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3rd class

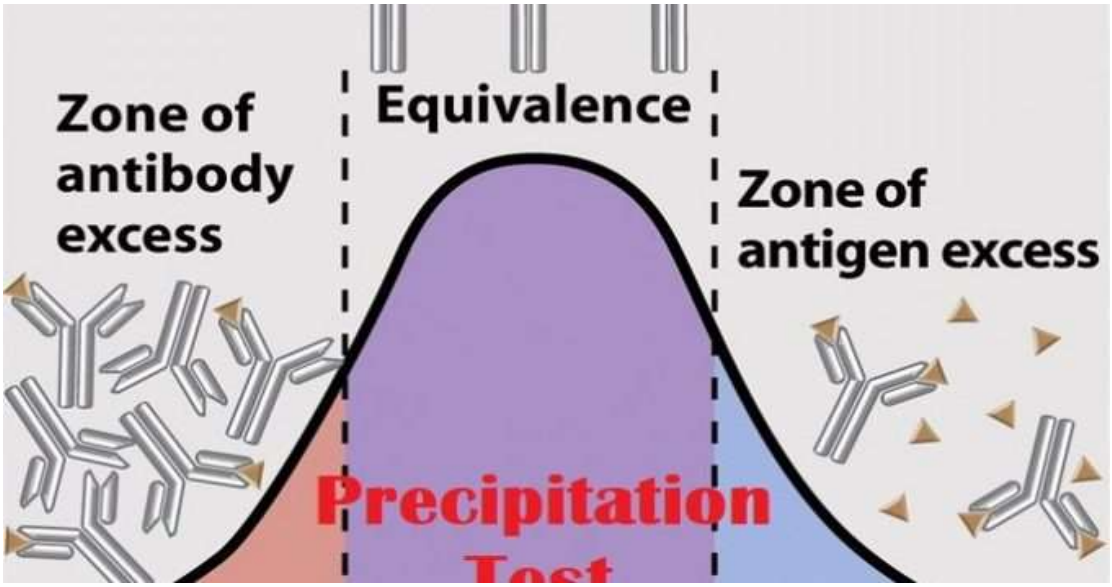
Precipitation Test

Principle:-

The precipitation technique requires the formation of a visible lattice that results from the combination of soluble antigen to its soluble antibody . It is usually works best when antigens and antibodies are at optimal proportions (equivalence)

Formation of an antigen-antibody lattice depends on the valency of both the antibody and antigen;

1. The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.
2. The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope or have different epitopes that react with different antibodies present in polyclonal antisera.



- When bivalent antibody combines with multivalent soluble antigen, visible precipitation is formed which is indicator of antigen-antibody reaction.
- If precipitate remains suspending instead of sedimentation, it is called flocculation test.
- In order to occur precipitation Ag-Ab must be in appropriate concentration.
- When antibody concentration is too high and antigen concentration is too low, visible precipitate is not found. This inhibition of precipitation by excess antibody is called prozone effect.
- On the other hand when antibody concentration is too low and antigen concentration is too high, visible precipitate is not formed.
- This inhibition of precipitates by excess antigen is called post-zone effect.
- Precipitation occurs only when antigen and antibody are in appropriate concentration Region is the graph where precipitate occurs maximally is called equivalence zone.

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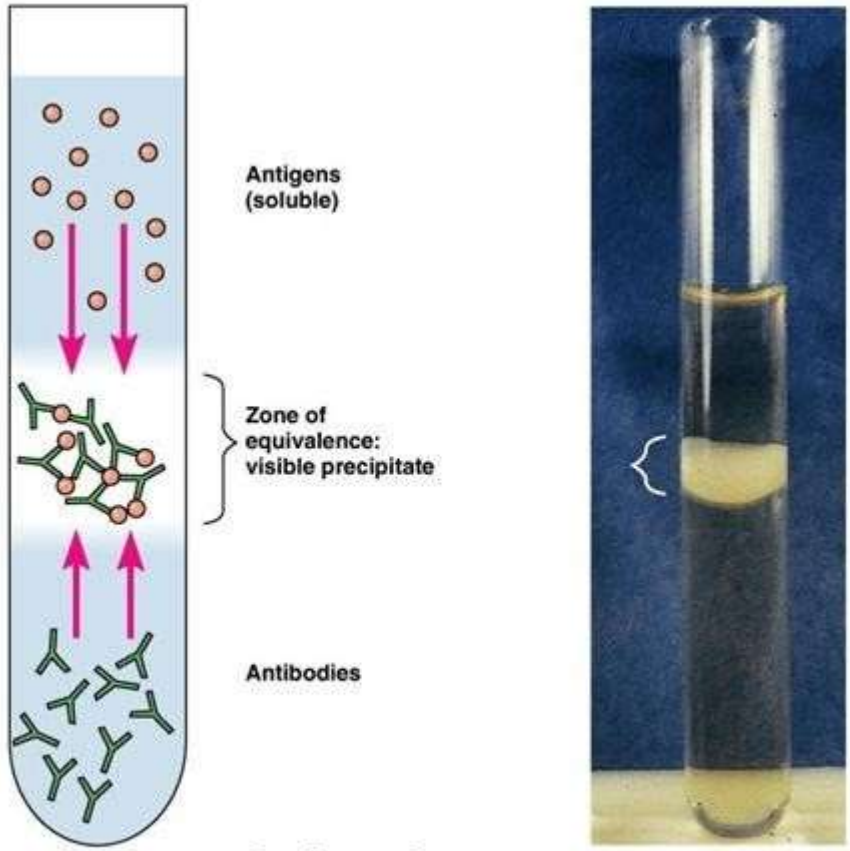
- Formation of precipitate can be described by lattice hypothesis.
- When antigen and antibody are in appropriate concentration, maximum cross linking of antigen by antibody occurs so that visible precipitate is formed.
- Either excess antigen or excess antibody prevents extensive cross linking of antigen by antibody so that visible precipitate is not formed.
- This is the reasons why the precipitate occur only in equivalence zone but not in prozone and post zone



Precipitation Test

Types of precipitation reaction:

Precipitation reaction can be broadly of three types;

1. Precipitation in solution
    - 1. Ring Test
    - 2. Slide Test
    - 3. Tube Test

2. Precipitation in agar
    1. Single diffusion in a single dimension
    2. Single diffusion in double dimension
    3. Double diffusion in a single dimension
    4. Double diffusion in double dimension
3. Precipitation in agar with an electric field.
    1. Immunoelectrophoresis (Immunodiffusion plus electrophoresis)
    2. Counter Current electrophoresis
    3. Rocket Electrophoresis

Ring test or interfacial test

It is one of the fast and simplest tests. It is performed in a test tube where the antibody solution to be detected in the sample is put at first and antigen solution is then poured. The test is confirmed as positive by the observation of a precipitate ring in the middle of the tube after a few hours.

- It is used in the Lancefield technique for grouping *Streptococcus* spp.
- It is also used in the detection of anthrax by Ascoli's test.

Ring Test Requirements

- Test tubes or capillary tubes, Serum containing reactant mainly antibody,
- Corresponding antigen solution, Chemicals such as glycerol.

Note: Glycerol can be used in the ring test to avoid the intermixing of antigen and antibody solutions.

Ring Test Result

An observable precipitate ring between antigen and antibody solution is seen after a few hours maybe about four which confirms the test to be positive.

Slide Test

This test is performed on slides such as cavity slides in the case of the VDRL test. In this test, the serum sample of the suspected patient is kept in the cavity of the slide and antigenic solution(already known) is mixed with it and shaken properly. Floccules are formed after a while in the case of the positive test.

Requirements

Glass slides (Cavity slides), A Serum sample, Known antigen or antibody solutions

Precipitation Reaction in Agar

It is performed on Agar or Agarose gel or polyacrylamide gel. It is also termed immunodiffusion. Agarose gel is generally preferred. Gels provide a medium for the diffusion of reactants through the pores.

It is more advantageous than in liquid medium as clear observable bands are formed in this type of precipitation. These bands are generally easy to preserve for a longer time and further use. Another advantage is the differentiation of individual antigens from the mixture of antigens.