Practical write up

B.Sc Life Sciences

Sem Vi

Paper: Immunology

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Aim: To perform a simple immune diffusion test (Ouchterlony test)

Requirements:

1.5% Agar, 1% Agar, Acetocarmine, Methylene blue, Glass slides, Micropiettes, Microtips, Blotting sheets, Tissue rolls, Plastic trays

Theory:

The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops into a visible precipitate. Excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so called equivalence zone, when the ratio of antibody to antigen is optimal.

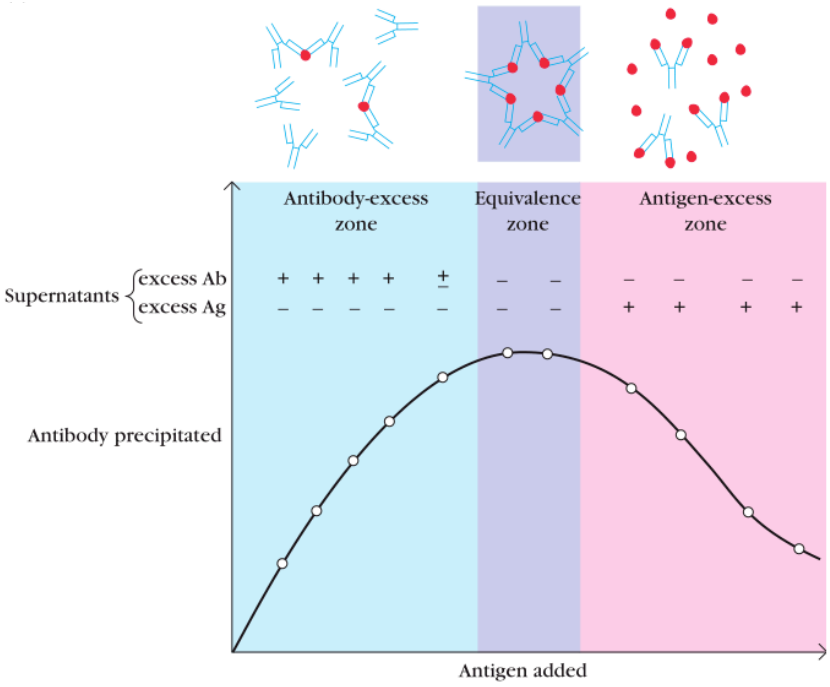


Figure 1: A precipitation curve for a system of one antigen and its antibodies.

Immune precipitates can form not only in solution but also in agar matrix. When antigen and antibody diffuse toward one another in agar, or when antibody is incorporated into the agar and antigen diffuses into the antibody containing matrix, a visible line of precipitation will form.

Two immune diffusion techniques are radial immunodiffusion (Mancini method) and double immunodiffusion (Ouchterlony method); both are carried out in a semisolid medium such as agar.

1. Radial Immunodiffusion (Mancini Method): The relative concentrations of an antigen can be determined by a simple quantitative assay in which an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation, a precipitin ring, forms around the well. The area of precipitin ring is proportional to the concentration of antigen.

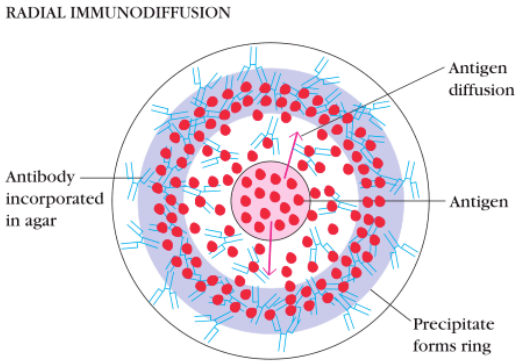


Figure 2: Radial Immunodiffusion

2. Double Immunodiffusion (Ouchterlony method): In this method, both the antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As the equivalence reached, a visible line of precipitation, a precipitin line, forms. This simple technique is an effective qualitative tool for determining the relationship between antigens and number of different Ag-Ab systems present. The pattern of the precipitin lines that form when two different antigen preparations are placed in adjacent wells indicates whether they share epitopes:

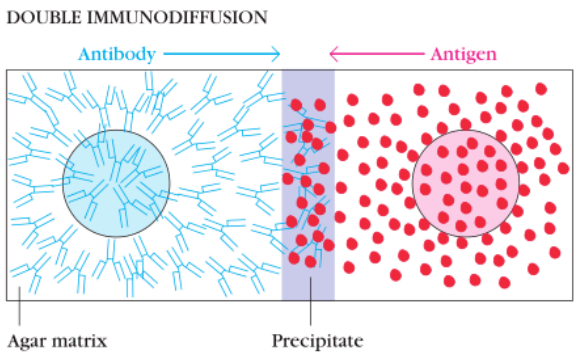
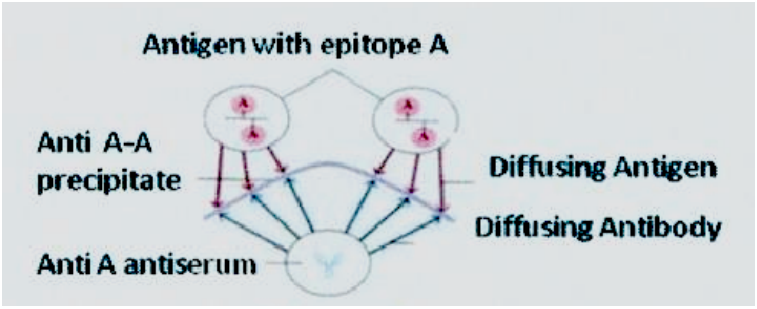
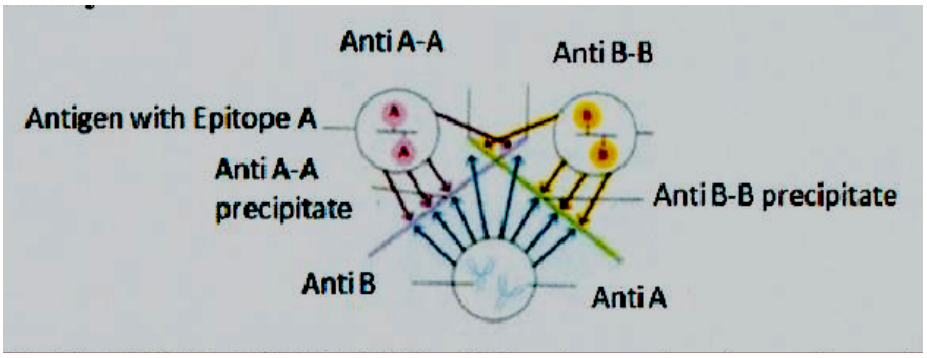


Figure 3: Double Immunodiffusion

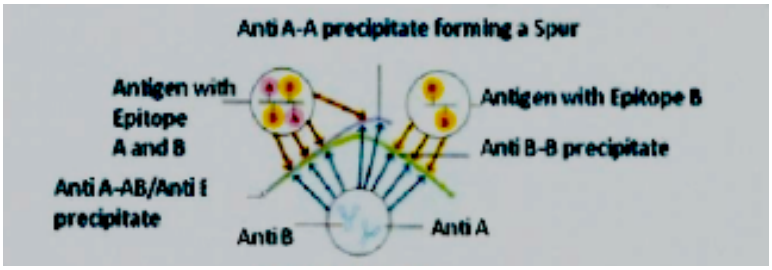
1. Identity: It occurs when two antigens share identical epitopes. As the antiserum forms a single precipitin line with each antigen, the two lines grow toward each other and fuse to give a single curved line of identity.



2. Nonidentity: It occurs when two antigens are unrelated (i.e. share no common epitopes). The antiserum forms an independent precipitin line with each antigen, and the two lines cross.



3. Partial identity: It occurs when two antigens share some epitopes but one or the other has a unique epitope (s). The antiserum forms a line of identity with the common epitope (s) and a curved spur with the unique epitope (s).



Procedure:

1. Prepared 1.5% agar and 1% agar and heated till the clearing of the solution and agar dissolved completely.

2. Overlayered about 1ml of 1.5% agar uniformly on the clean glass slide with the help of micropipette and left till solidification. This is called precoating.

3. Overlayered about 3ml of 1% agar uniformly on the solidified 1.5% agar layer with the help of micropipette and left till solidification.

4. Wells were bored in the solidified 1% agar layer with the help of microtip. A central well was bored and four wells were bored around it. Any kind of pattern of wells could be formed.

5. Wells were filled with about 2µl of methylene blue in the central well and adjacent wells were filled with acetocarmine dye. The pattern was also reversed with acetocarmine in the central well and adjacent wells were filled with methylene blue dye.

6. The sildes were left undisturbed for incubation at room temperature.

Observations: Dyes were diffused into adjacent agar from their respective wells. A precipitin line was formed in between the wells (i.e. in the zone of equivalence).

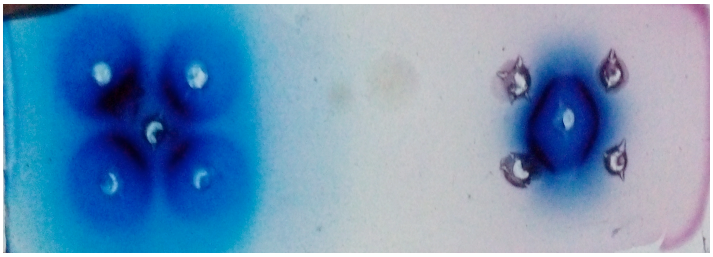


Figure: Methylene blue in wells as antigen and acetocarmine in wells as antibody, precipitin lines observed at the interphase of wells due to antigen-antibody interactions.

Discussion:

A precipitin line is formed at the interfaces of well where concentrations of both dyes were optimal. The number of lines of precipitate indicates the maximum number of distinct antigenic substance present in the antigenic solution. When the antigen and antibody in wells of identical shape and size, the curvature of precipitin depends on the relative molecular weight of antigen. Antibody line is straight in case of equal molecular weight otherwise the line tends to be concave towards high molecular weight.

The immunodiffusion reactions can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation.

Precautions:

1. Both agar layers (1.5% and 1% layer i.e precoat and main layer respectively) should be uniform.

2. There should not be any air bubbles in the agar layers.

3. Well should be in the 1% i.e. main agar layer and it should not puncture the precoating.

4. There should not be overflow of dyes from the wells.

5. During incubation slides should not be disturbed.