Experimental Biotechnology
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Lecture-28
Antibody-Antigen Interaction - Part-1

Hello everybody this is Dr.Vishal Trivedi from department of biosciences and bioengineering from IIT Guwahati. And today we are going to discuss about the in interaction of the antibodies with the antigen in the previous lectures we have discussed how to generate the antibodies we have discussed about the generation of the polyclonal antibodies and as well as the monoclonal antibody. So once you have generated the antibody you have generated the first immunological tool to use it for various purposes.

So one of the purpose of generating an antibody is to study the interaction of the antibody with the antigen. So let us see how the antibody and antigen actually interact with each other and how that can be exploited to design different types of experiments or different types of tools to study the various biological processes.

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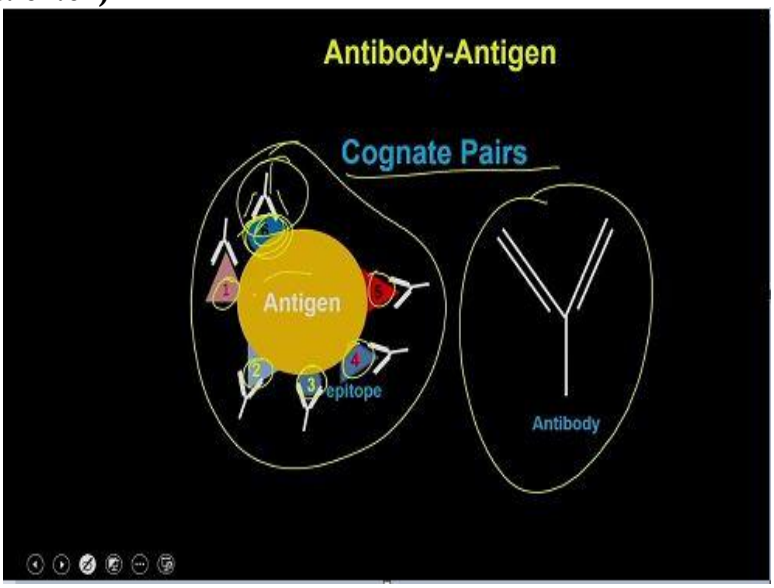

So as you can see that you have an antibody and then you have an antigen this antigen could be composed of one or more epitopic regions for example in this case we have the 6 epitopic regions like 1 2 3 4 5 and 6. And what you can see is all these epitopic region are actually

producing the antibodies or all these epitopic regions are antigenic in nature so they are actually producing the antibodies and all these antibodies are exclusive to this epitopic region.

Which means the antibody and antigens are actually forming a cognate pair where a antibody is exclusive to a particular antigen specifically antibody it is specific to that particular epitopic region. Which means if you have the antibody for the epitopic region 6 it is actually going to recognize this particular epitope and as a global it is also going to allow you to recognize the antigen as well. So why the antigen and antibodies are forming the cognate pair.

Because they are actually working on the exclusive recognition principles where you have the exclusive determinants which are allowing the antigen and the antibodies to interact with each other and that interaction is very specific and exclusive.

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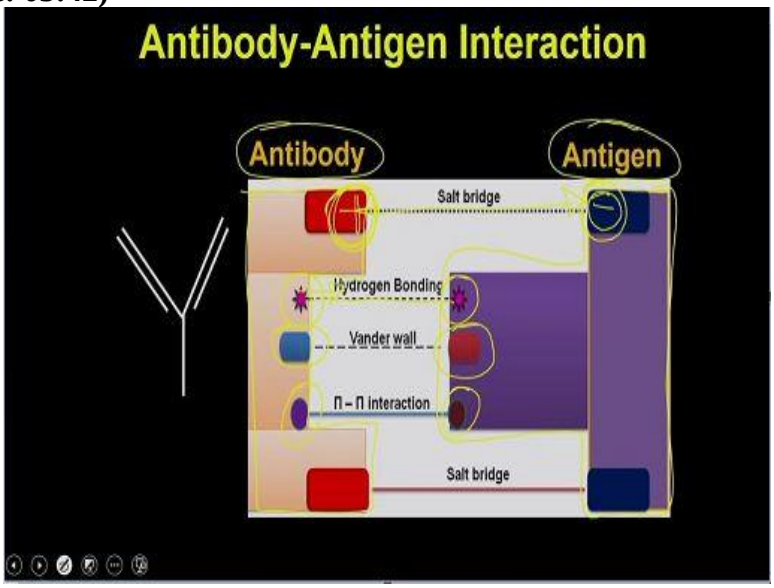

Now let us see that you have a antibody and then you have an antigen and how and why it is actually having the exclusive interactions or exclusive spasticity. Because the antigen is made up of a 3 dimensional structure. So that is you have a 3 dimensional structure which is comprised of the antibody. And what you can see is that it can actually allow the antigens to interact and the antigens which are actually mapping with the similar kind of 3 dimensional structures are actually been allowed to interact with the antibodies.

In addition to that the antibodies are also providing the various spaces where they are actually going to provide either the site for the electrostatic interaction or the hydrophobic interactions. For example in this case this site is actually having a positively charged residue so what it will happen is it actually will allow the antigen to have a negatively charged reidue. So even the 3 dimensional structure of an antibody as well as the antigen is matching. But the positive residues which are present in this side is not getting a negative residue present on the antigen.

It is not actually allowing this and particular antigen to bind which means there could be multiple antigens which are actually may be forming that similar kind of 3 dimensional structures. But until unless this interaction is not going to be satisfied the antibody is not going to recognize the antigen to form the stable complex. Similarly you have a site for the hydrogen bonding so the antibody could be a hydrogen donor that is why it is actually looking for an hydrogen acceptor on the antigen so that it will be able to form a stable hydrogen bonding.

Similarly you have the groups which are actually been participating into the wonder wall interactions and on the other hand it also has the groups which are actually been involved into the pipeline stacking interaction. So all these interaction as well as the 3 dimensional structures actually provide the specificity into the antibodies to recognize the antigen or the epitopic region present in the antigen.

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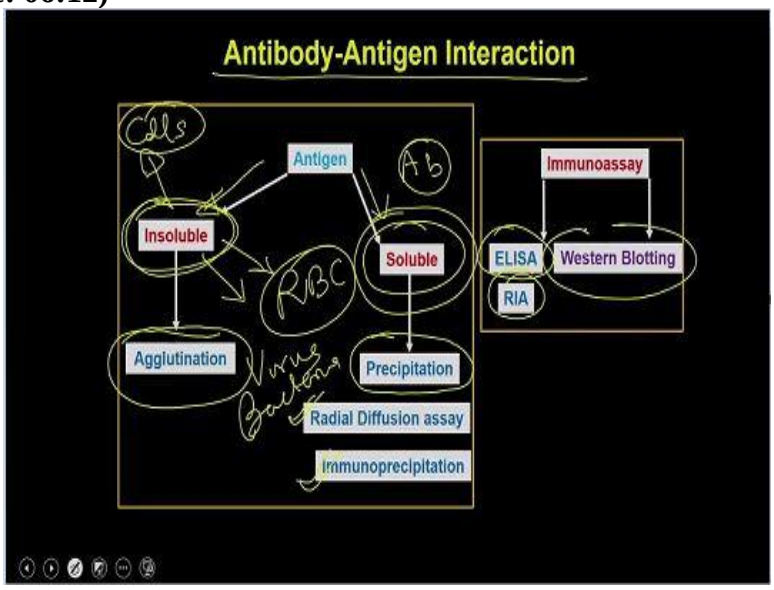

Now the antigen antibody interaction could be of multiple types because as you can see that the antigen could be of multiple types. For example antigen could be a insoluble antigens or it could be a soluble antigens so what is mean by the insoluble antigen is that. For example the cells like for example you have the RBE so RBE is a antigen which actually is not soluble in the solution which is actually going to form a particulate matter. For example if you keep the RBC into the any buffer it is actually going to settle down.

So that is why it is falling under the category of the insoluble antigens the other molecules which are also insoluble are like virus or some of the bacteria also are falling under the insoluble antigens. Soluble antigens are mostly the proteinaceous substances like proteins or the DNA or the RNA. And sometime also the lipids so the molecules which are soluble in aqueous media are falling under the soluble antigens. So if the antigen is insoluble it is actually going to interact with antibodies and will participate into the agglutination reactions.

Agglutination reactions are the reactions where the antibody is going to interact with the antigen and it is actually going to form a network or the mesh. Because of that it is actually going to clump it is going to clump all the antigen containing a particulate antigens. Whereas if the antigen is soluble in nature it is going to interact with the antibodies. And in a process its antibodies are going to precipitate the antigen from the vicinity.

The classical example in the precipitation reactions are the radial immuno diffusion assay as well as the immunoprecipitations. And apart from the antigen antibody interaction which are participating into the agglutination reaction or the precipitation reactions antigen antibody interaction is also responsible for development of different types of immunoassay such as the ELISA RIA or the western blotting. So in today's lecture we are actually going to discuss most of these techniques and then we will like also going to see how this technique can be utilized to solve the some of the scientific problems.

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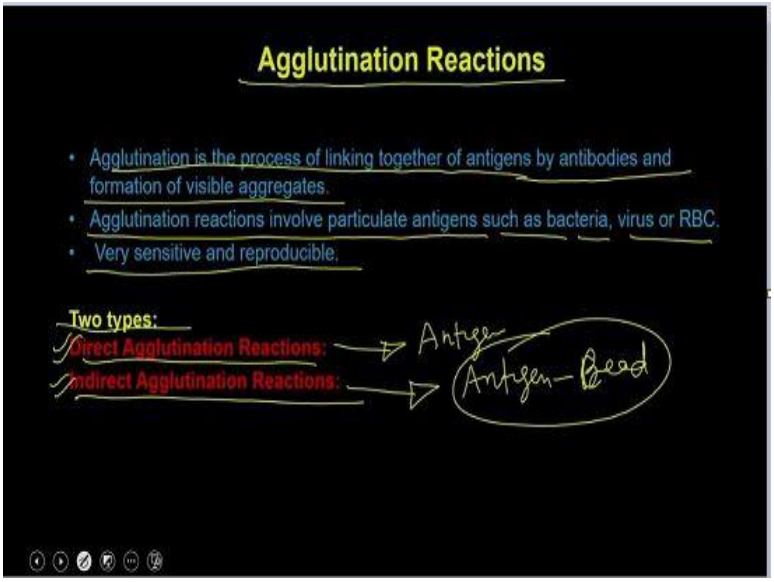

Agglutination reactions so agglutination is the process of linking together of antigens by the antibodies and the formation of visible aggregates the agglutination reaction involves the particulate antigens. Such as the bacteria viruses or the RBC the agglutination reaction is very sensitive and it is very reproducible. Do you can have 2 different types of agglutination reactions you can have the direct agglutination reactions or you can have the indirect agglutination reactions in the direct agglutination reactions.

You are actually going to use the antigen as such whereas the indirect agglutination reactions you are actually going to have the antigens which are actually going to couple to a bead or some support media so that it is actually going to form the particulate antigen which means if the antigen is soluble you can make it to insoluble simply by coupling it to a latex beads or amine beads or some cell so that it becomes the insoluble and then you can be able to perform the indirect agglutination reactions.

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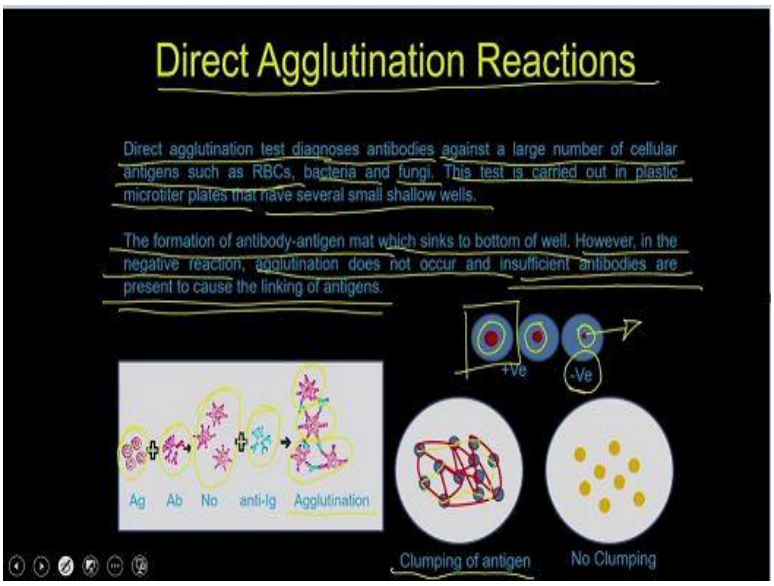

So in the direct agglutination reactions, direct agglutination reaction test diagnosis the antibodies against a large number of cellular antigens such as the RBCs bacteria and fungi this test is carried out in a plastic microtiter plate that have several small shallow wells the formation of the antibody antigen mat which sinks to the bottom of the well however in the negative reactions the agglomeration reaction does not occur and the insufficient antibodies are present to cause the linking of the antigens.

So what will happen is when you have a antigen which is a insoluble antigen when you are adding the antibodies the antibodies are binding to this antigen. But it is actually not causing the agglutination reactions because they are not been able to cross linked or clumped. Because the other antibody is missing but as soon as you add the anti-Igg antibody which is actually going to cross link the antibody which is attached to the antigen.

What will happen is all these antigens are going to be form a network and because of that it is actually going to form aggregate and that aggregate is going to be visible through the naked eye. And that is how it is going to have the agglutination reactions in the negative reactions the agglutination reaction will not occur and that is why it is not happen. Because you do not have the sufficient quantity of the antibodies which is not available to link the antigens. So until unless the end you do not have the sufficient quantity of antibodies.

The antigens are not going to be linked together to form a mesh or the mat like conditions and that is how it is actually not going to give you the agglutination reactions. What you can see is if you take the for example if you take the RBCs and if you add the antibodies. So what will happen is all these RBCs are actually going to be connected by a small antibodies through a network of antibodies. And because of that all these antibodies are going to clump and they will going to show you a aggregate.

Whereas in the negative control where you are not going to add the antibodies there will be no sufficient clumping. So that is why what you see is actually a RBC pellet so this is not actually a agglutination reaction. Whereas in this case or in this case you have the larger pellet and it is actually going to show you a mesh kind of network. Because of that it is actually different from this and so this is a very, very sensitive reactions or sensitive reactions where you can be visually you can be able to check whether the agglutination reaction is happening or not.

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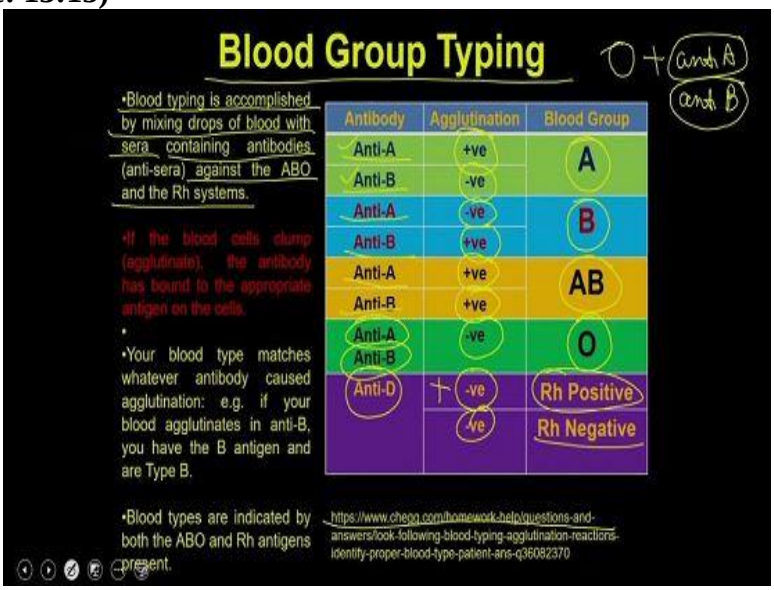

One of the classical example where you are actually using the agglutination reaction is the blood group typing so blood group you know that the human has the 4 blood group A B AB and O and all these blood groups are being classified by the reaction of the RBCs with the antibodies. So what you can do is that the blood group typing is accomplished by mixing a drop of blood with a serum containing antibodies against the ABO and the Rh system.

So what you in the experiment what you are going to do is you take a drop of blood and then you mix it with the anti A or with anti b antibody. So anti A is an antibody which is for the antigen A whereas the anti B is the antibody which is against the antigen B. So what will happen is you have taken RBC now you have added the those 2 sera and then you mix them into a slide and then you look for the agglutination reactions.

So what will happen is if you have the anti A antibody and anti B antibody what will happen is if there will be a positive agglutination reaction with anti A antibody but there is a negative agglutination reaction with anti B antibodies then the blood group is going to be A. Similarly if the agglutination reaction is negative for anti A but it is positive for anti B. Then you are going to have the B blood group now if you have the blood group anti A and anti B. If the both antibodies are giving you the agglutination reaction then the blood group is going to be AB.

Whereas if you are not getting the agglutination reaction for anti A or anti B then you are going to have the blood group O and for detecting whether it is A it is A positive or the negative you can actually also do a another reaction where you can add the anti D antibodies which are actually recognizing the Rh factor. So if the anti D is negative then it is Rh if anti D is positive then it is actually Rh positive if anti D is negative then it is called as the Rh negative.

So by doing this kind of analysis and just by simply doing the 3 reactions where you are going to add the blood drop with anti A anti B and anti D antibodies you will be able to identify and you will be able to classify a person with its blood group either it will be A positive B positive or AB or O positive. These blood groups are so i have given you a link in case you are interested to see all these reactions because you can be able to see visually how the reactions of the blood when it is mixed with the antisera is happening.

So you can just follow this link and it will actually going to give you the real experience of how this aggregation reaction actually look like.

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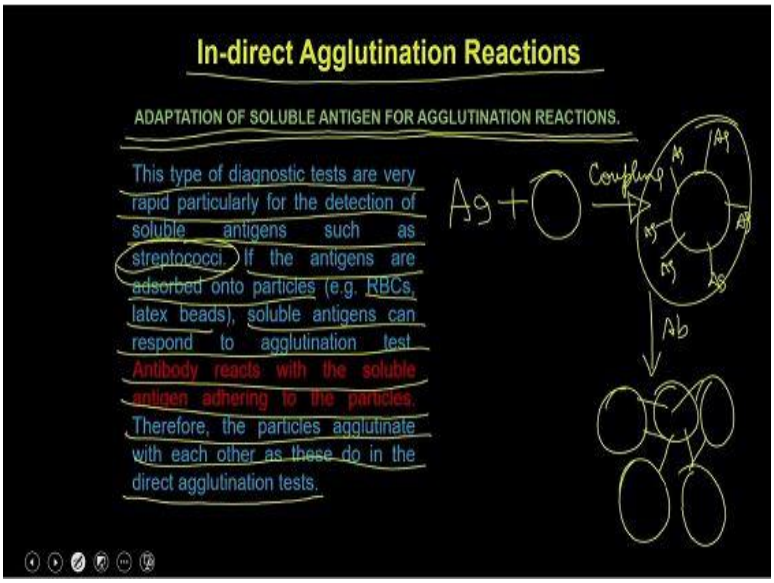

Now indirect agglutination reactions so indirect agglutination reaction is a adaptation of soluble antigen for performing the agglutination reaction as you can see that the agglutination reactions are only been done if the antigen is insoluble nature. Which means it is actually going to be a particulate in nature but in some cases suppose the antigen is soluble in nature but you would like to do the agglutination reactions. Because the one of the major advantage of agglutination reaction is that it is actually does not require a very expensive infrastructure.

Because what you can actually do is you just simply add the antibodies and then you wait for some time and then you will be able to visually see whether you are getting the clump of that particular cell or not or the clump of the antigen or not. And that is visually you can see in comparison to that if you perform any other immunological assay like ELISA RIA or radio immune assay or any other assay. It sometime it is actually requires more infrastructure number 1 number 2 it requires the specific spectrophotometer.

So that you will be able to see the readouts from the ELISA or RIA or the immunodiffusion assays. And in some time if you want to do a immunoelectrophoresis then it is also required the infrastructure whereas the agglutination reaction does not require the extensive or expensive infrastructures number 1 number 2. It is easy to perform you just add the antibodies and its work so that is why people are trying to develop the agglutination reactions even for all those antigens which are not insoluble.

But which are soluble in nature which means you can actually have the proteins which is soluble in nature and you can be able to perform the agglutination reaction simply by performing the indirect agglutination reactions. So this is the adaptation of soluble antigen for the agglutination reactions in this type of agglutination reactions what you are going to do is you are actually going to take the antigen then you are going to add the beads and you are going to run a coupling reactions.

If you remember when we were discussing about the coupling of the antigens to a beads or antigen to a beads to prepare the affinity column you can do simply the similar kind of experiments. And you can actually be able to couple the antigen to a bead or some spherical object. So that it is actually going to be insoluble in nature and that is how your antigen is going to be tagged to these particular beads.

And now your insoluble antigen is ready and then what you can do is you can simply add the antibodies and all these antigens are now going to participate into the agglutination reactions and they are actually going to give you the aggregates. And this type of this is actually a diagnostic text and it is actually allowing the rapid detection of the soluble antigens. Such as the steptococci which is actually a bacteria.

If the antigens are absorbed onto the particles like RBCs or latex beads the soluble antigens can respond to the agglutination test the antibody reacts with the soluble antigens adhering to the particles. Therefore the particles agglutinate with each other as these do not have a direct agglutination test so the indirect agglutination test is only for those antigens which are soluble in nature but you can actually evolve or develop the agglutination reactions for them for easy diagnosis purpose.

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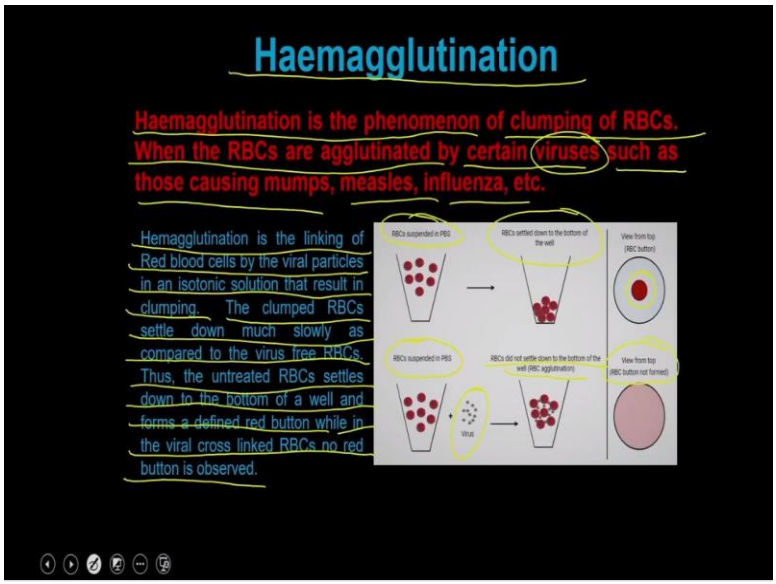

Now apart from the agglutination test you can also do the haemagglutination assays or hypnotization reactions so haemagglutination reaction is the phenomenon of clumping of RBCs when the RBCs are aggregated by the certain viruses. Such as the ndv virus those causing the mumps measles or influenza it these viruses can be detected simply by the doing performing the haemagglutination reactions. So in a typical haemagglutination reaction what you are going to do is you are going to take the RBCs then you add the viruses.

And these viruses are actually going to attach to some of the proteins which are present on to the RBC and that is how they are actually going to couple them. So hemagglutination is the linking of red blood cells by the virus particle in an isotonic solution that results in the clumping the clumped RBCs settle down much slowly as compared to the virus free RBCs. Thus the untreated RBCs settle down to the bottom of a well and forms a defined red button while in the virus cross-linked RBCs no red button is observed.

So what will happen is what you do is you take the RBCs suspended in PBS and then you are actually going to wash it. And then you prepare the RBCs and then once your RBCs are ready then you can just add the virus particles. And what will happen is the virus is going to cross linked these RBCs and that is how they are actually going to form the aggregates and these aggregates are actually going to show you the particulate pattern compared to that you are actually going to get the settling or down of the RBC.

Which is actually going to give you a smooth pellet where compared to that agglutinated RBCs are going to give you the particulate kind of appearances so in a control reaction what you see is that the RBCs are settling down as a smooth RBC button. Whereas in the case of the viruses they are not going to give you a button like appearance but a hazy pattern. Because all these RBCs are entangled with the virus particles and that is how they will actually going to form a meshwork and these meshwork are not going to give you a smooth button like appearances.

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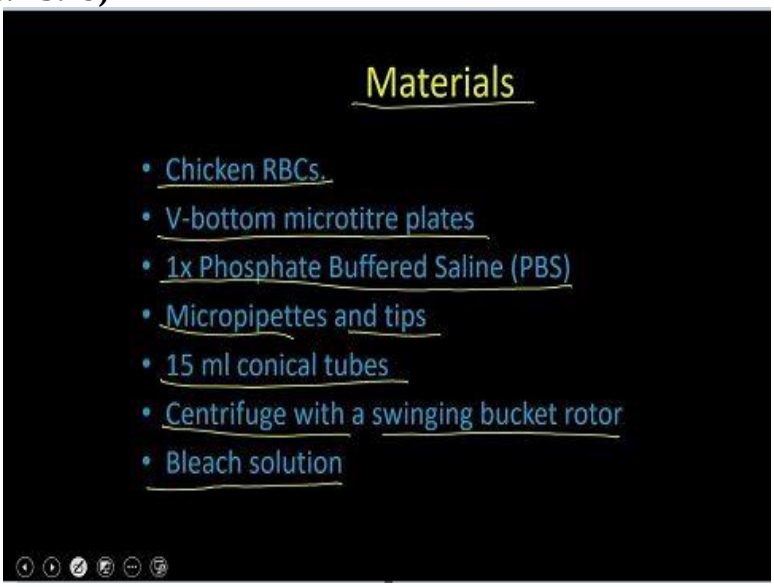

How to perform the hemagglutination assays the material what you required for doing the hemagglutination assay is the you require the RBCs you require the microtitre plate readers or microtitre plate you require the PBS. Then you require the micropipette and tips you require the conical tubes then you require the centrifuge with a swinging bucket rotor and you require a bleach solution so that you will be able to disinfect the virus containing articles so that there will be no cross contamination of the viruses to the other objects.

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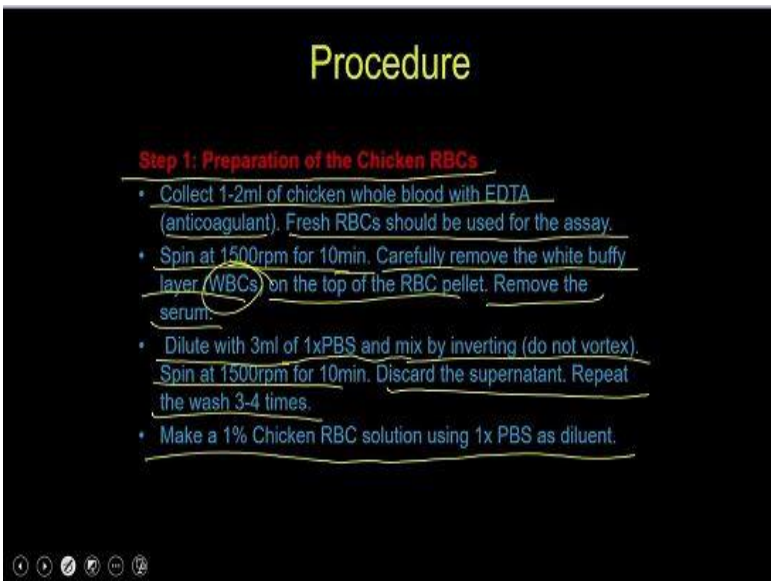

In the step 1 you have to prepare the RBCs so what you do is you collect 1 to 2 ml of the blood with EDTA so that it which contains the anticoagulant then the fresh RBCs should be prepared for this assay because the fresh RBCs are going to have the very high quantity of antigen as well as the fresh RBCs are not going to show any degradation of the antigen which is being expressed on their cell surface then you spin the this for 1500 rpm for 10 minutes carefully.

Remove the white buffy layer which means that you remove the WBSs on the top of the RBC palette and then remove the serum part then you dilute the resulting pellet into PBS and mix it with the inverting spin it at 1500 rpm for 10 minutes discard the supernatant repeat the wash 3 to 4 times and then you prepare a 1% RBC solution using the 1 x phosphate buffered saline as a diluent. Once your RBCs are ready then you can actually do the viral dilutions.

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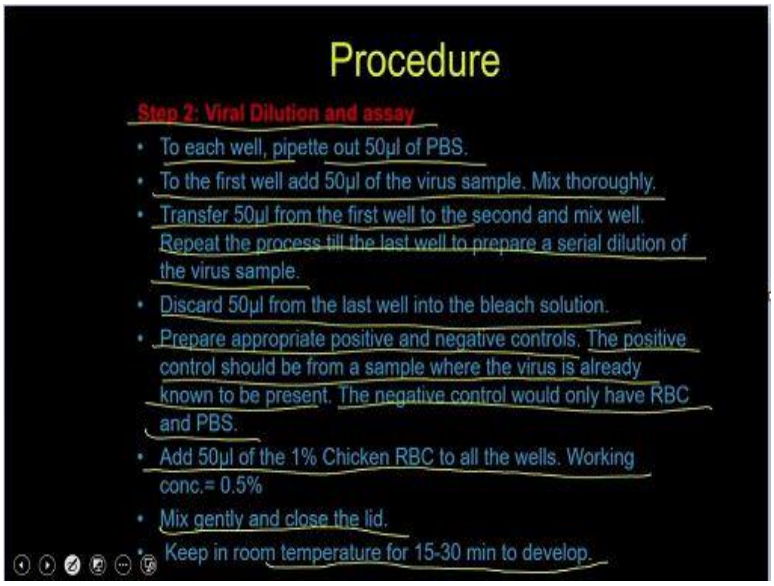

So viral dilutions you are going to do as a serial dilution so to each well you add the 50 microliter of PBS and then you are going to do a serial dilution of the viruses so what you do is you take the viruses first you add the virus into the first sample and then you are actually going to do a serial dilution simply by transferring the viruses from one well to another well and that is how you are actually going to prepare a serial dilution of the virus samples.

Which means you are going to prepare one fold dilutions discard the 50 microliter from the last well into the bleach solutions then you prepare the appropriate positive and negative control the positive control should be from a sample where the virus is already known to be present whereas the negative control would be only to have the RBC and the PBS then you add the 50 microliter of the RBC to all the wells the working quantitation would be the 0.5 percent RBCs.

Then you mix them gently and close the lid and then keep or incubate them for room temperature for 15 to 30 minutes to develop or to perform the reactions so that if it going to show you the hemagglutination assay.

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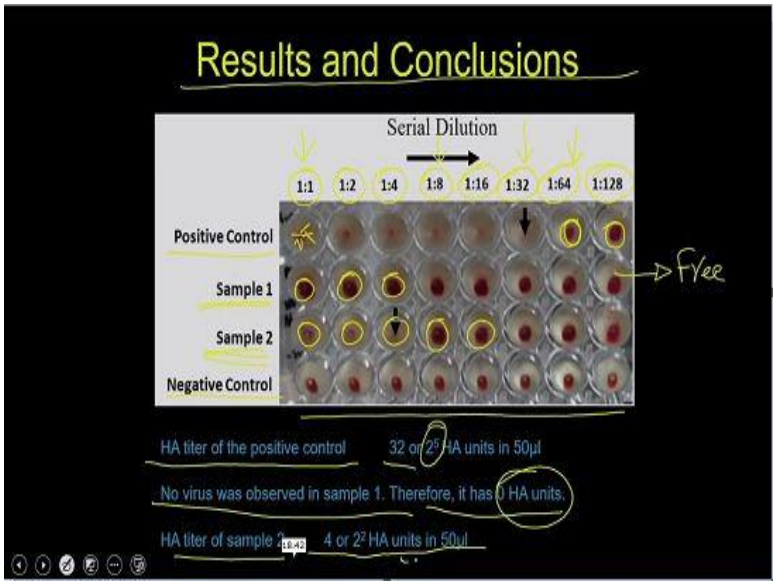

Ultimately the result what you are going to see is this so what you have done is you have just put the viruses into the different dilutions like 1 is to 1 1 is to 2 1 is to 4 1 is to 8 so it is every time it is diluting into half and ultimately you are going to have the multiple dilutions like up to 1 is to 128 then in the positive control what you see is that the up to the 1 is to 32 you are actually getting a RBC pellet with a hazy pattern so what you see is actually a cloudy pattern which is actually a representative with the indication of the hemagglutination assay.

Whereas you see this RBC this is a smooth RBC button that actually is indication of that the hemagglutination assay is not working in this particular concentration once the virus concentration will go down to a particular level then it will not be enough to agglutinate the RBCs and that is how you are going to see a smooth RBC button now you have a sample 1 and sample 2 so in the sample 1 what you see is that it is actually free of viruses because it is not showing a hemagglutination assay.

The RBC buttons are present from the first well itself whereas in the sample number 2 you have the agglutination reaction the hazy pattern here the hazy pattern here and the hazy pattern here but in the from the 1 is to 8 you are actually started getting the RBC button which indicate that the viruses has the titer of 1 is to 8 in the sample 2 whereas the negative control is showing you the RBC button from the top to bottom so what you see is that the hemagglutination title of the positive control is the 32 or the 2 to power 5.

Whereas the no virus was observed in sample 1 therefore it has the 0 HA unit and the HA titer of the sample 2 is 4 or the 2 to power 2 actually are in 50 microliters so this is actually going to tell you that which sample is having the more amount of virus and which sample has the lower amount of viruses and you can be able to quantitate the number of viruses present in each sample and this is a very useful essay to detect the virus in a particular sample and as well as you can be able to quantitate the level of viruses present in that particular solution.

Now let us come back to the antibody antigen interactions so far we have discussed about the insoluble antigens and we have discussed about the agglutination reactions in which we have also discussed about the hemagglutination and within the agglutination we have discussed about the direct as well as the indirect agglutination reactions now let us discuss about the soluble antigens so once the antigen is soluble it is actually going to form the precipitate by interacting with the antibodies.

And within the precipitation reactions you have the 2 assays like radial immune diffusion assay as well as the immunoprecipitations to study the interaction between the antigen as well as the antibodies.

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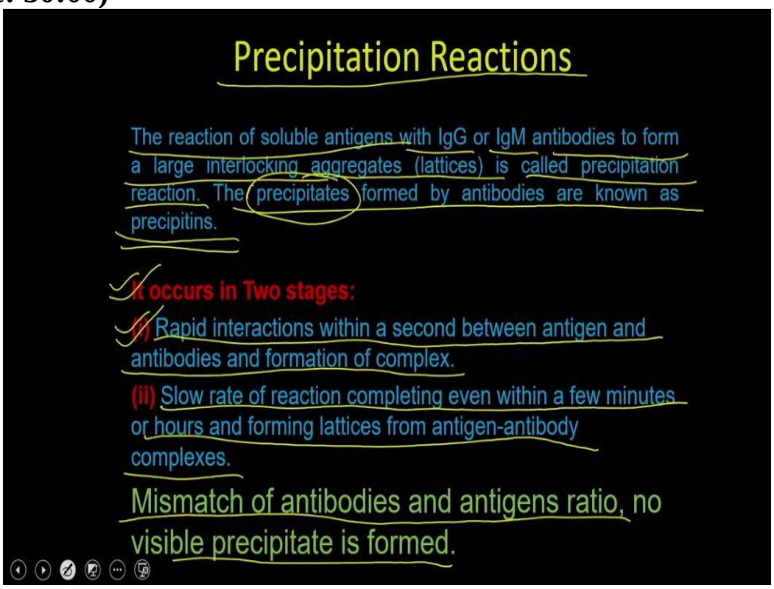

So precipitation reactions the reaction of a soluble antigen with the IgG or the IgM antibodies to form a large interlocking aggregates is called as the precipitation reactions. The precipitation reactions are formed by the antibodies are known as the precipitants so the precipitate what is

being formed by the antibodies are known as the precipitants it occurs in 2 stages so the antigen antibody interaction is actually occurs in 2 stage to form the precipitate in the stage 1 it actually is a rapid reaction.

Where as soon as you add the antibodies to the antigen there will be a rapid interaction within a second between the antigen and antibody to form the antigen antibody complex once this rapid interaction occurs and the antigen and antibody are forming a stable complex then they proceed to the stage 2 where there will be a slow rate of reaction completing even within a few minutes or hours and forming a lattice from the antigen antibody complexes which means now in the stage 2 which is actually a slow reaction.

All these antigen antibody complexes are going to form a clump or the lattice and by doing so the these complexes are going to be so heavy that they are actually not going to be soluble into the aqueous media and that is how they will actually going to be removed or they are actually going to form a particulate matter which is actually can be visible with the naked eye. Mismatch of the antibodies and antibody antigen ratio so what is very important for antigen antibody complex or antigen antibodies to form the precipitate.

Is that you are supposed to provide the adequate amount of antigen as well as the antibodies because if there will be a mismatch between the antigen or the antibody if you have too low antigen or too high antigen the antibodies are not going to form the optimal precipitate and that is all it is important that you should add them in a particular ratio to observe the optimal precipitates no visible precipitates is formed if you have the inadequate ratio of antigen and antibody.

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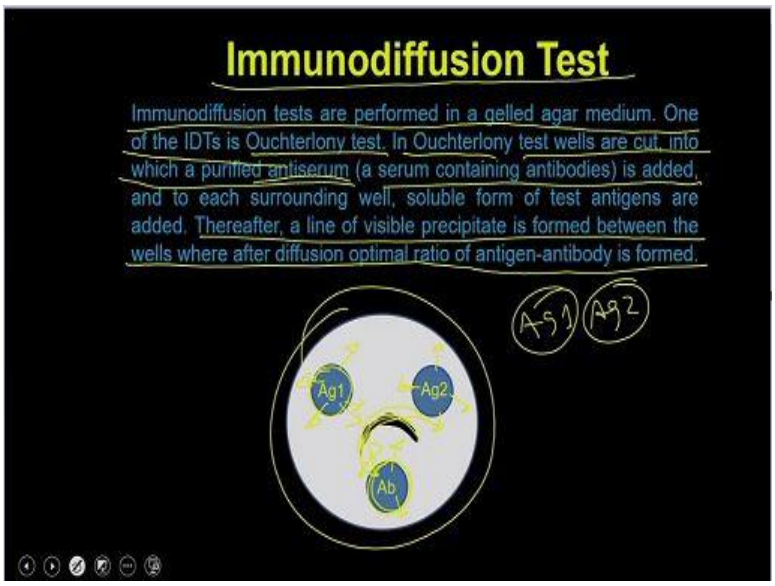

Now the first experiment what we are going to discuss is the immunodiffusion assays or immunodiffusion tests the immunodiffusion tests are performed in a gelled agar medium one of the immunodiffusion assay is called as the Ouchterlony test in the Ouchterlony test wells are cut so you have a agarose block and in this agarose block what you are going to do is you are going to cut the well and these wells are going to contain the antigen or the antibodies and then you are going to add the antibodies or the antigen into these well.

And what will happen is that the antigen is actually going to diffuse out from these wells in all the directions similarly you can imagine that antigen 2 is also inter is diffusing into this and the antibody is also diffusing into the agar and what will happen is well when they are diffusing they actually are going to meet with each other and that is how they at their meeting point these 2 are actually going to form the precipitate so what you see is that in this particular case the antigen 1 and as well as the antigen 2 are actually forming a continuous precipitate.

Which means the antigen 1 and as well as the antigen 2 are actually are identical to each other and they are similar to each other that is why they are actually showing the visible precipitate and they are actually forming a continuous precipitate when next to the antibodies so thereafter a line of visible precipitate is formed between the well where after diffusion optimal ratio of antigen antibody is formed so antigen and antibody is going to have a very high concentration here.

But when it is diffusing its concentration is diluting similarly when the antigen is diffusing it is also concentration is very high next to the well but when it is diffusing and that is all you are actually going to achieve optimal antigen antibody concentrations to see the visible precipitate.

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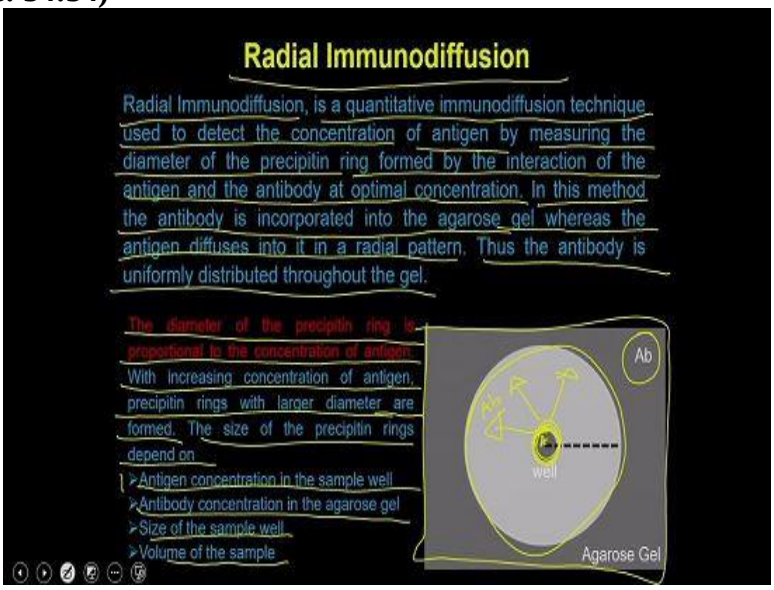

Now the next is radial immunodiffusion assay. Radial immunodiffusion assay is a quantitative immunodiffusion assay technique used to detect the concentration of antigen by measuring the diameter of the precipitant ring formed by the interaction of the antigen and the antibody at a optimal consideration in this method the antibody is incorporated into agarose gel whereas the antigen diffuses into it in a radial pattern thus the antibody is uniformly distributed throughout the gel.

So compared to the immunodiffusion assays in this one what you are doing is you are simply taking a agarose block and you are actually adding the antibodies into that this means the agarose block is going to have the homogeneous concentration of the antibodies so you have an agarose block which actually contains the antibodies in a homogeneous consideration so compared to the radial immunoassays in this one you are actually having in compared to the immunodiffusion assays.

In this one you are actually having the constant concentration of the antibodies throughout the agarose block and then what you do is you are actually going to cut a well and then you are actually going to add the antigen into that once you add the antigen; the antigen is going to

diffuse and it is actually going to achieve at a particular concentration it is actually going to keep interacting with the antibody which is present into this agarose block and then it is start making the precipitate depending on how much antigen you are having in this particular well.

It is actually going to give you a ring of precipitation proportional to the concentration of the antigen what you have added into the well the diameter of the precipitant ring is proportional to the concentration of the antigen with increasing concentration of the antigen the precipitate ring with the with will be a larger diameter are formed the size of the precipitate ring will depend on the following 4 factors number 1 it is actually going to be present on to the antigen concentration what you are taking into the sample well.

Number 2 it also depends on to the agarose antibody concentration what you are taking into this particular agarose block because if the antibody is going to be the limiting factor then the antigen is not going to be sufficient enough if the antibody is limiting then the antigen even in the antigen is more the precipitate ring will be proportional to the end the concentration of the antibody then you it will also depend on the size of the sample well so because the amount of the well what you are going to take the diffusion will start from there.

So the size of the precipitate ring also will be in proportion to that and then the volume of the sample is also very important to see the diameter of the precipitant ring.

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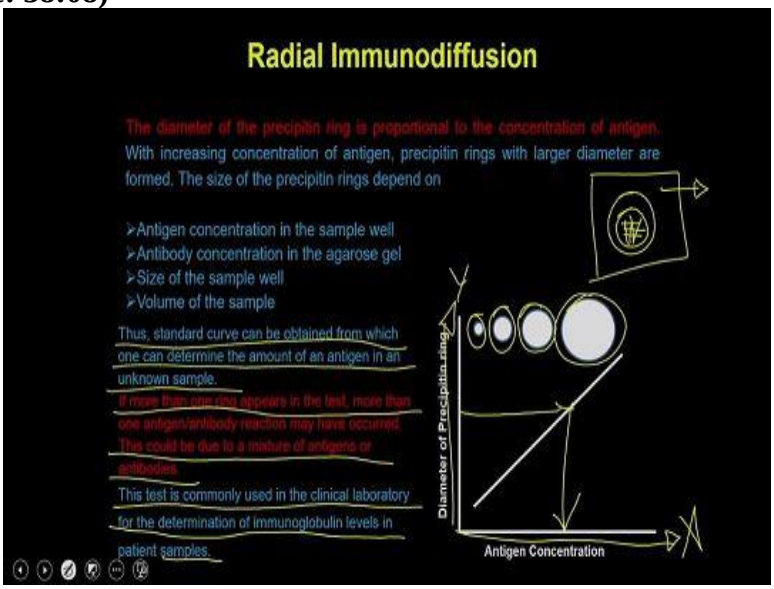

How to perform this essay the so thus the standard curve can be obtained from which one can determine the amount of antigen in a unknown concentrations for example you can actually do the unknown sample of different concentrations so you can do a antigen of different amount and that actually is going to give you the precipitation ring of different diameter. So what you can do is you can simply plot the diameters of these rings on the y-axis.

And you can actually plot the concentration of the antigen onto the x-axis and then what you can do is you can just simply use the unknown concentration and you can be able to determine the concentration of that particular antigen if the more than one ring appear in the test for example if you have the 2 rings like this then that actually implies that you have more than one antigen present into your reaction mixture.

Which means these antigens are actually having the differential reactions with the antibodies this could be the mixture of antigen or the antibody so this could be because you whatever the antibody you are adding is not pure so it is actually having the 2 different antibodies and that do these 2 different antibodies have the different types of interaction with the antigen and because of that you are actually getting the 2 rings one ring which is for the antibody 1 the other ring which is for the antibody 2 or in other case you have the single antibody.

But you have 2 antigens which are also having the interaction with the antibody so the smaller ring you are getting for the antigen 1 according to its concentration and the larger ring you are getting for the antigen 2 which is also according to its particular concentration this test is commonly used in the clinical laboratories for the determination of immunoglobulin label into the patients so with these radial immune assays you can actually be able to determine the antigens you can be able to determine the antibodies.

Because it is actually going to give you the ring of precipitant so you can actually be able to detect or you can be able to diagnose the presence of the antibody in the sample or the presence of the antigen.

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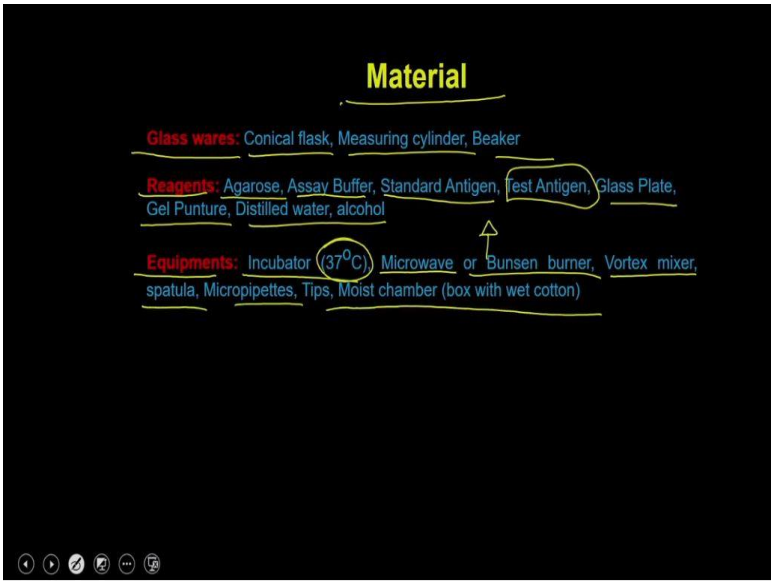

Now let us see how to perform this assay and whatever the material you are required the material what you required is the glasswares like conical flask measuring cylinder and beaker reagents you require agarose so that you will be able to prepare the agarose block then you require the assay buffer then you require the standard antigens then you require the test antigens glass plate gel punctures and the distilled water and alcohol the equipment what you require is the incubator the 37 degree Celsius incubator a microwave or the burner.

So that you will be able to prepare the agarose block then you require a vortex mixer spatula micropipette and all these kind of minor items.

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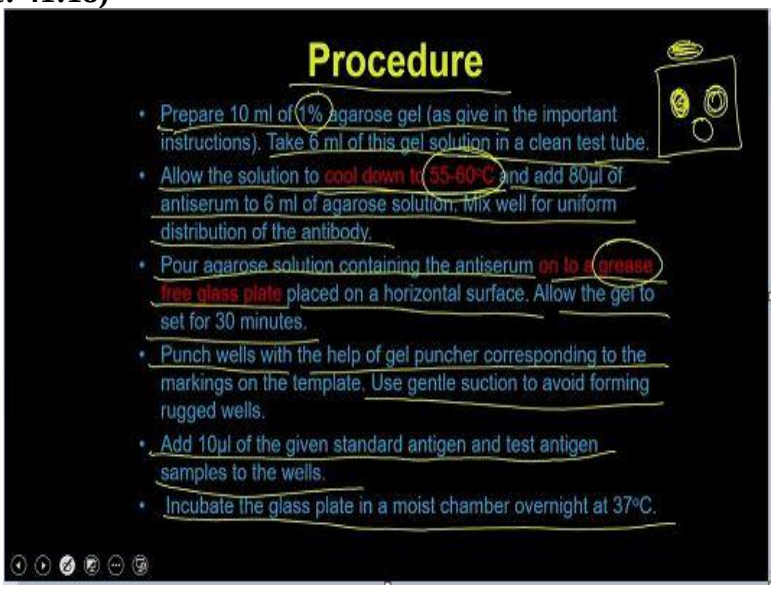

For the procedures first you have to prepare a 10 ml of 1 percent agarose gel so you can actually just weigh the amount of agarose what you require and then you put it into the 10 ml of the PPS then you take out the 6 ml of this test solution in a test tube allow the solution to cool down to 55 to 60 degree Celsius this is a very important step that you should allow this solution to cool down so that when you add the antibodies to the solution antibodies should not get denatured and add the 80 microliter of antiserum to 6 ml of agarose solutions.

Mix well for uniform distribution of the antibodies then you pour the agarose solutions containing the entasra on a non-greasy grease free glass plate placed on the horizontal surface allow the gel to settle for 30 minutes which means you are going to take a glass block and this glass block should not have the grease because the glass are coming where you have a shiny coating so if you have a shiny coating this a gross block will not going to stick to that.

So what you need is a glass which does not have the shiny coating and it should be rough actually so that it should be able to hold the gross block then you are actually going to pour the agarose onto this and then you let it let the agarose to be solidified once the agarose is solidified then you actually going to punch the wells with the help of a gel puncher which actually is going to you know just you have to make the holes so that you will be able to load the antigens into this use gelatins once you in the hole you can remove this agarose block.

So that there will be a well to load the samples then you can add the 10 microliter of the standard antigen and the test antigen into the well and then you incubate this glass plate into a moist chamber because if you allow the water to evaporate from this the agarose is not going to be remain as a thin sheet so you have to stop the evaporation of the water so what you can do is just simply add some tissue which is wet tissue into the water so that it should come keep the moisture content intact within this chamber.

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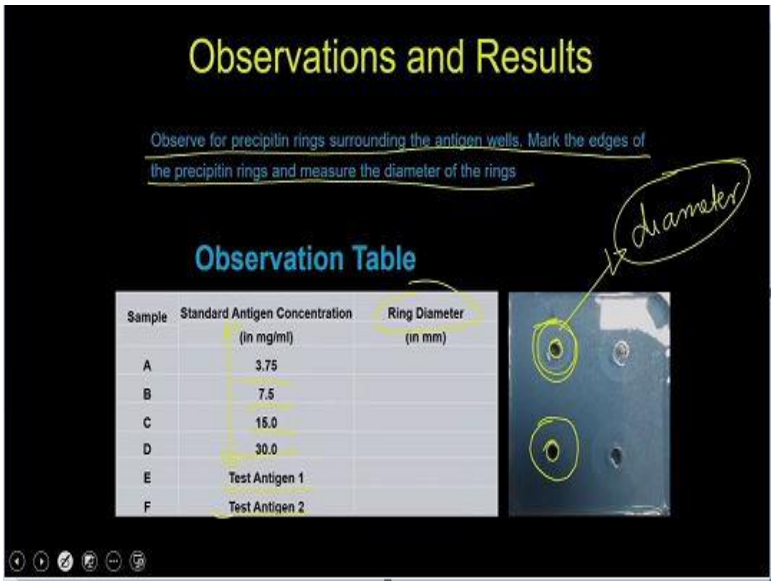

Now just incubate it for the overnight and then you are going to see the results then you observe the precipitant ring around the antigen well mark the edges of the precipitate ring and measure the diameter so what you are going to see is see for example this is the well and what you are going to see is a precipitant ring around this similarly this is the another antigen it is going to see the precipitate ring then what you are going to do is just outline this ring and then you are going to determine the diameter of this particular precipitation ring.

And what you are going to observe is that the against the concentration you have to note down the diameter and then you can also note down the diameter for the test samples then what you are going to do is you plot these in a curve.

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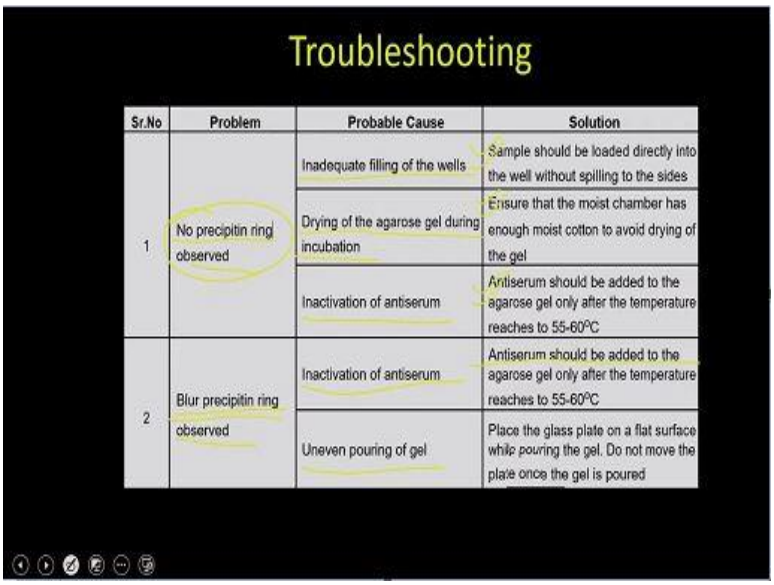

And you can be able to use that curve for calculating the concentration of the antigen in a unknown samples while you are performing this assay you might have to you might face the different types of problems and that is how you actually can do the troubleshooting as well. What is the problem you may not see a precipitation ring form in that case you might have not done the any adequate inadequate feeling of the wells or you can be able to the incubate the agarose gel got dried up while you were doing the incubation.

So while it will get dried up it is actually going to affect the diffusion of the antigen and that is how it is actually not going to give you the precipitant rings when you are actually adding the antisera into the agarose the agarose temperature was not 55 to 60. But it was slightly higher and that is how the antibodies got inactivated if you want to solve this problem what you have to do is you have to fill the adequate amount of the solutions into the well you have to ensure that there is enough moisturizer in the chamber.

And then you also have to ensure that the agarose got cooled down come to enough so that the antisera is not going to be inactivated sometime what you are going to also observe is the blur precipitant ring which means the precipitate ring is not going to be very clear cut. It is actually going to be hazy in that case you might the first reason is that it might be because of the inactivation of the serum or it could be because of the uneven pouring of the gel which means your gel thickness is uneven.

Which means it is not going to form the very smooth surface and because of that it is actually showing a blurry precipitant ring you can simply solve this problem either by ensure that the antisera has not been inactivated and the agarose is completely cooled down before you pour it into the glass plate. And then you can also place the glass plate on a flat surface while pouring the gel so that there will be no uneven pouring of the agarose onto the this glass plates so this is all about the radial immunodiffusion assays where we have discussed each and every detail of the protocols let us move on to the next slide.

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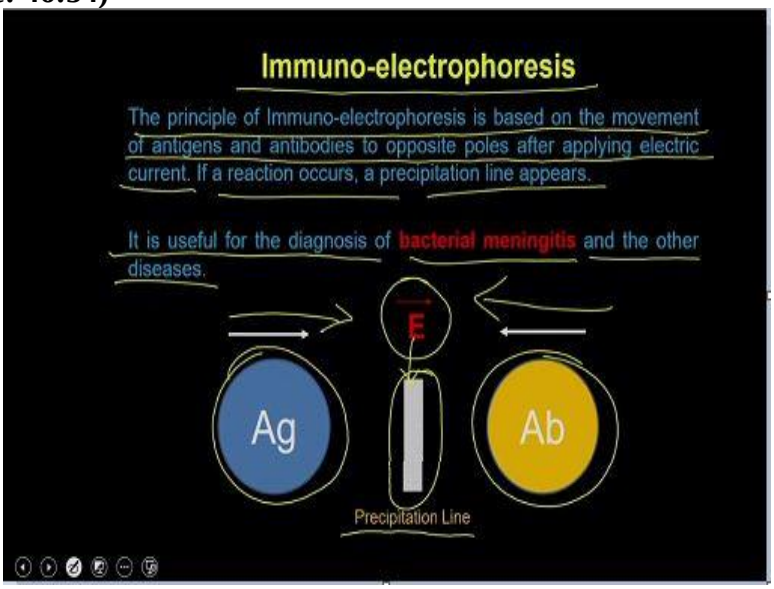

The next is the immune-electrophoresis the principle of the immune-electrophoresis is based on the movement of the antigen and antibodies to the opposite pole after applying the electric current if the reaction occurs you will see a precipitation line so far what we were doing is we were simply relying on to the diffusion of the antigen or the antibodies and in that case you are going to observe a precipitation ring or precipitation line but sometime the antigen or the antibody what you are handling are very big and they are there diffusion rate is very slow.

Because of that it is not possible for these antigens to be tested in a radial immuno assay or the immunodiffusion assays so in that case is what you do is you simply make a agarose gel and in that case you just you know pour the agarose pours the antigen into one well and the antibodies into another well and then you apply the electric current across them so what will happen is the antigen and antibodies are going to run in opposite direction and then while they are running they will interact with each other and that is how they will actually going to form the precipitate.

So what you are going to do is you are just simply going to add the antigen into one of the well antibodies into another well and then you are going to apply the electric field so once you apply the electric fields the antigen will run into this direction the antibody will run into this direction and when they will meet at the center point you are going to see a precipitation line so that precipitation line is going to be tell you that this antigen and this antibody are interacting with each other.

And this is this kind of assay is useful for the diagnosis of the bacterial meningitis and the other diseases so this is all about the studying the interaction of the antigen and antibody so far we discussed about the agglutination reactions we discussed about the precipitation reactions and in the precipitation reactions just we have discussed about the radial immune assays so with this i would like to conclude my lecture here in the subsequent lectures we are actually going to discuss about the immunoprecipitation reactions.

And then we are going to take up the immunoassays to discuss how to perform the immunoassays and how you can be able to use them for answering the some of the biological questions so with this i would like to conclude my lecture here thank you.