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IMMUNOCHEMICAL METHODS IN CLINICAL BIOCHEMISTRY

Annotation: In clinical laboratory practice, immunochemical methods are widely used to determine traditional biochemical objects-proteins, enzymes, hormones, mediators, pharmacological preparations, etc. Their advantage is their high sensitivity and specificity.

Key words: immunochemical methods, clinical biochemistry, medicine, diagnose, object.

The introduction of immunochemical methods is one of the ways to further improve the diagnosis of diseases of farm animals, since many serological and allergic methods currently used to diagnose a number of important diseases (tuberculosis, brucellosis, colibacteriosis, salmonellosis, etc.) do not have sufficient sensitivity and specificity. The basis of success in immunochemical studies is the production of immune serums of high titer and the desired specificity. Methods of obtaining immune serums to date remain mostly empirical. Differences in immunological reactivity in different animals may depend on age, sex, body weight, the state of the nervous and endocrine systems, the usefulness of the diet, conditions of maintenance and care, as well as a number of other factors that are sometimes very difficult to account for and standardize. Therefore, immune serums obtained even from animals of the same species and with the same immunization scheme can differ dramatically both in titer and in the set of antibodies. When receiving immune serums, the following basic conditions must be taken into account.

1. Preparation of the antigen. It should be as free from impurities as possible, which prevents the appearance of antibodies with a different specificity. At the same time, it is very important to take into account the lability of antigens, low

resistance to various factors and the possibility of their denaturation during purification.

2. The most appropriate immunization scheme for each specific case is selected empirically. Various adjuvants are used to stimulate antibody formation. It is better to take small doses of antigen, since in this case the consumption of valuable drugs is reduced and the probability of the formation of side antibodies decreases.

3. Evaluation of the obtained serum is mandatory, since it is impossible to predict its quality in advance. The antiserum titer, its specificity and avidity of antibodies are usually subject to verification. The immune serum should be as high titer and specific as possible, i.e. it should react only with those antigens that are being investigated. High avidity indicates a pronounced affinity of the antigen and the antibody. When conducting clinical and biochemical studies, the most widely used are the gel immunodiffusion reaction (IDR), immunoelectrophoresis, radioimmunological analysis (RIA), enzyme immunoassay (EIA) and some others.

The gel immunodiffusion reaction (IDR) is used for the analysis of multicomponent protein systems, comparative analysis of the antigenic structure of proteins and other antigens. This method is based on the ability of antigens and antibodies to diffuse at different rates, as a result of which equivalent ratios of certain antigens and antibodies are achieved in different parts of the gel, where the corresponding precipitation lines are formed.

According to this method, the analysis is carried out in a flat plate of agar, which allows you to place several different antigens around the reservoir with immune serum and thereby conduct their comparative analysis. The main provisions that need to be taken into account when setting up double diffusion in a gel are the following:

1. The formation of a precipitate occurs in a rather narrow equivalence zone corresponding to such a concentration of antigen and antibodies, at which both components are fully incorporated into the precipitate.

2. One antigen gives only one precipitation zone.

3. If not one, but several antigens are present in the solution, they behave independently of each other. As many precipitation lines are formed as there are antigen-antibody pairs.

4. The number of precipitation zones formed corresponds to the minimum of antigen-antibody complexes present in the system.

5. Antigens and antibodies having approximately equal diffusion coefficient form a straight precipitation band.

6. If both components are used in approximately equivalent amounts, then the precipitation band is located at an equal distance from the tank with the antigen and antibody.

7. If the concentration of antigens is higher than the concentration of antibodies, then the precipitation band is located closer to the reservoir containing antibodies and vice versa. With a significant excess of one of the components, the precipitation line can be "driven" either into a tank with an antigen or into a tank with an antiserum and not be observed at all.

8. If the precipitation line is formed by an antigen with a low molecular weight, then the precipitation band, all other things being equal, is located closer to the reservoir with the immune serum.

There are three main variants of the result of double diffusion in the gel: fusion of precipitation lines (identity reaction), crossing of precipitation lines (non-identity reaction), combination of the first and second cases (partial identity reaction - "spur" or "double spur").

Radioimmunological analysis (RIA). For the first time, this method was developed for the quantitative determination of insulin using a hormone labeled with a radioactive isotope of iodine. The method was based on competition between native plasma insulin and labeled 131I - insulin for a limited number of specific binding sites on insulin antibodies.

In addition to the classical RIA, it can be given in a solid-phase version. The principle of the method is that a hard surface (usually a plastic tablet) load with antibodies, and then add the test solution containing the determined antigen. To the

antigen-antibody complex formed on the carrier, after washing the unbound antigen, an excess of radioactively labeled antibodies is added. The amount of bound radioactive label will depend on the amount of antigen fixed on the solid carrier.

Solid-phase radioimmunological analysis can be carried out by adsorbing an antigen on a plastic panel that binds the antibodies under study (for example, Ig G contained in sheep blood serum). After washing the remaining proteins, the amount of bound antibodies is determined by adding labeled 125I rabbit antibodies to sheep Ig G, and then removing the excess labeled reagent. The amount of the radioactive label bound in the wells of the plastic panel is proportional to the amount of certain antibodies.

The radioimmunological method can also be used to determine low molecular weight compounds. In this case, the substances being determined are conjugated with proteins or other high-molecular compounds to give them antigenicity. It is in this way that radioimmunological methods for determining prostaglandins have been developed.

Currently, radioimmunological analysis is carried out using standard diagnostic kits, which include everything you need (reagents, dishes, instructions for use), manufactured in the factory. In the presence of such sets, the radioimmunological determination of biological objects is reduced to the sequential execution of certain operations and the measurement of radioactivity.

The method of enzyme immunoassay had a number of advantages over the method of radioimmunological analysis.

1. It did not use radioactive isotopes. The absence of radiation hazard greatly simplified the conditions of the event (special rooms, equipment, etc.).

2. Much greater stability of labeled compounds (in RIA it is determined by the half-life of isotopes).

3. The ability to quickly determine the results of an enzymatic reaction using conventional publicly available devices (photometers). The possibility of even a visual assessment of the reaction.

4. EIA is easy to automate.

Currently, many variants of EIA analysis have been developed. The choice of the analysis scheme depends on many factors, the main of which are the molecular weight and valence of the antigen, the required sensitivity limit, the composition of the medium in which it is necessary to determine the antigen, the properties of the enzyme, the possibility of obtaining antigens and antibodies in pure form, and much more. Methods of solid-phase (heterogeneous) EIA, the use of which is associated with the need to separate the bound antigens, have been developed for a number of different antigens and antibodies.

The principle of the method for determining antigens is that a solution containing the antigen being determined and the synthesized conjugate of the antigen with the enzyme is added to the immobilized antibodies. The identified and labeled antigens compete for antibody binding centers. After a certain incubation time, the enzyme conjugate is redistributed between the solution and the carrier. Measured in solution or in the solid phase after washing, the concentration of the label is proportional (quantitatively related) to the initial concentration of the antigen being determined and, after preliminary calibration of the system, serves as a characteristic of its content in the analyzed sample.

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