Electronic Systems for Cancer Diagnosis
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Lecture – 04
Cleanroom Equipments

Hi, Welcome to this particular module, and this is the last module for this particular lecture which is on Tissue and Cell Culture Techniques. So, in the last lecture or series two lectures actually or two modules I should say we have seen what is a cell, cell theory, incubator, biosafety hood, right and how to use the biosafety hood from the video that I have shown you as a part of the module.

Let us see today the few more equipment and chemicals that are required in this particular technique ok.

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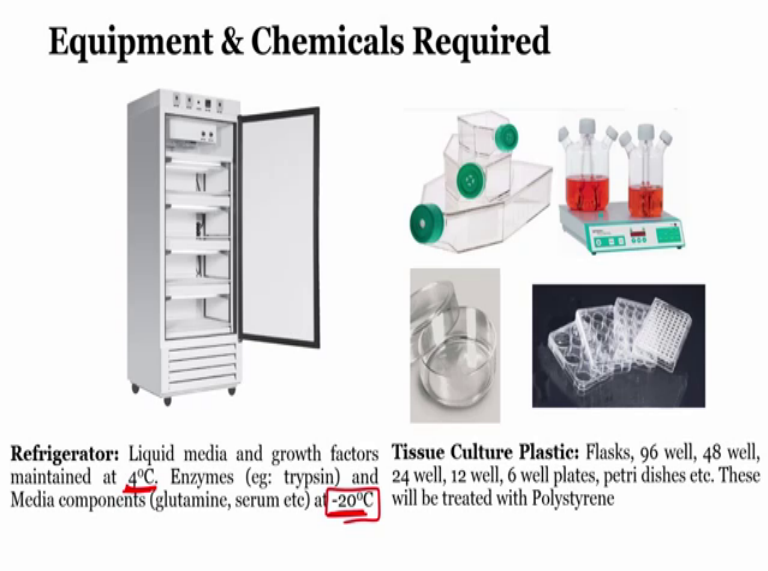

So if you see the slide, what I am showing you is a refrigerator, a refrigerator is not a general refrigerator that you can see at the home, but it is for maintaining the cells at 4 degree centigrade and as well as media, liquid media, growth factors, enzymes such as trypsin . But enzymes such as trypsin and media components like glutamine serum etcetera yeah we can use minus 20 degrees centigrade, right.

So, the another point is, not all the laboratories would have enough funds to maintain in this particular fashion. In that a norm normal refrigerator you can use to store, the freezer you can use to store the enzyme and glutamine; of course, you should make sure that there is no food along with the refrigerator. But as a alternative technique if you do not have a exclusive refrigerator for maintaining the cells you can use a normal refrigerator, freezer to keep yourselves and the media alive and in a proper shape. But it is always advised to use the refrigerator that is meant for cells and tissue, and for the media.

Make sure that you do not have food along with yourselves, not have any kind of other stuff along with the in the refrigerator, it is extremely dangerous to mix two things together. Bacteria, like I said is extremely harmful; in any case have a general practice if it is a wet laboratory where there are any chemicals or biological components or anything to do with any chemicals you should not you should not eat any food or drink water within the laboratory, right.

Make a practice it is a good laboratory practices, it is a good you know it is wise in terms of how to work in a laboratory. So, if it is a dry laboratory where there are only systems yeah, right you may prefer to drink or eat, depending how the lab works and how your Pi allows you to work in the laboratory. But what I feel is in generally you should avoid eating in a laboratory environment ok.

In particular for sure not in a wet laboratory all right. So, having said that there are several tissue culture plastics, as you can see here flasks, 96 well plates, right over here, 96 well plates, 48 well plates, 24, 12, 6 well plates and a petri dishes and there these will be treated will polystyrene. These are all treated with polystyrene, ok.

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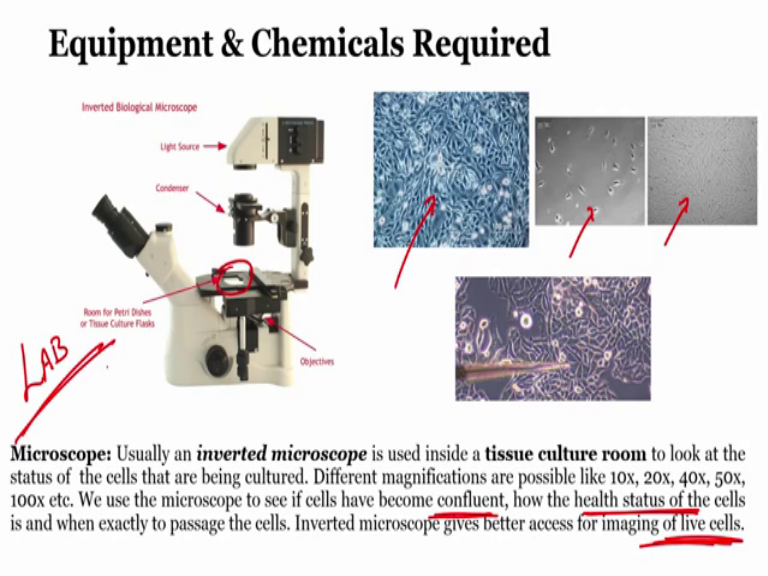

Let us see the another equipment and that is extremely important equipment which is our inverted microscope ok; it is called inverted biological microscope alright. So, what the microscope will consist of microscope, microscope consists of a light source, condenser, objectives you can change the objectives, and room for petri dishes like here right, or tissue cultured flasks ok.

Then, usually inverted microscope is used inside a tissue culture room to look at the status of the cells that are being cultured. Right, you can see here; the status of the cells you can see here, and you can see here. If the cells are confluent whether it has rich confluency we can use inverted microscope to understand that.

Different magnifications are possible from 10 x to 100 x. We use the microscope to see the cells have become confluent right, how the health status of the cells is and when exactly the passage these cells when exactly do passage the cells for the next cycle. Inverted microscope is a better access for imaging of live cells, alright. So, we have a inverted microscope in a laboratory.

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What is the next equipment? Next equipment is our autoclave we will see inverted microscope as a part of the lab component in detail, ok. How to operate inverted microscope, how to load the slide, how can we see the cells everything we will see the part of the lab component. If you see autoclave; autoclave is used to sterilize the equipment, utensils, etcetera that are used for tissue culturing for both the use and safe disposal.

So, you see whenever we work with any kind of equipment, where we have used bacteria or cells we and once the experiments are over we had to sterilize this equipment even you want to discard it in a biosafety bag. So, if you really see it looks like you know ultra-modern pressure cooker know, so it is actually it is same because it operates at a high temperature like 120-140 degree centigrade. And you can see there is a pressure gauge, there is a regulating device safety valve, autoclave lid, and there is a autoclave body, there is a vacuum release and steam release valve, there is an outer stain; and this actually boils down the bacteria or kills the bacteria by boiling it up, right.

If you see I do not know how much of you guys know the how to purify the water or once upon a time in early 90's, not every place in our country, where was facilitated with a clean drinking water. I am sure still it is not, ok. But the how to clean the water when the water is muddy, but it is a drinking water. So, what people used to do is, they used to boil the water and when you boil the water then the germs within the water will die.

Same thing if I boil the if I place the utensils in a boiling water, right what will happen the germs would die, right that is called sterilization, you make a sterile kill the bacteria kill any contamination from the device or from the equipment or utensils that we used in the biology laboratory, right.

So, like I said it is like a high-tech pressure cooker, and it removes microorganisms; actually it kills it is not just remove it, it kills like virus, bacteria, fungus, spores etcetera using high pressure and high temperature steam sterilization.

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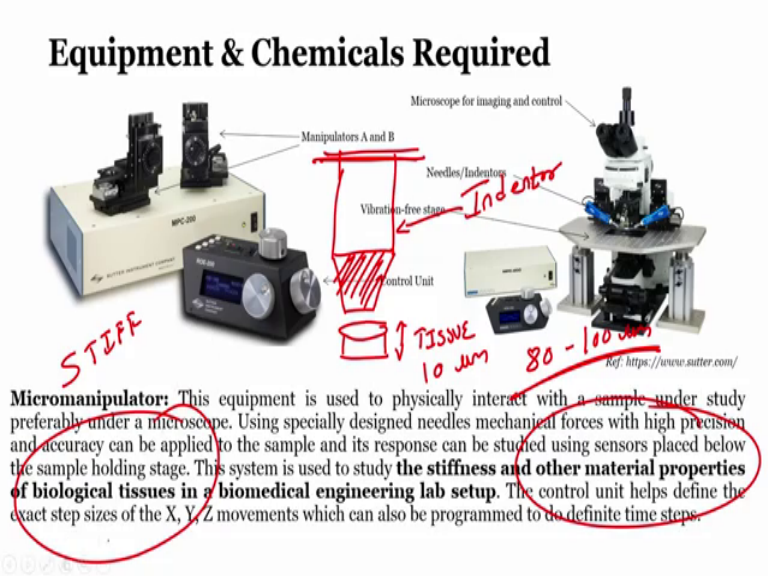

This is another equipment, very important and again you will see as a part of the lab component is the micro manipulator; micro manipulator. So, what exactly micro manipulator means? Micro manipulator is a equipment that is used to physically interact with the sample under study and preferably under the microscope. Using specially designed needles, mechanical forces with high precision and accuracy can be applied to sample and its response can be studied using sensors placed below the sample holding stage. The system is used to study the stiffness and the material properties of magical tissue, as well as it helps define the exact step sizes of X, Y, Z movements which can also be programmed to definite time steps.

Easy, no its confusing right. So, what does that mean, right micro manipulator is used so, let me give an example it becomes easier for you then, ok. I have a tissue here, right this is a tissue, I want to press this tissue, right. This is my indenter, it will indent will press,

right. Now, let us assume the issue is just 10 micrometer. Our hair size human hair size varies from 80 to 100 micrometers, they the thickness 80 to 100 micrometers. What is the tissue thickness? Only 10 micrometers; only 10 micrometers.

So, if I want to understand the stiffness of the tissue; how much stiff my tissue is if I want to know the stiffness of the tissue what will I do; I will indent the tissue, I will press the tissue and I had to indent the issue in by going down few microns in Z direction, right. So, in Z direction if I press the indenter and I have to go down few microns, I have to use an indenter. But how can I control few microns of you know pressing the tissue, few microns is possible with the help of a manipulator and that manipulator is my X Y Z manipulator it is also called micro manipulator, and that is why it is called micro manipulator ok. It can control the moment of my indenter in Z, in X or in Y right in terms of with a prison of few nanometers actually , but step size of your microns. We can and its really accurate.

So, you have to understand three different things it is your homework: resolution, accuracy, precision what is the difference, is resolution and accuracy same so, just go and understand these three terms and you will understand. So, that my point is, if I take this as a tissue, right and I have my it is the indenter if I want to press the tissue how can I press it with micron accuracy.

I can only press this with micron accuracy, when I have connected my indenter to a micro manipulator, that is how I am connecting my micro manipulator. Now another thing that is written here is that when you press the tissue you can place the tissue using sensor placed below the sample holding stage, where is the sensor here what I will do is if I so, let us understand one more term before we understand this thing.

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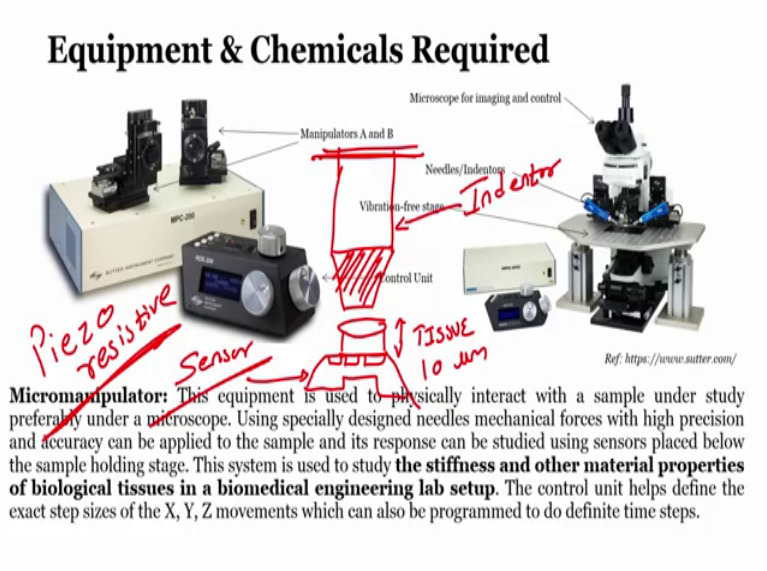

There is a term called piezo resistivity; what is called piezo- p i e z o resistive- r e s i s t i v e; piezo resistive when we apply a pressure there is change in resistance that is a piezo resistive.

So, suppose I have a sensor on which a, this is my sensor let us say right, this is my sensor on which I have placed the tissue, right. And now I am indenting the tissue I am pressing the issue with the help of indenter connected to a micro manipulator. When I press the tissue, what will happen the because of the stiffness of the tissue the force applied in the tip or the top of the tissue will be transmitted to the bottom of the tissue depending on the stiffness, right. It will not be same force, so whatever there is a change it that wherever apply a force there will be change in a resistance of the sensor; why because it is a piezo resistive sensor.

So, when the piezo resistive sensor is there when you apply a force that is engine resistance and that is engine resistance depending on how much force is transmitted from the indenter to the sensor and that depends on the stiffness of the tissue, you got it. So, for example, if my tissue is stiff and if I apply a force the force reaching to the sensor would be different compared to when my tissue is more elastic, right. So, the elasticity or a mechanical property of a tissue can be identified with the help of piezo resistive sensor on which there is a tissue placed and by pressing the piezo resistive sensor with the help of a indenter. And this you can do with micron accuracy with the help of micro

manipulator. That is a role of micro manipulator in a laboratory where we have to understand the tissue.

It is very important because, when you are when the cancer progresses, when the cancer progresses there is a change in the tissue property right, it is not just see the constituent of tissue changes, right. But what we have is all the code senate which is which is, right now used which are all biomarkers, HNE biomarker, progesterone biomarker, estrogen biomarkers, lot of biomarkers are there, right HER biomarkers. But, can we come up with an idea that if there is a change in the or if there is a progress in the cancer, right there is a change in the stage of the cancer the tissue property should change. And if we can use one example with a stiffness cancerous tissue would be more stiffer compared to the normal tissue depending on the stage of a tissue or vice versa, right.

So, if there is a change in the tissue property that is what my point is; if there is a change in the stiffness of the tissue property as the cancer progresses can we use the same platform micromanipulator with the indenter and a piezo resistor on which there is a tissue placed to understand the stiffness. So, if you understand stiffness, from the stiffness value we can say that this particular tissue from the patient belongs to this particular stage of cancer.

For example, if you were if I give you a very simple example if it is from normal region or benign or these from DCIS which is doctor carcinoma in situ or it is invasive carcinoma in situ, sorry it is invasive carcinoma so, are invasive ductal carcinoma I mean which is IDC so, it is like benign DCIS or IDC, then depending on the stage the tissue property should change. And that is what our idea is how can we measure this change in the tissue stiffness with the help of a given sensor, right. And can we develop a system around it, that we will see as a part of this course alright.

So, this is a micro manipulator that we have seen. Let us go to the next slide.

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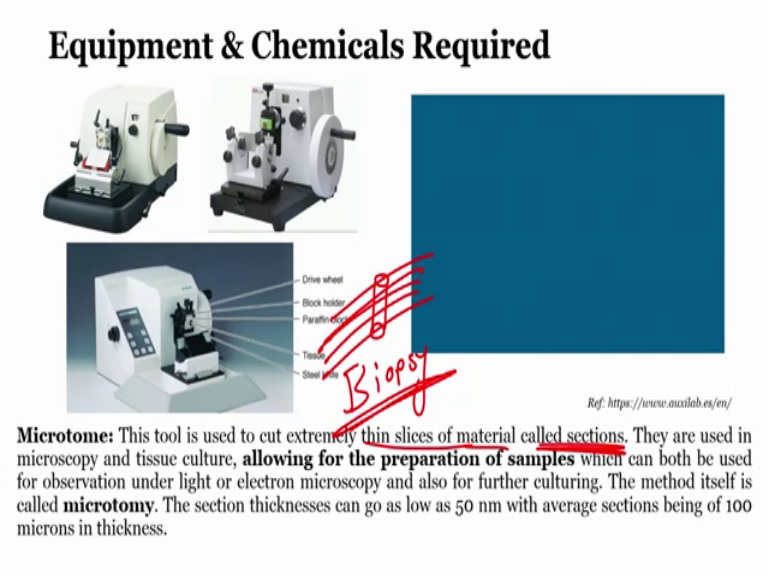

The next slide is microtome and that this tool is used to cut extremely thin slices of material called sections. They are used in microscopy and tissue culture allowing for the preparation of samples which can both be used for observation under light or electron microscopy.

So, if I take the tissue from the patient let us say using biopsy; biopsy- b i o p s y I am extracting the tissue from the patient using biopsy and then I have to slice the tissue. How can I slice the tissue? I can slice the tissue with the help of microtome. You see create thin slices of material, right you see here called sections. They are used in microscope and tissue culture arriving for the preparation sample which can be used for observation under light or electron microscope and further for culturing. The method itself is called microtomy and that is why we have microtome. The section thickness can go as low as 50 nanometer with every sections being of 100 microns in thickness. About 100 microns is the thickness average thickness when we slice the tissue and like it is written it can be used to understand the change in the properties of tissue under the light or electron microscope and it can also be used for further culturing the tissue.

So, if you in equipment looks as shown in the slide it is a drive wheel, it is a block holder, a paraffin block, a tissue, and a steel knife. So, when you want to store the tissue for a long time you can perifosine the tissue; paraffin block is there that is why it is written over here. And when the tissue comes to a laboratory if it is paraffin issue and

you want to deparaffinized it; so that means, you take out the tissue from the wax then you can use xylene- x y l e n e xylene ok. So, xylene is used to deeply refinze the para the tissue from the paraffin block. So, paraffin block is used to store the tissue for long time.

So, if I play this one then you will understand the details about how the microtome can used. Look at the video and let us see the next slide later.

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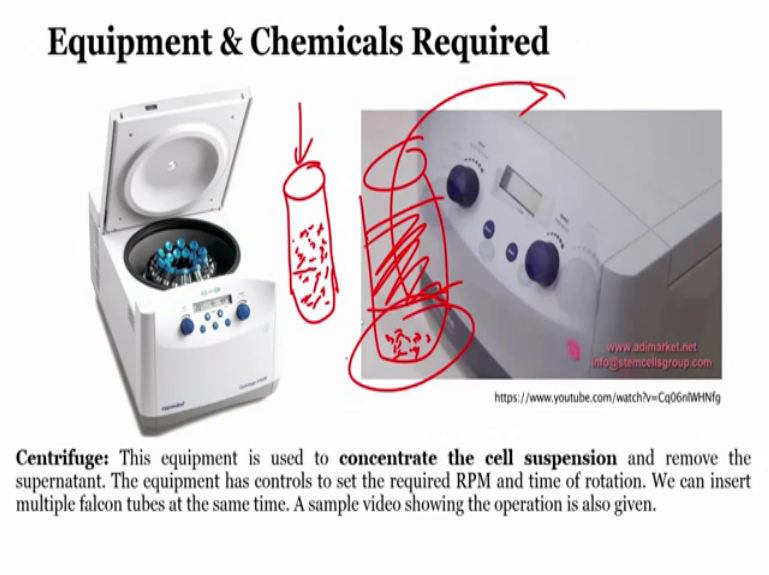

Now, let us go to the next set of equipment this is centrifuge, and the centrifuge is used to concentrate the cell suspension and remove the supernatant. So for example, if I have a tube in which I am placing the cells along with media right like this, now my idea is that I want cells to be only at the bottom of the tissue and remaining thing should be on the top here; cells should be at the bottom. What can I do? I can centrifuge this particular tube at high RPM, right just to separate or remove the supernatant.

Then this supernatant I just throw it out and I will have only cells left at the bottom of my tube. This thing we can do with the help of a system called centrifuge and the process is called centrifugation alright. So, the equipment is used to constantly the cell suspension and remove the supernatant, the equipment has controls to set the required RPM and time of rotation, depending how fast you rotate, how much time you take for rotation we can you can separate the suspension from the cells, we can insert multiple for falcon tubes these are called falcon tubes. And you can see there are array of , right over here. At the same time a sample video showing the operation is shown, right over here.

So, let us see how the centrifuge works right, in real time and then we continue the next slide.

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The executive series centrifuge is the newest addition to the elite product line of insight global biologics. And taking advantage of all aspects of superior separation, the executive series centrifuge comes fully equipped. A high performing machine with an enhanced look and smaller footprint is now here.

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When looking at the executive series centrifuge, you will first notice its large multifunction buttons and clear display. To the left is the time button where you will turn the dial to set the time and press start and stop centrifugation. To the, right you will find the speed button which also can be turned to set the RPM speed, and press the toggle between PRCF and RPM display.

These buttons are clear and easy to operate. The centrifuge comes with the control deceleration and soft breaking feature.

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This minimizes cell agitation or suspension during processing. Optimize separation is achieved without activation, providing the best achievable cell concentration for your procedure. To activate this feature press and hold the short button until you see the br of indicated one display. This machine has an automatic lock and release function for lid operation. To open the lid power up the machine and press the open button.

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You can manually open the load without ac power, by releasing the pull string at the base of the machine where you can pull to open. The lid automatically locks when closed.

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Looking inside you will find that the machine comes with the proprietary rotor in two customizable swinging buckets. These buckets are designed to swing horizontally and smoothly present to a full vertical position when completed. This prevents the buffy coat from re-suspending into the plasma and prevents the platelet slip phenomenon which depress the quality of the final product. With these new concepts and platelet and bone marrow separation the executive series centrifuge now makes its mark as it enhances the quality of these products. M cycles of the biologics remain committed to be a market leader of regenerative biologics, ok.

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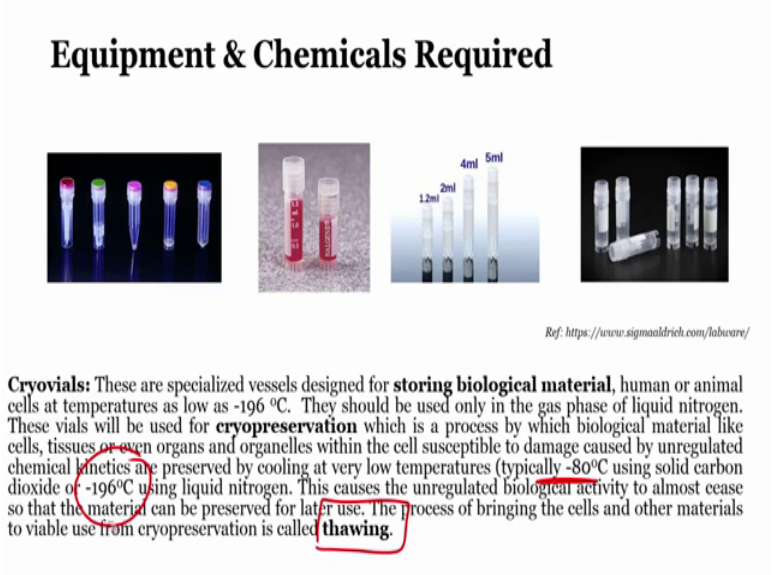

So now if you see this particular slide; what do you see here this slide shows the cryovials. Cryovials are this specialized vessel designed for storing biological material, human or animal cells a temperature as low as minus 196 degree centigrade. These are all cryovials exclusively made for storing the cells at extremely low temperature like cryopreservation, right. Now there are some cautions in or you can say the rules of how to use the cryovials.

So, first one is that you have to use only in the gas phase of liquid nitrogen. And this vials should be used for cryopreservation is a process by which the body material like cell tissues or even organs are organelles within the cell susceptible, to damage caused by unregulated chemical kinetics are preserved by cooling at very low temperature, right.

We have cryopreservation it is used to store these cells at low temperature. Thus, by preventing any kind of unregulated chemical kinetics.

Also we have to use solid carbon dioxide at minus 196 degree centigrade liquid nitrogen. So, because this causes the unregulated biological activity to almost cease right, why you have to use minus 196 degree centigrade liquid nitrogen or minus 80 degree solid carbon dioxide, because at this temperature the cell activity which is unregulated biological activity ceases down. And thus, we can use the or you can preserve the cell for a longer time.

The process of bringing the cells or reviewing the cells or reviving the cells is called the thawing; thawing- t h a w i n g. There we are bringing the cells back for our experiment from the cryopreservation; back to the laboratory environment and the process is called thawing cool. So, cryovials are used for what for preserving the tissue or preserving the cells, and what temperature it should be used it should be used at minus 80 degree if you are using solid carbon dioxide or minus 196 degree if you are using liquid nitrogen.

Now, cryopreservation is used for; why it is used because it ceases the chemical kinetics and preserves the cell, how can we revive it? We can revive it by the process called Thawing, right. So, this is what we have seen.

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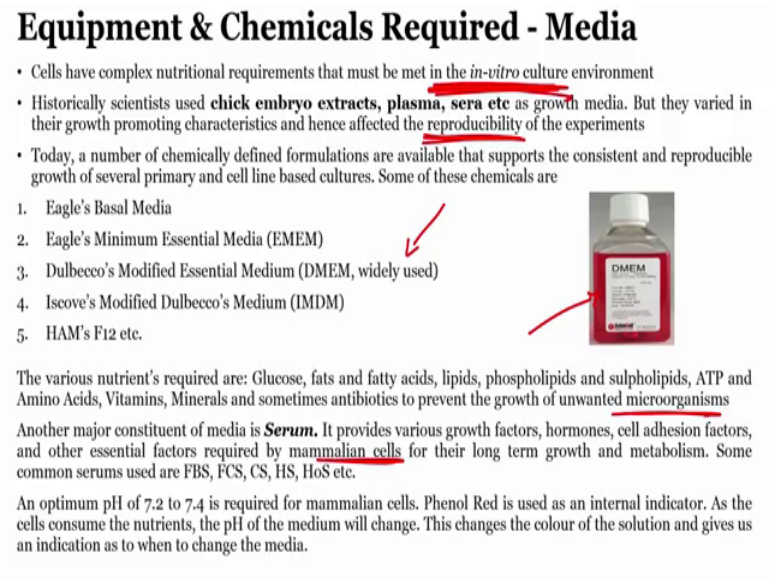

Now, let us see another very important nutritional requirement for the cells to be alive. And that is cells have a complex nutritional requirement that must be meet in the in-vitro culture environment. You see how we can keep the cells alive and we can also grow the cells in a laboratory environment. So for doing that, historically scientist used chick embryo extracts, plasma, sera etcetera as growth media. So, this is what is growth media, ok.

However, they varied in growth promoting characteristics and hence affected the reproducibility of the experiment; every time it was not reproducible. So, today a number of chemicals define formulations are available that supports the consistent and reproducible growth of several primary and cell lines based cultures. Some of the chemicals that are used in the laboratory are Eagle's Basal Media, Eagles Minimum minimal excuse me Eagle's Minimum Essential Media, which is called EMEM, we also use Dulbecco's Modified Essential Medium which is called DMEM which is extremely widely used, and then we have Iscove's Modified Dulbecco's Medium IMDM, and finally we have HAMs F12 etcetera, right.

But in most of laboratory will see the EMEM as a media which is used for preserving the cells or growing the cells as discussed earlier. The various nutrients required for the cells are glucose, fats fatty acid, lipids, phospholipid, and sulpholipids, ATP, and amino acids, vitamins, minerals, and sometimes antibiotics to prevent the growth of unwanted microorganisms. So, this is the combination of the media, another major constituent of media is serum. It provides various growth factors, hormones, cell addition factors in adults essential factors required for or required by the mammalian cells it is for the long-term growth and metabolism.

Some common serum used are FBS, FCS, CS, HS, and HoS. Now, what is the pH of the cell. The pH or optimum pH of the cell is from 7.2 to 7.4 and phenol red is used as an internal indicator. As cells consumes the nutrients the pH of the medium will change, when the pH of the medium is changes the color of the solution also changes giving indication that the it is time to change the media, right. So, when the constituents of the media is consumed by the cells then what happens; the color of the media changes and at that point we can see by looking at the color of the media it is time to change the media, got it.

So, this is the use of the media.

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Now, let us see what we have to show it to you or to offer you as a part of this particular laborate this particular course and this is the laboratory which we will show it to you, and we will show the details how the different things are used in the laboratory. As you can see, this is just a set of for developing electronic nose; E-nose; we will discuss sometime if time permits, right. And the idea is that can we understand the can we understand the disease from breath signature, and for that how can we develop different sensors. You can see here, we have a stereo microscope, we have a metallurgical microscope, then we also have a inverted microscope. So, this is an inverted microscope inverted- i n v e r t e d; inverted microscope, this is metallurgical- m e t t a l metallurgical sorry m e t a l t a t a l l u r g i c a l metallurgical microscope, this is sterio microscope s t e r i o; sterio microscope metallurgical microscope right, and we have inverted microscope. We will see each in detail when we see the lab laboratory as a part of laboratory class.

And then we have oven, we can you can see here, we have the autoclave, you can see here we have biosafety hood, you can see here we have to store different antibiotics, right over here, we have a co two incubator right over here. These are all positive pressure modules with HEPA filters HEPA- H E P A; HEPA stands for High Energy Particulate Air. High energy particulate air filters are used to create a controlled environment which is class 100 class 1000 class 10000 and so on.

So, there are different class cleanroom class; we will see the different cleanroom class in one of the lectures, right. And if you want to know more you can just go to my lab website and you can understand see what are the facilities that I have in my laboratory, or instead I should say we have as an institute as a department and as the institute for our students and for people who are interested to collaborate with the laboratory, ok.

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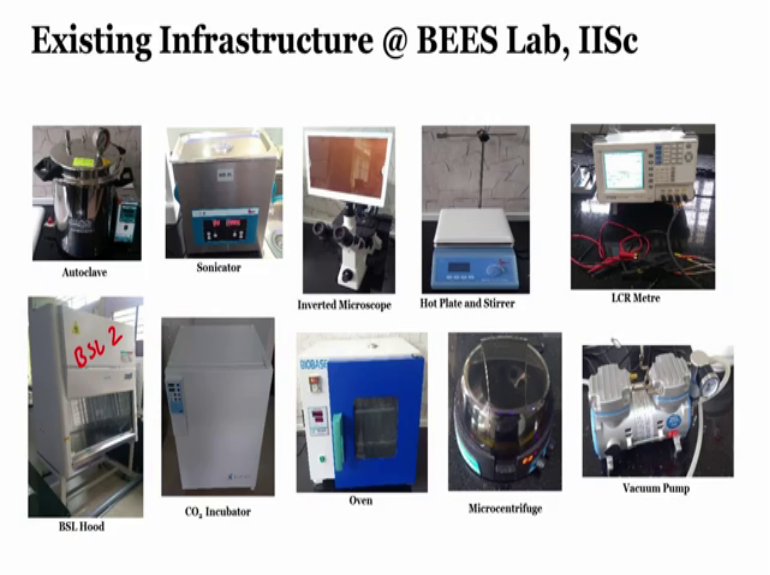

So having said that, if I go for another few of the things that we will discuss these are only few we have many I have just put few just to make you understand. We will discuss about autoclave that we have already seen, then we will go for sonicator; how to use sonicator. We will see inverted microscope, hot plate stirrer, LCR meter; we will see biosafety hood. This is biosafety hood two level two CO 2 incubator, oven, micro centrifuge, and we have vacuum pump.

These are like I said this very few of the equipment right over here, I will show it to you many more as a part of the lab.

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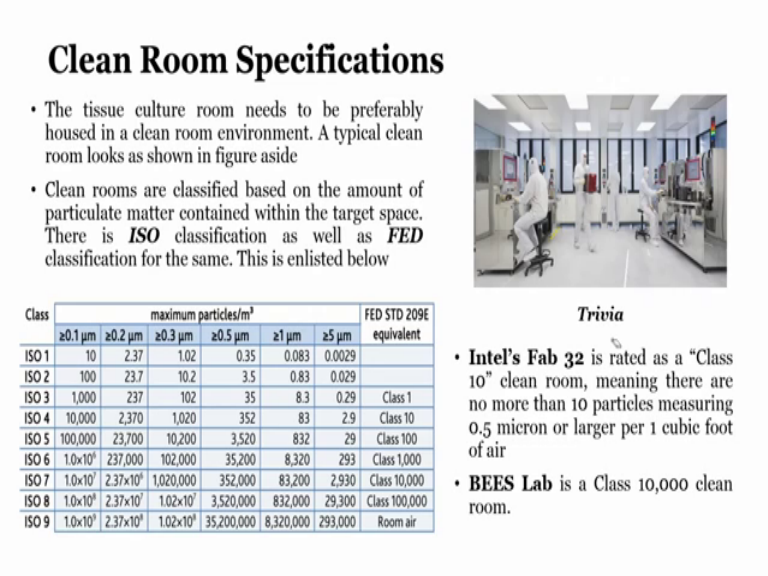

So, before so, this is the existing infrastructure, and in the end you can you can understand that; or let us let us conclude here because I think that the next class will be more focused on cleanroom technologies, and what are the cleanroom, and how the environment the cleanroom affects the fabrication of a device, and why we require a cleanroom, right. And we will discuss more on that, but as far as the cell and tissue culture is concerned and the application is concerned; an equipment used for maintaining the cell and tissue cultures are concerned this is the end of the particular this particular module and this particular lecture as well.

So, this lecture I have divided into three different modules, if you see all three modules you will have a basic idea of how the tissue and cell culture laboratory looks like, what are the equipment within the laboratory. And in next class let us see what we learn as a part of this particular course, which will be more on clean room, fabrication, and the design of electronic systems for cancer diagnosis, right.

Until then you take care, I will see you in the next class. Bye.