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MODERN FUNDAMENTALS OF ENZYME IMMUNOASSAY

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Abstract
Enzyme-linked immunosorbent assay (ELISA) is a laboratory immunological method for the qualitative or quantitative determination of various low molecular weight compounds, macromolecules, viruses, etc., based on the specific reaction of antigens (AG) with antibodies (AT). The detection of the resulting complex is performed using an enzyme as a label to visualize the reaction.

Keywords: antigen, antibodies, biological fluid, enzyme immunoassay, viruses, bacteria.

Introduction

ELISA appeared in the mid-60s and was initially developed as a method for antigen identification in histological preparations, as well as for visualization of precipitation lines in immunodiffusion and immunoelectrophoresis tests, and then began to be used for quantitative determination of antigens and antibodies in biological fluids. The method was developed by E. Engvall and R. Pelman, and independently by W. van Weemann and R. Schmidt. Van Veeman and R. Schuurs. ELISA is used to detect antibodies or antigens in biological fluids [1,2,3].
The ELISA consists of 3 mandatory steps:
1. The stage of binding of AG and AT and formation of immune complex.
2. Stage of formation of conjugate binding to the immune complex.
3. Visualization stage: transformation of the enzyme label into a detectable signal.
Immunologic reactions are based on the following principles:
1. Complementarity (“key to the lock”) - the interaction of AG and AT is strictly specific: binding of antigenic determinant with the active center of the Fab-fragment of AT occurs. The strength of

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binding is determined by the degree of spatial correspondence between the active center of AT and the epitope of AG.

2. Equivalence - immunologic reaction takes place at equivalent (corresponding to each other) ratios of AG and AT.

3. Immunologic reaction has a two-phase character: a) specific interaction - the "invisible" phase; b) non-specific phase - manifested visually (e.g., formation of flakes during agglutination, etc.).

Interaction between antigen and antibody subpopulation. Previously, a simple model of interaction between univalent AG and univalent AT was taken as a basis for quantitative description of the efficiency of AG:AT interaction. However, since the AT molecule has several antigen-binding centers and, in addition, is capable of interacting with several antigenic determinants of one AG molecule, this characterization of immunochemical complex formation is very simplistic. The term avidity or functional affinity (functional affinity) was introduced to describe the process of interaction of polyvalent AT with polyvalent AG, which is closer to reality. From the biological point of view, it is the functional affinity that plays the main role in the immune response to infection of the organism by viruses or bacteria that have repetitive antigenic determinants on their surface. The process of AG:AT complex formation of 1:1 composition, in which polyvalent interactions are realized, is also reversible and can be characterized by the stability constant of complexation. From the energy point of view, the formation of a polyvalent complex is much more favorable than a monovalent one [4,5,6].

ELISA variants Currently, a large number of different ELISA variants have been developed, with both fundamental and minor differences. There is no single clear classification of the variety of ELISA methods in the literature, which makes it difficult to identify common patterns and make a comparative assessment of the capabilities of different methods. ELISA methods are usually considered from the point of view of division into heterogeneous and homogeneous, i.e. according to the principle of carrying out all stages of analysis with the participation of the solid phase or only in solution. The primary process in ELISA is the stage of "recognition" of the analyzed compound by specific ABs. Since the process of formation of immunochemical complexes occurs in a strictly quantitative relationship due to affinity, concentrations of the components and reaction conditions, it is sufficient to quantify the formed immune complexes to determine the initial concentration of the analyzed compound [7,8,9].

In the case of AG assays, there are two approaches for making such an assessment:

1. Direct measurement of the concentration of formed complexes. 2;

2. Determination of the concentration of the remaining free (unreacted) AB.

Obviously, in the latter case, the number of immune complexes formed is determined by the difference between the total number of antibodies added and the number of antibodies remaining free.

The following variants of ELISA are distinguished according to the principle of conducting all stages of analysis with the participation of a solid phase or in solution:

- Homogeneous - if all 3 stages of ELISA take place in solution and there are no stages of separation of the formed immune complexes.

- Heterogeneous - when AG or AB are fixed on a solid phase (polystyrene plates or spheres).

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- Homo-heterogeneous - if stage I - formation of immune complexes - takes place in solution, and then a solid phase is used to separate the components.

Heterogeneous or solid-phase ELISA is the most widely used in clinical practice. According to the type of immunochemical interaction at the first stage of analysis (in which binding of AG and AB occurs), non-competitive and competitive ELISA are distinguished. If only the analyte compound and its corresponding binding centers (antigen and specific antibodies) are present in the system, the method is non-competitive. If, however, in the first step, the analyte compound and its analogue (enzyme-labeled analyte compound or analyte compound immobilized on a solid phase) are present in the system at the same time, competing for a limited number of specific binding centers, the method is competitive. In a competitive ELISA, the antigens or antibodies to be detected compete with similarly labeled conjugate antigens or antibodies for binding sites on the immunosorbent. Assays of this type are often used to detect antigens present in high concentrations or hormones that have only one antigen binding center. Among the non-competitive solid-phase ELISA schemes, there are three main formats: direct, indirect and sandwich. The differences between these variants are as follows. In the indirect ELISA variant, antigen is sorbed onto the surface of the wells of a polystyrene plate at the first stage. After removal of unbound antigen molecules, a sample containing antibodies specific to the antigen is added. The resulting antigen-antibody complexes are detected using anti-species antibodies conjugated to a label. Labeled anti-species ATs allow detection of ATs to different antigens. In the direct embodiment, the detection of the sorbed antigen is performed directly by specific antibodies conjugated to the tag. Since the added specific tag is bound to the antibodies, it means that the concentration of the stained reaction product is equal to the antibody concentration. And the antibody concentration is equal to the antigen concentration. The advantage is the small number of steps. In sandwich immunoassays, in the first step, antibodies specific to the antigen being analyzed are sorbed onto the surface of the plate rather than the antigen. After removal of unbound antibody molecules, a sample containing antigen is added. To detect the formed antibody-substrate-antigen complex, a second antibody specific to another, spatially distant, epitope of the antigen conjugated with a label is added. At the stage of detection of a specific immunocomplex, the antigen appears as if sandwiched between immobilized and labeled antibody molecules, which gave rise to the name "sandwich" method. Test systems for antibody detection work according to a similar scheme, but they use antigen as an immunosorbent, while the conjugate contains a solution of enzyme-labeled antigen. The use of "sandwich-type" antibodies specific to two different epitopes of the antigen in immunoassays allows to achieve high sensitivity and specificity in antigen detection even in such heterogeneous samples as blood plasma [4,8,9,10,11].

Among the competitive solid-phase ELISA schemes, there are two main formats: direct and indirect. In the direct competitive ELISA format, specific antigens immobilized on a solid phase are used, and enzyme-labeled and unlabeled antibodies compete for binding to the immobilized antigen. After incubation, two types of immune complexes are formed: those containing the enzyme tag (labeled) and those without it (unlabeled). The more detectable (unlabeled) antibodies the sample contains, the greater the competition with labeled antibodies and, therefore, the fewer labeled immunocomplexes are formed. Then, after washing the carrier from unbound components,

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a substrate-chromogenic reagent is added and the enzymatic activity of specific immune complexes formed on the solid phase is recorded.

Thus, the detectable signal obtained by direct competitive ELISA is inversely dependent on the antigen concentration. The advantage of the direct scheme is the small number of steps, which allows easy automation of the assay. The disadvantages of the scheme include the complexity of methods for synthesizing enzyme conjugates, as well as the possible influence of sample components on enzyme activity [11].

The indirect competitive ELISA format uses enzyme-labeled antispecies antibodies (specific or secondary) and an antigen-protein conjugate carrier immobilized on a solid phase. The antigen-protein conjugate is immobilized on the surface of the carrier, a solution containing the antigen to be detected and a fixed concentration of unlabeled specific antibodies is added, and incubated. After removal of unbound components, a fixed concentration of labeled secondary antispecies antibodies is added. After incubation and washing of the carrier, the enzymatic activity of the specific immune complexes formed on the solid phase is detected. As with the direct competitive method, the detectable signal is also inversely proportional to the concentration of the antigen to be detected [7,10].

The use of a universal reagent - labeled antispecies antibodies - makes it possible to detect antibodies to different antigens. In addition, the analyzed sample and the labeled reagent are introduced into the system at different stages, which eliminates the influence of different effectors contained in the sample on the catalytic properties of the enzyme label. However, this scheme complicates the analysis due to the introduction of additional stages. This method is used for the qualitative and quantitative detection of, for example, opiates (morphine, heroin), cannabinoids (marijuana, hashish), amphetamines and methamphetamines, barbiturates.

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