Experimental Biotechnology
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Module-VIII
Cell Biology Techniques (Part-I)
Lecture-32
Cell Culture Medium

(Video Starts: 00:23) (Video Ends: 00:59) Hello everybody, this is Dr. Michelle Trivedi from department of biosciences and bioengineering IIT Guwahati, and today we are going to start our new topic and that new topic is on the cell biology. So, when we talk about the cells, we are actually going to talk about the different types of cells, whether it is prokaryotic cell or the eukaryotic cell. The first question comes, how, and different types of cells are present in the world and how we can be able to utilize them for answering some of the questions, or scientific problems.

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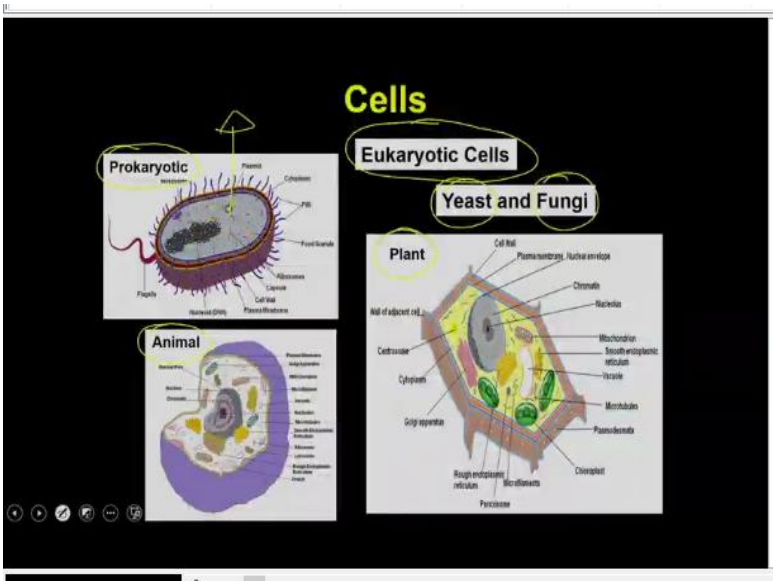

So, you have the 2 different types of cells like prokaryotic cells, such as the bacteria or eukaryotic cells within the eukaryotic cell, you have the animal cells, you have the plant cells, you have the yeast, and you have the fungi and all these cells are having the different types of applications as far as the scientific experiments are concerned, but the one of the major issue is that all these cells are required to be replicate or required to propagate before they can be used for scientific experiments. So, how and what are the different steps some can be used to propagate, and multiply the cells.

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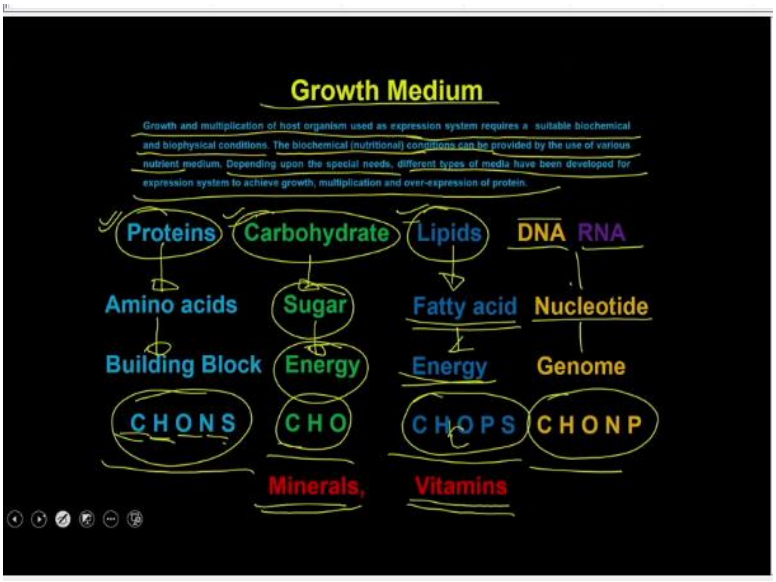

So, the growth media, what you are actually can be used to propagate whether it is a prokaryotic cell, or the eukaryotic cell is different, but the basic requirement of these different types of cells are same. So, the growth media is required for the growth and multiplication of the organisms for performing a biochemical as well as the biophysical under a suitable biochemical and biophysical conditions.

What is mean by the biochemical means that the nutritional conditions such as provided by the use of various nutrient medium, depending on the special needs the different types of media have been developed for the expression system to achieve the growth multiplication and over-expression of the proteins. For example, if an organism is need to be grow and multiply in number.

It required the proteins, which are actually been supplied or which are actually been formed by the amino acids and these amino acids are considered to be the building block which means it requires the proteins to build the new. It requires a protein for building the new organelles or new structures. Similarly, it requires the carbohydrates, the classical example of the carbohydrate is the sugar.

And the sugar is the source of the energy. So, it requires this carbohydrate for driving the energy, this energy it required for running its metabolic reactions or some other cellular processes. Similarly, it requires the lipids that lipids are actually been present in the form of the fatty acid and the fatty acids are also been required for providing the energy. And then it also requires the DNA and RNA which are made up of nucleotides.

And these nucleotides are being responsible for either forming the genome, or it responsible for the messenger RNA, what you see at the end, is that irrespective of whether it is requiring the protein, carbohydrate, lipids, DNA or RNA and their constituents are different, but for synthesizing these molecules, it requires the building blocks. For example, in the case of protein require carbohydrate.

It requires the carbon, hydrogen, oxygen, nitrogen and sulfur. So, if you provide a organism, a source, through which it can actually be able to withdraw the carbon, hydrogen, nitrogen, oxygen, and sulfur, it could be able to utilize those sources and could be able to synthesize the proteins with the help of by synthesizing the different types of amino acids. Similarly, it requires the carbon, hydrogen, and oxygen to synthesize the sugar.

And similarly you can see that for the lipids, it requires a carbon, hydrogen, oxygen, phosphorus and sulfur, and it also requires the carbon, hydrogen, and oxygen, nitrogen and phosphorus. Apart from these it also requires the minerals such as the different types of minerals, and also as well as the vitamins to run its metabolic reaction. Because all these minerals as well as the vitamins means are part of some of the enzymes, which are required to run the metabolic reactions like glycolysis, Krebs cycle and electron transport chain **to** not only to produce the energy.

But also to produce different types of metabolites and all these metabolites are going to be utilized in different types of reactions. So, that it will be able to synthesize the new compound and these new compounds are going to be used for different types of purposes, such as building the new building blocks, or it is going to be used for defense x purposes or it is also been used in synthesizing the protein which are going to take part in multiplying the organism from one organism to another organism. So, considering all these requirements, so people have developed different types of growth media for different types of organisms. So, let us discuss about all that.

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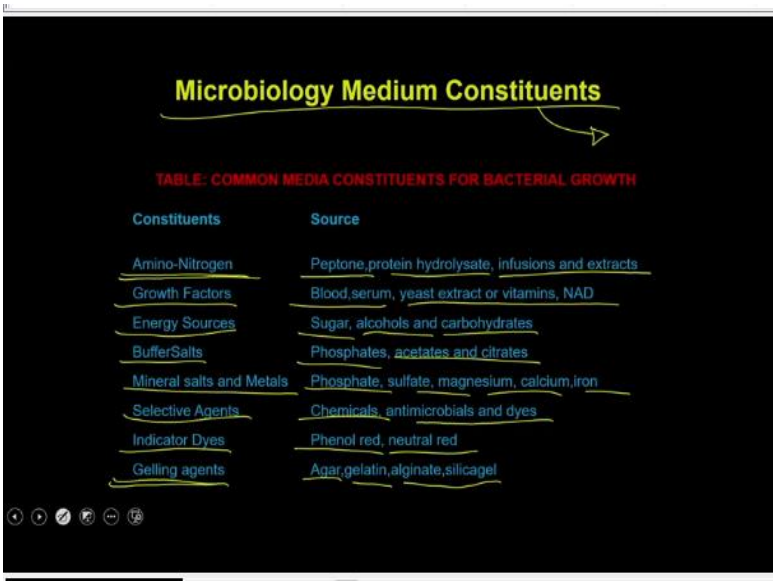

So, regarding the different types of microbiology media like microbiology media means the media which is required to run the propagation of the prokaryotic cells, what you need is the amino acid, so amino acid nitrogen, that you will get from the peptones or protein hydrolysates, or infusions and extracts, then you require the growth factors which is acquired from the blood, serum and yeast extracts.

Then you require energy source like carbon, so it require alcohol and carbohydrates, then you require the buffer because if you prepare a media its pH also should be important. That is why you require the phosphates, acetates and citrates. Then you require the minerals and metals. So, phosphate, sulfate, magnesium and calcium and then you require the selective agents like chemicals, anti microbials and dye.

Then you require some time the indicator dyes, so that you will be able to monitor the change in the pH of that particular microbiology media. So, in that case, you add the phenol red as well as the neutral red, and if you want to make the solid media, then you also have to add the gelling agents. So, gelling agent could be a agar, gelatin, or the silica gel and based keeping all these into permutation combinations. So, people have developed the different types of microbiology media.

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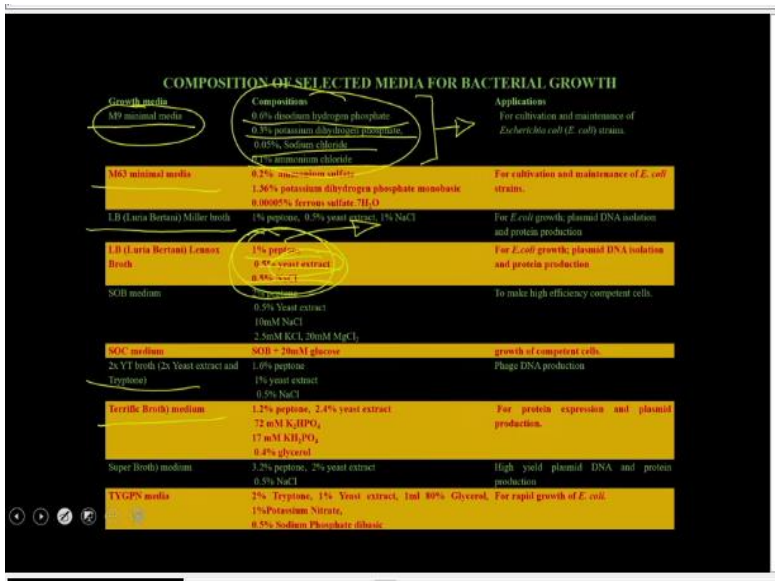

So, these are like defined media like M9 media or M63 media, or they are the composite media or the more complicated media like the LB media or the TB media or YT media. The difference between the defined media versus the complicated media is that the defined media has a defined composition in terms of the, what is the level of hydrogen phosphate, potassium phosphate and sodium chloride or ammonium chloride.

Compared to that, if you see the LB media, it actually contains the peptone yeast extract and NaCl, which actually means that you cannot control the composition of that particular media because you do not know what are the components are present in that peptone, what are the components are present in the yeast extract and so on. So that is why in a defined media you use only when you are actually would like to optimize some process.

Because the one of the major issue with the define media is that it is not very rich media, it requires. It is very you know it only contains the buffers and salt and sugar source. So, it only provides the constituents atoms or constituent salts, which actually the organism has to process to synthesize, whereas in this case, you are actually providing the metabolites. So, they are the organism is going to take up these metabolites and then actually can bypass multiple pathways to synthesize the molecules in a very quickly way.

Apart from that these medias are very, very no defined media versus the rich media is also being used in a controlled way. So, that you will be able to ask the different questions. For example, defined media can be used if you want to understand the uptake of different types of sugars sources. So, that cannot be done with the rich media.

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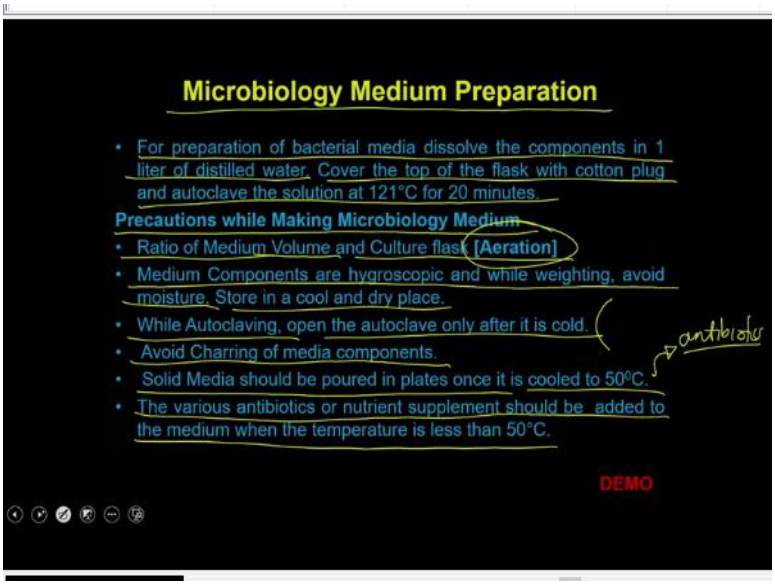

So, how to prepare a microbiology media for preparation of the microbiology media dissolved the component in a 1 liter of distilled water, covered the top of the class with the cotton plug, and then you autoclave the solution at 121 degrees Celsius for 20 minutes, you can take the precautions while making the microbiology media. What you can do is you have to maintain the ratio of media volume and the culture flask, which is actually required for the aviation.

Because every organism when it grows, it requires a certain amount of oxygen for its respiration. So, for that you have to maintain a certain proportion, or certain. So, the flask in which you are going to prepare the media has to be in proportion to the amount of media what you are going to prepare. Then the media components are hygroscopic and while weighing you avoid the moisture, the store in a cool and dry place.

Similarly, while autoclaving open the autoclave only when it is cold because if it is not cold, then it is actually going to have the hot water as well as the hot vapours. And that actually can cause the burn injuries. Similarly you can have the charring of the media components so you should not heat too much or you should not do too much autoclaving. Solid media should be poured in place once it is cooled down to 50 degrees.

That is important if you are actually adding the antibiotic. So, if your media is containing the antibiotics, then you should let the solid media to be cooled down enough so that when you add the antibiotics, the antibiotic should not be get inactivated, the various antibiotics or

nutrient supplements should be added to the media when the temperature is less than 50 degrees Celsius.

So, this is all about the theoretical way of making the microbiology media for the purpose of giving you a virtual experience. **(Video Starts: 12:12)** I would like to take you into my lab, and this clip is being prepared in our laboratory to demonstrate you how to prepare a microbiology media. And this clip is been prepared by the one of my student whose name is the Suram Banish.

And what you will see is that the Banish has explained to you the many aspects of preparing the microbiology media, and as well as what are the different precaution you should take. In this video, we are demonstrating how to prepare bacterial culture broth, preparing culture broth we need 3 components, one is the peptone, yeast extract, and sodium chloride. For 100 ml of culture broth we need 1 gram of peptone, 0.5 grams of yeast extract and 1 gram of sodium chloride.

I am going to weigh individual components and dissolved it with double distilled water. Then we have to autoclave the media. Before weighing care should be taken, spatula is clean and the balance is taken. After weighing we have to clean the spatula and keep it in original position. And during weighing care should be taken to avoid contact with the any of this media company.

After weighing the media components we have to dissolve them in double distilled water. So, initially we are dissolving in 80 ml of distilled water. Once the components completely dissolve we have to make up the volume up to 100 ml. While it is stirring we have to prepare cotton plugs for the flask, for preparing cotton plugs we have to take 1 thick layer of sheet up cotton with your 2 hands hold like this must the media dissolution is complete we have to pour into the flask.

We have to pour up 100 ml so we use only one third of the less remaining space is empty. This is used for aeration purpose and also ensure proper autoclaving. In order to check whether the components are autoclaved or not, the media is autoclaved or not will use sterility indicator. This is paper is sterility indicator. We have to paste on to the flask. And we

have to autoclave. If the autoclave is properly finished then we will see the white strips, coming into the black one.

So, this is the indication of autoclave process is complete. Now the media components are completely dissolved. Now we have to pour into the flask plug the mouth with cotton plug and wrap this aluminium coil, tie it with rubber band. Now this is ready for autoclave. Once the media preparation is completed we have to stabilize the media in order to use further applications.

This is a typical autoclave where you can see temperature and pressure indicator. And these are the pressure knobs. And this one is quick pressure release knob, you can use when you, are in a hurry you have to use this one, but I would prefer not to use this one, let it go and explain. We have to turn on the autoclave. So, you can see here the bulb is glowing, before keeping the media components to autoclave.

Make sure that the heater inside the autoclave submerged with water. Now, I am going to keep the media components in the basket, which we use for the autoclaving. Keep this one inside the autoclave. While closing the autoclave make sure that you are closing in opposite direction once the pressure and temperature reaches 121 degrees Celsius and 15 lb pressure. You have to hold on that for 20 minutes.

Then you have to turn off the machine let it cool down and remove the components. The same procedure you have to while opening, you have to open in a opposite direction. To conclude the video demonstration, we have discussed how to prepare bacterial culture media and how to prepare cotton plugs and autoclave it. During culture weighing the media we have to make sure that the media component should not be exposed to air.

Because those substances observed in moisture, and become liquefy. So, another thing is that for cotton plug preparation we have to take a single layer of cotton, then we have to fold it. And after autoclaving we should not release pressure in a single short. Let it go and come to normal pressure, then we have to open autoclave. **(Video Ends: 21:29)** With this demo the student might have explained you all the steps in detail. So let us move on to the next topic.

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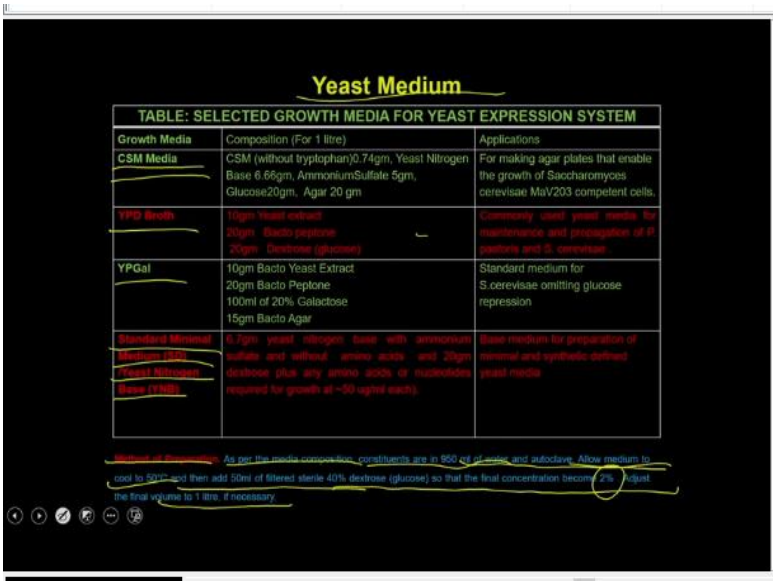

So, next is the yeast. So, the yeast is the yeast media. So, these are the different yeast media what you can use, and all yeast media are actually also having the similar kind of thing that you have required, how to prepare the yeast media as per the media composition you can take those constituents, you can dissolve them into a 950 ml of water. Then you autoclave, allow the media to cool down to 50 degree.

And then you add the 50 ml of filters trial 40% dextrose. So, that the final concentration will become the 2%, you adjust the final volume to 1 liter, and since you are adding the reactions under the aseptic conditions there is no need to do another round of autoclaving or other kind of sterilizations. Why we are doing the 2 step addition because the sugar is very sensitive for getting the deactivated or denatured while if you are doing it for the autoclaving.

That is why you have to prepare the media, you have to filter style the sugar solutions. And then, as per the requirement you add the sugar into the media, after the media is cooled down to 50 degrees Celsius.

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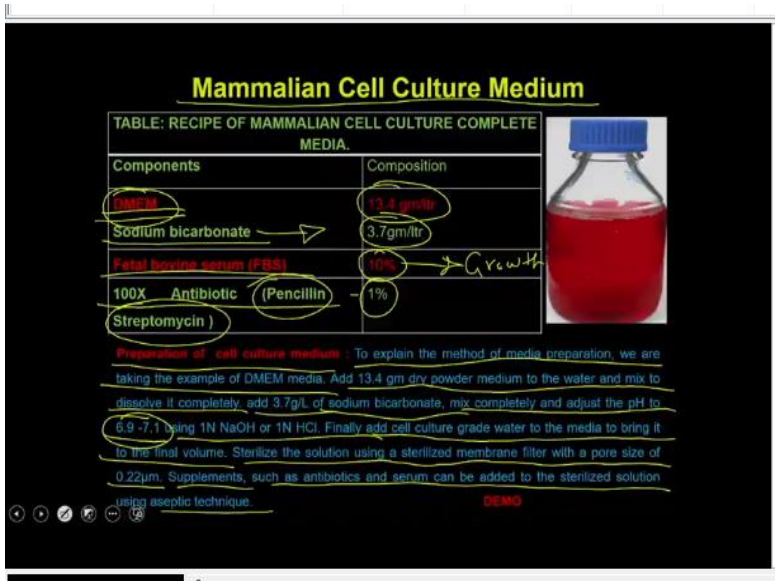

After this also we are going to discuss the mammalian cell culture media. So, the mammalian cell culture media requires the different types of components like you for this one we are discussing about the DMEM media. So, DMEM media is DMEM powder what you require is 13.4 grams, then you require the sodium bicarbonate 3.7 grams. Sodium bicarbonate is required to maintain the pH.

And to provide some buffering capacity, then you require the fetal bovine serum which is actually at the 10%, and that is required because you want to provide the growth factors. And then you required have to add the antibiotic cocktail, which is at the 1% this is just to inhibit the bacterial as well as the fungal growth. So, that there will be no growth of the bacterial system into this.

And for preparation of the cell culture media what you have to do is you have to first prepare. We are taking the example of DMEM, add 13.4 gram dry powder media into the water and mix it to dissolve it completely then you add the 3.7 grams of sodium bicarbonate, mix completely and adjust the pH to 6.9 to 7.1 using the 1 normal NaOH or KCL. Finally you add the cell culture grade water to the media to bring it to the final volume.

It sterilize the solution using a sterilized membrane filter with a pore size of 0.22 micron. Supplements such as antibiotic and serum can be added to the sterilize media using the aseptic conditions. So, this is the theoretical way of explaining you the media preparations, what the precautions you have to take and how to prepare the media under the aseptic conditions. **(Video Starts: 24:42)**

I would like to take you to my lab to explain you the precaution as well as the crucial steps what you supposed to do and what are the common mistakes, what people are doing when they are preparing the mammalian cell culture media. In this video we are going to demonstrate how to prepare cell culture media from mammalian cell, For preparing cell culture media there is a step by step process.

First we need to weigh the components of the media, and dissolve it in required amount of water, then we need to set the pH using ph strip. And then we need to filter the media using 0.22 micron filter to make it aseptic, for further use we can also allocate the media and store it in 4 degree centigrade. So, let us get the video started. In this video we will be demonstrating how to prepare mammalian cell culture media.

For that purpose we need cell media, which is DMEM Dulbecco's Modified Eagle's medium (()) (25:43), and we need FBS fetal bovine serum. And we need antibiotics cocci comprised of streptomycin and antistreptomycvin. The basal media provides inorganic materials, amino acids, which are required for basic development of cells, and the FBS is used for providing both factors to the cell.

We cannot autoclave this media because it might degrade the components of the media, for that purpose we use 0.22 micron filters. This is 250 ml bottle top filter. Now I will be demonstrating how to prepare filters from the beaker. We have to pack it closely so that it does not allow any leakage. And after this we have to put it for autoclave. This is a autoclave bottle for filter.

After we pack the filter we have to keep it for autoclave. For that purpose we use indicator to check whether the filter has been autoclaved or not. When the lines on the strip turns black, it means that the filter has been autoclaved okay, 1 second, 1, 2, 3 go. In order to prepare media now we will be adding basal media to the already autoclaved double distilled water. We can use double distilled water or milliQ water for that purpose.

After adding media we need to stir it on a magnetic stirrer for the components to dissolve completely. We can either use double distilled water or milliQ water, but double distilled water is more preferable as it contains more ions than milliQ water. After the media

components have dissolved completely, we need to set the pH of the media. For that purpose either we can use pH meter or pH strips.

In this case they cannot use pH meter. As the bulb of the pH is sensitive the media components and may get corroded. After the media components has dissolved completely this will be able to set the pH of the media. After the components of media can be dissolved completely we now need to adjust the pH of the media. The bright red color indicates that the condition that media is in the range of 7.2 to 7.4.

If the color of the media turns purple, then it indicates that the media is basic. If the color of the media turns yellow and it indicates that the media has become acidic. Now checking whether our media falls in the range of 7.2 to 7.4. After the media has been set we now need to filter the media inside the biosafety cabinet, as we have added the constraints in the non aseptic condition.

After the media components has been completely dissolved and the pH has been set we now need to sterilize the media using membrane filter media. For that purpose we use glass to biosafety cabinets, which are used for handling mammalian cell cultures. So, this is the typical biosafety cabinets in which we perform the filtration for media, this is the control panel which is used to operate this machine, this is the on and off switch.

This is the switch for normal light. This is the switch for UV light. Now I have been demonstrating how to filter the media. Now, we are going to filter the media. For that purpose we need a suction pump, which can be connected in the bottle top filter, this suction pump is for the purpose of extracting the air from the bottom top filter. So, that we can filter the media. Initially we need to check the media, we need to check the bottle top filter with less media to check if there is any leak or not.

For that purpose we are going to add around 50 to 100 ml of media. As we can see that there is no leakage in the filter, we can proceed with the filtration, After the media has been filtered we now need to add a BS and antibody in order to make it complete media. The complete media comprises of serum, whereas the incomplete media does not contain serum. We are adding 100 ml of fetal bovine serum, in order to make it 10% BS containing serum.

With this we have prepared 1 ml of DMEM complete media, comprising of 10% FBS and 1% antibiotic solution. So, far we have seen how to prepare cell culture media from mammalian cells, although there are some precautions to be follow like when we are setting pH of the media we need to use pH strips, instead of using the pH meter. There are some combines in the media that can block on to the bulb of the pH meter and reduce its efficiency.

Secondly, when we use the media we need to all the media from 4 degree to 27 degree, but we need to find first room temperature and then to 27 degrees to avoid change in the pH of the media. And also if they are producing the media in larger quantities we have to allocate as per our requirements, and then use in order to avoid contamination and change in pH of the medium. **(Video Ends: 35:12)** So in the demo the students have explained to you how to prepare the media and what are the precautions you should take, and let us discuss those in detail.

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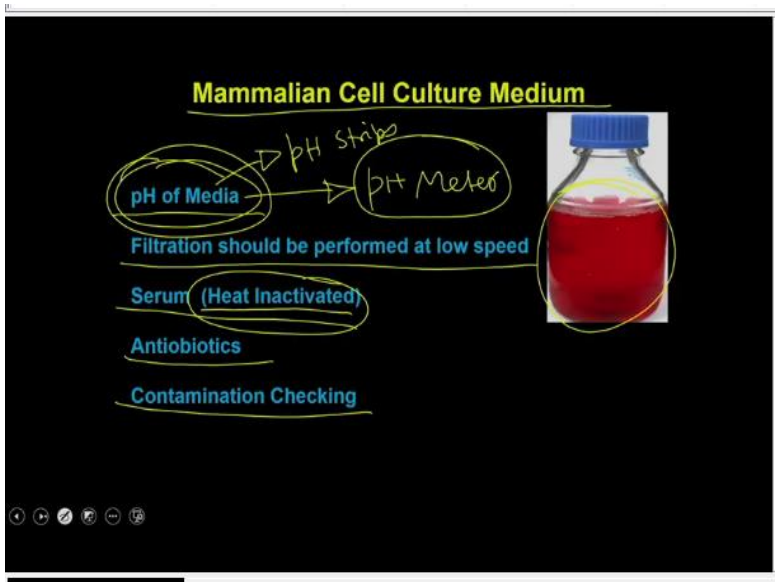

So, when you are preparing a mammalian cell culture media, the first thing what you have to be very, very cautious about is the pH of the prepared media or the filtered media. So, the filter media, the pH you should not never use the pH meter because if you use a pH meter, it is actually going to destroy the probe of the pH meter. Instead, you can use the pH strips, because a pH strips allows you to measure the pH.

And you should always maintain the pH after it has been filtered. So, that you just verify that the pH what you require is it the pH of the cell culture media, the filtration what you are going to do is you have to do it at a very low speed. So, that you should not take up the air

because if you do it very high speed filtration, that actually compromises the pore size of the membrane filter what you are using.

And that actually is going to add up the bacteria and other kind of infectious organism or the opportunistic organism and then ultimately the media is going to be contaminated. Then you have to use a serum which is heat inactivated. So, the serum is a liquid part what is present in the blood, which means if you coagulate the blood, you are going to get the serum, but serum is still contains many type of immunologically active agents such as the complements.

So, these serum is only been required for providing the growth factors, not for the compliment because if you add the complement the complement are going to destroy the cells and they were not allowed the cells to propagate. So, you have to remove the complements and for removing the complement, you have to either buy the serum which is heated activated, or you buy the serum which is not inactivated then you can do a heat inactivation simply by heating the serum at 60 degree for half an hour.

And then you will see that there is a precipitate being formed into the serum, and then you just filter that serum with a 0.2 micron and that is going to remove the precipitate, and that theorem, you can use for preparing the cell culture media. Antibiotics also you have to add, and then before you start using the serum, or the media for your cell culture purposes, you always have to check that it is free of contaminations.

How to check that is that you take out a 10 ml or 20 ml of your media, keep it in a petri dish, without the cells and keep it in a 37 incubator for 2 days. If you do so, if there will be a contamination like bacteria or fungi, it is actually start growing and that actually is going to give you the idea whether the cell culture media what you have prepared is free of contamination or not.

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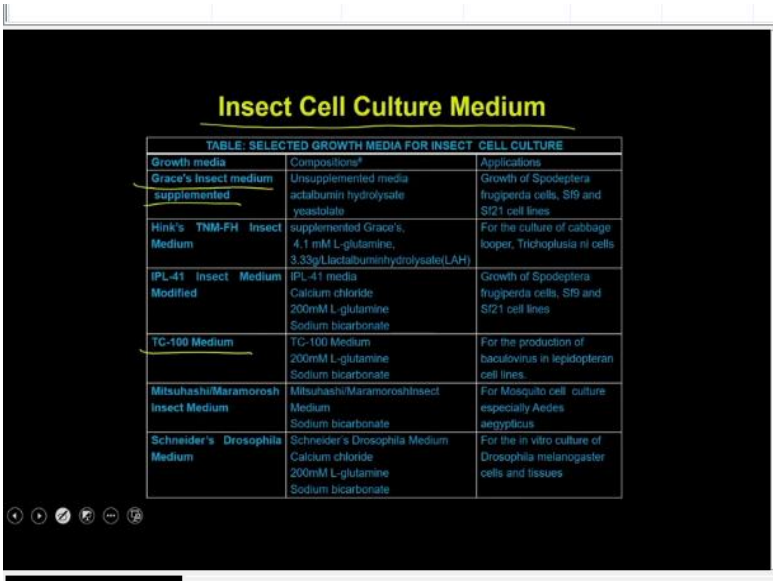

So, this is the another thing is thus insect cell culture media because the mammalian cell culture media is only for the mammalian cells, but it is not suitable for the insect cell that say the people have also designed the different types of insect cell culture media. So, insect cells are still different, and they require the requirement for the nutrition is also different. So what you have is the different types of the insect cell culture media and the preparation is you can prepare some of these medias to propagate the insect cell lines and that can be used for your experimental purposes.

So, with this, I would like to conclude my lecture here and in this video, or in this lecture we have discussed about the different types of cell culture media as well as the microbiology media which you can use for propagating the different types of host cells or different types of cells for using them into the experiments. So, with this I would like to conclude my lecture here. Thank you.