



IMMUNOLOGY FIRST COURSE

Ag – Ab reaction: Agglutination test

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المرحلة : الثالثة

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Practical

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Agglutination Reactions

Lecture Topics :-

1. Definition of Agglutination Reactions

2- Agglutination Types.

Agglutination Reactions :

- ❖ The interaction (immune reaction) between antibody and a particulated antigen resulting a visible clumping called **agglutination**. Antibodies that produce such reactions are called **agglutinins**.
- ❖ The agglutination tests are done either on the slide (**slide agglutination test**) or in tubes (**tube agglutination test**).

A. **Slide agglutination test** is simple and easy to perform and it needs few minutes only to perform the test.

B. **Tube agglutination test** is used to quantitate the amount of serum antibodies against a microbe by serially diluting the serum and then mixing the diluted sera with constant quantity of microbial antigens.

After appropriate incubation the highest dilution of the serum showing visible agglutination is taken as the titer of the serum

For example, **Widal tube test, Brucella tube test**).

The titer reflects the concentration of antibodies in the serum; higher the titer, more is the concentration of antibodies

Prozone phenomenon: is a false negative responses a false negative response resulting from high antibody titer which interferes with formation of antigen- antibody lattice.

Agglutination Types:

1. Direct Agglutination Test

Uses of Direct Agglutination Test:

A- Bacterial Agglutination:(. Diagnosis of microbial infections).

It is used to diagnose bacterial infection, when the immune system starts the production of serum antibodies specific for surface antigens on the bacterial cells.

These Abs can be detected by bacterial agglutination reactions.

Agglutination Types:

B. ABO blood grouping of human erythrocytes (by using antisera).

Principles:

Recognizing the type red blood cells (RBCs) by typing A and B antigens

2- Indirect Agglutination Test

In the indirect agglutination test, known soluble antigens are coated on to other cells (e.g., sheep or turkey erythrocytes) or inert particles (e.g., latex, polystyrene), which act as passive carriers of antigens.

Many antigens can be coated onto erythrocytes directly or after treating the erythrocytes with **formalin**, **tannic acid**.

There are two type of this agglutination:

- a. Agglutination of antigen-coated erythrocytes. It is called Hemagglutination test.
- b. Agglutination of antigen-coated Latex beads.

A. Hemagglutination test (Agglutination of antigen-coated erythrocytes)

Principles:

1. Red blood cells (sheep RBCs are mostly used) in this test are used as carriers of antigens and coloring agents. Antigen-coated red blood cells are prepared by mixing a soluble antigen with tanned red blood cells (that have been treated with tannic acid or chromium chloride to make them sticky), the Ag will be adsorbed onto the surface of the red cells.

2- Diluting serum containing antibody serial dilutions into microtiter plate wells, and the antigen-coated red blood cells are then added to each well.

3-Agglutination can be noticed by the size of the agglutinated red blood cells on the bottom of the well.

4-The formation of a red spot in the well is a marker for no agglutination.



A. Latex agglutination (Indirect agglutination test)

Clinical applications:

1. **Pregnancy test** is of a highly sensitive assay for small quantities of antigen in urine samples. The latex particles coated with prepared **Human chorionic gonadotropin antibodies** (anti-HCG) in pregnancy test kits. When urine from a pregnant female, which contained HCG mixed with latex beads reagent, if agglutination occurs that will indicate for pregnancy, if absence means no pregnancy.
2. **ASOT Anti-streptolysin O (ASO or ASLO)** .
3. **Widal Test**

Any questions?

*Thank
You*