Basics of Biology
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Lecture 32
Immune System (Part 2)

Hello everyone, this is Dr. Visual Trivedi from Department of Biosciences and Bioengineering IIT Guwahati. And what we were discussing, we were discussing about the defense response into the living organisms. And in that context in the previous lecture, we discuss about the general overview of the immune response or general overview of the immune system.

And in that we have discussed about the different components which are taking place taking important role in terms of the surveillance of the infectious organisms or to the causing the robust immune response against the infectious organisms. So, I mean, while discussing about that, we have discussed about how the antibodies can be produced by the immune response and how that can be produced even in the lab conditions, so, that you can be able to utilize that for the different types of applications.

So, while we were discussing about the antibodies, we discuss about, we discussed that the antibodies can be of two different types, it could be a polyclonal antibody or the monoclonal antibodies. So, what is made by the polyclonal antibody, the polyclonal antibody is antibody which comes from the multiple clones, whereas, the monoclonal antibody is antibody which is going to be produced by the single clone. So, in the previous lecture, we discuss about the polyclonal antibodies and how the polyclonal antibodies can be produced into the animals.

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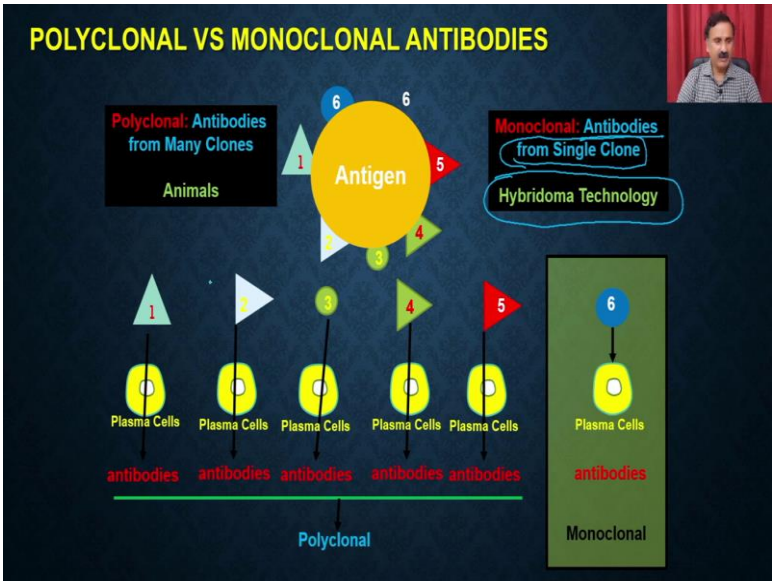

Now, in today's lecture, we are going to discuss about the monoclonal antibody. So, as the name suggests, the monoclonal antibodies are going to be produced from the single clone of the B-cells or the plasma cells and these antibodies are going to be produced by a technology which is called as hybridoma technology. So, in a hybridoma technology, we are actually going to follow many steps. So, what are these steps, let us see, what are the different steps what you are going to use for the hybridoma technology.

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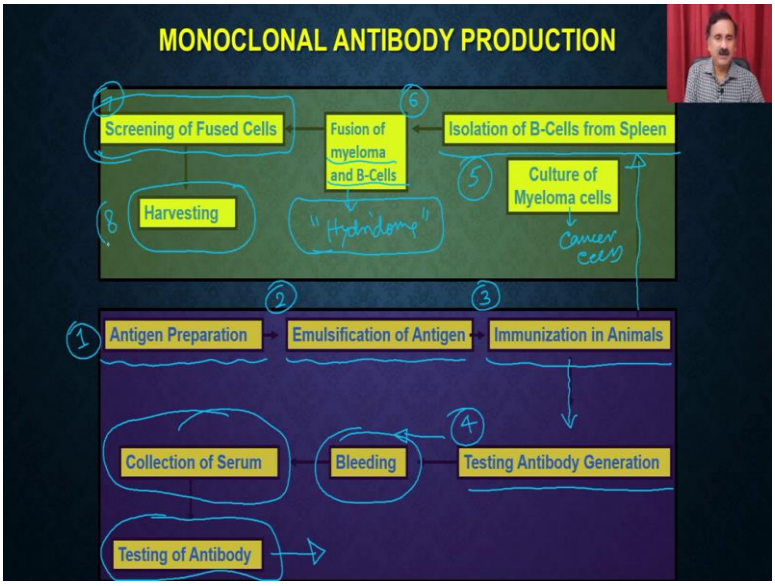

So, in the hybridoma technology, you are going to have the steps what is we have discussed just in the previous lecture about the, when we were discussing about the polyclonal antibodies. So, the first step is that you are actually going to produce the antigen. So, you are

going to prepare the antigens, then you are actually going to do the emulsification of the antigen. So, this means you are actually going to prepare the antigens for the injections. And in the step 3, you are actually going to do the immunization of the animals.

So, when you do the immunization of the animal at that stage, it is actually going to split into the two-part, one part which goes into this section and the other part is where you are actually going to. So, in the step 4, then you are going to test whether the antibodies are being produced in the animal or not. And if the steps in the, if the antibodies are produced, then you are actually going to take out the blood and you are going to collect the serum and then once you collect the serum you are actually going to test whether the robust antibodies are produced.

So, by the time when the antibodies are produced, what you are going to do is you are going to take these immunized animals. And from these immunized animals, you are actually going to start isolating the B-cells from the spleen. So, in this stage, when the antibodies are being produced into the animal, you are actually going to dissect this animal and then you are actually going to isolate the B-cells from the spleen and then you have to culture.

So, this is the step 5, where you are actually going to isolate the B-cells from the spleen. And then you are also going to culture the myeloma cells to myeloma cells are actually the cancer cells. So, and then you are actually going to have the step 6 where you are actually going to do the fusion of the myeloma cells and the B-cells. And when you do the fusion of the myeloma cells and B-cells, it is actually going to form the hybridoma cells.

So, it is going to give you the hybridoma cells. So, hybridoma means it is going to give you of hybrid cells, where you are going to have the properties of the B-cells, so that it can actually be able to produce the antibodies. And then it also going to have the properties of the cancer cells, so they will be keep growing in definite period. So then what you are going to do, you are going to do the in this step 7, you are actually going to do the screening of the fused cells, or you are going to do the screening of the hybridomas.

So that is actually going to give you the individual hybridomas. And individual hybridomas are going to give you the monoclonal antibodies, and that you are going to do the harvesting in the step 8. So, let us see what are different steps in a schematic diagram.

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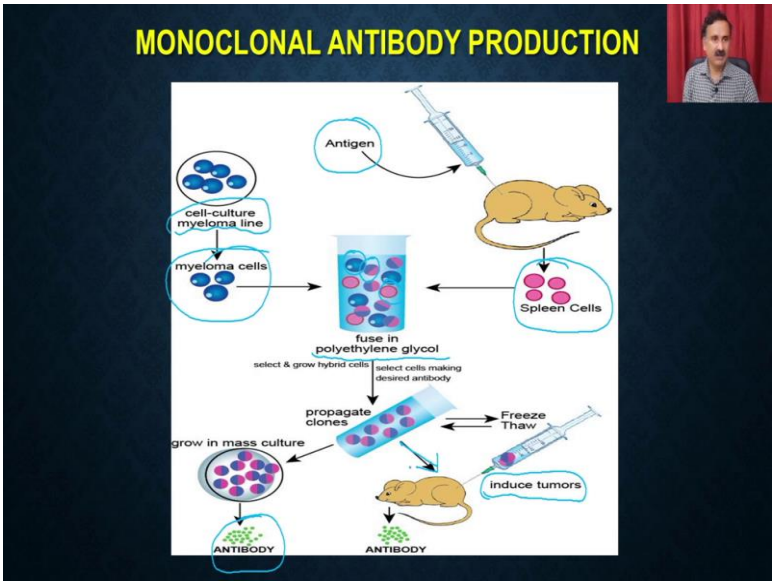

So, in the schematic diagram, what you are going to have is you are going to have the antigens, what you are going to do is you are going to prepare the antigen and then you are going to immunize the animals. And then from the animal you are actually going to isolate the spleen cells and these spleen cells are actually going to have the B-cells. And on the other hand, on the parallel, you are going to do the cell culture of the myeloma cells.

And then these myeloma cells as well as the spleen cells are going to fuse and that is how they will you are going to do the fusion in the presence of with the help of the polyethylene glycol, and then you are going to get the some of the fused cells or some of the individual myeloma cells or some of the individual spleen cells.

So, then you are going to do the selection, and when you are going to do the selection, you are actually going to get the hybridomas and these hybridomas then can be utilized for the production of the antibodies. In some of the cases what we will do is they are actually going to take the hybridomas and then they are actually going to freeze these hybridomas for the future work, but when they require these hybridoma to be utilized again, they sometime actually going to induce the injector hybridomas back into the animal and that is how they are going to get the antibodies from the animal as well.

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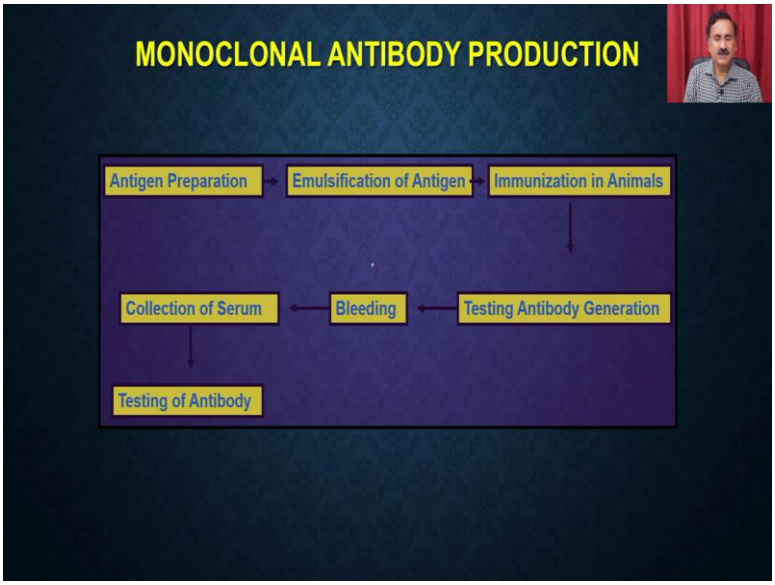

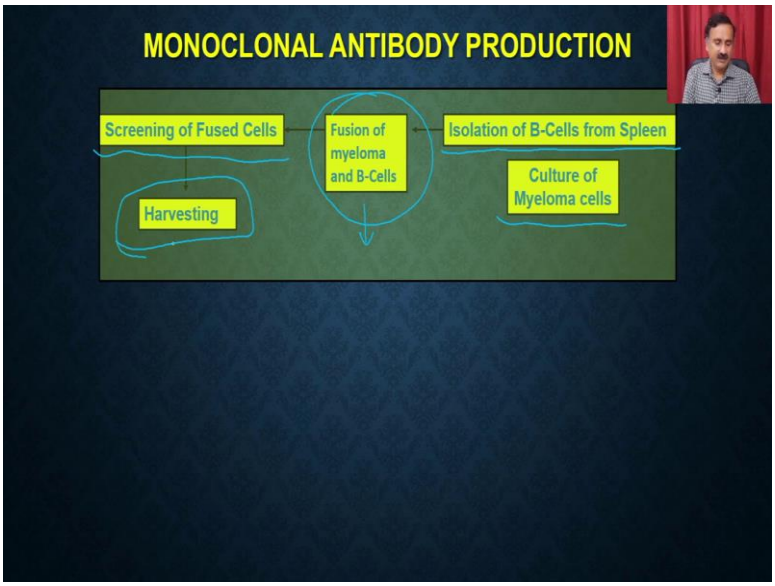

So, these are the steps we have already discussed when we were discussing about the polyclonal antibody productions. And now, we are going to discuss, we are going to discuss about these steps where we are going to see how you can be able to do the isolation of the B-cells, how you are going to culture the myeloma cells, and how you are going to fuse the myeloma and B-cells so that you can be able to produce the hybridomas and then you can be able to screen the hybridomas and then you can actually be knowing the harvesting on the antibodies.

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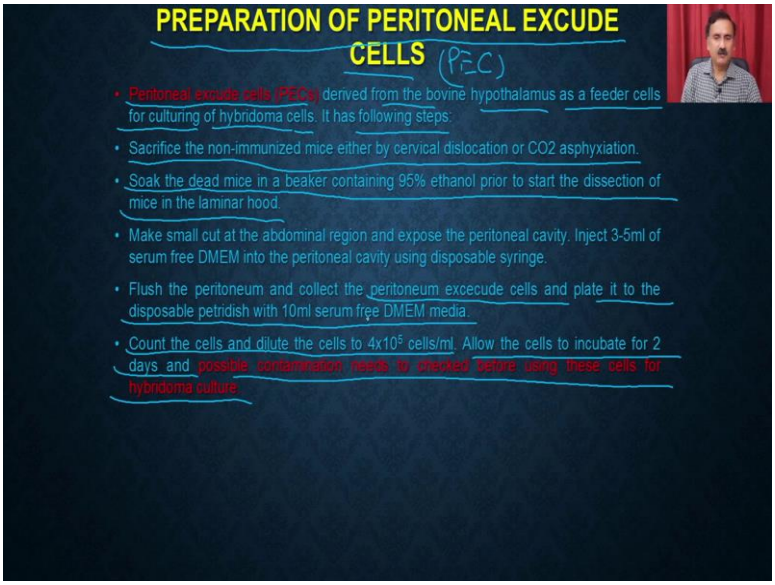

So, for doing the hybridomas you have to first do some preoperative steps. So first you have to prepare peritoneal execute cells or these are also called as the PEC cells. These PEC cells are required because they are actually being used as a feeder cell for the hybridomas. So they are, these peritoneal excute cells are being derived from the bovine hypothalamus as a feeder cell for culturing the hybridoma cells, it has the following steps.

You first have to sacrifice the animals, either by the cervical dislocation or the CO_2 asphyxiation. Then you soak the dead animal in a beaker containing 95 percent ethanol prior to start the dissecting of the animal in the laminar hood, you are actually going to dissect the animal and then you are going to isolate the cells peritoneum execute cells and then you plate it on to the disposable fatty dish with 10 ml of serum free DMEM media.

You count the cells and dilute the cells 4 into 10 to the power 5 cells allow the cells to incubate for two days and the possible contamination need to be checked before the cells for, you can use these cells for feeding the hybridomas.

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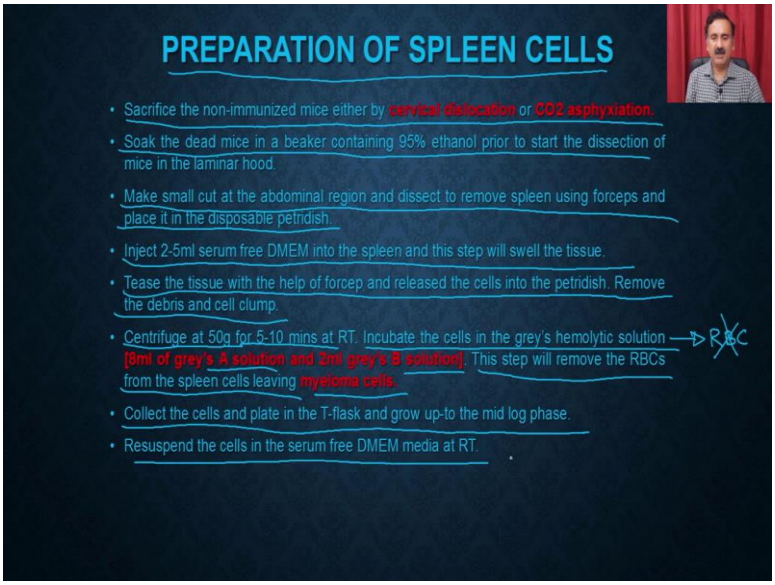

Then you have to isolate the B-cell. So, you have to prepare the spleen cells. So, you sacrifice the non immunized mice either by the survical dislocation or CO_2 asphyxiation. Then soak animal in a beaker. Then you make a small cut at the abdominal region and dissect to remove the spleen using the forceps and place it in a disposable petridish. You inject 2 to 5 ml serum media into the spleen and step will swell the tissue.

Tease the tissue with the help of forceps and released the cells into the petridish. Remove the debris and cell clump. The centrifuge at 50 g for 5 minutes to 10 minutes. Incubate the cells in grey's hemolytic solutions. So, grey's hemolytic solution is a combination of the A solution and the B solution.

And the purpose of the solution is that it actually allows you to lies the RBCs and that is how it is actually going to remove the RBCs. So this is terrible remove the RBC from the spleen cells leaving the myeloma cells and then you collect the cells and plate in the T-flask and grow up to the mid log phase. Resuspend the cells in the serum free media at room temperature.

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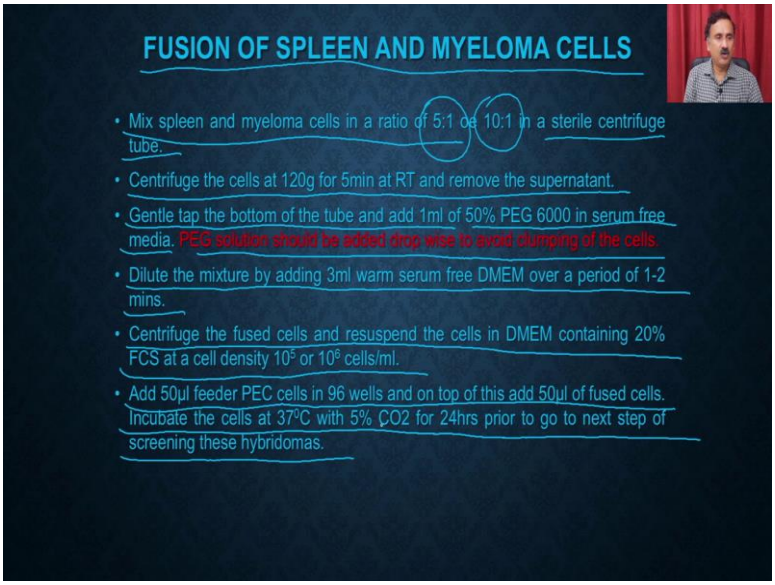

Then you are going to perform the fusion of the spleen and the myeloma cells, so makes the spleen and the myeloma cells in a ratio of 5 is to 1 or 10 is to 1 in a sterile centrifuge tube. Centrifuge the cells at 120 g for 5 minutes at room temperature and remove the supernatant. Gently tap the bottom of the tube and add 1 ml of 50 percent PEG 6000 in serum free media. PEG solution should be added drop wise to avoid the clumping on the cells.

And the PED is a fusing agent, so you can actually fuse the plasma membrane and that is how it is actually going to give you the fused cells. Dilute the mixture by adding the 3 ml warm serum free media over a period of 1 to 2 minutes. Centrifuge the fused cells and resuspend the cell in DMEM containing 20 percent FCS at a cell density of 10 to power 5 or 10 to power 6 cells per minute per ml.

Then you add the 50 microliters of feeder peritoneal execute cells in 96 wells plate and on top of this add 50 microliters of fused cells. Incubate the cells at 37 degrees Celsius with 5 percent CO2 for 24 hours prior to go to the next screening these hybridomas. Now, how you are going to do the screening of hybridoma?

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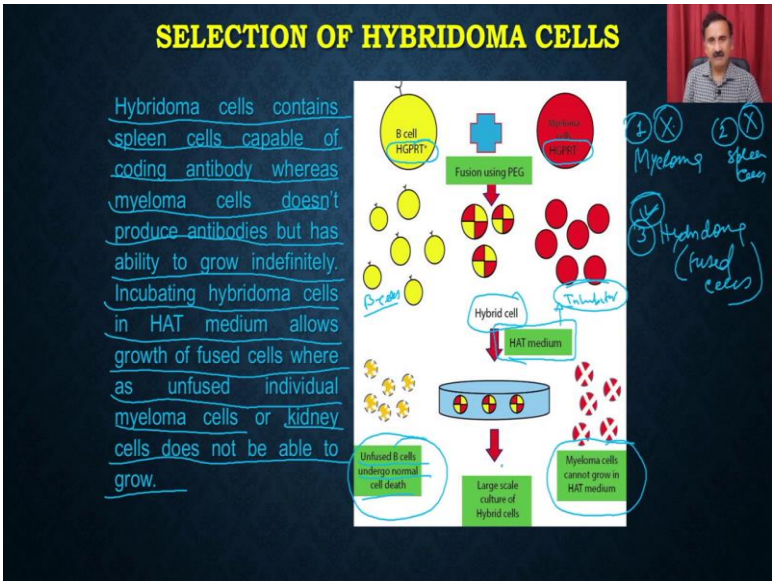

So, in the hybridoma, you are going to have, when you are going to do the fusion you are going to get the three cell type, you are going to get the myeloma cells, you are going to get, so number 1, you are going to get the myeloma cells, number 2, you are going to get the spleen cells, and the number 3, you are going to get the hybridoma, which means you are going to get the fused cells.

So, you have to use some property which actually is going to destroy the myeloma cells which are going to destroy the spleen cells and then it will actually allow the only the hybridoma cells to survive. So, hybridoma cells actually contains the spleen cells and capable of coding the antibodies, whereas the myeloma cells do not produce the antibodies, but has the ability to grow indefinitely. Incubating the hybridoma cells in a HAT media allows the growth of the fused cells, whereas the unfused individual myeloma cells or to the kidney cell do not be able to grow.

So, what will happen is that you have a B-cell, which is actually a GPRT positive. And then you have a myeloma cell, which is the GPRT minus. So, when you fuse them, you are actually going to have the three-cell type, you are going to have the B-cell, you are going to have the B-cell, which is actually a GPRT positive, but it cannot grow for an indefinite period of time, because it has a limited time period. Then these cells which are GPRT minus, so they will not be able to survive in the presence of this HAT media.

And then you also going to have the fused cells. So when you have the fused cells or hybrid cells, and you put them into the HAT media, HAT is actually going to contain the inhibitors.

And these inhibitors are actually going to inhibit the DNA synthesis into the both cells. And because the B-cell is can recover from that DNA synthesis or DNA innovation of that DNA synthesis because it is a GPRT positive cells, but the B-cells can only grow for a very limited period of time, so that is why eventually they will die.

But the myeloma cells will not be able to survive, because they are a GPRT minus. So, unfused myeloma cells are going to be die in due course of time, whereas the hybrid cells are actually going to be survived because they are going to have the DNA synthesis potential from the B-cell. And they are actually going to have the indefinite period of multiplication ability from the myeloma cells, and that is why these hybridomas are going to survive.

So, unfused B-cells undergoes normal cell death. So, they will go under the apoptosis and that is how they are actually going to die, whereas the myeloma cells cannot grow under the HAT media but because the DNA synthesis is going to stop, whereas the hybrid cells are going to survive. So, why they, why it is so?

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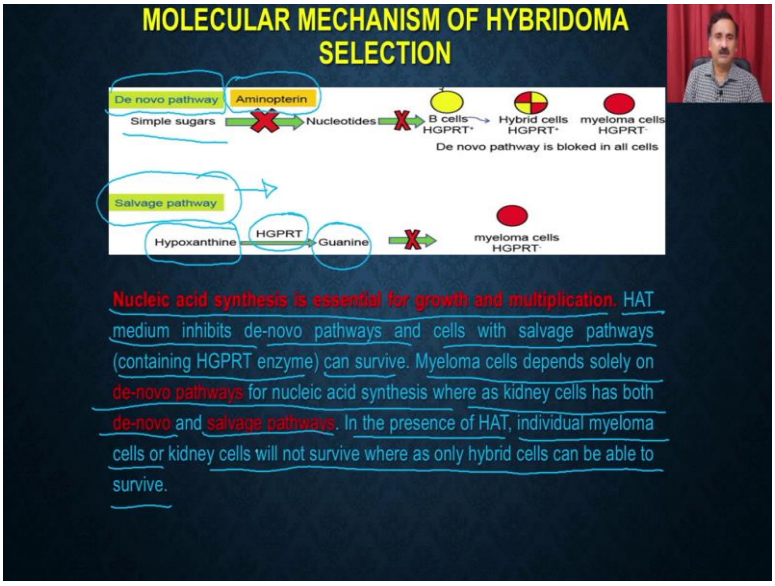

Because the HAT media is actually going to have the inhibitor which is called as the Aminopterin. So, HAT media is actually going to have the Aminopterin media, and that Aminopterin is actually going to destroy the de novo DNA synthesis pathway. So, the simple sugar which you can actually use for the de novo pathway can be utilized to synthesize the nucleotide, but that pathway is going to be destroyed.

So, now, the cells are only going to be dependent on to the salvage pathway. And the salvage pathway is not present into the myeloma cells, whereas the salvage pathway is very actively

present into the B-cells. So, for running the salvage pathway, the HAT media also has the hypoxanthine. So this hypoxanthine is then going to be processed by the GPRT enzyme, and then it is actually going to synthesize the guanine and this guanine can be utilized for the production or the generation of the nucleic acids.

So, the nucleic acid synthesis is essential for the growth and multiplication. I am sure you when we were discussing about the cell division and cell cycle, you might have seen that when the cells are entering into the S phase, they go for the DNA synthesis, and then you have the two copies of the DNA. And then these two copies of DNA are been distributed among the daughter cells. So, that DNA synthesis is important for the cell to enter into the division phase.

And that is how it is actually going to multiply. But in the presence of HAT, which is actually going to destroy the DNA synthesis, it is the HAT media contains the inhibitors for the de novo pathway and the self with the salvage pathway can survive. So, the GPRT enzyme, the cell which can only have the GPRT enzyme can survive.

So, the myeloma cells depends solely on the de novo pathway for the nucleic acid synthesis, where as the kidney cells has the both de novo as well as the salvage pathway. In the presence of HAT, the individual myeloma cells or the kidney cell will no longer survive, as only the hybrid cell can be able to survive. Now, once you have got the hybridoma, you have to screen the hybridoma for the production of the monoclonal antibodies.

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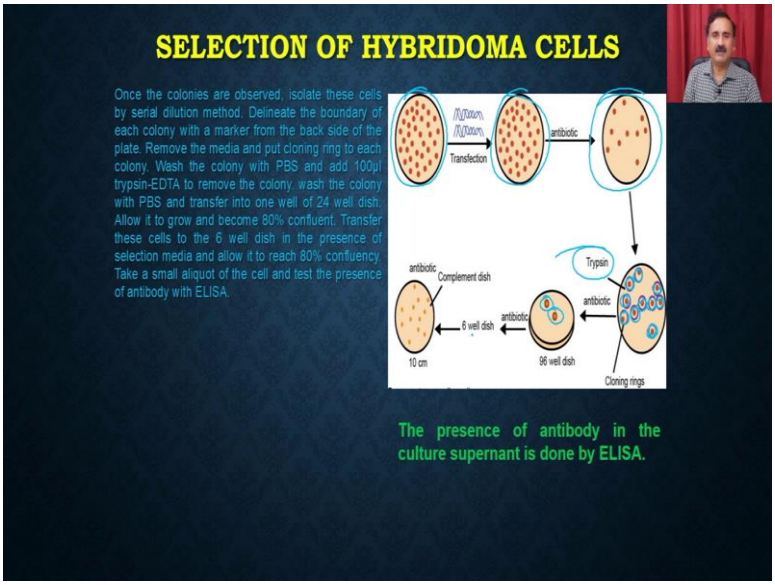

So, how are you going to do that, you are going to have the hybridomas. So what you are going to do is you are going to have the serial dilutions. So, you are going to have the serial dilution, and that is how you are going to have the individual hybridomas. And then you are going to use the selection circles.

And that is how you are going to collect these individual hybridomas with the help of trypsinization. And that is how you are going to have the individual hybridomas. And these individual hybridomas then can subculture and it will give you the complete dish and that can be used for the harvesting of the antibodies.

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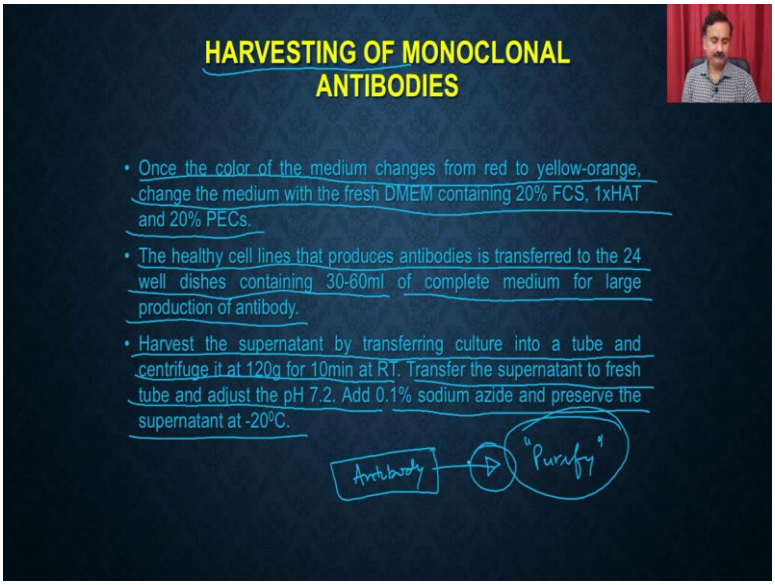

So, how you are going to do the harvesting? So, once the color of the media changes from red to yellow-orange, you change the media with the fresh media containing 20 percent FCS, 1xHAT and 20 percent the peritoneal execute cells. The healthy cells line that produce antibody is transferred into the 24 well dish containing 30 to 60 ml of complete media for the large production of the antibody.

Harvest the supernatant by transferring the culture into a tube and centrifuge it at 120 g for 10 minutes at room temperature. Transfer the supernatant to the fresh tube and adjust the pH to 7.2 Add the 0.1 percent sodium azide and you can preserve the supernatant at minus 20 degrees Celsius.

Now, once you have produced the antibodies, you are actually also need to purify the antibodies so that you can be able to use only the antibodies not the other biomolecule. So, when you have the antibodies been produced into this system, you are going to have the antibody and the other biomolecules like the cell culture components and all that. So, you have to purify these antibodies for the other biological applications.

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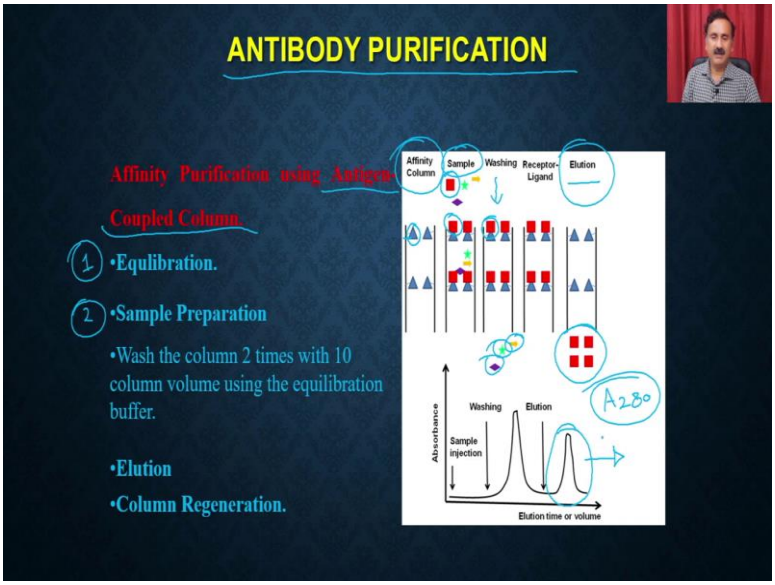

How you can do the antibody purification. So what you have to do is first you have to prepare a column where you are going to have the coupled the antigen. And then you are going to follow the following steps. You are going to do first step is incubation, then you are going to do the sample preparations, then you are going to wash the column. So, this is what you are going to do.

You are going to first prepare the affinity column so that you are going to have the antigen attached to the column. Then you are going to prepare the sample, so this sample is going to have the different types of biomolecules. It is going to have the antibody also. So, you can imagine that the antibody will go in bind to these antigens, whereas other molecules will not.

So, then you are going to do the washing, once you do the washing only the antibody which is bound to the antigen will remain onto the column whereas all other bio molecules are going to be removed. And then you are going to have the illusions. So, when you do the illusions, the antibodies will come out that you can actually be able to monitor. So, if you monitor that at absorbance at 280 nanometer, you can be able to monitor this and you can get the pattern and that is how you can actually collect the fraction where the antibodies are been eluted.

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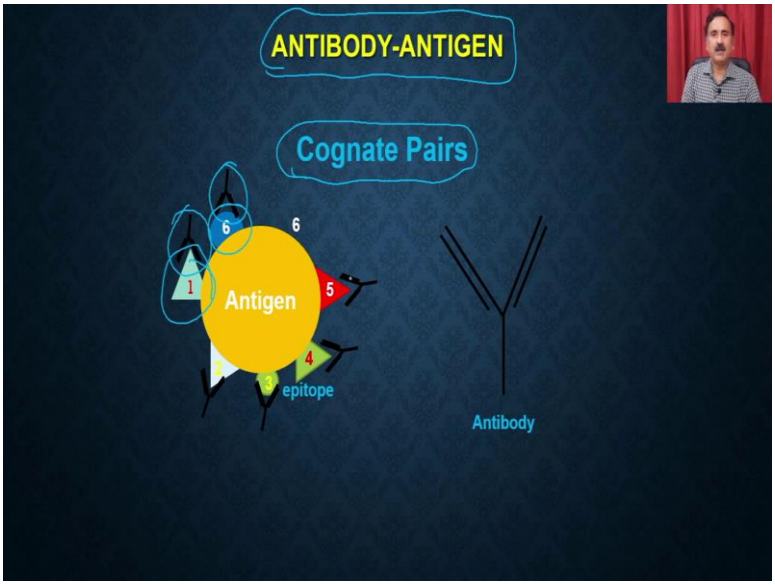

Now, once you have and purified the antibodies, you can actually be able to do a lot of experiments, you can actually be able to detect the antigens. So, remember that the antibody and the antigens are actually a part of the cognate pair. So which means every epitope, every epitope is actually going to produce an antibody and that is how this antibody is actually going to have its cognate antigen or I will say the antigenic region on to the big antigens.

So, that is why the antibody can be used for detecting this particular antigen because the antigen and antibody are actually forming a cognate pair. Now, why they are forming a cognate pair? Because they are very, they are forming a very exclusive pairing and they are forming a exclusive interaction.

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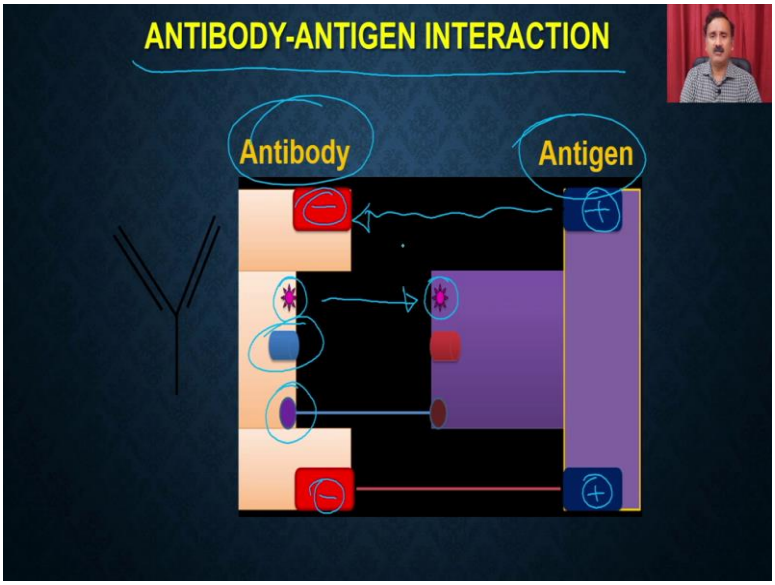

So, you can imagine that if this is the antibody and this is an antigen, the antigen antibody are actually having a very well defined three dimensional conformation. So, because of that the antibody is going to fit into the antigen. Apart from that it may actually have the interaction sites which are also going to be complementary to each other. For example, in this case, this is going to be negatively charged, it is going to be positively charged. So, it is going to be actually having a interaction.

Similarly, you can have the negative charges here, which is actually going to have the positive charges onto this. Similarly, you can have the hydrophobic interactions that are also going to have the hydrophobic groups onto the antigen and that also is going to form an interaction. Similarly, you can have the salt bridge interactions, you can have the Hydrogen bonding, you can have the Vander wall interactions.

And because of these interactions, the antibody and antigen are forming a very tight complex and that is how they can be utilized for developing the different types of assays and different types of reactions. What are different reactions and assays an antibody and antigen interaction can produce?

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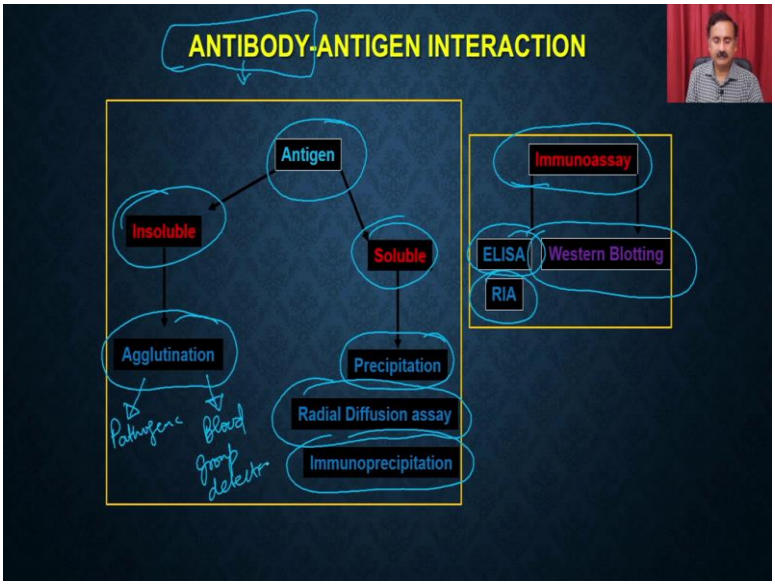

As an antigen, you can have an antigen, you can have the insoluble antigen or you can have the soluble antigen. So, as far as the immunoassay is concerned, you can have the different types of immunoassays you can have the ELISA, you can have the RIA or you can have the western blotting. Whereas, in the case of antigens antigen can be insoluble or antigen could be soluble.

If your antigen is insoluble you can have the agglutination reactions and these agglutination reactions can have the very robust applications in terms of the detection the different types of pathogenic organisms or it can also have the role in the other kinds of applications. Whereas, if the antigen is soluble, it actually can be responsible for precipitation reactions, radial immunoassays and as well as the immunoprecipitations.

So, let us discuss how we can be able to exploit the antibodies and that can we actually going to use for the generation of the some of the immune responses. So, the first thing is, you are going to use for the agglutination reactions, because that agglutination reaction is actually going to help in the detection of the pathogenic organisms or it can also be used for the other kinds of application for itself for example, it can be used for the blood group detections.

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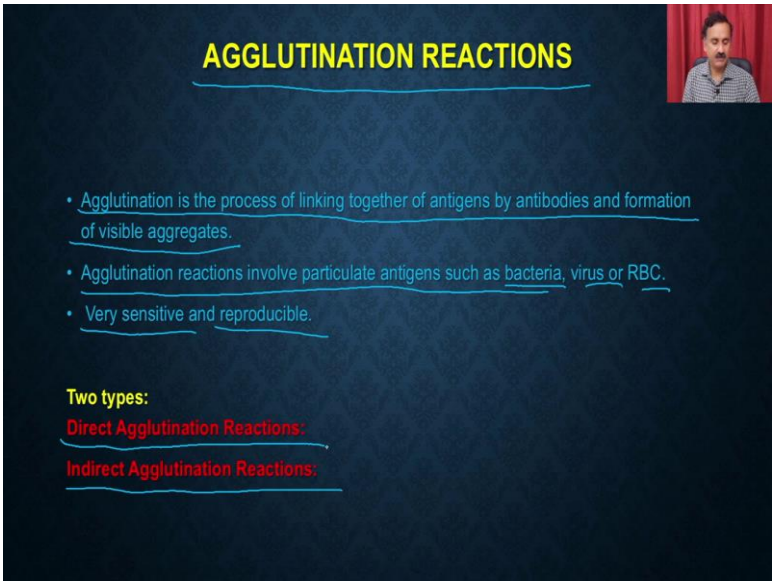

Now, what is the agglutination reactions? Agglutination reaction, agglutination is the process of linking together of antigen by antibody and forming of a visible aggregates. Agglutination reaction involves a particular antigens such as bacteria, virus, or the RBC, it is very sensitive and reproducible. You can have the two different types of agglutination reactions, you can have the direct agglutination reactions or you can have the indirect agglutination reactions.

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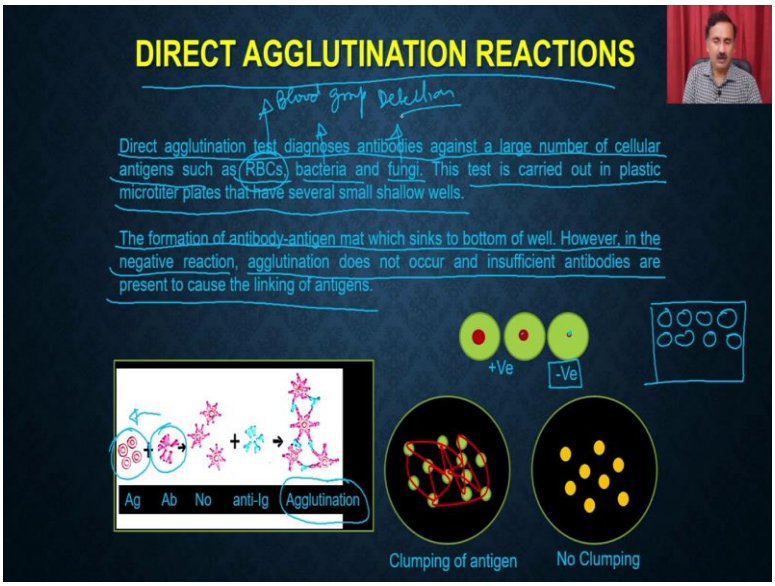

In a direct agglutination reactions. The direct agglutination test diagnose the antibodies against a large number of cellular antigens such as the RBCs, bacteria and fungi. This test is carried out in a plastic microtiter plate and that has the several small shallow wells. So, it can

be used in a 96 well plate, you can have a different type of wells and all these wells can be used for the different types of samples.

The formation of antibody-antigen mat, which sinks to bottom of the well. However, in the negative reactions, agglutination does not occur and insufficient antibodies are be present causing the linking of it. So, you can see that this is the, these are the positive signal, where you can actually have the antigens, which are actually going to connect with each other with the help of the antibodies, and that is how it is actually going to form a clump.

So, you can see that this is the antigen that you have, if you add the antibodies, and these antibodies are reacting with these antigens, they are actually going to bind on to this antigen and that is how they are actually going to form a big mesh. And these are actually going to form the agglutination reactions, that is how you are going to have a precipitate like this. But if you are actually going to see the unprecedented material, then this is going to be called as the negative control.

Now, how you can actually be used the direct agglutination reactions? You can use a direct agglutination reaction, because the antibodies are going to react with the RBCs, bacteria and fungi. So, you can actually be able to detect the bacteria, fungi or you can actually be able to detect the different types of RBCs and that was the basis of the blood group detection. So, blood group detections, how you can do the blood group detections, you can actually do the direct agglutination reactions.

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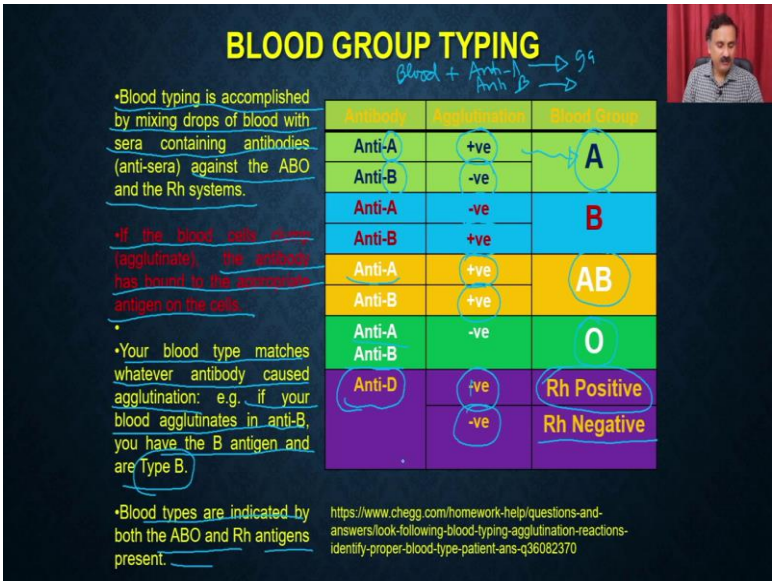

So, the blood group typing is accomplished by the mixing a drop of blood with a sera containing the antibodies against the ABO system and the Rh system. So, you can have the antibodies which can be against the A antigen or you can have the antibody which can be against the B antigen. So, what you can do is you take the blood and then you mix it with the anti-A antibodies or you can actually mix it with the anti-B antibodies and then you look for whether the agglutination is happening or not.

So, you can imagine that if you have taken the sample and you have mixed it with the anti-A or anti-B. So, if it is forming a tradition reaction with anti-A, but it is not forming your agglutination reaction with anti-B, then the blood group is going to be A blood group. Same is true for the anti-A, if you do the sample mixing with anti-A or anti-B, and if the anti-A is not forming sample or it is not forming the agglutination, but the anti-B is forming the agglutination then the blood group is going to be B.

Similarly, if you have the agglutination for the anti-A and anti-B both then the blood group is going to be AB. And if you have the absence of agglutination for the anti-A or anti-B, then the blood group is going to be O. And apart from that, you can also have the Rh factors, so you can have the antibodies against the Rh factor. So if you have the Rh factor negative, then it is going to be called as the Rh, so it is going to be positive, then it is going to be called as the Rh positive. If it is going to be negative, then it is going to be called as the Rh negative.

So, if the blood cells are going to clump, the antibody has to bound to the appropriate antigen onto the cell, your blood type matches whatever the antibody cause the agglutination. For

example, if your blood agglutination in the anti-B, you have the B antigen, and that is why the blood group is B. Blood types are indicated by both the ABO and Rh antigen which are present. So, you can easily see how the blood group typing can be worked in the following this paper actually.

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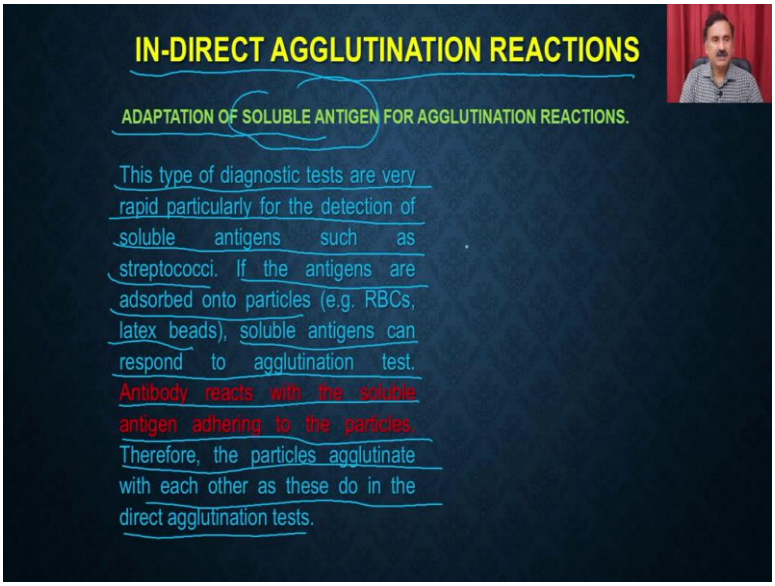

Apart from that, you can also do the indirect agglutination reactions. So, in the indirect agglutination reactions, you are going to have the, you are going to make the insoluble antigen to, you are going to make the soluble antigen into an insoluble antigen. So, these types of diagnostic tests are very rapid particularly for the detection of the soluble antigens such as the streptococci. So, if the antigens are adsorbed onto the particle, for example, the RBCs or to the latex beads, the soluble antigen can be responded to the agglutination test.

Antibody react with the soluble antigen adhering to the particles. Therefore, the particles accumulate with each other as those dough in the direct agglutination test. So, whether it is a directed agglutination test or the indirect agglutination test, both of these tests are utilizing the antibodies against the antigen and that is how they are actually can be used for the detection of that particular pathogenic organisms.

And apart from that, they can also be used for the blood group typing as well. So, with this brief discussion about the monoclonal antibody and with the brief discussion about how you can be able to utilize these antibodies under the agglutination reactions and how you can be used these antibodies for the detection of the different types of blood groups. I would like to

conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the immune response. Thank you.