**Fabrication Techniques for Mems - based Sensors: Clinical Perspective**

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**Lecture – 46**
**Introduction to Equipments: Bio-safety Hood**

So, we are discussing about bio safety Cabinets. A bio safety cabinet has its origins in there is a within the research in microbiology. So, actually they are conventionally found in microbiology labs and their main intention or main aim of using bio safety cabinet is to protect the person who is working with in the cabinet from any contaminating pathogens.

So, as a name implies bio safety cabinet it is basically for safety of the person who is working with any kind of samples, biological samples from contamination and possible hazard from pathogens.

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So, if you see the equipment, we have an emblem here yellow color. So, this is this is usually the sign for biohazard. So, what this emblem means is that this equipment, it does not mean that this equipment is a bio hazard, what it means is that this equipment deals with things is used to deal with stuff that may potentially be a bio hazard.

So, it is warning people. Suppose, is a new person enters a lab. He does not know anything. He is just visiting the lab. He simply goes and he is he is a very inquisitive type let us say curious person. He goes ever and touches things, opens things and all, for such a person, this should serve as a warning that please be careful with this equipment and do not open or close it at your wingman fancy. So, they should be very careful. So, this emblem is for that.

Now, bio safety cabinet so, on similar lines of bio safety cabinet, you can also talk about another equipment called Fume hood. Fume hood as a name implies what does is suppose we are doing some experiments say let us say in generally if you say chemical biological experiments. While you do the experiment and while the samples in the experiment interact with each other, they will generate some chemicals, mostly in the vapor form.

So, these chemicals have to be taken out from the immediate surrounding. A fume hood we will do only that a fume hood what it does is it will take suck out these chemicals and then exposit off. A bio safety cabinet on the other hand, does much much more than that. As per US standard bio safety levels are classified into different types like class 1, class 2, class 3 etcetera and there are set guidelines for each of these bio safety levels.

As per each of these bio safety levels, there are also different types of bio safety cabinets also. So, this bio safety level or BSL, bio safety level by BSL and the classes are called 1 2 3. So, it is called BSL 1, BSL 2 BSL 3 like that BSL 1, BSL 2, BSL 3. So, this bio safety cabinet or bio safety hood is a BSL 2 equipment, bio safety level class 2 equipment.

What are these classes? I will get into it shortly. Now, one of the main things is to make sure that there are 2 things I mean there are 2 things to make sure; one is a person works on a sample; there are two things one is the person should not get affected by anything contagious or dangerous in the sample. Another thing is while the person works on it the sample which he works on must also not get contaminated or disturbed by the surrounding environment.

Depending on these 2 criterions also this bio safety class levels are defined. We will get into this shortly. We have understood the basics of a bio safety cabinets what are the

different types of containment facilities available. Now, let us look at actually what is this bio safety cabinet or bio safety hood all about.

So, if you can see this is the control part of this whole bio safety cabinet, we have powered it on. So, now, this is as I have told this is for a BSL 2 environment. So, there is enough levels of protection that needs to be taken care of and the precautions that need to be done, before we before we even start using it to be exact.

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So, there are few things that are provided on the bio safety cabinet that we need to do before we start using it. So, if you see the knobs, there are 1 2 3 4 5 knobs provided. Major ones are say if there is a UV knob at the middle, that is before we start using it or actually whenever we do not use it, we need to have the UV light on. So, if you see the UV light has come on, the color inside has changed. What does this UV light do?

Whenever we are not using the bio safety cabinet, UV light should be on so that, it will sterilized and kill any pathogenic growth or a bacterial growth that might happen inside the bio safety cabinet. So, that that is sterility all sterile environment is maintained sufficiently well throughout the inside the bio safety cabinet at all times whenever we are not using it. But as you all know, UV light we should not get exposed to UV light.

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So, this bio safety cabinet provides that protection also. Ideally what we should do is that when assignment, we start using it we should switch off the UV light. So, there is we should switch off the UV light and use it, but then suppose we forget about it and we start using it. To start using it, we need to lift the lid. This is a glass lid that is provided for our applications.

So, ideally we should be switching off the UV light like this. So, let me switch it off; we will switch it off and then we have to lift this lid, lift the lid and work. We should work like this inside, but then and you make sure that please never lift the lid fully. This lid is not meant for that, why this lid is provided has a reason.

Everything has a reason. So, we should keep only at our working height and then work why this is so? Because this is because suppose something happens there, some chemicals get released from there, they should not reach us reach our eyes.

So, that protection is provided by this glass; that protection is provided by this glass and you can safely work with only your hands inside and you can reach and then cabinet size is such that you can reach throughout the region of the cabinet. Hope this is clear.

Now, let us see let us get back to our discussion. Suppose now our UV light is on; let me switch on the UV light again. So, our UV light is on again, but we forgot to switch it off

before we started, then we are starting to use; please look I look here, we are starting to use and I am lifting the lid. Immediately that UV light goes off.

So, this is the protection provided because you cannot, you will not get exposed to UV light, it can cause diseases like cancer. So, it has we have to be very careful that you are not expose UV light. So, the cabinet gives that protection also. Any bio safety cabinet that works on BSL 2 facility will have these features installed. Let us see it again. So, I am see let us say I try to switch on the UV light while the lid is up that also that that equipment will not allow you to switch it on. See the lid is up right now, this gap is there, I am trying to switch on. It is not switching on. Now, I am closing it.

Now, it is closed if I, now if I switch on the UV light will come. Let us say we now again we try to open it, the moment it crosses a very small area itself the light will switch off. So, this is the safety precaution that is provided in case we forget to switch off the UV light. Now, we understand about the UV light right. There is another, the second option that is provided is for normal visible light.

So, yeah, we can see it now here, the second option is for normal visible light; we will shortly see the normal visible light and then, the first knob is for circulation fan and exhaust.

So, before we start using the bio safety cabinet, the protocol is this that which will always have the UV light on; the UV light is on. This fan should be running. So, when the fan when you switch on the fan, the alarm will ring here. The alarm is ringing because it needs some time to reach that circulation speed. So, it is telling that I have not reached my set speed. So, please wait so, I can actually because I know that this is the issue, we can actually mute the alarm. So, the alarm will go.

So, if any other and toward incident, unexpected incident happens also this alarm will ring. So, there is a circulation happening inside. So, this circulation will constantly flush in good quality air through the hood and also take out any chemicals that gets secretary. Now let me.

So, while you work on the hood, it is advised that always you have the white light on. So, let us switch on the white light. So, I have switched off the fan because now it has been properly clenched. So, you can work without the fan running. So, what we can do is

once the work is once your work is over, we can put on the UV light, put on the fan and leave it.

So, that will sterilize a environment inside. Now we have switched off, switched on the working light; this is the working light. Now with the working light on, we can lift the lid; keep our hands inside and work. This way we can work; this is how we should work in a bio safety cabinet. Now I think it is very much visible now inside.

These knobs see these 2 inlets are for distilled water and any other chemical or circulation that you need; water you can bring in even air at high pressure air if you want to clean some vapors with nitrogen gas that also you can bring in; that is your own wish. You can always connect things in the other side and bring them bring them here.

Now, here you have a pressure indicator showing what is the pressure inside the hood right now. So, right now it is showing the default atmospheric pressure only. Now, let me open it fully. The cabinet also provides to plug points has maximum output of 220 volt 3 amps. In case so, what happens is while you work we will have to use other equipments also; other equipments like centrifuge, cytocentrifuge and let us say a hot plate. So, this hot plates cytocentrifuge etcetera, I have to be kept inside right, but we have to power them also. So, for powering those equipments this plug points are also provided.

Now, the tube here which you see here is the UV source. So, this will generate the UV light source. Our white light source is kept inside here. It is there in this areas and the light comes on falls on it. The light is not kept there because you will not get the glap from that light. So, light comes from here, it floods the bio safety cabinet nicely and properly. So, let us close this again. So, especially when you are working with so, these are all equipment level protections that are provided right; these are anywhere man made.

So, you cannot always be very sure that these equipments may not malfunction. So, they may malfunction at different points. So, always where any UV protection glasses with you like I am wearing now. So, that in case this protection mechanism does in work. So, that UV light is on you can see the UV light there.

So, but by default this protection is there; but in case if protection stops working because of some control circuit failure inside the equipment, you should still be protected. So,

that is why you should wear UV protection glasses. Again, if you see the glass bottom, they are they are also providing proper cautions. See here. So, this is at the bottom of this in glass.

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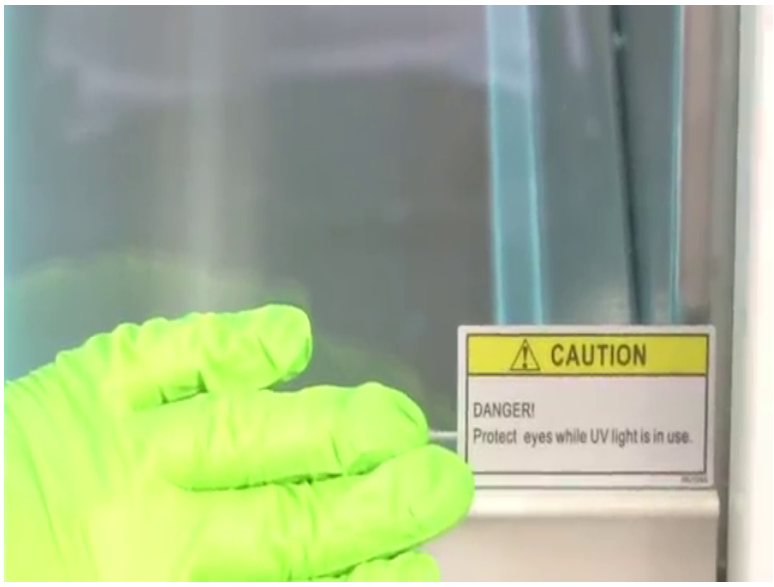


So, Caution Danger Protect eyes while UV light is in use. So, even through this glass, they are advising you us that we should use UV light to protect us because anyway even though, we are not exposed we will be we are actually seeing the UV light right. So, they are asking us to protect us from the UV light and for this table this is a SUS table. So, for this SUS table also they are giving caution like, the SUS table must be cleaned up while it contacts with caustic medium or the work is finished.

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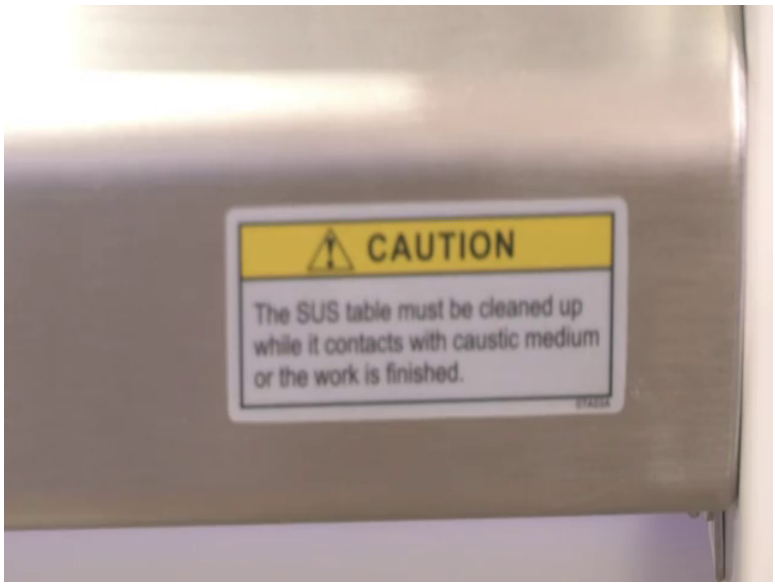


So, every time you finish the work, you should clean up this table with nice distilled water or even acetone or IPA you can wipe the surface clean it either when work is finished or while you are using any caustic medium and this is the safety limit that is provided. So, these are the major features that are available inside a bio safety cabinet. Shortly, I will show you after keeping some basic biology equipment inside how we have to actually work with things inside.

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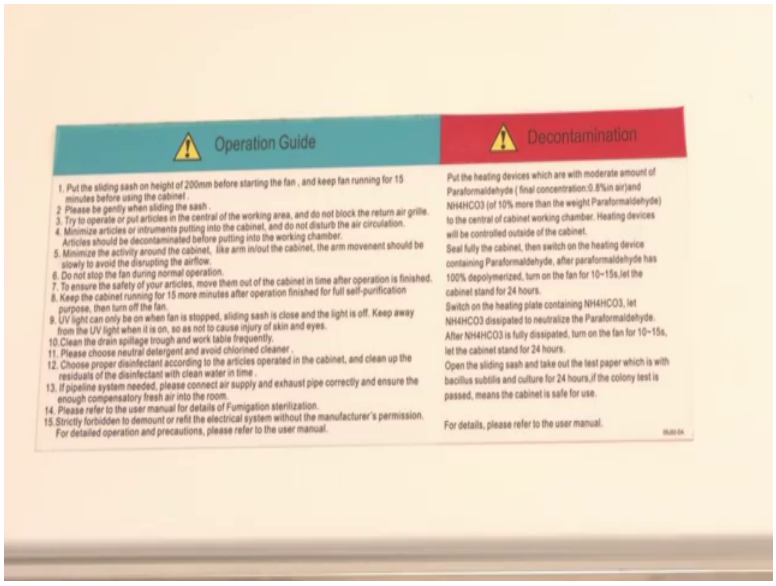

So, one more thing if you come up, there is one more knob provided here, which is the fourth knob that is power socket off. So, what this does is that. So, we have seen that there are two power sockets right inside, we may not need that power on that sockets all the time. So, we can actually switch this on to make sure that power comes there; otherwise power need not come that is a whole idea.

So, the power is separately provide that we need not separately power those sockets. From the power line that powers this cabinet, those sockets will be powered and you can actually switch off power output from the sockets through this option and now, you see that this is the working area right. This much is only the working area and then what is this much area. So, this is the fume hood that is provided that processes whatever fumes are coming from the sample, it will clean that fumes remove all the contaminating equipment and then exhaust it out to the environment.

So, the air that comes out of the environment will be free of any contaminant or pathogenic material; that is what is there in this much area about the bio safety cabinet. Now, let us look at the manufacturers themselves as per their guidelines of manufacturing, they have provided few operation guides. We will just quickly go through what are the operation guides that they have provided which will give us a better idea about how to use the bio safety cabinet over and above what I have just described now.

So, what you are telling we will quickly go over them. First one put the sliding sash on height of 200 mm before starting the fan and keep fan running for 15 minutes before using the cabinet. This is what I had told. So, before we start using the cabinet, you should switch on like a fan keep it running for some; you should switch on the fan keep it running for some time. So, that the air within the cabinet is purified and the ultraviolet light also cleansers the environment say like this. So, I have switched on the fan, it will cleanse the environment.

So, if you keep our eye hand inside, I have switched on the fan. So, these vents are there here from there air is coming. So, if you look at this paper, you will feel that air is coming see. Actually it is that whatever is air is inside right it is being sucked in at this region and it is recirculated from another portion. From here it is getting sucked and it gets re circulated from top. So, this circulation is maintained here.

So why because it is sucking the air from here we need to close it so that properly so that it can cleanse the inside properly. Then next one, please be gentle when sliding the sash. So, what is sash? Sash is this glass window that we know that is called the sash.

So, it is very we have to handle it gently because one or ones or two times when I was pushing it up, this is going away. So, it will go and hit in a slightly fast way. So, you should make sure that you are sliding it up very slowly. Keep your hands on the slide do not leave it like push it and leave it; keep it within in your hand slide it till the top.

While you are using it you will not have to slide it till the top, mind it. This is only when you want to clean the area; otherwise you will use it only with your working distance, working height. Keep it like this; sit on your chair in a comfortable position; keep your feet nicely inside; put your only your hands inside and then work; once your work is finished, slide the door closed, switch on the UV light and leave.

Before using switch on the circulation; clean the air inside; switch on the UV light, sterilize; then, use. This is the protocol. Now, next one try to operate or put articles in the central of the working area and do not block the return air grills.

We had seen the air grills. So, we should not put anything on top of those grills. We saw the grills where air has been sucked right; do not put any equipment on top of it and once you start using it, this table will get consumed by lot of equipment and samples. While you do that also make sure that you do not clog the entry points or exceed points of the air.

Next, minimize articles or instruments putting into the cabinet and do not disturb the air circulation. This is again a reputation of the point where we told; even though we might have to use some equipment inside, try to keep it minimum and the things that you can do outside the bio safety cabinet, the less pathogenic or non pathogenic work. You can do outside the bio safety cabinet.

Next, article should be de compared contaminated before putting into the working chamber. This is a very important many important fact because say suppose, we will be using different types of samples, equipment, different devices etcetera inside the bio safety cabinet.

If you do not sterilize them properly, whatever protection we are providing through this very costly equipment we will come to naught or 0 if our equipment or the tools that we are using inside the cabinet are not sterile. So, we have to use, we have to as much as possible the items that can be sterilized through equipment called Autoclave, which we will see shortly, should be autoclaved.

Other big equipment should be properly sterilized and cleaned before they are used inside the hood that is what they are telling. Next, minimize the activity around the cabinet like arm in out the cabinet, the arm movement should be slowly to avoid the disrupting the air flow. This is self explanatory. Next, do not stop the fan during normal operation.

So, the fan should always be running. I mean do not abruptly stop the fan. Then to ensure the safety of your articles move them out of the cabinet in time after operation is finished; keep the cabinet running for 15 more minutes after operation finishes for full self purification purpose and then, turn off the fan. So, the fan should be running for at least 15 minutes.

So, let this siren go off. Let us see if this siren stops after sometime. Now, I have muted it because for 15 minutes this siren will run because it is telling us that it is purifying. Then UV light can only be on when fan is stopped, we have seen it.

If I try to switch on the UV light, when the fan is on it will not switch on. See I am trying to switch on, it will not switch on. UV light can only be on, when fan is stopped; sliding sash is closed and the light is off. When all the situations are satisfied only, only then can the UV light be switched on; when or the sash or the sliding sash should be closed, the fan should be off, then only the UV light can be switched off.

Keep away from the UV light when it is on so as not to cause injury of skin and eyes. Clean the drains spillage trough and work table frequently. Please we have to click keep this area clean. Please choose neutral detergent and avoid chlorined cleaners because they can react with the surfaces that are there inside the bio safety cabinet and cause corrosion.

Choose proper disinfectant according to the articles operated in the cabinet and clean up the residuals of the disinfection with clean water in time. So, we it is very important that

we use very clean water; preferably distilled water or di water which is even better which is called deionized water.

Such a water we will have with no ionic content in it. So, it will not be reactive even to a very small extent, by default also water is not that reactive. But if you have, but it is still have lot of dissolved salts and ions in it. So, we have to use de-ionized or distilled water so that any unknown ions dissolved in the water do not react with your sample or your cabinet.

If pipeline system needed, please connect air supply and exhaust pipe correctly and ensure the enough compensatory fresh air into the room. For that we make sure that we have our HEPA filter arrangements which will constantly push in very clean air into the room. If please refer to the user manual for details of fumigation and sterilization.

Sterilization, we have told you about. So, fumigation will be carried out by this top part. What is fumigation? Fuming so, whatever exhausts chemicals that come out of the sample, they will be burnt that gases will be burnt again. So, that they are sterilized. So, this is sterilization through burning, fumigation is that.

Let us say you are, you have cut down some trees and they are actually going back, you actually burn them right. So, that is fumigation. So, fumigation is sterilization through burning; fumigation and sterilization then, strictly forbidden to demand or re fit the air taking system without the manufacturers permissions.

These are the major precaution measures that you should take care. Shortly, we will show you how to use a few equipments inside the bio safety cabinet. So, you get a hang of it. These bio safety levels we will get into shortly. Before that, we I have mentioned that bio safety cabinet is used for mainly for protection of the worker or the researcher from pathogens.

So, pathogen is a very generic word; what are pathogens are any foreign body that tries to get in your body that is a pathogen, that could be a harmful bacteria, that could be a virus, that could be a protozoa; there are several protozoa in to protozoic diseases also. So, we need to protect ourselves well, while we are working from this harmful foreign bodies. So, bio safety cabinet this is mainly used for that. Now, class 1 bio safety cabinet well class 1 bio safety cabinet we will provide protection.

So, there are many 3 mains; 3 different types of protection. Personal protection, personal is us, person who is working on the cabinet that is personal protection. 2- is Environmental protection that is protection from harmful agents of the environment that I am or anybody is working on that is this area and this lab in general.

And also to the building or surrounding places and the third protection is a product protection. Product protection is a protection of the sample which we are working on from being contaminated or changed by factors not under our control. So, we should now if it is something that we have introduced and we want that sample to change that is different. But suppose the environment itself is making the sample to change in a way that we do not desire it.

Then, that product is not protected, but we need to protect the product from it. So, that is called Product protection. So, 3 mains for protections are there; personal protection, environmental protection and product protection. So, a class 1 bio safety cabinet provides basically personal protection and environmental protection; product protection is not provided. What it means is that the air that is used. So, the in this cabinet you can you can anyway see; so, there is a enclosed area inside right. So, you can actually if you see inside there are 2 nozzles. So, I will just lift it now.

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So, there are 2 nozzles. So, these nozzles can be used to supply deionized water or distilled water, very clear water and also to circulate air inside the bio safety cabinet.

Now, in a class 1 bio safety cabinet as I told there is no product protection provided. So, what it means is that the air that comes in contact with the sample might not necessarily be free of contaminates, that is what it means.

What is also means is that you will just be circulating a normal air within your cabinet. So, that is class 1 and class 2 bio safety cabinet; class 2 bio safety cabinet would include BSL 2 and BSL 3 bio safety level 2 and 3. A class 2 bio safety cabinet would provide all 3 types of protection that is Personal protection, Environmental protection and Product protection.

How it does this? It does this through HEPA filters we have already covered what HEPA filters are; what are tower modules. We have tower modules in this lab, we have seen it. So, what HEPA filters do is, first of all the air that get circulated within the bio safety cabinet would it themselves be HEPA filter; that means, HEPA is what high efficiency particulate air. The number of particulate matter greater than 5 microns is very much controlled in a HEPA filter environment. So, if it is a class 1000 clean room; that means, there will be only 1000 number of particles greater than 5 micron size within your enclosed space. Class 10 clean room would mean there will be only 10 particles.

So, you have huge amount of control in the quality of air that you would be circulating in your bio safety cabinet. So, when your bio safety cabinet has provision to circulate HEPA filtered air and also the chemicals that come out while the product while your sample interacts with the chemicals that you add, the vapors, this vapors will also be HEPA filtered before it end us the environment.

This way, it provides environmental protection and personal protection and because we are using HEPA filtered air, very clean air only to circulate around the sample, the sample is also protected from unwanted changes. Thereby, we get yes, product protection; so, product protection, personal protection and environmental protection. So, this is class 2. So, class 3 is a very special type of bio safety cabinet. This is not that there are mainly used for BSL 4 containment. So, see if you see this class 1 class 2 class 3 is a separate US government or a US standard based classification, parallelly we have the bio safety level classifications also.

So, bio safety level 1 2 3 4 up to up to 4 are there. What is bio safety level 4? Bio safety level 4 are applications where we have to use highly guarded containment facilities for

very very dangerous viruses and contaminants like, HIV virus Ebola virus. So, such very very very dangerous viruses, if you want handle; you need to use a class 3 or a BSL 4 environment.

There the protections provided are much more in detail. We can go to the details of it at a later time. So, you just understand that there is class 1 class 2 different types of classes of bio safety cabinets and their specific applications are for different different purposes. And how they are differing is through the arrangement of the air and the extraction of fumes that come out; how they are process before there put back into the environment.

So, BSL 4 is the highest level of protection because once you have to be very careful while handling such infectious agents. Now, we will look at some of the knobs that are present in the equipment.

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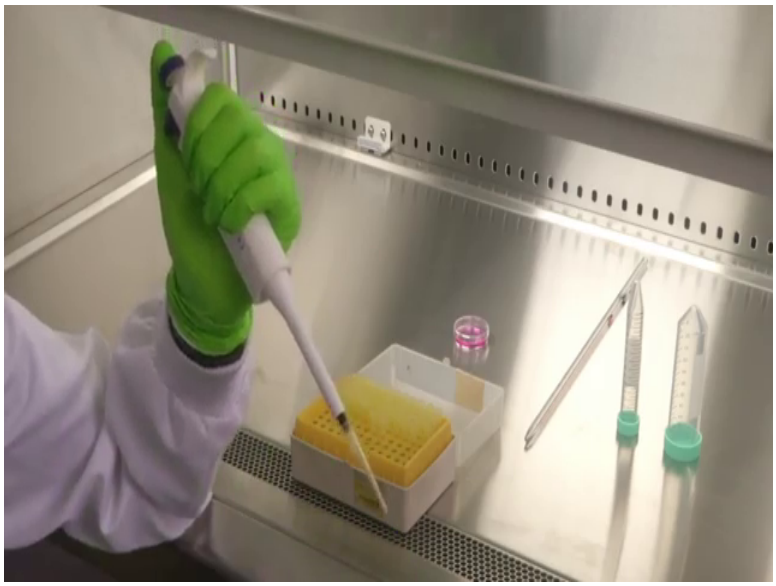

So, 100 micro liter it is means set now let us see how this operates actually. So, there are basically 2 knobs in this; this 2 violet you have to see. So, this is where you take out the sample. So, here it will closely observe my hand, I press it; if this will create vacuum fair here. Then when I take it back, it will take exactly whatever volume we have shown right here in that; that much volume it will take in the pipette tip. It is called type tip; I will show the pipette tip shortly.

So, like this, then after this you can actually if you are transferring such let us say we are we take it and we are transferring it somewhere else. If that is the case, if you want to finally, transfer all the droplets you have to push it further like this. There is an extra push that can be provided, that is only for transferring whatever is there already in the tip back to the in second container, but the volume measurement will be through this only; do not push it further like this.

What is this used for? This is used for finally, discarding the tip like this. So, let me show you have a pipette tip looks like. So, this is a micropipette tip box. So, tips can be arranged in this box. So, tips. So, this is how a micropipette tip looks like.

So, this is be 200 micro liter. So, it can hold maximum 200 micro liter of sample volume. So, let us say now how to take this tip? So, you take your micropipette, putting in the hand you are comfortable with; put it like this, give few tabs and your tip is already engaged into your micropipette. Now if you have to take sample volumes, you have to press it like this and then it will take the sample volume and it will then you can go and release it somewhere else.

Now, let us say we have to remove it. So, ideally we will be handling. So, cell culture suspensions; cell culture suspensions right that you might have to transfer from one dish to another. Let us say at that point you do not want to actually touch this thing the tip. So, what you do is you will do everything like this, you will transfer it from there and then transfer it back and then there will be a waste box kept there or below just below your here or just below your bio safety cabinet and then, what we will do? Just press this press this and that tipple fall into the waste box. I will just show it again, I put it in the tip box.

So, I am taking a tip. So, tip is tightly put; you will take samples, we will transfer it and then we will discard it like this. Now, let us see how actually sample volumes change. Now, let us see how we are actually transferring sample volumes. Say I have my pipette tip. So, what I have here is culture of cells in this dish which is purple in color.

The cells are in suspension that is they are suspended in a medium which is DMEM which is Dulbecco's Minimum Essential Medium. This medium gives the necessary nutrients required for the cells to continuously grow; how the cells are grown using CO 2 incubator, we will introduce to you soon. So, this is a dish containing cultured cells. So, now, I want to transfer 100 micro liter of these cells to this dish. How do I do that? So, I

will first show you how much the sample volume differs when we take 200 micro liter and 100 micro liter.

Let me just adjust it back to 200 micro liter which is the highest volume. So, as you can see it is 200 micro liter now. So, I will now take; so, you see how I am using only my hands are inside and the hood is almost closed. So, that my face is protected by this glass shield. Now I select I take a tip I open the lid of the dish, now. So, I press it. So, when I press and then I introduce and release, it will take in the sample volumes. So, I have kept the other dish ready.

So, if I release it, you can see that 200 micro liters taken. You can see it in the tip, but I do not want 200 micro liter. So, let me put it back like by pushing it like this. Now, see one drop is hanging; now to drop that drop only we have to press it further so, the drop will go. Now, I do not want 200 micro liter, you guys saw that the volume came till here, but I want to transfer only 100 micro liter. So, let us reduce it. So, now, I have set it to 100 micro liter.

Now, let me see how much volume comes in the tip. See earlier it had come till here, now it has come only till here that means, only 100 micro liter is transferred here. Let me show it you again; let me show it to you with very less volume; let me show it to you with 20 micro liter let us say. So, I am reducing it further. So, let us not go to the 20, let us go to 50 micro liters. So, that it is clearly visible to you. Now, I have kept it to 50 micro liters. Now, I am taking 50 micro liter only.

So, we have seen how to take 100 micro liter, 200 micro liter; how it came in the pipette tip. Now, as I have shown we have set it to 50 micro liter. Now let us see how much volume comes when I set it to 50 micro liter. See as you can see 100 micro liter had come till here, this is the ring is there know, till that ring it had come.

Now, 200 micro liter had come till here; now 50 micro liter has come only till here. So, that we do not worry about how much volume we have to take. We just need to set it here, now that liquid also we will not flow out. So, it is already there unless we press it, press it and drop it; it will not go away, it will be inside the tip only.

Now, let me take it back to 100 micro liter. So, this is suspension of cells in DMEM as I had told before. Now it, I have set it to 100 micro liter; here 100. So, we are taking it 100 micro liter is taken. This is the next dish to which we have to transfer it.

So, I am transferring it; see, make sure that all drops within the tip are dropped. They are not dropped, please make sure that it is dropped by pressing it further. So, 100 micro liter measured volume is now transferred to this dish.

100 micro liter is now transferred; see, it is 100 here. We have transferred from here to here 100 micro liter. Let us say we want to transfer another 100 micro liter; I will just press here, take it 100 micro liter is taken, just come to this dish, press it, 100 micro liter is transferred. So, 2 times we have transferred 100 micro liter to this dish. So, we have 200 micro liter in this dish; 200 micro liter or 0.2 ml. Now the use of this tip is over. For each and every time you have to use, you have to use a fresh tip because as you have seen it is very easy for this tip to get contaminated.

These tips micro pipette tips are not meant for reuse. As soon as you use it you have to throw it away. So, we have to discard it later. So, like that we should keep it a set or discard it in a discard box next to our place, as far away from more experiment as possible.

Finally, we have to take it out and clean the place with tissue paper and maybe acetone or IPA or ethanol some alcoholic solution. This is how pipetting is done in tissue culture dishes these are as I had shown before these are the same 25 mm dishes we had seen when we were studying the inverter microscope. This is the tip box where you can arrange tips so that you can easily take them out.

So, that you your use of your hands should be avoided you this what I am doing right now, this should never be done. This is banned inside a biological lab should never do this; we should do like this best, I am showing in to show you that you should not do it. Best lab practices you will have a tip box, your tips will be there; you take your pipette, take it do your experiments, minimum environment of your hand.

No contact with the tip because the tip carries the samples. Now, neither the sample should get contaminated nor should you get any kind of pathogenic infection from the

sample because you do not know the sample might have viruses, HIV virus, Ebola virus anything could be there.

So, this is how you should handle tips. Next I will just show you few other equipment that are used. This is just like we saw micro pipette tip, which is this one, this is a 2 ml bigger pipette tip this is not a micro; this is just normal pipette tip 2 ml micro vapor tip. So, in case you want to transfer huge volumes, you can use this with another equipment call a (Refer Time: 40:28) to pipette that is a different pipette, which we will show it to you later. So, this is another 2 ml tip, you can see that volumes are marked here nicely. This is borosil, made up of borosil glass.

Now, to transfer so if you want to centrifuge yourselves like centrifuging is making yourselves come down and supernatant to be separated. Just so, that you can the separate only the cells out, you can use tubes like this. So, this is 15 ml tube; this is 50 ml tube as you can see. So, 15 ml tube, 50 ml tube.

So, this can be used for very varied purposes and for this conical part also there are what you called sub calibration so that you know what you are taking. Here also for the conical part, you have sub calibrations. So, this is starts it 2 ml here. This will be 1.5 ml and within 1.5 ml also you have sub calibrations here; see 1.5 up to 0.1, you can actually measure out in this tube.

So, students, I think now it is clear to you how you should use the bio safety cabinet; how you should keep minimum parts of your body inside and how to use this. This is the crux of how bio safety cabinet is; at later point in the course in case we are doing any other further experiments, those experiments also we will show in the bio safety cabinet.