Welcome students of T.Y.B.Sc.

The title of the Unit: Unit

8: Immunological techniques.

Module name: Principle of

Immunoelectrophoresis. I'm

Dr. Carolina Fernandes,

Associate Professor from P.E.S's

RSN College of Arts and

Science, Farmagudi, Ponda,

Goa. Outline of this module:

What is immunoelectrophoresis?

Its principle, methodology and

applications. The learning outcomes:

The student will be able to:

define immunoelectrophoresis,

explain its principle, methodology

and applications.

Immunoelectrophoresis is a technique

that involves combination of the

principles of electrophoresis

and immunological reactions.

Electrophoresis is the movement

of charged particles

(ions) in an electric field resulting

in their migration towards the

oppositely charged electrode.

Molecules with a net positive charge

(cations) move towards the negative cathode,

while those with net negative charge

(anions) migrate towards positive anode.

Electrophoresis is a widely used

analytical technique for the

separation of biological molecules,

such as plasma proteins,

lipoproteins and immunoglobulins.

The rate of migration of ions in an

electric field depends on several

factors that include shape, size,

net charge, and solvation of the ions,

viscosity of the solution and

magnitude of the current employed.

These assays are done in semisolid gels,

into which holes are cut for

antigen and/or for

antibody and diffusion occurs

until antigen and antibody are

at equivalence and precipitate.

Immunoelectrophoresis is useful

for the analysis of complex mixtures

of antigens and antibodies.

Immunoelectrophoresis constitutes

refinement of diffusion and

precipitation in agar.

Grabar and Williams devised this

Technique. This involves the

electrophoretic separation of

composite antigen into its

constituent proteins,

followed by immunodiffusion

against its antiserum,

to produce separate precipitin lines.

It is performed on an agarose gel with an

antigen well and antibody trough cut on it.

The test serum is placed in the antigen well and electrophoresed for about one hour. Antibody against human serum is placed in the trough and diffusion allowed for 18 to 24 hours. Antibodies that react with specific serum proteins form reaction arcs specific for each protein. When serum samples contain several antigens, immunoelectrophoresis can be used to detect separate antigen-antibody complexes. The advantage: ability to separate several antigens that might be present in a serum sample. The complex proteins of biological samples are subjected to electrophoresis. The antibody is then applied in a trough parallel to the electrophoretic separation. The antibodies diffuse and when they come in contact with antigens, precipitation occurs, resulting in

the formation of precipitin bands,

which can be identified.

Precipitin bands form wherever matching

antigen and antibody precipitate.

These are the steps of immuno

electrophoresis.

Electroimmunodiffusion:

The development of precipitin lines

can be speeded up by electrically

driving the antigen and antibody.

There are two types: counter

immunoelectrophoresis,

also called one dimensional double

electroimmunodiffusion and

rocket electrophoresis,

also called one dimensional

single electroimmunodiffusion.

The first one,

counterimmunoelectrophoresis,

uses an electrical current to

speed up the migration of antigen

and antibody, is a newer technique

for identifying bacterial and

viral antigens in blood.

This involves simultaneous

electrophoresis of antigen and antibody

in gel in opposite directions,

resulting in precipitation

at a point between them.

They produce precipitation

lines within 30 minutes.

The clinical application: detecting

antigens like alphafetoprotein in

serum, antigens of Cryptococcus

and Meningococcus in the CSF.

These are the results.

Second, rocket electrophoresis is used

for quantitative estimation of antigens.

The antiserum to the antigen to be

quantitated is incorporated in

agarose gel on a slide. Antigen

in increasing concentrations, is

placed in wells punched in the set

gel. The antigen is electrophoresed

into the antibody-containing agarose.

The pattern of immunoprecipitation

resembles a rocket.

Laurell’s two dimensional electrophoresis:

is a variant of rocket electrophoresis.

The antigen mixture is electrophoretically

separated in a direction perpendicular

to that of the final rocket stage.

It is used to quantitate each of

the several antigens in a mixture.

These are my references and credits.

Thank you.