AGGLUTINATION AND PRECIPITATION

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AGGLUTINATION REACTION

When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. Agglutination is more sensitive than precipitation for the detection of antibodies. The same principles govern agglutination and precipitation. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions. The zone phenomenon may be seen when either an antibody or an antigen is in excess. 'Incomplete' or 'monovalent' antibodies do not cause agglutination, though they combine with the antigen. They may act as 'blocking' antibodies, inhibiting agglutination by the complete antibody added subsequently.

Slide agglutination:

Procedure:

1. When a drop of the appropriate antiserum is added to a smooth, uniform suspension of a particulate antigen in a drop of saline on a slide or tile, agglutiation takes place.

2. A positive result is indicated by the clumping together of the particles and the clearing of the drop.

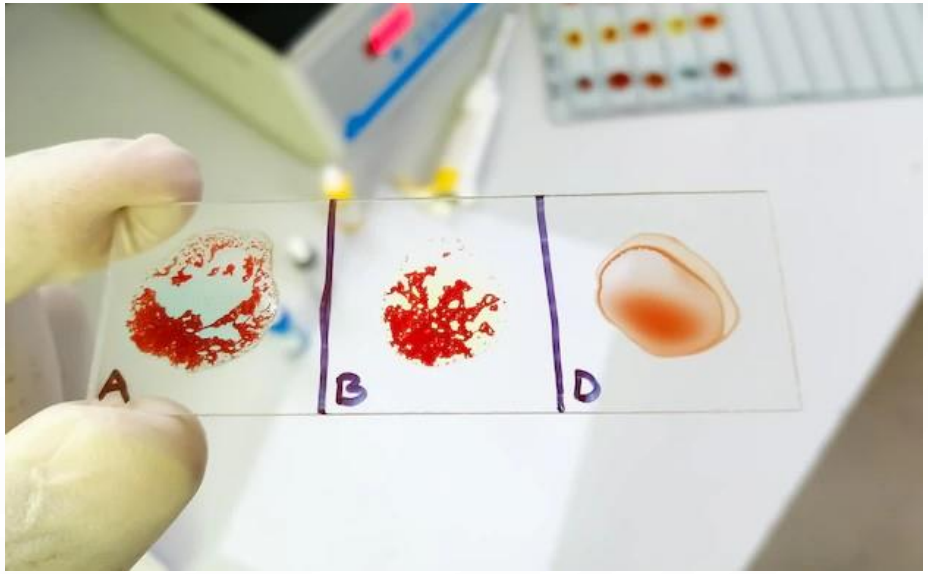
3. The reaction is facilitated by mixing the antigen and the antiserum with a loop or by gently rocking the slide. Depending on the titre of the serum, agglutonation may occur instantly or within seconds.

4. Clumping occurring after a minute may be due to drying of the fluid and should be disregarded.

5. It is essential to have on the same slide a control consisting of the antigen suspension in saline, without the antiserum, to ensure that the antigen is not autoagglutinable. Agglutination is usually visible to the unaided eye but may sometimes require confirmation under the microscope.

Uses:

A routine procedure for the identification of many bacterial isolates from clinical specimens.
It is also the method used for blood grouping and cross-matching.

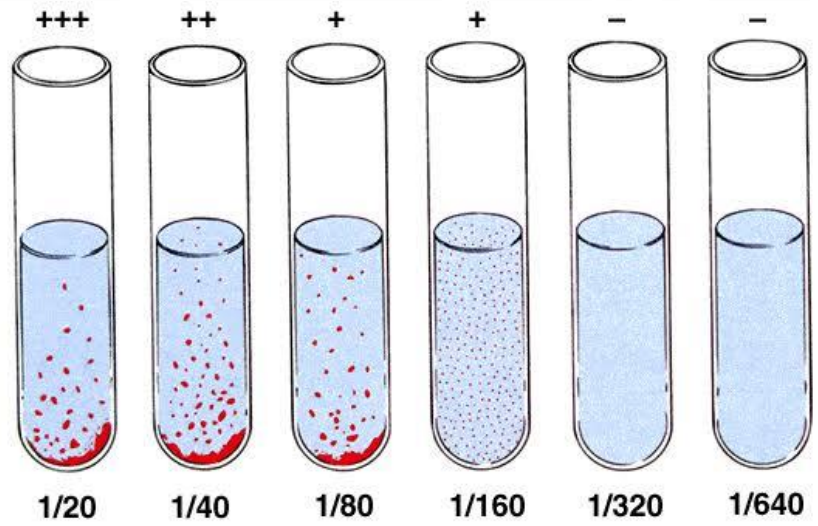


Tube agglutination:

This is a standard quantitative method for the measurement of antibodies. When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilutions of an antiserum in test tubes, the agglutination titre of the serum can be estimated.

Uses: Routinely used for the serological diagnosis of typhoid, brucellosis and typhus fever. In the Widal test used in typhoid, two types of antigens are used. The 'H' or flagellar antigen on combining with its antibody forms large, loose, fluffy clumps resembling wisps of cotton wool. The 'O' or somatic antigen forms tight, compact deposits resembling chalk powder. Agglutinated bacilli spread out in a disc-like pattern at the bottom of the tubes.

Complications: The tube agglutination test for brucellosis may be complicated by the prozone phenomenon. Several dilutions of the serum should be tested to prevent false negative results due to the prozone of 'blocking' antibodies. Incomplete or blocking anti-bodies may be detected by doing the test in hypertonic (5%) saline or albumin saline, or more reliably by the antiglobulin (Coombs) test.



Heterophile agglutination test:

The **Weil-Felix reaction** for serodiagnosis of typhus fevers is a heterophile agglutination test and is based on the sharing of a common antigen between typhus rickettsiae and some strains of proteus bacilli. The Streptococcus MG agglutination test for the diagnosis of primary atypical pneumonia.
Examples of agglutination tests using red cells as antigens are the **Paul-Bunnell** test and the cold agglutination test. The former is based on the presence of sheep cell agglutinins in the sera of infectious mononucleosis patients, which are adsorbed by ox red cells but not by guinea pig kidney extract. The cold agglutination test is positive in mycoplasmal (primary atypical) pneumonia. The patient's sera agglutinate human O group erythrocytes at 4°C, the agglutination being reversible at 37°C.

Antiglobulin (Coombs) test: This was devised by Coombs, Mourant and Race (1945) for the detection of anti-Rh antibodies that do not agglutinate Rh-positive erythrocytes in saline.

Principle: When sera containing incomplete anti-Rh antibodies are mixed with Rh-positive red cells, the antibody globulin coats the surface of the erythrocytes, though they are not agglutinated. When such erythrocytes coated with the antibody globulin are washed free of all unattached protein and treated with a rabbit antiserum against human gamma globulin (antiglobulin or Coombs serum), the cells are agglutinated

Types:

Direct Coombs test:

The sensitization of the erythrocytes with incomplete antibodies takes place in vivo, as in hemolytic disease of the newborn due to Rh incompatibility. When the red cells of eryth-roblastotic infants are washed free of unattached protein and then mixed with a drop of Coombs serum, agglutination results. The direct Coombs test is often negative in hemolytic disease due to ABO incompatibility.

Indirect Coombs test:

Sensitization of red cells with the antibody globulin is performed in vitro

Uses: Originally employed for the detection of anti-Rh antibodies, the Coombs test is useful for demonstrating any type of incomplete or non-agglutinating antibody, as, for example, in brucellosis.

Passive agglutination test: The only difference between the requirements for the precipitation and agglutination tests is the physical nature of the antigen. By attaching soluble antigens to the surface of carrier particles, it is possible to convert precipitation tests into agglutination tests, which are more convenient and more sensitive for the detection of antibodies. Such tests are known as passive agglutination tests.

Hemagglutination:

A special type of passive hemagglutination test is the Rose-Waaler test. In rheumatoid arthritis, an autoantibody (RA factor) appears in the serum, which acts as an antibody to gamma globulin. The RA factor is able to agglutinate red cells coated with globulins. The antigen used for the test is a suspension of sheep erythrocytes sensitised with a sub agglutinating dose of rabbit anti-sheep erythrocyte antibody (amboceptor)

Latex agglutination test: Polystyrene latex, which can be manufactured as uniform spherical particles, 0.8-1.0 μm in diameter, can adsorb several types of antigens. Latex agglutination tests (latex fixation tests) are widely employed in the clinical laboratory for the detection of ASO, CRP, RA factor, HCG and many other antigens.

Co-agglutination test: Passive agglutination tests are very sensitive and yield high titres, but may give false positive results. When, instead of the antigen, the antibody is adsorbed to carrier particles in tests for the estimation of antigens, the technique is known as reversed passive agglutination. This method is used to diagnose bacterial antigens like Legionella, Streptococcus pyogenes and N.gonorrhoea in clinical samples. It is based on agglutination of the antibody sensitised protein A-bearing Staphylococcus aureus in the presence of bacterial (Legionella) soluble antigens in clinical specimens.

PRECIPITATION REACTION

Precipitation:

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex forms an insoluble precipitate.

Phases: The amount of precipitate formed is greatly influenced by the relative proportions of antigens and antibodies. If increasing quantities of antigens are added to the same amount of antiserum in different tubes, precipitation will be found to occur most rapidly and abundantly in one of the middle tubes in which the antigen and antibody are present in optimal or equivalent proportions. In the preceding tubes in which the antibody is in excess and in the later tubes in which the antigen is in excess, the precipitation will be weak or even absent. For a given antigen-antibody system, the optimal or equivalent ratio will be constant, irrespective of the quantity of the reactants. If the amounts of precipitate in the different tubes are plotted on a graph, the resulting curve will have three phases:

1. **Prozone phenomenon:** This is caused by excess antibody in the test system. Failure of a visible reaction is due to inhibition of lattice formation by the excess antibody.
2. Zone of equivalence: Here, the antigen and antibody are in optimum proportions. Lattice formation and visible reactions are enhanced.
3. 3. Post-zone phenomenon: This is caused by the presence of excess antigen in the test system. No visible reaction will occur.

Applications: The precipitation test may be carried out as a qualitative or quantitative test. It is sensitive in the detection of antigens and as little as 1 µg of protein can be detected. It is relatively less sensitive for the detection of antibodies. Precipitation tests have several applications:
Forensic application in the identification of blood and seminal stains.
Testing for food adulterants.
Grouping of streptococci by the Lancefield technique.
The VDRL test for syphilis
To standardise toxins and toxoids.
To test toxigenicity in diphtheria bacilli.

The following types of precipitation and flocculation tests are in common use:

Ring test: This, the simplest type of precipitation test, consists of layering the antigen solution over a column of antiserum in a narrow tube. A precipitate forms at the junction of the two liquids. Ring tests have only a few clinical applications now. Examples are Ascoli's thermoprecipitin test and the grouping of streptococci by the Lancefield technique.

Slide test: When a drop each of the antigen and the antiserum are placed on a slide and mixed by shaking, floccules appear. The VDRL test for syphilis is an example of slide flocculation.

Tube test: The Kahn test for syphilis is an example of tube flocculation test. A quantitative tube flocculation test is used for the standardization of toxins and toxoids. Serial dilutions of the toxin/toxoid are added to the tubes containing a fixed quantity of the antitoxin. The amount of toxin or toxoid that flocculates optimally

Properties	Precipitation reaction	Agglutination reaction
Definition	It is the antigen antibody reaction where the antibody reacts with the soluble antigen to form precipitin	It is the antigen antibody reaction where the antibody reacts with the soluble antigen to form agglutinin
Size of an antigen	larger	Comparatively smaller
Solubility of antigen	Soluble form	Sedimented form
Sensitivity	Less sensitive	More sensitive
Media used	Either liquid or gel matrix	Does not require
Types	It is of three types: Precipitation in solution, precipitation in agar by diffusion and electrophoresis	It is of two types: Active and passive agglutination
Matrix	Precipitation reaction can be performed on glass slides, petri plates and test tubes	Agglutination reaction can be performed on microtitre plate, glass slides and test tubes
Resulted compound	Precipitins	Agglutinins
Formation of resulted compound	Either found as suspension or sink to the bottom	The end product sinks to the bottom
Appearance of end product	Appear as large, insoluble mass of visible precipitate	Appear as large visible aggregates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