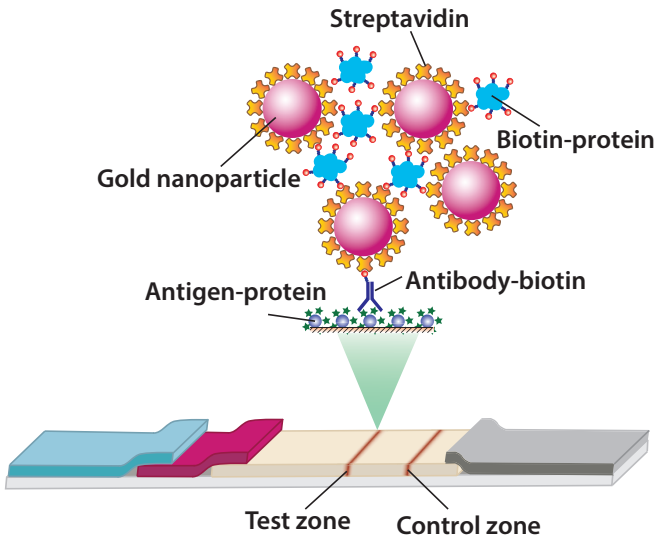

Figure 5. Signal amplification in immunochromatography via the functionalized nanoparticles aggregation

to form various surface coatings. However, the choice of the optimal coating for the immunochromatographic purposes has not yet been grounded.

Signal amplification methods in immunochromatography

A promising idea for reducing the detection limit in immunochromatography is to enhance the signal from the detectable marker after it has bound as part of the immune complex in a certain area of the test strip. However, the choice of marker is limited by the requirements for its size. Larger particles, although they give a larger registered signal, are less acceptable as participants in immunochromatography processes. With increasing size, the risks of incomplete washing out of the applied marker, its binding to the membrane before reaching the binding zone, etc. increase. However, we can carry out initial interactions with nanoparticles of a suitable size, and then increase them in the binding zone or ensure that they generate an additional amplifying signal.

Such amplifying processes can be the complex formation of several variants of conjugates. nanoparticles functionalized with complementary interacting combinations of molecules: biotin — streptavidin, additional pairs of antigens and antibodies, etc. The principle of such amplification is shown in Figure 5 and can provide gains in detection limits of up to two orders of magnitude.

Of the various options for increasing the size of nanoparticles, the most promising seems to be the reduction of metal salts on their surface. Such processes, quickly and effectively implemented on immunochromatographic membranes, include, in particular, the widely known silver mirror reaction. In recent years, many new solutions in the field of immunochromatography have proposed using nanozymes as markers — nanoparticles that have enzymatic activity. Advantages of nanozymes bioanalytical markers — ease of production and modification, low cost, high catalytic activity, stability during storage, resistance to inactivation. The most studied types of nanozyme catalysis are oxidase, peroxidase, catalase, superoxide dismutase, and for oxidation-reduction reactions that can be realized using a large number of chromogenic substrates. It should only be taken into account that the optimal conditions for transformations of chromogens (pH, composition of the reaction medium) can be different for natural enzymes and nanozymes. Literature data [59–64] allow asserting the possibility of using nanozyme amplification in 1–5 minutes to achieve a decrease in the detection limits of immunochromatography from one to three orders of magnitude.

When moving from laboratory prototypes to production technologies, the issue of reproducibility of amplification results becomes critical. Systems with multiple additional conjugates are associated with significant risks, since variability of results is significantly increased due to both differences in the properties of new reagents and discrepancies between strips in the dynamics and completeness of washout. For amplification by particle growth and nanozyme catalysis, the variants (not yet debugged) with amplifying reagents dried on additional membrane components, which are tightly pressed to the working membrane after immunochromatography, would be preferable.

Multianalytical immunochromatographic systems

An important opportunity to expand the information content of immunochromatographic testing is the simultaneous detecion of several compounds using one test strip. The use of several markers for this purpose, registered in one binding zone and distinguished by optical properties, is not very popular because minor nonspecific signals can significantly worsen the differentiation and quantitative assessment of specific signals. Variants with geometric separation of binding zones of different specificity are preferable for this purpose [65–68]. In this case, different

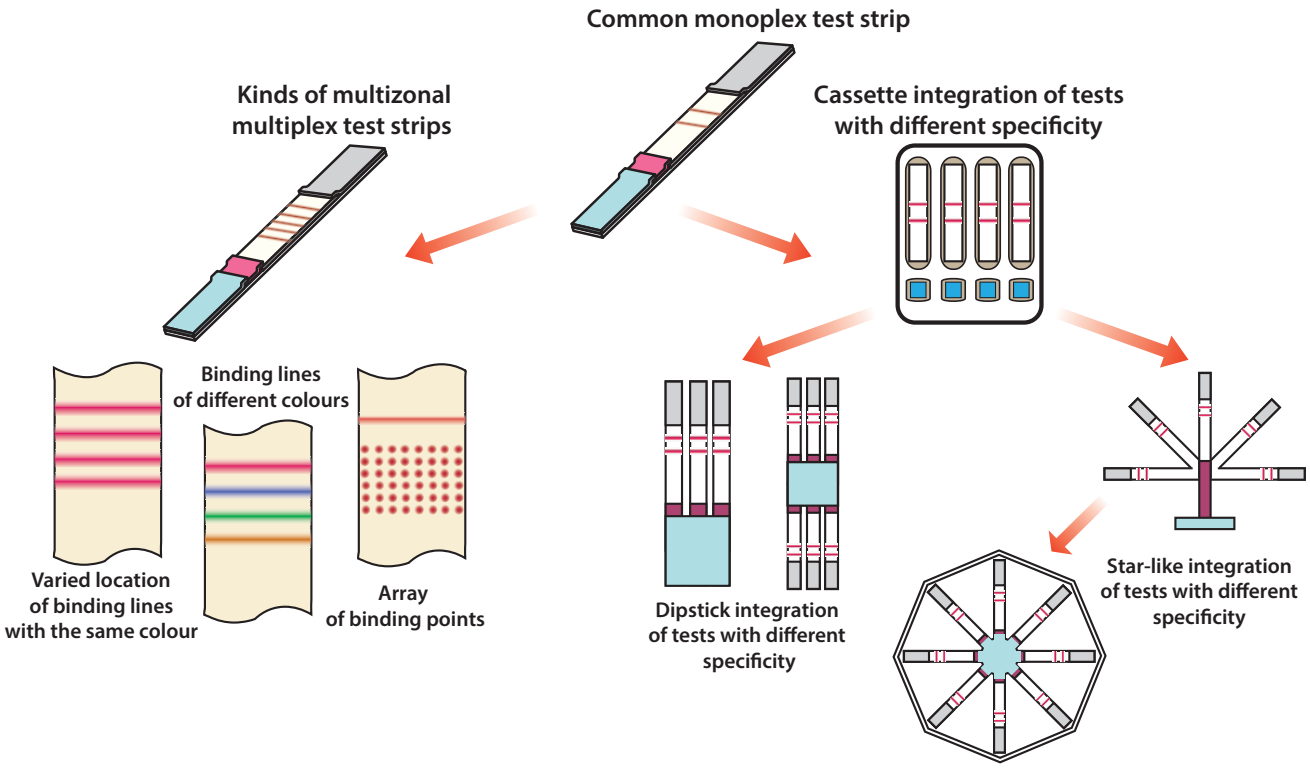

Figure 6. Variants of immunochromatographic test systems for simultaneous detection of several compounds

labels can be used in these zones for ease of identification. The geometric diversity of multiplex test systems is shown in the Figure 6.

In practice, variants with cassette combination of individual test systems and application of several lines with reagents of different specificity to the working membrane are already successfully used. It should be noted that combining immunoreagents of different specificity on one test strip is not a trivial task. Depending on the location of the binding zones, the duration of reagent interactions, the blurring of the liquid front moving along the membrane, and the dynamics of its intersection with the binding zones change. Therefore, it is necessary to evaluate the properties of the reagents and select their location on the membranes so that the analytical characteristics of the multitest do not deteriorate in comparison with the monotest.

A significant gain is achieved by transfer to a two-dimensional geometry for applying the immunoreagents of different specificity to a test strip [68]. Standard test strip sizes allow for the simultaneous, high-precision control of the content of approximately 30 analytes in a sample. However, the productivity of test system manufacturing in this variant is significantly reduced, even despite the availability of automation tools for this process.

Similar approaches can be used for semi-quantitative testing when monitoring a single analyte. By selecting the concentrations and reactivity of the immunoreagents applied to various binding zones on the working membrane, it is possible to ensure that the number of colored zones on it changes in accordance with the concentration of the analyte and is characterized by several threshold levels — Figure 7.

Application of test strips outside the scope of immunochromatographic recognition: field molecular genetic diagnostics

In monitoring pathogenic contaminants of food products and raw materials, an effective tool is the possibility to highly selectively recognize regions of nucleic acids that are characteristic namely of these pathogens. The first kind for the successful implementation of this con-

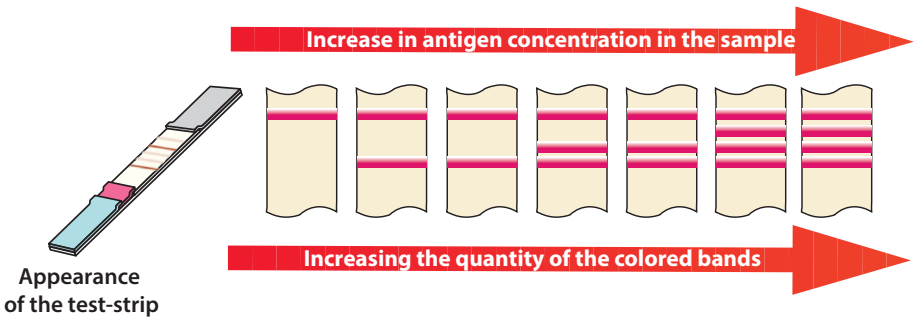

Figure 7. Immunochromatographic test system for semi-quantitative characterization of the target analyte content — changing number of visually observed colored binding zones

cept was the polymerase chain reaction (PCR) and analytical systems based on its application. Currently, PCR is widely used and is considered as the gold standard of molecular genetic diagnostics. Special devices, thermal cyclers, have been developed and are mass-produced to perform PCR in real time (with direct detection of the resulting target products). They provide cyclic modes for the formation and dissociation of nucleic acid complexes, enzymatic synthesis of complementary DNA chains, and registration of the reaction product by the glow of the fluorophore label.

PCR continues to be the main method for laboratory diagnostics, since it requires special sample preparation, the use of stationary equipment, and testing in specially equipped rooms with sterile conditions that exclude contamination of samples.

A promising alternative to PCR is isothermal amplification methods. In them, the production of multiple copies of target unique regions of nucleic acids is provided by special enzymes and combinations of oligonucleotide probes. The test sample, after simple and rapid sample preparation, is mixed with reagents for amplification, and all subsequent reactions occur simultaneously at a constant temperature. The exclusion of a thermal cycler in such testing brings it closer to field use. For this purpose, the use of simple non-device analytical means for the final stage of assessing the presence of a specific reaction product seems reasonable. Such proven means include immunochromatographic test strips, which is what determines the interest in their integration with isothermal amplification methods. The number of developments implementing this idea (the general principle of analytical processes is shown in Figure 8) has increased sharply in recent years [69–71], although there are only a few examples of commercialized products for quality control of agricultural products and food.

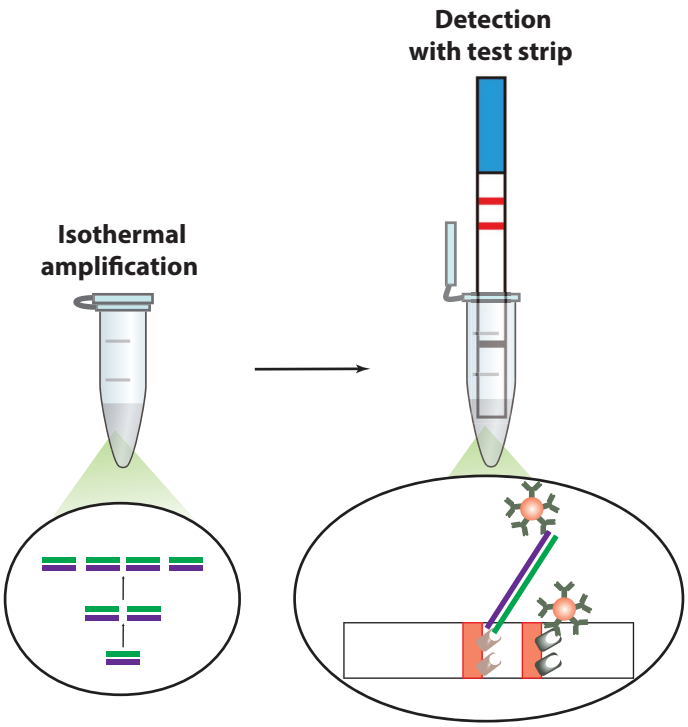

Figure 8. Scheme of the combination of isothermal amplification and detection of its products using immunochromatographic test strips

What features should be taken into account when considering the implementation of such analytical systems into practice?

1) The most promising testing protocols should be those that involve simple and rapid sample preparation at the initial stage. Today, there are a significant number of solutions developed for working with biosamples in medical diagnostics that can also be applied to various food matrices.
2) Despite the exclusion of the thermal cycler, when switching to isothermal amplifications, there remains a need for thermostatic instrumentation, since the enzymes used for these processes are capable of effective catalysis only at certain temperatures in the range from 37–42 °C to 60–70 °C. In this regard, low-temperature amplification variants are most interesting, such as recombinase polymerase amplification, a shift of interactions to suboptimal lower temperatures, or the search for/design of new enzyme preparations for amplifications.
3) Direct binding of the products of amplification processes on the test strip by complementary interactions of nucleic acids is impossible in most cases, since the product is a double-stranded DNA fragment without sticky ends. Therefore, most successful developments involve the use of primers with low-molecular labels, while additional receptors with high-affinity affinity to labels are used to form colored zones on the test strip — biotin — streptavidin, various combinations of antigens and antibodies specific to them.
4) A new promising tool for molecular genetic diagnostics are nucleases of the Cas family in complex with guide RNAs, activated strictly after highly selective recognition of nucleic acid regions complementary to the guide RNAs. The results of the investigations demonstrate the possibilities of their successful combination with isothermal amplification processes.

Registration of markers by various physical parameters

An actively developing trend in immunochromatographic developments in recent years is the creation of systems with marker's registration based on several physical parameters [72,73]. Thus, variants of test systems with combinations of colorimetric and fluorescent, optical and magnetic, optical and photothermal registration, a combination of traditional colorimetry and registration of giant Raman scattering have been described. Systems with three and even four registration methods are also proposed. Such an expansion can significantly reduce the detection limits of labeled immune complexes. Corresponding gains of several orders of magnitude in

comparison with traditional colorimetry are observed in a number of systems using the same markers and immunoreagents. In addition, magnetometry detects marker particles with equal efficiency regardless of their location in the pores of the test strip and the distance from the surface. This minimizes the dispersion of signals typical of colorimetric detection.

However, it is necessary to keep in mind that alternative methods of registration require the development of appropriate instrumentation — portable detectors [74,75]. Given the availability of various prototype solutions in the literature, the question of their serial production remains open (with the exception of photometric detectors). Technical solutions used for other tasks with large objects and large distances require significant adaptation for use in immunochromatography. The analytical characteristics of portable detectors can still be estimated approximately. Volumes of their production and cost will be determined by the variety of controlled analytes and the scale of demand.

The question of the practical implementation of the integration of two detection methods in one test system remains controversial. It is unclear how two types of measurements should be organizationally combined and how to solve problems arising from discrepancies in results. Moreover, when rechecking all negative results of a screening test with a more sensitive method, the role of the initial testing becomes unclear. Perhaps for some specific tasks such two-level control will be justified, but these issues have not yet been worked out.

As noted above, the situation with colorimetric registration and processing of immunochromatographic testing results can be considered successful today. There are a number of mass-produced (including in Russia) portable detectors, programs and attachments for smartphone cameras and other communication devices, software for transforming the resulting images of test strips into analyte concentrations [76]. So the practical prospects of this area of developments can be assessed optimistically. However, the question of the competitive advantages of specialized detectors compared to the adaptation of widely produced general-purpose communication devices remains open. With the formal economic preference of the second option, it remains uncertain how reproducible the results obtained using different cameras are. Metrological certification of detectors of standard configuration seems more realistic.

The development of modern information technologies and Internet resources makes it possible, in the near future, to consider field diagnostic tools (such as immunochromatographic tests) as elements of information systems that ensure the collection, processing and transmission of test results with their inclusion in integrated databases [77,78]. This makes it possible to conduct prompt comprehensive monitoring of the situation with products contaminations, analyze observed trends and formulate reasonable and well-grounded recommendations.

Control of toxic contaminants in food: assessment of the actual situation in Russia

After analyzing idealized systems, it is appropriate to move on to consideration of existing immunochemical analytical systems, their diversity and capabilities. The possibilities of their use in different countries vary significantly and require separate detailed research. Within the framework of this publication, we will limit ourselves to information characterizing the practical implementation of monitoring and application of immunochemical test systems in Russia.

Among the toxic contaminants of food products, the most popular objects of control are antibiotics. Chloramphenicol, the tetracycline group, streptomycin and zinc bacitracin are still widely used as antibacterial drugs in veterinary medicine due to their low cost and high efficiency. Immunochemical test systems for mycotoxin control are just beginning to be widely distributed, and immunodetection of pesticides is very limited.

The main method used to control antibiotics in food is chromatographic, and its results indicate the prevalence of situations with the simultaneous presence of several antibiotics in samples [79]. In this regard, in Russia, the legislatively established control of antibiotic residues is focused primarily on this method. The "List of regulatory means and methods of testing for the application and implementation of the requirements of the Technical Regulations of the Customs Union 021/2011"[1] contains mainly methods for determining antibiotics based on the principles of HPLC–MS/MS and enzyme immunoassay.

According to the decision of the Eurasian Economic Commission[2], all groups and classes of antibiotics previously regulated by Technical Regulation of the Customs Union 034/2013 "On the Safety of Meat and Meat Products"[3] are included in the Technical Regulation of the Customs Union 021/20114, the scope of which also concerns processed products. At the same time, verification of raw materials supplied by third-party organizations, although it assumes its assessment within the framework of production control, but not with such a frequent frequency as for microbiological indicators. Usually, raw materials are

[1] List of international and regional (interstate) standards, and in case of their absence — national (state) standards containing rules and methods of research (testing) and measurements, including rules for sampling, necessary for the application and implementation of the requirements of the technical regulations of the customs union and the implementation of conformity assessment of objects of technical regulation. For Technical Regulation of the Customs Union "On the Safety of Food Products" (TR CU 021/2011). Retrieved from https://www.gostinfo.ru/trts/List/45 Accessed October 09, 2024 (In Russian)

[2] "On amendments to some decisions of the Customs Union Commission and the Council of the Eurasian Economic Commission" Decision of June 23, 2023 No. 70 Retrieved from https://www.alta.ru/tamdoc/23sr0070/ Accessed October 09, 2024 (In Russian)

[3] TR CU 034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from http://docs.cntd.ru/document/499050564. Accessed October 09, 2024 (In Russian)

[4] TR TU 021/2011. "Technical Regulations of the Customs Union On food safety (as amended as of July 14, 2021)" Retrieved from https://docs.cntd.ru/document/902320560#8Q20M0. Accessed October 20, 2024 (In Russian)

controlled once every three months. The decision of the Eurasian Economic Commission does not impose obligations on manufacturers to identify the current wide list of monitored antibiotics. Enhanced control remains the prerogative of state control carried out by specialized departmental laboratories.

Low levels of permissible antibiotic content in animal products necessitate the use of highly sensitive and high-throughput methods for their detection. However, the number of chromatographic methods that allow the simultaneous extraction and determination of a wide range of antibiotics in animal products is very limited. One of the causes for this limitation is the difficulty of simultaneous extraction of various antibiotics. Therefore, the development of a unified pre-treatment based on the general properties of the isolating antibiotics is in great demand [80].

High sensitive instrumental analytical methods such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC–MS) are not suitable for monitoring bulk samples due to the high cost of equipment, the complexity of pre-treatment, and the need for qualified personnel. This situation determines the demand for alternative simple and productive methods, such as immunochemical ones.

The "List…"[1] includes about 30 immunochemical methods, but almost all of them implement the principle of enzyme immunoassay and are oriented towards imported test systems. Rapid immunochemical analysis is practically not represented in the "List…". For effective monitoring of food contaminants, it is necessary to develop and start the production of immunochromatographic test systems, both in traditional formats and using the improvements described in the review.

The public interest in immunochromatographic tests for medical purposes caused by the COVID-19 pandemic has led to the expansion of manufacturing bases for large-scale production of such tests and increased awareness among potential end users about them [81]. This opens up great prospects for the expansion of the application of immunochromatography in the field of food safety in the nearest future.

Conclusion

The examples of new developments in the field of immunochromatographic systems, reviewed herein, demonstrate the opportunity of overcoming the limitations, traditionally attributed to these systems: low sensitivity and the inability to quantify the analytes content. This allows us assuming the new prospects for the immunochromatography application to solve the issues of food safety ensuring. The application of new markers, signal amplification systems, portable detectors and the immunochromatography results processing tools significantly increases the competitive potential of these test systems, their information output and objectivity of the test results. However, the capabilities, demonstrated in the laboratory developments of the prototype test systems, do not guarantee the success in starting the production and subsequent wide use. The inclusion of new components and stages into immunochromatographic devices requires additional time- and labor-consuming technological developments in order to ensure the reproducibility of the new test systems properties, their long-term stability, and confirmation of absence of the negative effects of the biosamples components. The approaches versatility is also important, allowing combination of the new testing principles with the monitoring of wide range of analytes — in food and agricultural technologies, in veterinary and medical diagnostics. The wide range of new developments makes it possible to assume with high degree of probability, the upgrading the immunochromatographic testing tools that will be capable to detect significantly lower analytes' content while maintaining the rapidity and methodological simplicity of the testing.

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