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MODERN ENZYME IMMUNOASSAY METHODS IN MEDICINE: THEORETICAL FOUNDATIONS, APPLICATIONS, AND PROSPECTS

Turakulov Zhavlon Sodik ugli
Assistant of the Department of Clinical Laboratory Diagnostics and with a course in clinical laboratory diagnostics PGF
E-mail: sammi@sammi.uz
Phone: +998937034142

Rabbimova Dilbar Jaloliddinovna
Cadet of the Department of Clinical Laboratory Diagnostics and with a course in clinical laboratory diagnostics PGF

Khusanova Moxichexra Nodir kizi
Student of the Samarkand State Medical University

Annotation. The article examines the history of development, theoretical foundations, and practical significance of the enzyme immunoassay method, which is widely used in modern medicine and toxicology. The author describes the transition from radioimmunoassay to enzyme labels and details the differences between homogeneous and heterogeneous assay formats. Particular attention is paid to the advantages of ELISA in the detection of drugs and narcotics in biological fluids, as well as factors affecting the accuracy of results.

Key words: enzyme immunoassay, ELISA, antigen, antibody, biological fluids, drug monitoring, toxicological screening.

Introduction. Instrumental analytical methods such as chromatographic, electrochemical, and spectrometric techniques are widely applied for the determination of medicinal substances in biological fluids; however, they usually require complex preliminary sample preparation. Immunochemical analysis methods, based on specific antigen-antibody interactions, enable selective and sensitive detection of analytes directly in biological media without additional isolation or purification steps. These methods allow high-throughput analysis and are well suited for rapid diagnostic applications. Enzyme immunoassay is one of the most widely used immunochemical techniques for the determination of drugs and their metabolites [10].

The development of immunoassay began in 1959 with the introduction of radioimmunoassay by R.S. Yalow and S.A. Berson for quantitative insulin determination. Despite its high sensitivity and specificity, radioimmunoassay has limitations related to the use of radioactive labels, including safety concerns, isotope instability, and restricted automation, which stimulated the development of alternative immunoassay methods [11,12].

In 1971, E. Engvall, R. Perlmann, V.K. van Weemen, and A.N. Schuurs introduced enzymes as highly sensitive and versatile labels for immunoassays, which marked the development of enzyme immunoassay. Enzyme labels possess significant analytical advantages due to their pronounced catalytic activity and protein nature. One enzyme molecule is capable of converting numerous substrate molecules into reaction products within a short time, providing signal amplification and high assay sensitivity. In addition, enzyme molecules contain multiple functional groups that facilitate stable conjugation with antigens or antibodies [2,7].

Depending on the need for separation of bound and free components, enzyme immunoassays are classified into heterogeneous and homogeneous methods. Heterogeneous ELISA involves the separation of antibody-bound and free antigen fractions, followed by measurement of enzymatic activity in the bound fraction. This approach is characterized by high sensitivity and specificity and includes both competitive and non-competitive assay formats. In contrast, homogeneous

immunoassays do not require separation of reaction components. In such systems, enzymatic activity changes as a result of antigen–antibody interactions occurring directly in solution. Homogeneous ELISA methods allow rapid analysis and are particularly suitable for express diagnostics. Overall, enzyme immunoassay techniques combine high sensitivity, specificity, and practical convenience, making them widely applicable in biomedical and pharmaceutical analysis [4,8].

Sequential heterogeneous immunoassay is performed in two stages. At the first stage, antibodies immobilized on a solid support interact with the target antigen or another analyte. After incubation, unbound components are removed by washing, and enzyme-conjugated antibodies are added. At the second stage, excess unbound conjugates are washed off, followed by the addition of a specific substrate to initiate the enzymatic reaction [6].

In competitive heterogeneous immunoassays, enzyme-labeled antigen competes with free antigen from the sample for a limited number of antibody binding sites immobilized on a solid phase. After incubation and removal of unbound components, the enzymatic reaction is carried out by adding the appropriate substrate. The accuracy and sensitivity of heterogeneous immunoassays depend on several critical factors, including the nature and preparation method of the solid support, the type and concentration of the enzyme conjugate, the sequence of assay steps, incubation conditions, and the potential influence of matrix effects [8].

To date, numerous ELISA technologies have been developed that combine enzyme labeling with efficient detection systems. Contemporary studies on ELISA focus on immunogen design, including hapten structure and carrier protein selection, the type of antibodies used, and the choice of assay format. A critical step in the development of any immunoassay is the production of highly specific antibodies, as assay sensitivity and specificity largely depend on antibody quality. Antibodies are commonly generated by immunizing laboratory animals such as mice, guinea pigs, or rabbits with the target antigen [1,3].

Purification of non-specific immunoglobulins from serum is typically performed using sequential protein fractionation methods, including ammonium sulfate precipitation, gel filtration, ion-exchange chromatography, and affinity chromatography. Antigen-specific antibodies are isolated primarily by affinity chromatography, in which the antigen is immobilized on a solid matrix, allowing selective binding and subsequent elution of purified antibodies using appropriate buffer systems. This method provides a high degree of purification, often achieving enrichment levels of up to 1000-fold or more [5].

Despite the high efficiency of affinity chromatography, complete homogeneity of antibody preparations cannot be achieved. This limitation can be overcome by the production of monoclonal antibodies, which possess a single specificity and recognize only one antigenic determinant. Monoclonal antibodies are obtained using cell engineering techniques through the hybridization of immunocompetent B lymphocytes with myeloma cells, resulting in hybridomas capable of unlimited proliferation. Monoclonal antibody preparations are characterized by stable composition, consistent physicochemical properties, and minimal cross-reactivity with unrelated antigens, making them highly valuable for immunoassay applications. However, their limitations include relatively lower affinity toward certain antigens and high production costs [2].

An effective immune response is generally induced by compounds with a molecular weight exceeding 3000 Da. Consequently, the generation of antibodies against low-molecular-weight substances is challenging, as such compounds lack intrinsic immunogenicity. To overcome this limitation, small molecules are converted into immunogens by conjugation with carrier proteins or by aggregation. Human or bovine serum albumin is most commonly used as a carrier protein for hapten conjugation [11]. The selection of an appropriate ELISA format is determined by the specific analytical objectives. In many chemical-toxicological studies, qualitative detection confirming the presence or absence of a target substance is sufficient. However, in certain cases, precise quantitative determination of analyte concentration is required [4].

Numerous studies have demonstrated the effectiveness of ELISA in the detection and measurement of narcotic and medicinal substances in various biological fluids. Using this method, minimal detectable concentrations have been established for sulfonamide drugs, lisinopril, enalapril, barbiturate and benzodiazepine derivatives, morphine, amphetamines, and cannabinoids. ELISA has also been successfully applied to post-mortem blood analysis for the detection of cocaine and opiates [9].

Currently, commercially available ELISA reagent kits are widely produced by companies such as Syva, F. Hoffmann-La Roche Ltd, Abbott, and research institutions within the CIS. These kits provide reliable detection limits in the range of 300-500 ng/mL. Most commercial assays are based on solid-phase ELISA principles and are implemented using microplates or test tubes. Polyclonal antibodies are predominantly used due to their lower production cost and adequate analytical performance [12].

Errors in the determination of medicinal substances using ELISA may arise from several factors related to the properties of biological samples and analytical conditions. The composition of biological fluids, particularly salt content, can influence enzyme-labeled activity by altering pH and ionic strength. Endogenous enzymes, protein-bound metabolites, or salt forms of analytes may reduce competitive binding in immunochemical reactions, thereby affecting assay accuracy. The presence of enzyme inhibitors in samples represents another important source of error. Heavy metal salts, including mercury-containing preservatives, as well as anticoagulants such as EDTA and certain drug metabolites, can significantly suppress enzyme activity. For instance, preservatives such as sodium azide and sodium benzoate, commonly used for sample stabilization, inhibit horseradish peroxidase, potentially leading to false-negative ELISA results [3].

A specific challenge in immunochemical analysis is cross-reactivity caused by structurally related compounds. Therefore, potential cross-reacting substances must be evaluated, and manufacturers usually provide relevant information in assay documentation. In post-mortem samples, decomposition processes result in the formation of biogenic amines that may cross-react with antibodies, increasing the risk

of false-positive results. Consequently, all positive ELISA findings should be confirmed using alternative analytical methods, such as chromatography or mass spectrometry [13].

Conclusion. ELISA is a sensitive and specific immunochemical method for detecting and quantifying medicinal and narcotic substances in biological fluids. Its advantages include minimal sample preparation, high throughput, and applicability for rapid screening. The accuracy of results depends on sample composition, enzyme inhibitors, and potential cross-reactivity, highlighting the need for careful handling and interpretation. ELISA results should be confirmed by complementary analytical methods when necessary, ensuring reliable detection and enhancing its value in biomedical and toxicological studies.

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