

Serology Lec. 2

Definition: Is the science that deals with the measuring antibody or antigen in body fluids.

Application of serological tests

Antigen tests

Antigen tests often enable an early diagnosis or presumptive diagnosis of an infectious disease through:-

- Identification of a pathogen that has been isolated by culture
- Identification of pathogens in different samples of the patients.

Antibody tests

These tests are used mainly:-

- To diagnose a microbial disease when the pathogen or microbial antigen is not present in routine specimen or if present is not easily isolated and identified by other available techniques.
- To screen donor blood for different infectious diseases
- To monitor the effectiveness of a given treatment by measuring antibody titer
- To diagnose autoimmune disorders.

Agglutination Reactions

The interaction between antibody and a **particulate antigen** results in visible clumping called **agglutination**. Antibodies that produce such reactions are called **agglutinins**.

In district laboratories, agglutination tests are frequently used because they are simpler to perform, require no special equipment, and are usually less expensive.

Agglutination reactions depend on the cross linking of polyvalent antigens. An excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the **prozone effect**.

Several mechanisms can cause the prozone effect. First, at high antibody concentrations, the number of antibody binding sites may greatly exceed the number of epitopes. As a result, most antibodies bind antigen only univalently instead of multivalently. Antibodies that bind univalently cannot crosslink one antigen to another.

The prozone effect can also occur for another reason. The antiserum may contain high concentrations of antibodies that bind to the antigen but do not induce agglutination; these antibodies, called **incomplete antibodies**, are often of the IgG class. At high concentrations of IgG, incomplete antibodies may occupy most of the antigenic sites, thus blocking access by IgM, which is a good agglutinin.

Agglutination tests can be performed:

- A. On slides
- B. In tubes
- C. In microtitration plates

Hemagglutination Is Used in Blood Typing

Agglutination reactions are routinely performed to type red blood cells (RBCs). In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide.

Determination of which antigens are present on donor and recipient RBCs is the basis for **matching blood types for transfusions**.

Bacterial Agglutination Is Used To Diagnose Infection

A bacterial infection elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions. Serum from a patient thought to be infected with a given bacterium is serially diluted in an array of tubes to which the bacteria is added. The last tube showing visible agglutination will reflect the serum antibody **titer** of the patient. The agglutinin titer is defined as the reciprocal of the greatest serum dilution that elicits a positive agglutination reaction.

The agglutinin titer of an antiserum can be used to diagnose a bacterial infection. Patients with typhoid fever, for example, show a significant rise in the agglutination titer to *Salmonella typhi*. Agglutination reactions also provide a way to type bacteria. (different species of the bacterium *Salmonella* can be distinguished by agglutination reactions with a panel of typing antisera).

Passive Agglutination Is Useful with Soluble Antigens

The sensitivity and simplicity of agglutination reactions can be extended to soluble antigens by the technique of passive hemagglutination. In this technique, antigen-coated red blood cells are prepared by mixing a soluble antigen with red blood cells

that have been treated with tannic acid or chromium chloride, both of which promote adsorption of the antigen to the surface of the cells. Serum containing antibody is serially diluted into microtiter plate wells, and the antigen-coated red blood cells are then added to each well; agglutination is assessed by the size of the characteristic spread pattern of agglutinated red blood cells on the bottom of the well, like the pattern seen in agglutination reactions.

Over the past several years, there has been a shift away from red blood cells to synthetic particles, such as latex beads, as matrices for agglutination reactions.

Agglutination Inhibition

A modification of the agglutination reaction, called **agglutination inhibition**, provides a highly sensitive assay for small quantities of an antigen. For example, one of the early types of home pregnancy test kits included latex particles coated with human chorionic gonadotropin (HCG) and antibody to HCG. The addition of urine from a pregnant woman, which contained HCG, inhibited agglutination of the latex particles when the anti-HCG antibody was added; thus the absence of agglutination indicated pregnancy.

Agglutination inhibition assays can also be used to determine whether an individual is using certain types of illegal drugs, such as cocaine or heroin. A urine or blood sample is first incubated with antibody specific for the suspected drug. Then red blood cells (or other particles) coated with the drug are added. If the red blood cells are not agglutinated by the antibody, it indicates the sample contained an antigen recognized by the antibody, suggesting that the individual was using the illicit drug. One problem with these tests is that some legal drugs have chemical structures similar to those of illicit drugs, and these legal drugs may cross-react with the antibody, giving a false-positive reaction. For this reason a positive reaction must be confirmed by a nonimmunologic method. Agglutination inhibition assays are widely used in clinical laboratories to determine whether an individual has been exposed to certain types of viruses that cause agglutination of red blood cells. If an individual's serum contains specific antiviral antibodies, then the antibodies will bind to the virus and interfere with hemagglutination by the virus. This technique is commonly used in premarital testing to determine the immune status of women with respect to rubella virus. The reciprocal of the last serum dilution to show inhibition of rubella hemagglutination is the titer of the serum.