Welcome students of T.Y.B.Sc.

The title of the Unit,

Unit 8: Immunological Techniques, Module name:

Principle of immunodiffusion

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The outline of this module:

What is immunodiffusion?

Its principle, methodology and

applications.

The learning outcomes: the student will

be able to describe Immunodiffusion,

an in vitro serological reaction.

Explain principle,

methodology and applications.

Introduction: any molecule that

induces production of antibodies

when introduced in the body of

an animal is called an antigen.

Example bacteria, viruses, pollen, etc.

Antibodies are proteins produced

by the immune system,

which help defend against antigens.

Antigen-antibody reactions in vitro

are known as serological reactions.

Antigens

and antibodies combine with

each other specifically and in

an observable manner. In the body,

they form the basis of antibody-mediated

immunity in infectious diseases,

or hypersensitivity and autoimmune

diseases. In the laboratory,

they help in diagnosis of infections,

in epidemiological surveys, in the

identification of infectious agents,

enzymes.

Stages of antigen-antibody reactions:

Primary stage:

initial interaction between

antigen and antibody is invisible.

It is rapid,

occurs at low temperatures and

obeys the general laws of physical

chemistry and thermodynamics.

Reaction is reversible,

Antigen and antibody is bound to

each other by weak Van der Waal's

forces, ionic bonds and hydrogen bonding.

Secondary stage: consists of

demonstrable events - precipitation,

agglutination, lysis of cells,

killing of live

antigens, neutralization of

toxins, complement fixation,

immobilization of motile organisms

and enhancement of phagocytosis.

General features of antigen-

antibody reactions:

The reaction is specific.

Entire molecules react and not the fragments.

There is no denaturation of the antigen

or antibody during the reaction.

The combination occurs at the surface,

so surface antigens are

immunologically relevant.

The combination is firm but reversible.

The firmness is influenced by the affinity

and avidity of the reaction.

Both antigens and antibodies participate

in the formation of agglutinates

or precipitates.

Antigens and antibodies can

combine in varying proportions.

Both antigens and antibodies

are multivalent.

Affinity-refers to the intensity

of attraction between the antigen

and antibody molecules.

It is the function of closeness

of fit between the epitope

and antigen binding region of its

antibody. Avidity is the strength of

the bond after the formation of antigen-

antibody complexes.

Measurement of antigen and antibody-

maybe in terms of mass or

more commonly as units or titre.

The antibody titre of a serum is

the highest dilution of the serum,

which shows an observable reaction

with the antigen in a particular test.

Two important parameters in serological

tests are sensitivity and specificity.

Sensitivity is the ability of the

test to detect even very minute

quantities of antigen or antibody.

When the test is highly sensitive,

false negative results

may be absent or minimal.

Specificity is the ability of the test

to detect reactions between homologous

antigens and antibodies

only, and with no other.

In highly specific test,

false positive reactions are absent or

minimal. Types of antigen-antibody reactions:

Examples are precipitation, agglutination,

complement fixation, immobilization,

opsonization, neutralization, immuno

fluorescence, radioimmunoassay,

enzyme immunoassay.

Immunodiffusion is precipitation in gel.

Precipitation: Principle - when a soluble

antigen combines with its antibody

in the presence of electrolytes

at a suitable temperature and pH,

the antigen-antibody complex

forms an insoluble precipitate.

Precipitation can take place in

liquid media or in gels such as agar,

agarose, or polyacrylamide.

They used to study specificity

of antigen-antibody reactions.

These assays are done in semisolid gels,

into which wells or holes are cut

for antigen and/or for antibody and

diffusion occurs until antigen and

antibody are at equivalence and precipitate.

It can be carried out as either a

qualitative or quantitative test.

It is sensitive for the detection

of antigens.

Antibody participates in

precipitation is called precipitin.

The antigen participating in precipitation

is called precipitinogen.

Advantages of immunodiffusion:

The reaction is visible as a

distinct band of precipitation.

It is stable and can be stained

for preservation.

It indicates identity,

cross reactions,

non identity between different antigens.

Types: 1. Single diffusion in one

dimension also called Oudin procedure.

Here, the antibody is incorporated

in agar gel in a test tube and the

antigen solution is layered over it.

Antigen diffuses downward through

the agar gel,

forming a line of precipitation.

2. Double diffusion in one dimension, is

also called Oakley-Fulthorpe procedure.

Here, the antibody is incorporated

in the agar gel, above

which is placed a column of plain agar.

The antigen is layered over it.

The antigen and antibody move

towards each other through the

intervening column of plain agar

and form a precipitate.

Here, are the results.

3.

Single diffusion in two dimensions,

also called radial immunodiffusion

or Mancini technique.

Here, the antisera is incorporated

in a gel and poured on a flat

surface. Wells are cut on the surface,

to which antigen is added.

Antigen diffuses radially out of the

well into the gel and interacts

with the antibody, forming ring

shaped bands of precipitation

concentrically around the well,

the diameter of which is related to

the concentration of the antigen.

These are the results.

4. Double diffusion in two dimensions,

Ouchterlony procedure: It is

called double diffusion,

because it involves diffusion of

both antigens and antibodies.

The test is performed by punching

a pattern of small wells into an

agar medium and filling them with

test antigens and antibodies.

Agar gel is poured on a slide

and wells are cut.

Antiserum is placed in the central

well, while different antigens in

the surrounding wells. A band forming

between two wells indicates that

antibodies from one well have met

and reacted with antigens from the

other well. Helps to compare different

antisera and antigens directly.

When a well containing antibody

is placed near two antigen

wells, lines of precipitate that

form between the antigen-antibody

wells are indicative of various

characteristics of the test antigens:

A single, fused, precipitin band/

or arc, indicates a distinct

antigen-antibody reaction,

shows that the antigens are

identical - called reaction of identity.

When two lines cross or intersect

each other, indicates a lack of

identity between the antigens/

lack of relatedness.

The formation of a spur on one

side only demonstrates that the

two antigens share one antigenic

determinant but differ in other

antigenic characteristics, indicating

partial identity between antigens.

These are my references.

and credits. Thank you.