COUNTER CURRENT IMMUNOELECTROPHORESIS

AIM: To learn the technique of Counter Current Immunoelectrophoresis.

PRINCIPLE: **Immunoelectrophoresis** is the electrophoresis of a determined antigen mixture in an agarose gel that allows the separation of different antigens along the gel slide, and then the lateral diffusion of an antibody in the gel. **Counter Current Immunoelectrophoresis** is a modification of immunoelectrophoresis in which antigen and antibody move in opposite directions and form precipitates in the area between the cells where they meet in concentrations of optimal proportions.

In this method, immunoprecipitation occurs when antigen at the cathode is caused to migrate in an electric field through a suitable medium of diffusion against a stream of antibody migrating from the anode because of endosmotic flow. This is one-dimensional double electroimmunodiffusion in which antibody and antigen are moved towards each other by an applied electric field, because the gel is buffered at a pH between the isoelectric points of the antigen and antibody. The antigen and antibody are placed in separate wells in an Agarose gel plate. The gel is alkaline and antibody (Immunoglobulin) at pH 7.6 has a charge-nearing zero. During electrophoresis, the agarose matrix absorbs OH- ions on the surface resulting in a net increase in positive ions at a distant from the matrix. These positive ions migrate towards the negative pole with a solvent shield, resulting in a net solvent flow called endosmosis. Hence, antibody molecules, which have no charge, move towards cathode along with solvent shield due to this phenomenon.

MATERIALS: Agarose, Antigem, Test antiserum, Positive antiserum, Assay Buffer, Electrophoreis apparatus, Glass slides.

PROCEDURE: Prepare 10 ml of 1.0% Agarose (0.1 g/10 ml) in 1X Assay Buffer by heating slowly till agarose dissolves completely. Take care not to froth the solution.

2. Mark the end of a glass slide as +ve and -ve, so that when placed the glass slide in electrophoresis apparatus, the +ve mark faced towards anode and the negative mark faced towards cathode.
3. Place the glass plate or slide on a horizontal surface. Pipette and spread 5 ml of agarose onto the glass slide. Allow to solidify for 15 minutes. Take care that the slide is not disturbed and allow the gel to solidify.

4. Cut wells of according to the template using gel puncher. The distance between the two wells should not be more than 0.5 cm.

5. Place the slide in the electrophoresis tank and fill the tank with 1X electrophoresis buffer till the buffer just covers the gel surface. Do not add excess of buffer.

6. Add 10µl of antigen in each of the two wells towards cathode (Negative electrode) and 10µl of positive control antiserum and test antisera in wells towards anode (Positive electrode) as shown.

7. Connect the power cord to the electrophoretic power supply according to the convention: red : anode and black : cathode.

8. Apply 50 V and allow the electrophoresis to continue for about 45 minutes.

9. Observe for precipitin line between the antigen and antisera wells.

**INTERPRETATION:**

a) Precipitin line indicates the presence of antibody for the antigen in the test sera.

b) The absence of the precipitin line indicates the absence of any antibody for the antigen in the test sera.

**RESULT:** The presence of precipitin line between the antigen and antibody indicates its specificity while the absence of precipitin line indicates non-specificity.

**NOTE:** *Don’t include this in record*

<table>
<thead>
<tr>
<th>Antigen well 1: BSA</th>
<th>Antibody well 1: Anti BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen well 2: IgG</td>
<td>Antibody well 2: Anti BSA</td>
</tr>
</tbody>
</table>