A SHORT THEORETICAL OVERVIEW OF THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Summary. The article provides a theoretical overview of the enzyme-linked immunosorbent assay (ELISA). The concept of IFA is defined, the main stages of the analysis are given, two groups of IFA methods are considered in terms of the way they are performed, and a brief description is provided. The importance and role of ELISA methods in modern clinical biochemical and laboratory research are considered, the advantages and disadvantages of the methods are indicated.

Keywords: enzyme-linked immunosorbent assay (ELISA), antigen, antibody, specific antigen-antibody reaction, immunochemical methods of analysis, homogeneous methods, heterogeneous methods.

Enzyme-linked immunosorbent assay (ELISA) is a laboratory immunological method of qualitative or quantitative determination of various low-molecular-weight compounds, macromolecules, viruses, etc.

ELISA is based on a specific antigen-antibody reaction. The theoretical foundations of ELISA are based on modern immunochemistry and chemical enzymology, knowledge of the physicochemical laws of the antigen-antibody reaction, as well as on the basic principles of analytical chemistry [1 - 6].

ELISA is one of the directions that is most actively developing and widely used in clinical biochemistry and laboratory diagnostics. Due to the diversity of research objects, there are a large number of variants of this method for determination: from low-molecular compounds, peptide and steroid hormones, pharmacological drugs, pesticides to viruses and bacteria, and even to other antibodies; variety of binding principles and variety of IFA conditions.

According to only one variant of registration of enzymatic activity, it is possible to use photometric, fluorometric, bio- and chemiluminescent methods.

To date, a large number of different variants of ELISA have been developed, which have both fundamental and secondary differences.

The entire process of enzyme immunoassay can be conditionally divided into
several main stages:

1) formation of a specific «antigen-antibody» complex;
2) introduction of a label into the formed complex;
3) visualization of the linked label.

Regarding the method of execution, all ELISA options can be divided into two groups: 1) systems that do not require separation of the components of the reaction mixture (homogeneous methods); 2) systems for which separation is necessary (heterogeneous methods).

**Heterogeneous ELISA in microplate version**

In order to analyze the efficiency of complexation, it is necessary to completely purify the complexes from free components. This task turned out to be easy to solve if one of the components of the antigen-antibody pair is firmly bound (immobilized) on a solid carrier.

The use of immobilization of antibodies on a solid support gave rise to solid-phase (heterogeneous) ELISA methods. Heterogeneous (solid-phase) ELISA in the microplate version has become the most widespread in test systems for clinical laboratory research. For the solid phase, the surface of the wells of a polystyrene microplate is used, on which known antigens or antibodies (immunosorbent) are adsorbed. In the course of the specific reaction of the immunosorbent with antibodies or antigens determined in the test sample, immune complexes are formed, which are fixed on the solid phase. Substances that did not participate in the reaction, as well as excess reagents, are removed by repeated washing. Such a scheme allows to simplify the process of effective separation of reaction components [1 - 6].

There is a direct and indirect heterogeneous ELISA.

**Direct heterogeneous ELISA**

In the direct enzyme-linked immunosorbent assay, the introduced material (antigen) is fixed during incubation on the surface of clean wells. The amount of the researched material is detected with the help of antibodies to detect the antigen, connected with a specific label, which provides an enzymatic reaction.

**Structure of the analysis**

Biological material (blood, buccal mucosal scrapings, smears) is placed in clean wells for some time (usually 15-30 minutes), long enough for antigens to stick to the surface of the wells. Next, antibodies to the detected antigen are added to the wells. This means that by detecting antigens, for example, syphilis, antibodies against syphilis antigens are added. This mixture of the research material and antibodies is left for some time (from 30 minutes to 4-5 hours) so that the antibodies can find and contact the corresponding antigen. The more antigens in a biological sample, the more antibodies will bind to them. Since antibodies are added in excess, not all of them will bind to antigens, and if there is no antigen in the sample, then, accordingly, no antibody will bind to the antigen. In order to remove «excess» antibodies, the contents of the wells are poured (or washed by decantation). As a result, all «excess» antibodies are removed, and those that have bound to antigens remain, since the antigens are «glued» to the surface of the wells.

The next stage is an enzymatic reaction. A solution with an enzyme is added to the washed wells and left for 30-60 minutes. This enzyme has an affinity for the
substance (specific label) to which antibodies are bound. An enzyme catalyzes a reaction that converts this specific label (substrate) into a colored substance (product).

Since the added specific label is associated with antibodies, it means that the concentration of the colored reaction product is equal to the concentration of antibodies, and the concentration of antibodies is equal to the concentration of antigens [1 - 6].

**Indirect heterogeneous ELISA**

In indirect enzyme-linked immunosorbent assay, antibodies to the identified antigen are used, connected to a specific label. This specific label is the substrate of the enzymatic reaction.

According to the type of immunochemical interaction at the first stage of the analysis, non-competitive and competitive variants of the analysis are distinguished among heterogeneous methods.

If only the analyzed compounds and their corresponding binding centers (antigen and specific antibodies) are present in the system, then the method is non-competitive.

If, at the first stage, the analyzed substance and its analogue (an enzyme-labeled analyzed compound or an analyzed compound immobilized on a solid phase) are simultaneously present in the system, competing for a limited number of specific binding centers, then the method is competitive [1 - 6].

**Homogeneous ELISA**

In 1972, scientists developed a new approach with conducting the entire analysis without a solid phase. The method was named homogeneous ELISA (Eng. «EMIT» - enzyme multiplied immunoassay technique) and was based on accounting for differences in the catalytic properties of the enzyme label in its free form and in the immunochemical complex. Based on this approach, kits were developed for the determination of a wide range of toxic, narcotic and medicinal products. A significant advantage of the EMIT analysis is the possibility of using small volumes of the analyzed sample and a high detection speed (2-5 min), due to the absence of a separation stage of the free and labeled analyzed compound. The disadvantages of the method include lower sensitivity than in heterogeneous ELISA, and the possibility of determining only low-molecular-weight antigens [1 - 6].

Of course, the ELISA method, like any immunochemical methods of analysis, can give false positive and false negative results.

For example, false positive results when determining antibodies to various infections can occur due to the rheumatoid factor, which is immunoglobulin M against the person’s own immunoglobulins G; due to antibodies that are formed in various systemic diseases, metabolic disorders or taking medicines; in newborns, such false positive reactions can occur due to the formation of M-antibodies to the mother’s immunoglobulin G in the child's body.

Falsely negative results in the determination of antibodies can be due to immunodeficiency conditions, as well as technical errors in the preparation of the reaction [1 - 6].

But, due to undoubted advantages, namely:

- convenience in work;
- speed;
- objectivity due to automation of accounting of results;
- possibilities of researching immunoglobulins of different classes (which is important for early diagnosis of diseases and their prognosis) currently ELISA is one of the main methods of laboratory diagnostics.

The ELISA method can be used for diagnosis:
- viral diseases (HIV infection, viral hepatitis, cytomegalovirus infection, Epstein-Barr virus, herpes infection, coronavirus disease);
- to diagnose sexually transmitted infections (syphilis, chlamydia, trichomoniasis, gonorrhea, ureaplasmosis);
- hormone levels in endocrinology;
- autoantibodies and markers of oncological diseases in oncology;
- general IgE and specific IgE antibodies in allergology.
- medicines, drugs in biological samples.
- serum proteins (ferritin, fibronectin, etc.).

References: