I am Ruella D'souza.

Today we shall be covering Unit

8 immunological techniques.

Under which we will be covering

principles of precipitation and

agglutination in the upcoming module.

The outline.

At the conclusion of this presentation,

the student will be able to comprehend

the principles behind the precipitation

and agglutination reactions.

And their role in various

immunodiagnostic tests.

Principle of precipitation.

Interactions of antibodies and

antigens in the form of various tests.

Have been developed to determine their

presence or absence in a sample.

A type of interaction between

soluble antigen with its specific

antibody in a suitable medium.

That results in the formation of an

insoluble complex that precipitates.

Is termed as a precipitation reaction.

Precipitation tests.

In the diagram you can see tubes

containing antigen and antibody.

The first two tubes, labelled A

indicate a zone of antibody excess.

The next three indicate a zone of equivalence

which will demonstrate precipitation.

The last two tubes are called post zone

or zone of antigen excess which will

not demonstrate precipitation very well.

Precipitation is governed by

the formation of a lattice.

The zone of equivalence has optimum

concentration of antigen and antibody,

thus resulting in crosslinking

and precipitation of the antigen.

In the zone of antigen excess,

there is incomplete crosslinking.

As is seen also in a zone of antibody excess.

The types of precipitation

reactions that we will study.

A) The ring test used to group Streptococci

by the Lancefield technique.

B) the slide test. Example,

the VDRL test used to detect syphilis.

C) the tube test example.

The Kahn test also used to detect syphilis.

Let us now study the ring test.

As you can see in the image.

The antibody is placed in a test

tube and overlaid with the antigen.

There occurs diffusion of the antigen

and antibody towards each other.

It is visualized as a precipitate ring

at the junction of the two layers.

It is the simplest form of the test.

This slide test.

You can see three images of non

Reactive, weakly reactive and strongly

reactive antigen- antibody samples.

The test is carried out by

placing one drop each of antigen

and antibody on the slide.

It is then mixed by shaking

gently and floccules will appear

as a result of binding.

The tube test.

A fixed amount of serum is

added to multiple test tubes,

each containing antigen.

Optimum concentration of antigen

antibody will result in precipitation.

And will be seen at the bottom

of the test tube.

The tube test is used to

standardize toxins and toxoids.

We now move to the

principle of agglutination.

At the bottom of your screen you have an

image demonstrating tube agglutination.

Agglutination occurs when an immune

complex is formed by crosslinking cells

or particles with specific antibodies.

This results in the creation of

visible aggregates or clumps that

can be seen with the unaided eye.

Direct agglutination.

Is the agglutination,

for example of typhoid bacilli

When mixed with serum

containing typhoid antibodies.

This is the basis of the Widal test.

Hemagglutination results from

the crosslinking of antibodies.

To RBC’s or erythrocytes.

As a result of attachment

to surface antigens.

Hemagglutination is routinely

used in blood typing.

We also know that certain

viruses can accomplish what we

call viral hemagglutination.

Take a look at the image.

you have RBC’s and

measles virus mixed together.

As a result of viral hemagglutination,

there is crosslinking between

the RBC’s and the measles virus.

B) RBC;s when mixed with the

measles virus and with antiviral

measles antibodies from serum

Will not demonstrate hemagglutination.

This is because the measles virus is

neutralized by the antibodies and thus

incapable of agglutinating the RBC’s.

This indicates a positive

test for measles virus.

It is used to diagnose influenza,

mumps and other viral infections.

The Coombs test is an

indirect agglutination test.

For the detection of cell bound

or incomplete antibodies.

Certain Sera,

such as RH antibody containing sera.

Not only failed to agglutinate

corresponding antigen.

Rh, positive RBC but also inhibit it.

These are called blocking

or incomplete antibodies.

They do not agglutinate RBC's,

but they most certainly bind to them.

We have an image for the direct Coombs test.

Antibody coated cells from a patient.

Are allowed to react with the Coombs reagent

which is anti immunoglobulin antibody.

This demonstrates agglutination as a

result of the Coombs reagent binding to

the FC region of the immunoglobulin.

This is used to detect anti D antibody as

in the case of erythroblastosis fetalis.

We also study the indirect Coombs test.

Here, the patient serum which will contain

the antibodies is mixed with donor RBC's.

Then anti immunoglobulin

antibody Coombs reagent is added.

Which will demonstrate agglutination.

The indirect Coombs test is performed

if an RH negative woman is married

to an RH positive man.

And has developed anti RH antibodies.

It may also be performed following

transfusion of RH positive blood

to an RH negative individual.

Or if RH positive baby is conceived

to an RH negative woman.

Or the abortion of an RH positive

fetus in an RH negative mother.

to summarize this module.

We studied three types of precipitation

reactions and the tests conducted.

We also studied agglutination reactions.

Direct, indirect and

viral hemagglutination type.

The references for this

module includes Roitt’s,

Esential immunology,

Kuby's immunology, Janeways,

immunobiology.

Basic and clinical immunology

or immunology by Geoffrey.

Thank you.