Basics of Biology
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Lecture 31
Immune System (Part 1)

Hello everyone, this is Doctor Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati. And what we were discussing, we were discussing about the living organisms. So, so far, we have discussed different aspects of the living organism, we have discussed about the classifications, then we have discussed about the evolution of the living organisms.

And subsequent to that, we have also discussed about the different types of cells, cellular structures, where the cellular structures are for the prokaryotic or the eukaryotic cells. And then we have also discussed about the structure and function of the different types of biomolecules. And in the previous module, we were discussing about the central dogma of molecular biology or the central dogma of life.

And in that discussion, we have discussed about the DNA dependent DNA polymerase activity and which is also been known as reapplications. Then we also discuss about the transcriptions and we also discuss about the translations. And apart from that, we have also discussed about the post translational modifications and the post transcriptional modifications. So, the central dogma of molecular biology or the central dogma of life is required to run the different types of activities of a cell, but these cells are also been participating into the different types of functions.

So, in today's lecture, we are going to discuss about the some of these additional cellular processes. And by discussing this, we could understand how these processes are been helping the organisms to overcome the different types of difficulties or different types of adaptations. So, they are actually going to help the organism for the adjusting into the local environment or adjusting to the new environment.

And apart from that, some of these processes are also been essential for the survival of the organism, because it protects the organism from the different types of infectious organisms or different types of other organisms. So, let us start discussing about these important aspects of the living organisms, where we are going to discuss about the different processes.

And what we are going to discuss? We are going to discuss about the immune responses and we are also going to discuss about the cell death and how the apoptosis is happening in the cell. And on the other hand, we are also going to discuss about the vesicular transport within the cell. So, let us start our discussion with the immune system. So, what is immune system?

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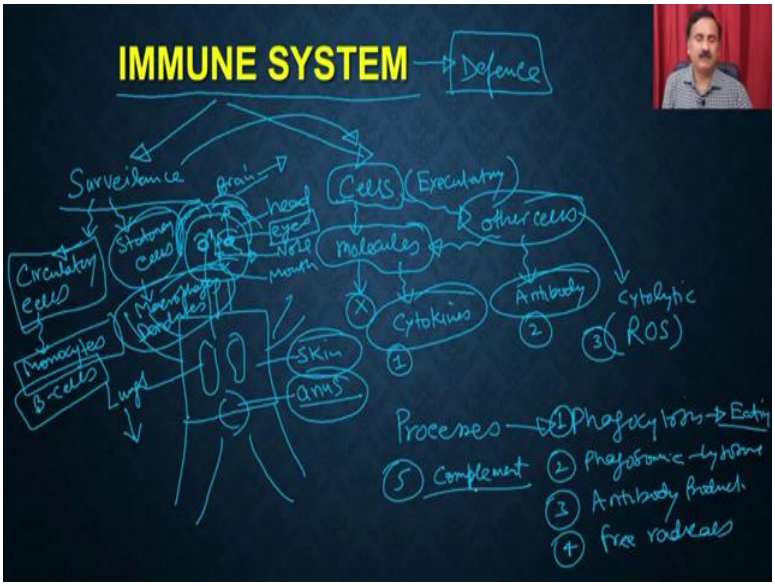

So, immune system is defense mechanism which is present in most of the multicellular organisms and the immune system is required for maintaining a local environment or a global environment where the organism is going to protect himself from the other organism. So, the other see, majority of the other organisms are trying to take the nutrition.

So, you can have the two different types of organisms, one which are going to have their own mechanism to acquiring the nutrition or they are actually going to prepare the food from utilizing the chemical or the natural products like for example the plants so the plants are actually going to prepare their own food and by utilizing the sunlight and as well as the carbon dioxide and water from the environment, but apart from the plants all other organisms are dependent on the other, dependent on the plants for the direct or the indirect way to provide the nutrition.

But in that category also some of the organisms are having their own machineries. So, that they can be able to take the raw material from the environment and that is how they can be able to prepare their own, they can be able to take up the nutrition. But apart from that, you can also have the organisms which actually grows on the other organism and these organisms

are called as the infectious organisms or the parasites, these parasites are actually taking the nutrition from the other organisms.

So, there is defense mechanism which actually is going to protect the organisms so that it does not allow the other organisms to grow and that is how it is actually going to avoid the other organism to take up the nutrition. So, this particular defense system is called as the immune system. So, how the immune system is actually going to have? See, so, first is that you have to understand that since the immune system's main job is that it is actually going to provide the defense against the incoming infectious organisms or the other organisms.

It should have a very well defined system. So, what it should have? So, immune system should have a surveillance system. So, it should have a surveillance system or it should have some way to you know some so that it can be able to monitor. Then it can also have the cells, which actually can be able to perform the actions on to the infectious organisms and then it also has, so it could have the cells which are actually going to secrete the molecules and these molecules are having the function that they are actually going to kill the other organisms.

So, the surveillance system is going to be distributed throughout the body. For example, if we take a example of the human being that the human being, within the human being we can have or in the even the other vertebrate animals you can have the different routes through which you can actually be having the entry of the other organisms. So, for example, if you take a human being, you can have, this is the human being, you can have the right this.

So, here what you have is you have eyes, you have the nose and then you have a mouth, then on the top you are going to have the hairs and you are going to have the brain. So, this is the head. So, this is the head. These are the eyes. These are nose. And this is a mouth. Apart from that you can have the, so this is, apart from that you can have the internal organs like so, now, if you see these are the four different or places or opening apart from that you can have the opening at the here. So, you can have the anus, which is also going to be opening.

So, these are the some of the places so which and then throughout the body you can have the skin. So, that is the, these are the major places from or major routes through which the other organism can enter into the body. So, for example, it can actually if it enters through the eyes actually it can enter into the eyes and then from here, it can go into the brain or it can go into the other part of the body. So, just within the eyes, you can have to have a surveillance system so that you can be able to monitor.

Similarly, you have the nose. So, nose is actually, if you see the connection, the nose is connected. Then you have a respiratory system and then it goes into the lungs. So, you can have the lungs. So, nose is like the air passages, so these whatever the it comes through the air, it is actually going to travel all the way and then it goes into the lungs. So, you can also should have a surveillance system to monitor that.

Then in the mouth, mouth is going to be connected to the elementary canal and then it is actually going to be connected to the other organs like the liver, it can be connected to the pancreas and all other places. So, that also should have a surveillance system. So, how the immune system is going to do a surveillance? It is actually going to have the two types of surveillance system one is it is going to have the circulatory cells and the circulatory cells are actually going to keep circulating throughout the blood and these cells are actually going to keep looking at the cells which are not from the same human beings.

Then it can also have the stationary cells like the cells which are being present into the tissue, and that is how it is actually, or the organs, and that is how they are actually going to continuously monitoring. So, within the circulatory cells, you can have the monocytes which are actually going to function as the surveillance system or you can also have the B-cells, which are also going to function as the surveillance system. So, that they are going to monitor the other organisms whereas in the stationary cells, you are going to have the macrophages or the dendrite cells.

So, you can have the dendrite cells or you can have the macrophages. So, in every place you can actually have the macrophages. For example, you can have the macrophages in the brain, you can have the macrophages in the lungs, you can have the macrophages in the liver (12:01) so, in majority of the organs you can have the macrophages and these macrophages job is to actually going to do the surveillance.

Now, once the surveillance system is actually going to found different organisms, then what we are going to do is this surveillance system is actually going to inform the other type of cells. So, these are going to be called as executory cells, which means, they are actually going to act on to the information what they are going to receive from the surveillance system. And that is how these cells are then going to secrete the molecules or they are actually going to activate the other cells. So, that they are actually going to have a robust defense response.

And that robust defense response is always been governed by the different types of molecules. What are different molecules? You can actually have a secretion of the different types of cytokines, you can also have the secretion of the antibody and you are also going to have the secretion of cytolytic substances. So, you can have the like, you can actually have a secretion of the reactive oxygen species and so on.

So, all these molecules whether they are being cytokines or antibodies or the free radicals, they are actually going to clear up or they are actually going to kill the microorganisms or they are going to kill the other organisms. And that is how they are actually going to provide you the protections. Now, in this particular executory cell, you are actually going to have the multiple processes, like in these executory resells you can have the processes like you can have the phagocytosis, because how these executory cells are actually going to function.

Even in the surveillance system also you can have these processes. So you can have the phagocytosis. Phagocytosis means the eating. So, it is going to be called as eating which means that these cells are going to eat that particular other organism and that is how they are actually going to sense that whether these organisms are, what kind of this organism is and then how what could be the defense response I should produce. Then apart from that, it can also have the phagosome-lysosome fusion.

So, that all we are going to discuss in this course. And then it also can have the antibody production and it can also have the production of the free radicals like the Ross, and it can also have the production of the difference or it can be also having the activation of the complement system.

So, since this is a course of the basics of biology, we are not going to discuss in detail about the immune system or the defense response, because this whole thing you can actually be able to study by going through with any of the immunology MOOC course, in any of the MOOC courses, whatever is available, but we are going to discuss very briefly we are going to discuss this about the how the cells are phagocytosing the other cells and how they are actually doing the phagosome-lysosome fusion, and how the antibodies are being produced by the immune cells and that is how they are actually going to cause robust response.

And because, these systems are existing into the host organism or into the human beings, the system can also be exploited for different types of applications as well. Like for example, the antibodies can be utilized for different types of acids and all those kinds of things.

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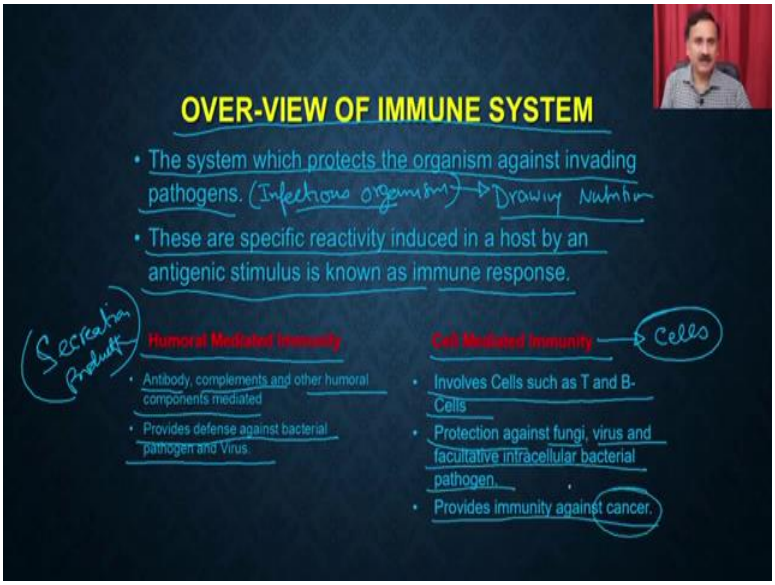

So, let us talk about the immune system. So, you can have this, this is the system which actually protects the organism against the invading pathogen or the infectious organism. And we have already discussed about the infectious. So, infectious organism why, it is so because the infection organism is going to start taking down nutrition, so, it is going to start drawing the nutrition from the other organism and that is why it is not required.

These are specific reaction induced by a host in antigenic stimulus and it is called as the immune response. And when you have the immune response, like just now we have discussed, you can have the surveillance system and then you can have the excretory cells. And once the surveillance system is going to detect the pathogenic organisms, or it is going to detect the infectious organisms, it is going to give that information to the executory cells and then executory cells are actually going to have the secretion of the different types of biomolecules.

So, considering the what component of the immune system is going to activate, and what component is going to participate into their defense responses, the immune system can be of two types, it can be a humoral immune mediated immunity or it can be a cell mediated immunity. In the humoral immunity, you are going to have the liquid part or it is going to be a cell, the secretion products of the secretion products, which are going to have their action on to the infectious organisms. So, what you have?

You have the antibodies, you have the complements, and the other humoral mediated immune responses, like the cytokines, interferon gammas, and all those kind of things. And it

actually going to provide the defense against the bacterial pathogen as well as the virus actually. So, it is going to provide the infection protection against the bacterial as well as the viral infections. Whereas in the cell mediated immune response, the cell is actively going to participate into the causing the defense response.

So, that involves T-cells, such as the T-cells and B-cells, and it actually going to provide the protection against the fungi, viruses facultative intracellular bacterial pathogen and it also provides the immunity against cancer cells. Now, the central theme of today's lecture is that we are going to discuss about the antibodies and how the antibodies are being formed inside the cell and how we can be able to produce the antibodies so that you can utilize that for providing the protection or providing the immunity into the other patients or other animals.

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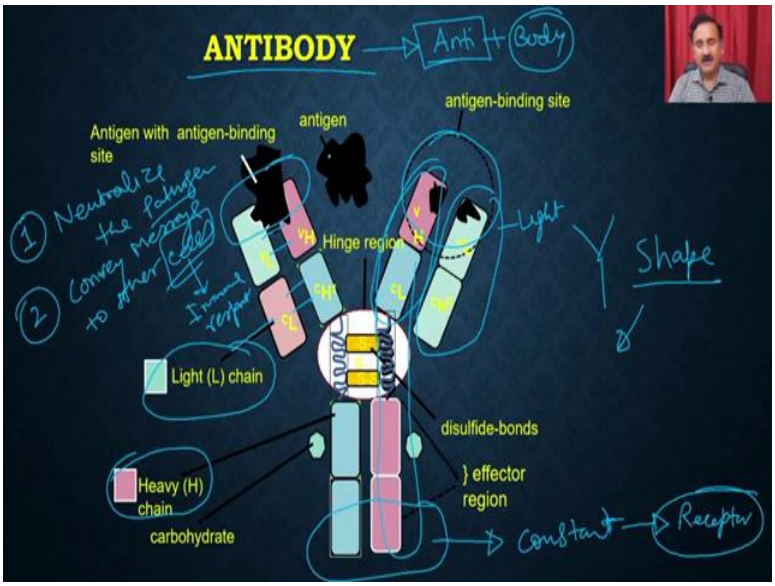

So, if you see the antibodies, antibody is a is a bow molecule, which is going to be so, antibody means anti plus body, so it is going to provide going to act against a body. So, it is going to be called as, that is why it is called as antibodies. An antibody is a Y shaped structure or Y shaped molecule where you have the two chains what is called as the light chain. So, this is the light chain, you are going to have the light chain. And then you also going to have the heavy chain and this is a heavy chain.

So, this total what you see here is called as the heavy chain. So, this is the light chain. So, this is the light chain and then this whole thing is what you see here is called as a heavy chain. This light chain and heavy chains are binding to each other with the help of the disulfide linkages between the light chain and the heavy chain, and then the light chains are also you

know. And that is how the antibody molecules have the two important regions one is called as the antigen binding region.

So, this is the antigen binding region of the antibodies are this is the antigen binding region or the antibody, and then this is the region which is actually going to be called as the constant region. So, and this constant region is always having a receptor for which the antibody is can be able to relay the signal to the other cells. And that is how the antibodies are actually going to be utilized for the two purposes one, it is actually going to neutralize the pathogen or neutralize the pathogen.

So, it is actually going to bind the pathogen and that is how the pathogen is not going to be having a free mobility and that is how they will be confined to a localized environment. The second is because it is going to bind the pathogen on one end, and it is going to bind the other cells onto the other end, it actually can convey the message to the other cells, so it is going to be convey the message to the other cells, and that is actually going to allow these other cells to cause the immune response.

So, if they are actually going to be used for two purposes, one is they are actually going to restrict the movement of the pathogen. So, they are going and so they are actually going to confine the infection at very localized area. And then the second is they are actually going to provide the information about this particular pathogen to the cellular machinery and that is how they are actually going to allow the production of a very robust immune response. And that is why these antibodies are playing very huge role in terms of the immune responses.

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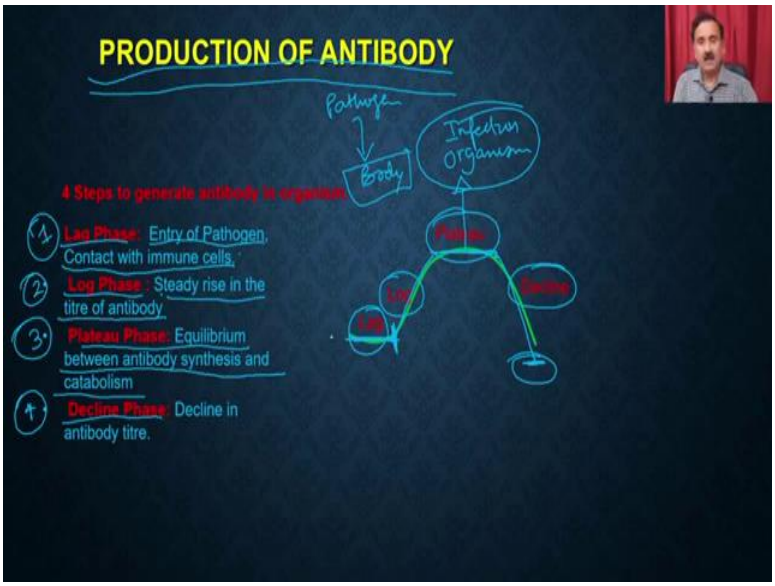

There are different steps through which the antibodies are being produced in the human body. So, there you have the four different stages, you have the lag phase, you have the log phase, you have the plateau, and then you have a decline phase. So, once microorganisms are entering into the body, they are actually going to be processed by the cellular machinery, and that is how they are actually going to be production of antibody against that particular pathogen.

So, you are going to have the entry of the pathogen into the human body and that is how the day the pathogen is going to enter into the body, it will actually going to have these four events, that is how the antibody is going to be produced. So, what are these events? You can have the lag phase, so first is you can have the lag phase. So, in the lag phase, the pathogen is going to enter into the body and then it is actually going to be processed by the immune cells.

Then you can have the log phase. So, in the log phase there will be production, there will be a production of the antibodies. And there will be a steady rise in the titre of the antibody. So, in the first phase, you are actually going to prepare the immune response or you are going to keep the, you will train the cellular machinery and that is how they are actually going to be start producing the antibodies.

So, up to the log phase, they are actually going to have steady rise in the antibody productions. After this there will be equilibrium. So, after that, it will enter into the plateau phase where there will be an equilibrium between the antibodies, what is going to be formed

and the what is going to be degraded. So, that is how it is actually going to form a flat shape. And ultimately after this, you are going to enter into the decline phase because by this time, the organism is or the infectious organism is actually going to be disappear or it is going to be cleared from the infection site. So, that is why after this there is no need to have the antibody.

So, once there is no need to have the antibodies, then the antibody producting cells are actually going to stop secreting the antibodies and that is how there will be a decline in the production of the antibody. That is how it is actually going to come back to the normal positions. And again, they will be ready for producing the antibody for the next organisms or next threats, what is going to happen. So, that is how if you see these are the four major events, what is going to happen when there will be a production of antibodies.

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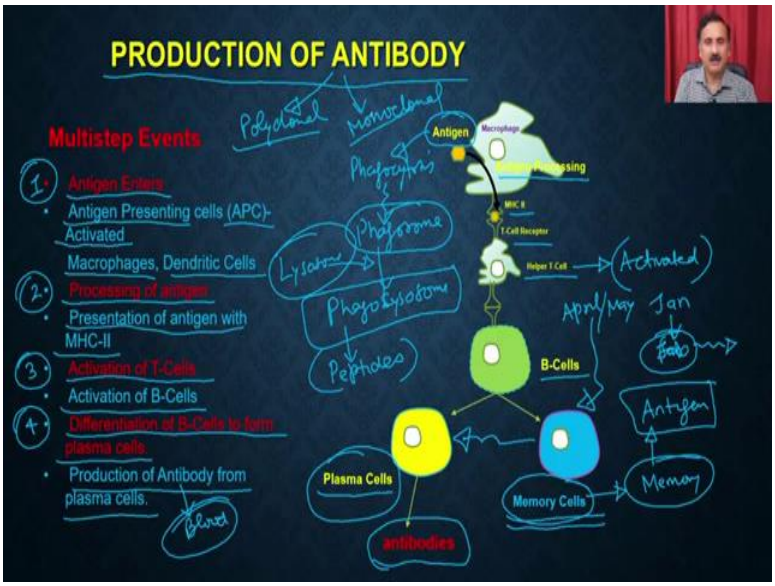

Now, how this is going to happen, there are different steps which are going to be involved into the production of antibodies. So, first is during the lag phase, you are going to have the entry of the antigens. So, as soon as the microorganism or the pathogenic organism is going to be present into the circulatory system or it is going to be present at the different types of organ sites, it is going to be received by the surveillance system.

So, it is going to be received by the macrophages into the tissue sites or it can be received by the monocytes or it can be received by the dendrites or it can be received by the B-cells and the and those all these cells are going to be called as the antigen presenting cells or antigen processing cells. So, the antigen is going to be received by the antigen processing cells, which can be either the macrophages, dendritic cells or the B-cells, and then they are actually going

to be processed, so the antigen presenting cells or APCs are going to be activated because once the antigen is going to come into the vicinity of the cells, they are actually going to be activated.

And then these macrophages or the dendritic cells are going to do the processing of the antigen, which means, see in the step 2, you are going to have the processing of the antigen. So, this antigen is going to be internalized into the cells and then the cells are actually going to process, so they are going to be internalized by a process which is called as the phagocytosis and then following the phagocytosis there will be a formation of the phagosomes and these phagosomes are actually going to fused with the lysosomes and that is how they are actually going to form the phagolysosomes.

And these phagolysosomes is actually going to have the content from the lysosomes and it is going to have the antigen into these phagosomes and that is how within the phagolysosomes the enzymes what is present into the lysosomes are actually going to process the antigen. So, it is going to degrade the antigen and the antigen is going to be present in the peptide form. So, if, and within the phagosomes, the antigen is now going to be converted into the small peptidic fragments.

So, these peptidic fragments are then going to be presented on to the antigen presenting cell with the help of the MHC class 2. So, then these are antigens are going to be presented along with the MHC class 2 molecules, and that is going to provide a signal for the executory cells. So, they are actually going to provide the signal for the other cells like the T-cells. So, and especially like helper T-cells. So, these T-cells are actually going to interact with the MHC class 2 bound antigen with the help of the T-cell receptors.

And by doing this engagement, the T-cell is going to be under the activated state. And once the T-cell is going to be under the activated state, the T-cell is going to provide this information to the downstream cells. And that is how these downstream cells are called as the B-cells. So, the B-cells are going to interact with the T helper cells. And that is why in the third event, you are going to have the activation of the T-cells and then you are also going to have the activation of the B-cells.

And the T-cells are actually going to activate the B-cells and these B-cells are then going to differentiate into the two different types of cells. They are actually going to form the plasma cells or they are actually going to form the memory cells. The plasma cells are actually going

to have the ability to produce the antibodies by utilizing the information what the T-cell is going to convey to the B-cells and that is how, they are actually going to start producing the antibodies.

Now, the B-cell is also going to differentiate into the other type of cells, these cells are called as the memory cells. And these memory cells are actually going to keep a memory of this whole event. So, it is going to keep a memory of this antigen. So, that whenever these antigen is going to enter into the future. So, for example, like suppose I got an infection in January. So, if I got the infection in January, probably these events are going to happen and that is how there will be a production of deeper antibodies by the Feb I am going to start producing the antibodies.

And then I also develop the memory cells. So, memory cells for this particular antigen. Now, what happened is that if there will be an infection in, for example, in April or May again, then the system will not go through this long procedure of the different types of events like again the next time is antigen is not going to be processed and all that. Then ultimately we know that there are memory cells.

So, what will happen is that in April or May, these memory cells will actually going to get the signal from the immune system and that is how the memory cells are actually will differentiate into the plasma cells, and that is how the memory cells will start producing the antibodies. So, the plasma cells will start producing the antibodies.

So, in the step 4, you are going to have the differentiation of the B-cells to form the plasma cells and these plasma cells are actually going to start producing the antibodies, which is actually going to be secreted from the cells and that is how the antibodies are going to be present into the blood. So, it is going to be present into the blood and that is how the antibody is going to be secreted going to be distributed throughout the body and that is how it is actually going to provide the protection.

Now, you might have seen these, the hook all the events, what is responsible for the production of antibodies. So, based on this scheme, the antibodies could be of two types antibodies could be of the polyclonal antibodies or it could be a monoclonal antibody, which means the either you are using the single type of plasma cells, then it is going to be called as the monoclonal antibodies and if you are using the multiple plasma cells, then you are going

to be called as a polyclonal antibody. So, what is the major difference between the polyclonal antibody versus the monoclonal antibodies?

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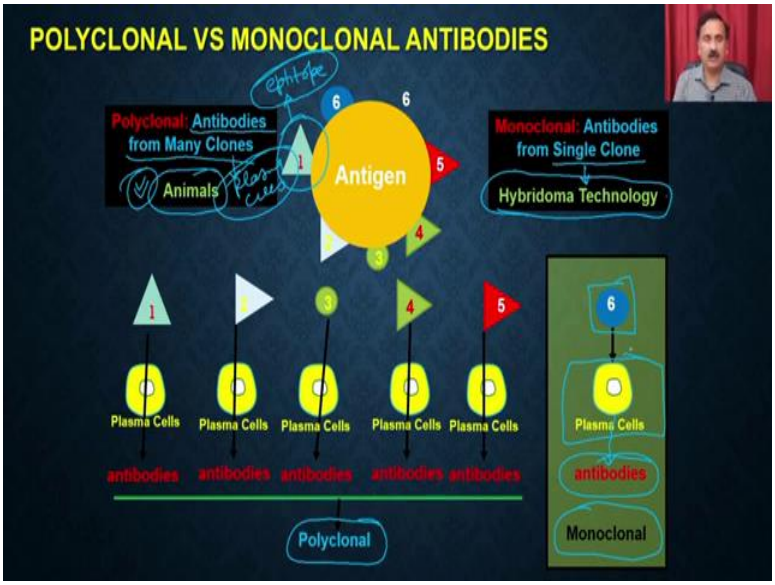

In a polyclonal antibodies, as the name suggests, the antibodies are going to be produced by the many clones of the plasma cells. So, it is going to be from the many clones of the plasma cells. And the polygonal antibodies are going to be produced into the animal. So, even imagine that if I have an antigen and it has the different region, which can be utilized for the antibody production. So, it can have the 1 2 3 4 5 6 all these different types of regions and these regions are actually being called as the epitope.

So, these epitopes are actually been responsible for the production of the antibody. So, it is all these epitopes pick region and the corresponding claws are actually going to produce the antibodies, the collection of these antibodies are going to be called as the polyclonal antibodies, whereas, if the you can have the monoclonal antibody, which is actually going to be from the single cell and the technology what you are going to use to produce the monoclonal antibody is called as a Hybridoma Technology.

And the monoclonal antibody means that the epitopic region 6 is actually going to activate only a one clone of the plasma cells and that clone is actually going to start producing the antibody. So, that is why it is monoclonal antibody is actually going to be produced by the single clone whereas the polyclonal antibody is going to be produced by the many clones.

And polyclonal antibody can be produced into the animal whereas the monoclonal antibody require the hybridoma technology where you require the many more steps and that is how

you can be able to select the single clone and that is how you are going to be able to produce monoclonal antibodies. So, let us see how you can be able to produce the polyclonal antibody under the experimental lab conditions.

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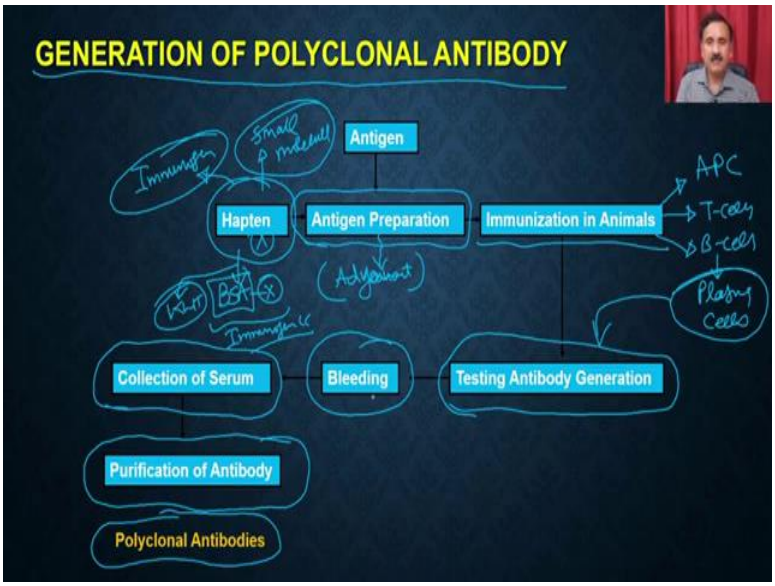

In the experimental lab conditions, if you want to generate the polyclonal antibodies, you have to prepare these or you have to follow these following steps. So, what you can do is first you have to have the antigen what you have to prepare for the immunizations. So, antigen can be of two different types either it can be a hapten. So, you know that the haptens are the antigens, which are actually non immunogenic.

So, then you might have to convert hapten into a immunogen. How you can actually be able to do the conversion off and happen to the immunogen is that you can actually couple the hapten to big molecules, because majority of these haptens are the molecule which are small molecule. So, small molecules and because they are small molecule, they are not having the immunogenic potential to activate the immune cells.

So, because of that, what you have to do is you take the haptens and then you couple it to a big protein such as the BSA or the KLH. And if you couple them, the BSA is actually suppose this is a X, hapten what you have is X, then if you couple the X to the BSA, then the hapten is going to be immunogenic molecule and then it will get converted into immunogenic molecules. And now, what you have to do is you have to prepare the antigens.

So, in the antigen preparation you have to mix the antigens with the adjuvant so that you can actually be able to get the robust immune response. So, once you prepare the antigen with the

adjuvants, it is good enough to get the immunizations and then you are going to do the injections into the animals.

Once you do the injection into the animal, all those events are going to take place it is going to first going to be processed by the antigen presenting cells, then it is actually going to be processed by the T-cells, then it is going to be processed by the B-cells, and then ultimately the B-cells is actually going to convert into the plasma cells, and then the plasma cells are start producing these antibodies.

So, once the plasma cells will start producing the antibodies, then you can be able to, first you have to do is you have to test whether the antibodies are producing or not, if the antibodies there is sufficient antibodies into the blood, then what you can do is you can do a bleeding, so you can do bleeding into the animal and that is how you can be able to collect the serum.

So, this serum is actually going to have the antibodies, and then you can actually be able to purify the antibodies utilizing a (())(36:47) column. And so, you are going to get the polyclonal antibodies. So, let us see how these events you can do and what are the different steps what you have to do in the lab.

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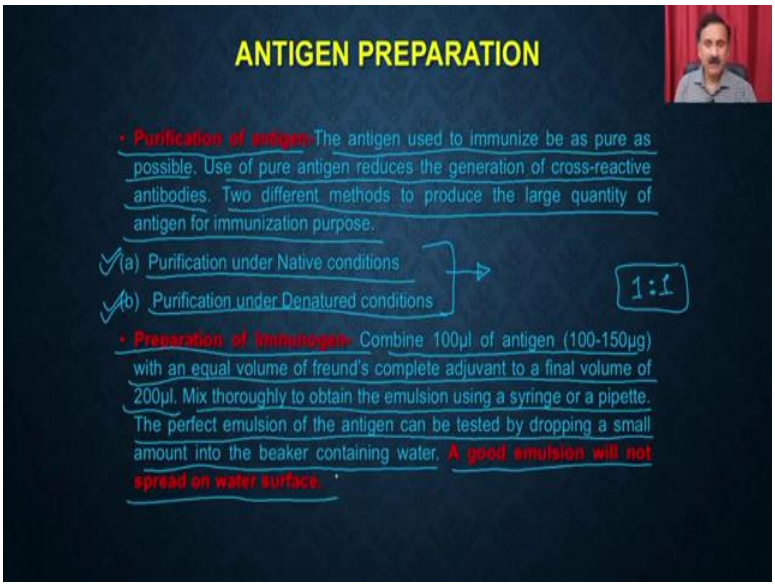

So, the first is, the first step is that you will have to actually prepare the antigen for the injections. So, the antigen used to immunize be as pure as possible because an use of the pure antigen reduces the generation of the cross reactive antibodies to different matter to produce a large quantity of antigen for immunization is that you can actually make the antigen under the native conditions or you can actually prepare the antigen under the denaturing conditions.

So, these I am not discussing because, these two steps I have already discussed in some of my earlier MOOC courses and that you can actually go through if you are interested to know how you can be able to purify the antigens under the native or denatured conditions. Then you have to prepare the antigen or immunogen for the injections.

So, for this what you have to do is you have to combine the 100 microliters of the antigen which is going to be into the range of 100 to 150 micrograms total amount with an equal amount of the freund's complete adjuvant to a final volume of 200 micrometer, which means, you have to take the antigen and the freund's complete adjuvant into the 1 to 1 ratio.

Then you mix thoroughly to obtain the emulsion using a syringe or the pipettes. The perfect emulsion of the antigen can be tested by dropping a small amount into the beaker containing the water. A good emulsion will not spread onto the water surface.

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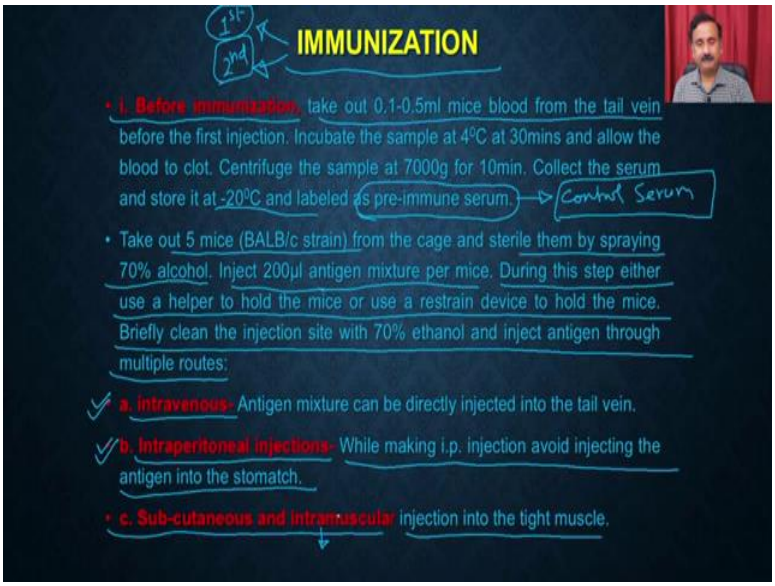

Then you are going to do the immunizations. So, you are going to do the immunization. So, you remember that nowadays we are actually going through the immunization steps were we are immunizing the people for the COVID. And in the COVID Also, what we are doing is we are doing the two injections. So, these two injections are actually providing the robust immune response against the Corona virus.

Same is true here when you are doing the immunization into the animal you are going to do the immunization two times. But here since we are going to do the experiments, we are actually going to take out the blood before immunizations, so that we can be able to detect whether there is a production of antibodies or not. So, before we do the immunization, you can take out the 0.1 or 0.5 ml blood from the mice.

So, that you can be able to and you can collect it and prepare the serum and then you keep it at minus 20. And this is actually going to be called as the pre immune serum which means it is actually going to serve as the control serum and it is going to be used for the comparison purpose because when you are to see whether the antibodies started producing or not, you also should have the serum before you got the, you have immunizations.

Then what you can do is you do the take out the mice and sterile them by spraying with the 70 percent alcohol and then you inject the 200 microliter antigen mixture per mice during this step either use a helper to hold on mice or use a restraint device to hold the mice. So, you can briefly clean the injection site with the 70 percent alcohol and inject the antigen through the

multiple routes. So, what are the different routes what you can use, you can use the intravenous route, which means you can directly inject the antigen into the blood.

Then you can have the intraperitoneal injections, which means you can actually do the injections into the belly. So, it is going to be called as the intraperitoneal injections. So, while making the IP injections avoid injecting the antigens into the stomach. So, this intraperitoneal introduction means you are actually going to make the injections into the space between the stomach and the, between the stomach and the outer body.

So, that is intraperitoneal injections are, and that is how you have to keep very good precaution that you should not inject the antigen into the stomach. And then you can also do the injection as the subcutaneous or intramuscular. So, that you can actually do into the thigh muscles. Remember that the intramuscular injections are what we are getting when we are getting further COVID vaccines.

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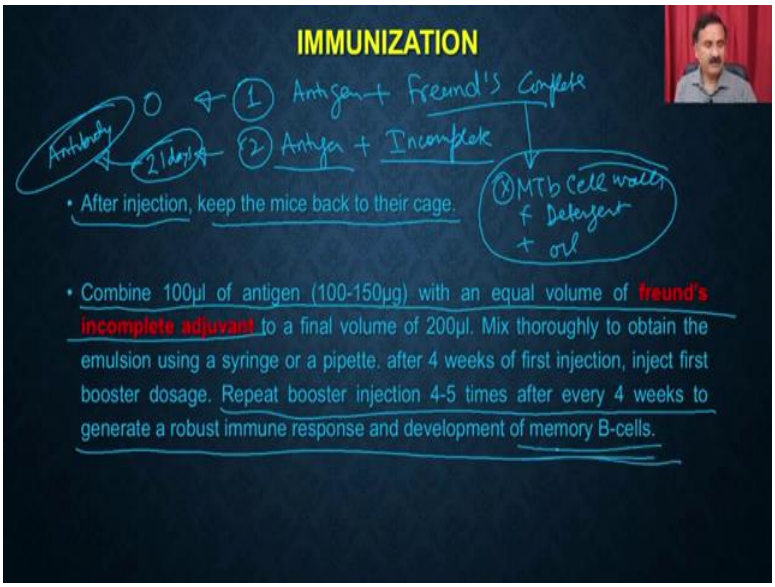

Then what you have to do is you are actually going to after injections to keep the mice back into the case. And you are actually going to do the immunization two times, first if you are going to do immunization where you are actually going to mix the antigens with the freund's complete adjuvants. And the second time you are going to have the antigen plus you are going to have the incomplete adjuvant.

So, in this case, if you going to combine the antigen with a equal volume of freund's incomplete adjuvant then you are actually going to have the you know, so this injection you are going to do the time at 0. And this injection you are going to do after the 21 days. And

that is, what is the difference between complete adjuvant versus incomplete adjuvant. So, a complete adjuvant is actually containing the mycobacterium cell wall and it went to contain as the detergents and they are also going to contain as the oil. So, this mixture is actually activating the immune system.

And that is how it is actually bringing the large quantity of the B-cell and T-cell at the site of injections, whereas when you are injecting after 21 days the incomplete adjuvants. The incomplete adjuvant does not contain the mycobacterium tuberculosis cell wall. So, that is how that time whatever the machinery come at the site of injections, it is actually going to process the antigen of your antigen and that is how after the second injection, there will be a enhanced production of antibody which is going to be against the antigen what you have injected.

So, you repeat the booster dose after 4 to 5 times after 4 weeks. So, if there is a not a robust immune response, you can actually repeat injecting the booster response so booster injections after every one month and that is how you can actually be keep checking whether the antibodies are producing or not. And ultimately you are going to have degeneration of the memory B-cells.

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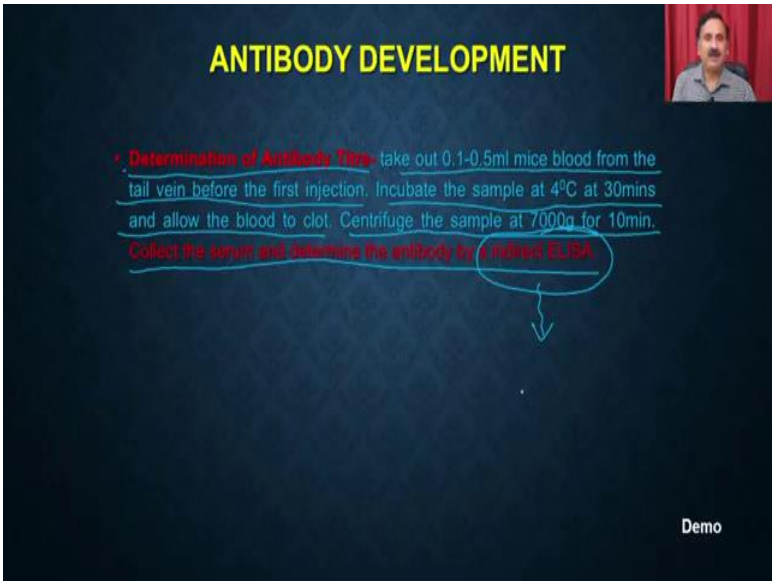

So, when you do, how you are going to determine, how you are going to detect whether the antibodies are produced or not, you can determine the antibody titres, so you can take out two 0.1 to 0.5 ml blood from the tail vein before the injections. Incubate the sample at 4 degree at 37 degrees Celsius and that will allow the blood to clot and then you centrifuge the sample at

7000 g for 10 minutes you collect the serum and determine the antibody by the indirect ELISA.

So, to give you a real life experience, how you can be able to utilize this, how you can be able to go through with these procedures and steps I have prepared a small demo clips where I can be able to show you all the steps and then with this clips, you will be able to understand how you are going to prepare the you know, how you are going to make the antigen with the fluids complete adjuvants and incomplete adjuvants, how you are going to prepare, how you are going to do the mixing of these two and you are going to prepare for the, how you are going to perform the emulsifications and all that and how you are going to check whether the antigen what you have prepared is perfect or not. Let us see the demo.

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I am Doctor Amogh Anant Sahasrabuddhe, I work in CSIR-CDRI Lucknow. And in today's demo, we will be discussing different steps involved in generation of antibodies. So, for the first step, we require several things like trans complete adjuvant, here it is from sigma. You need a micro emulsifying needle which has two openings connected with a fine needle. We need antigen which is purified and filtered.

So, there are no contaminations, it is sterile solution of antigen. Then we take out some of the freund's complete adjuvants independent of and then mix them together. Since this adjuvants is oil based it does not meet easily with the watery system like antigen is in previous. So, therefore, we mix them rigorously, vigorously and forcefully.

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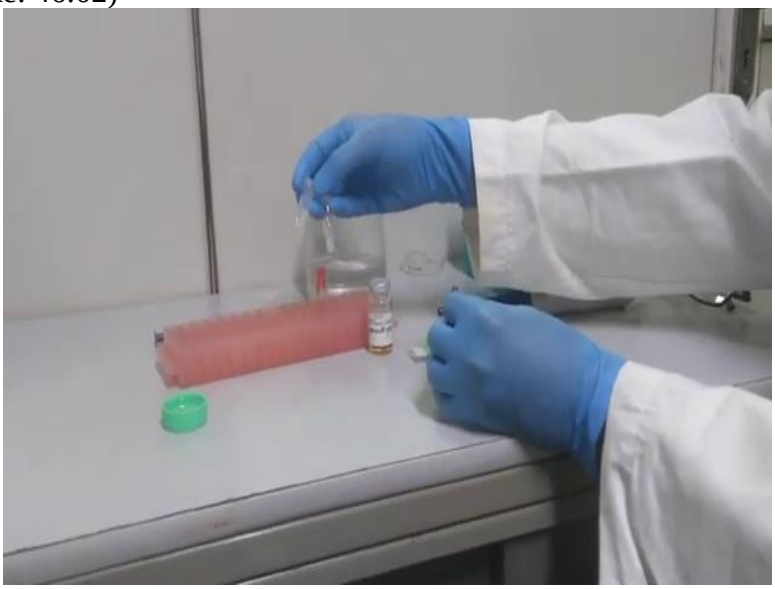

For that purpose we take these two, we mix this emulsion and we mix this previous and previous containing antigen and the adjuvant, always adjuvant. After mixing them we take out in a needle, using a needle we take out in a syringe like this and then we fix the macro emulsifiers needles into it, attach another syringe into it like this.

So, once you have filled your antigen and the adjuvant in this needle, you push it here and then you keep pushing from one side, keep pulling from another side, keep pushing from one side, keep firm from another side. So, this process forcefully pushes your material I mean the oil and the antigen through this fine needle and with that, in the process that emulsion is formed, emulsion can be called as water in oil or oil in water because both are in the same concentration same volumes. So, you can call them anyway. So, it is the emulsion, by this method emulsion is formed.

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So, for ready reference, we have already prepared the emulsion. This emulsion looks like white, initially it was two phase and then slowly it has turned into single phase, now we can push this emulsion from one needle to another side and from another this syringe to another syringe. So, this process creates very good emulsion. This does not separate out later when we are ready to inject. So, how do we check them?

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So, for checking purposes, we drop one of this emulsion, a drop of immersion on a water surface like this. If emulsion is not formed perfectly this will spread out otherwise it will not spread. So, this is, check that your emulsion is formed correctly. So, once you find that this drop is now spreading your emulsion is actually ready for injection. So, this was the process by which you prepare the emulsion for injection.

So, this is the first step of preparing emulsion. So, now, let us understand why we prepare the emulsion. We have check that emulsion is formed. Now the purpose of making the emulsion because we have antigen and through antigen you can raise antibodies, but after emulsifying them you actually make the antigen releases slowly. So, it is a sustained release kind of preparation.

So, that the antigen is exposed to system in a systematic manner so that more and more memory cells can be generated. And that is the sole purpose of having emulsion. Otherwise, if you inject antigen as such in PBS or in other watery system it will be spread out in the body and it will be cleared up by the immune system and no memory cells will be generated. So, these are, this is the main purpose of preparing the emulsion.

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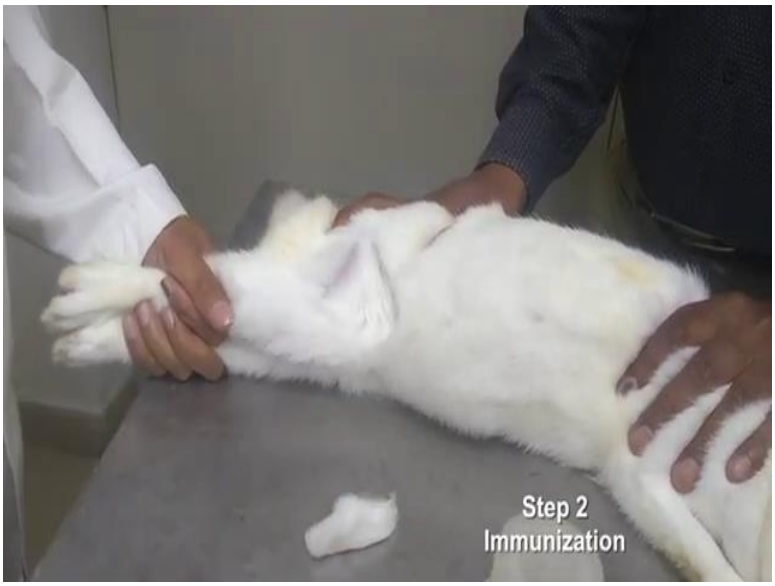

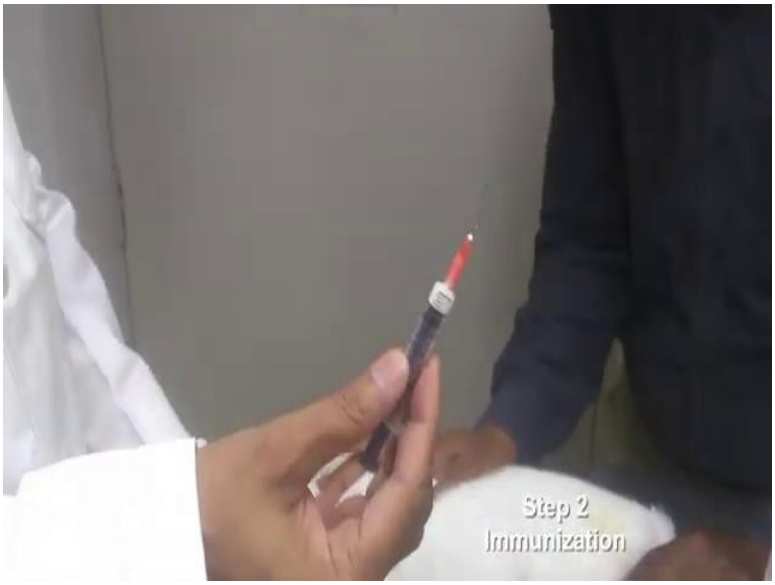

So, now we have prepared the emulsion, we have to animal house, this is a rabbit which will be utilized and before immunization we have to take pre human breed, so that we can compare better the serum and the anti serum. So, now we will start how the we utilized it. So, now we are preparing very nice. Here very first important thing is in all this several processes is we have to avoid that the (())(50:13).

So, for that purpose we have to strain the animal because we have to inject. So, we strain the animal in a way that it has less and less pain and the moment is also less. So, we will inject this into the, emulsion into the thigh. We have to catch both the legs and we have to sterilize the area using alcohol.

We have to look at the thigh muscles, they should be cleaner, cleanly visible, skin should be cleanly visible. There are two kinds of injection that we give, one is intradermal and another

one is subcutaneous. So, today we will be doing subcutaneous injection. This is our emulsion that we are prepared by micro emulsifying, we have seen ideas.

This is the area where we would like to inject, you have to take out all the airs from syringe and the needle. We have taken out, clean the area again. And then we apply anti septic powder, here it is Butadiene powder so that the infection cannot develop later on. We sprinkle some of the area of injection. And the slowly leave the animal relax and it is immunized.

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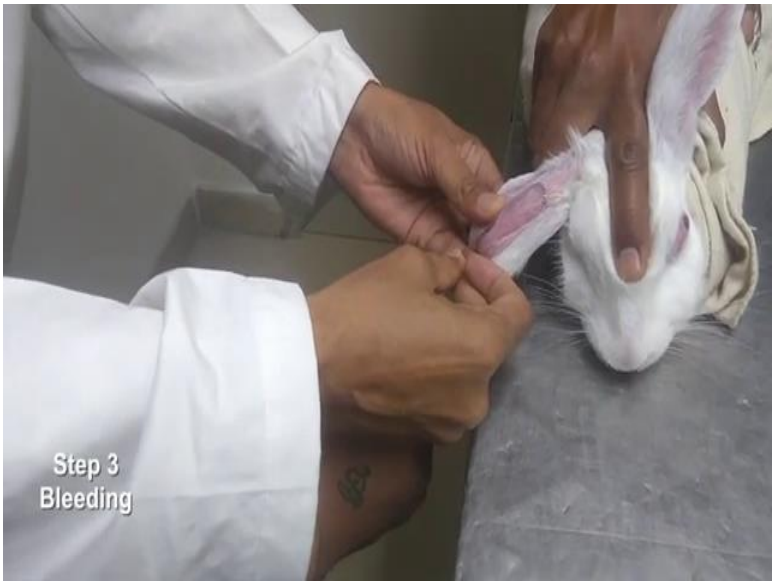
Step 3
Bleeding


Step 3
Bleeding

To strength animal in a towel or this kind of cloth. So, the advantage of having this cloth to strength, if the animal has its claw inside outside of this cloth and then it cannot moved. So, we have to restrict the moment in similar in sterilized on this cloth so (()) (53:33). Now, we can strain the animal in this cloth, to keep the animal relaxed on this cloth administry.

Make sure that the ear are outside and the animal is staying properly, so as to reduce its movement. And now, it is ready to bleed. We will bleed the animal from this mid ear vein, and we rub it so that it gets heated up and the circulation is faster. (())(54:36) also expand and more and more flow will be there. This is the method, this is normally we apply. When the vein is properly visible, this is the mid ear vein from which we bleed.

We will slightly sterilize it using alcohol and using a 20 gauge needle which is wide enough to give sufficient bleed. We will pick the vein and collect the bleed, then we need to stop it we just less and less pain you can collect the bleed like this. Now, we have to make sure that no further bleeding occurs and we will wipe out whatever bleed is outside using steroid water, we wipe out outer blood here and then if (())(56:44). And we wipe out all over using water so that the vein becomes cool and gets shrunk. We will just check, still it is bleeding so keep it pushed until the bleeding stops.

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Now, I think the blood has stopped coming out and then now we apply some antibiotic. Here, in this case it is Butadiene powder so that there is no further infections or inflammation in the rabbit and this also ensures that there is, this is your (())(57:59) and if there is an

inflammation it will have something, so it will avoid that kind of (())(58:07). We have the isolated approximately 10 to 12 ml of blood.

This will give us approximately half of the blood serum. And this will be coagulated, the blood will be coagulated in fact the serum is integrate for (()) (58:26) for butadiene. So, the clog is shrunken properly and the serum is taken out and then so it is serum and it is in preservative like (()) (58:38) and keep it as minus 20 or minus 18 for the fragment and we can also test simultaneously the title of it and testing the using (()) (58:53) of test and do the symbol of test specifically.

Now, we will see this animal is relaxed, it has, I think it has previous thing of a metrological blade thing and see this is very important (()) (59:11) later the step you go through is a animal, animal should be ensured not to have a pain but you can (()) (59:19) internal procedure is pain for that is how it is painful procedure you can decide it since this procedure is not painful it had now preferring is the so this is the very important step to ensure that animal has (())(59:33), it should be relaxed. So, in whole of the process, I think you have got to know all the steps of and you are ready to pre utilized it and where we prepare medicine we injected the emulsion, we isolated the blood after giving sufficient booster doses and handful of the process this environment. I think you have used most of these processes and you like it.

And this demo we have discussed about how you can be able to mix the prepare that antigen for the injections, how you can be able to prepare the injections, how you can be able to bleed the animals for producing for preparing the serum and so on. So, with this detailed explanation about the each and every steps I am sure you could have understood the all the processes. So, with this I would like to conclude my lecture here. In our subsequent lecture we are going to discuss some more aspects related to the immune system. Thank you.