Immunochemical methods

Immunochemical methods are based on antigen-antibody interaction. Using these methods, we determine the presence of pathogens or demonstrate whether or not a sample contains specific antibodies to a given antigen . An **antigen** is a macromolecular substance of natural or artificial origin that the organism recognizes as foreign. When an antigen (in this case an immunogen) is introduced into an organism, it elicits an **immune response** . Lymphocytes are stimulated , which after differentiation produce plasma cells capable of secreting antibodies. **Thus, an antibody** is a molecule that is able to bind to an antigen and thereby trigger a defense response. We distinguish **polyclonal antibodies**(directed against multiple epitopes of a particular antigen), **monoclonal antibodies** (directed against a single epitope of an antigen) and **recombinant antibodies** (a combination of the two).

Immunoprecipitation methods

Immunodiffusion methods

Simple radio-immunodiffusion method

Antigen samples are applied to the wells on an agarose gel containing the specific antibody. During 2-3 days of incubation at room temperature, the **antigen diffuses radially in this gel** . Upon reaction of the antigen with a specific antibody, an **immunoprecipitation ring is formed, the area of which is proportional to the amount of antigen** . We evaluate the results of the samples using a special ruler, where we measure the area of the rings and thus determine the concentration of antigen in the samples.

Immunodetection by optical methods in solution

Immunoprecipitation associated with immunoturbidity

Immunoprecipitation is based on antigen-antibody interaction and **immunoprecipitate** formation . The condition is the presence of a polyvalent antigen (antigen reaction with multiple epitopes). **If we put a constant amount of antibody and an increasing amount of antigen in several tubes , those immunoprecipitates will be formed.** The essence of the immunoprecipitate is the spatial lattice, where the epitopes (binding sites A) of the antigen are connected with the paratopes (binding sites P) of the antibodies. After a while, it reaches a certain size, when it finally precipitates. The expression of the amount of immunoprecipitate on the amount of antibody and antigen is described by the so-called **immunoprecipitation curve** .

If we determine the concentration of antigen in an unknown sample (eg plasma proteins), we use the method of **immunoturbidimetry** . The reaction of the antigen with the antibody produces turbidity. Using **spectrophotometry** , we measure the intensity of light that was not scattered by turbidity and determine the concentration of antigen in an unknown sample (we use only the ascending part of the curve, where the concentration of antigen is directly proportional to the concentration of precipitate).

Immunonephelometry

The method is based on measuring the intensity of scattered light coming from the solution in all directions . It is measured at an angle that is different from the direction of the incident radiation (usually 45 ° or 90 °). We determine the antigen concentration in the same way as in immunoturbidimetry. We use a nephelometer , where the radiation source is usually a laser .

Immunoanalytic methods with labeled reactants

The methods are based on **labeling** an antigen or antibody with a substance that is more sensitive than immunoprecipitate detection. The label can be an **enzyme (enzyme immunoassay), a radioisotope, but also a fluorescent or chemical substance**

Enzyme immunoassay

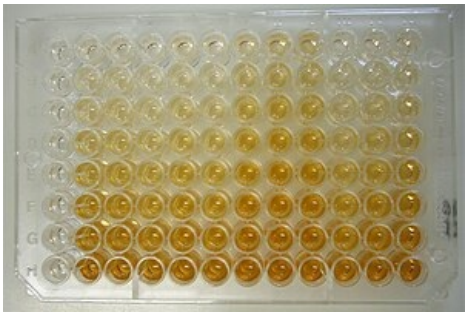
These methods use enzymes to label an antigen or antibody. Peroxidase or alkaline phosphatase serve as a marker. There are two main techniques, **ELISA** (Enzyme-Linked Immunosorbent Assay) and EMIT (Enzyme Multiplied Immunoassay Technique) .

Homogeneous enzyme analysis

A sample containing the antigen of interest is mixed with a known amount of the same antigen with bound enzyme (conjugate) and the appropriate antibody in limited amounts. In an immunochemical reaction , the unlabeled antigen competes with the conjugate for binding to a limited amount of antibody . Upon binding of the antibody to

the conjugate enzyme, a conformational change in the enzyme associated with loss of activity may occur. The more unlabeled antigen in the sample, the more antibody molecules react with it and the more enzymatic activity in the conjugate is retained. This method is used to determine low molecular weight substances (drugs, hormones).

Heterogeneous enzyme analysis


ELISA

One of the methods is the so-called **ELISA** , where we determine the amount of antigen or antibodies. Here, the antigen or antibody is **firmly anchored to a solid phase** , which may be either the surface of a microtiter well, a tube, or a magnetic particle. ELISA methods are divided into **competitive and non-competitive (sandwich)**

Competitive technique

In this type of assay, unlabeled ligand competes for binding to a limited number of binding sites on the solid surface of immobilized antibodies with the enzyme- labeled ligand . After a short incubation, the complex is separated from the free. After washing (removal of unbound molecules), substrate is added and the enzyme present in the bound fraction converts it into a colored product. The amount of product formed is inversely proportional to the concentration of unlabeled ligand in the test sample. The absorbance of the samples is measured. Wells that contain only the ligand-enzyme conjugate are most intensely stained and the color loss in the wells is proportional to the amount of unconjugated antigen. Standard curves are obtained by plotting antigen concentration and absorbance. **We determine the concentration** from the curve antigen samples (or we have it done by PC - Elisa reader).

Non-competitive (sandwich) technique

Using this method, we determine antibodies or antigens that have at least two different determinants. A specific antigen is bound to the surface of the wells of the microtiter strips. In unknown samples that we add to the wells, we investigate the presence of antibodies against this antigen. If the samples contain a given antibody to a given antigen, they bind to the antigen and form an immunocomplex. After washing the unbound components of the sample, the immune complexes are detected by the **conjugate** (enzyme-bound protein). A **so-called "sandwich"** is created, which consists of **antigen in the strip wall, antibody and conjugate** . The amount of bound labeled antibodies is visible by the enzymatic reaction . After washing, add the **substrate** which is converted to a colored product by peroxidase. It stops the **enzyme reaction** by adding STOP solution (weak acid). According to the intensity of the staining, we evaluate whether the samples contained antibodies or not.

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http://che1.lf1.cuni.cz/html/imuno.pdf

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