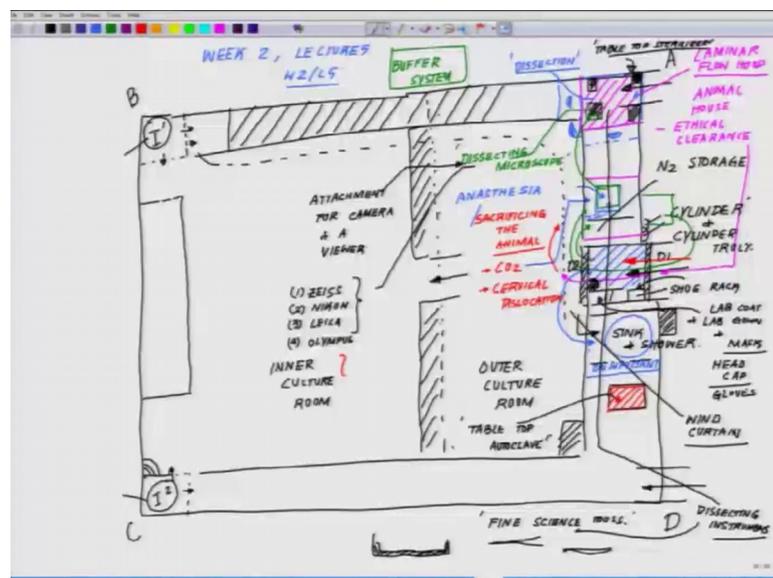


Cell Culture Technologies
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Lecture – 10
Precutions during Designing the Lab Layout – III

Welcome back to the lecture series in Cell Culture Technology. So, today we are into the fifth lecture of the second week.

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So, we are into now week 2 lecture 5 w 2 1 5. So, in the last class, we talked about that in this corner if we put a laminar flow hood with a sitting arrangement like this or sitting arrangement like this or maybe a sitting arrangement like this we cut the. So, before lamina flow hood design, let us decide one more stuff you get the animal inside right. So, here you are getting the animal inside the facility, now you have to have a set up for sacrificing the animal.

So, your sacrificing the animal means you to kill the animal and in that case say for example, we consider that you will be using mice or you will be using rats or something. So, you remember in the last class I told you that you needed the animal ethics committee clearance. So, that is where you have to get your clearance how first of all that clearance will come with how many animals you will be using. One where you will be housing the animal, two in the housing what all things will be providing in terms of

you know food the kind of food you will be supplying, the water supply the air conditioning and all those paraphernalia, but what is very critical is that how you are going to sacrifice the animal. If you are sacrificing and taking other tissue what kind of mode are you going to use, are you going to kill them by carbon dioxide or you will be doing something called cervical dislocation or you will be using something some other mode.

So, there are different modes by which you can sacrifice the animal, and these different techniques are getting revised and updated from time to time, through the years there are several improvisations which are taking place and the veterinarians or people concerned or aware of it, and they can literate you on that that you know this is the technique which is best suited for you or for this kind of animal or that kind of animal or this kind of or say for example, you wanted to do an anesthesia say for example, you are not sacrificing the animal let us put an alternative you want to do is an anesthesia because say for example, you wanted to implant something in an animal and you know you want to take the surrounding tissue.

So, in that case what kind of anesthesia you are going to follow. See if you are not in veterinarian will not be aware of it because there is no reason for you to know this additional piece of information. So, this part is very critical and whatever you do should tally with what you have promised with animal ethics people, should there should not be any discrepancy promise something and you do the stuff different way. So, let us assume in this situation then you wanted to do a CO₂ sacrificing. So, that again brings us that you needed a supply of CO₂, which most of the labs currently across the world does you know.

So, and you have to have a chamber this kind of a chamber like you know where you keep the animal, and you have to have a gas supply something like this and we allow the animal to have a peaceful death and then whatever way you wanted to dissect it out or you decapitating the head or you wanted to remove some kind of a gut tissue or some kind of muscle or you wanted to do a isolate the cardiac tissue you want to isolate the brain. So, driest will follow, but this part is very important. So, in this facility you should have a small corner and prefer a corner and close to the sink. The reason being because this is something which is kind of you wanted to you know, do it very cleanly you do not to mess up things here.

So, you have to have a facility in the case of course, these are all assuming that you are doing primary culture, because if this is clear to you then the secondary cultures and all will be very easy to you. So, you have to have a facility somewhere and these this animal sacrificing should (Refer Time: 05:58) deep inside the lab even in the outer facility. You should not have something of that kind out here or out here or somewhere out here right you prefer to the have this very close to the door somewhere out here, or some people would they do which I do not know I mean how good it is they even have something outside they just sacrifice the animal and just bring the sacrifice animal inside instead of doing the sacrifice inside the facility.

Well I mean it has it is advantage and disadvantage because the disadvantage is that then you need another additional kind of enclosure, which is much more exposed and the disadvantage is that you do not have to do this stuff inside the outer facility of your lab. So, I will leave it to your wisdom which one you prefer, but some such facility has to be there and my need this one this will never be written in textbook how you do it, and this is what I am telling is from my experience of setting up labs design and layout how we really have to think, beyond because when I said the first class I still remember back in probably 1997 or maybe earlier than that, I was not aware of it and there is no textbook which could tell me.

So, it is more of like you know and this was not the www dot edu of you know internet and all those things, these where like you know and. So, there are handful of textbook here and there, but not really telling you how really you set up the whole thing what are the points you have to keep in mind. So, you can see it was very trial and error you know talking to people and seeing the facility and figuring this is another right way, this is like you know. So, whatever I am telling you is from share from my experience there is no textbook which is going to tell you all these things that how you should, but you have to think in advance because if you think in advance and put you sure you can think far better than what I am putting out here for you ok.

And if you think it right and there are people who are very good they can tell you that you know if we put it here this is the problem, they may not be knowing your science and they do not need to even, but they can give you a better insight how you really can design the whole thing in a way. What I am trying to tell you are those rate limiting points which most of the time we missed out, and then we do some hanky panky job you

know or do it like that in a fitted fit things like that and that really a bad design, bad way of like you know putting things together. So, please be careful whenever you set up the thing think, think calm quite think over it how you really want the facility, because the your facility looks nice you can bring people and finally, the end user they will be happy you know this is a very systematic facility, and one more thing these kind of facilities takes a lot of soul to be put there.

So, those whose server uses this such facilities should carry that spirit of that soul, that is you know we are not going to you know mess up with. Because any any any negligence could destroy the work of many other people because you are handling biological samples, which are prone to contamination they can destroy your whole facility in no time. And once the contamination takes up, it is very tough to contain them so, coming back. So, you have to have a animal sacrificing facility and you take the tissue out whatever concern tissue and now you have to do everything in the hood.

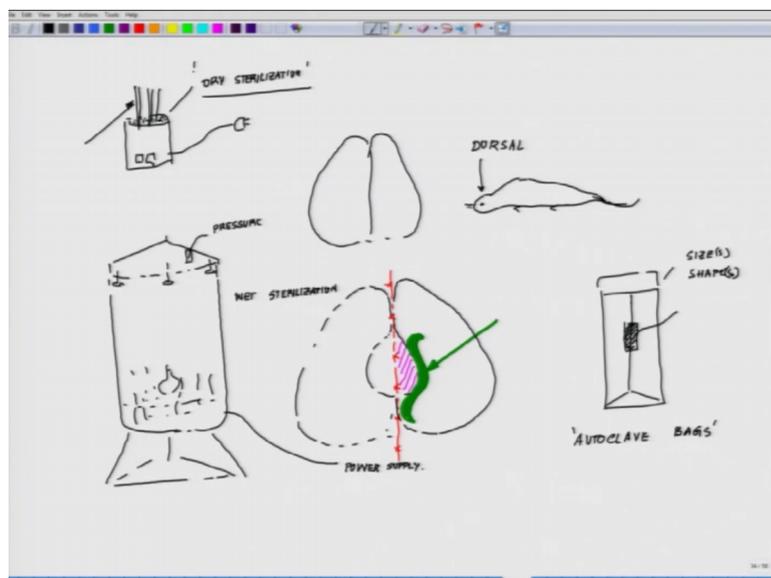
Now, when you get into the hood with the tissue, you have to have a buffer system, this part we will come in the medium development, you have to have a buffer system which will be helpful to ensure that the tissue remains alive during our dissection part the animal is dead anyway, but it has to be supplied with necessary sometimes if the tissue is very prone to know oxidative damage you have to ensure that you know how you can handle it what kind of additional material you main love to put, some kind of antioxidant and whatever, you know we will come later into this part while we will talk about medium developments, but keep this in mind this is very important you know.

So, out here inside this laminar flow hood, you may prefer to have a permanent fixture or a not a fixture a permanent location for dissecting microscope, you will be needing this pretty frequently. If you are dissecting big animals you may not need it, but if you are dissecting embryos or features, you will be needing it. So, see through the catalogs on through it comes about the microscope and get a good quality dissecting microscope and here is my what of like in a caution, do not because this dissection process. Especially for the embryo is a tricky process and do not is spoil your eyes by having a bad microscope for that purpose because it is a pain, because you would not be able to locate the tissue right if you have a bad microscope look through the good microscopes of Zeiss olympus or you know leica. So, where these are some of the giants in the field or Nikon as a matter of fact you know to have a good dissecting microscope.

And if you are really good in that, then I would recommend you something which is a very good teaching aid, is that many of these dissecting microscope comes with an attachment to put a camera which could be put on a screen and you can record it. So, let us think of a situation your lab works on say hippocampal neuron, hippocampus is a very specialized part of the brain which is involved in learning and memory. And most of the people who work on hippocampal culture, cannot rely on cell lines they isolate hippocampus, mostly from rat or mice embryo this is a standard practice once you will come for the primary cell cultures in depth we will talk about it.

So, now in the brain isolating hippocampus is not something very easy.

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So, the structure is something like this, if you look at the structure and of the brain. So, for example, you take out the brain you remove the skull and everything, you are having the dorsal view or the top view of the brain it sink with the. So, what does that mean; that means, that animal is you know sitting like this, and you are seeing the animal from the top. So, now, that is called a dorsal view. So, this is the dorsal view of the brain and the ventral view if we kind of twist or make the brain upside down like this, like this this is the dorsal view right and you just look it like this, you just make it upside down.

So, if this part of my hands are dorsal view and these are the ventral views. So, if you roll it down like that, the ventral view is something like this of the brain. Now hippocampus is an organ, you would not be able to see from the top unless you cut it like

this and you remove this part. So, you have to scoop it out that underneath this, you will see a. So, which will be organ which will be sitting like this and it is deep inside, this is not something unless your a very thorough with the brain anatomy and you really can it is not so easy to isolate this, this is where they hippocampus is line.

So, coming back, why I mentioned this. So, say for example, your lab has been working on this area. So, somebody is an expert who can isolate hippocampus, but this gentleman or this lady is going to leave the lab. So, what do you want when they dissect, why do not you record it. So, you have a system or this could be used as a teaching aid for you know practical training of dissection and isolation of a specific kind of tissue, what you will be using in your culture right.

So, it is a good practice to have a good dissecting microscope, with possible attachment or camera and viewer and this viewer could be in computer you can you know interface the camera with the computer and the computer screen you can see and you can record it. There is a good dissection you can record it and that could be a tool for cell culture facility, that you know this is how we isolate the tissue. So, I will recommend careful while buying a dissecting microscope and go for like I personally have use Zeiss you are really good ones really good ones I have used Nikon I have even used Leica. So, these are some of the good ones olympus I have an used, but you know I have heard good things about it.

So, but try to get a good dissecting microscope my first suggestion. So, you have to have a dissecting microscope inside not here where all your dissection will take place in the corner, you prefer to have a table top sterilizer what is a table top sterilizer. So, for dissection you will be needing dissecting instruments. So, be careful about the dissecting instruments again a tool which you will be using very frequently, if you are a primary cell culture lab.

So, try to get the best quality of it, there are global companies which are really good like fine science tools fine science tools. This is Swiss company they are the maker of some of the finest tools I have ever used till this date, and if you do not find them and if you cannot afford those ones your lab is not ready to afford those ones. You can always go to the local goldsmith, and they can really curve out or the watchmakers they can really curve out very nice instruments for you, but you know you have to you know valley up

with them in order to tell them this is your requirement because most of the watchmaker in our country they use very very fine you know fine tercets scissors.

So, you can get those things fabricated which is not a big deal trust me, or you can get a coarse one and then you can gold plate it and you know make it sharper, and several things you can do there a lot of zone for innovation tremendous zone for innovation what you can work through. But you have to think how you really can make these things happen. So, get the best quality dissecting instrument, and there are dissection instrument box it is something, like those of you have seen in other lunch boxes which are made up of aluminum or steel or something these are box similar to that underneath it you have a kind of a cushion like thing which is a polymer or rubber kind of thing keep a rubber on the side so that say for example, you have the box like this with a cover.

So, you have the rubber bed like this, this rubber bed ensures that the instrument which are there. So, you have see this fine sharp you know forceps, you have very fine should say very fine you know scissors, capsules and you did not want these to get damaged. So, what you do? You can even fabricate you can get a box and you can you know you can put very nice rubber lining. So, even if the tip of the instrument kinds of get hit should not get damage. So, again these are the experience which is telling you, because I have seen things getting damage, destroyed just because we did not take proper care, which you could have done all right if you have been just little bit of farsightedness.

So, you have to dissecting box before you get the dissecting box, what they do you sterilized it. So, you have to have one more thing, you have to have an autoclave somewhere out here, now this autoclave can be outside the lab also. So, autoclave is something like a pressure cooker, where at high moisture and high pressure you sterilize anything. So, you will be needing autoclaves and ensure that these autoclaves are according to the requirements or number of end users you do not want to get a very huge ones, which is kind of tough there are modern days facilities where they have centralized autoclaving. So, just before I again get back to the dissecting thing, let me talk about this autoclave stuff.

So, it is the autoclaves could be multiple types, like you know you could have autoclaves like this, you will see any of these labs they will have like this huge you know autoclaves sitting on the corridors, you will see most of the time on I stand like this. So, they are

bolted on to your fore side like this, and here connected to the power supply and here you keep your stuff and there is pressure gauge, which ensure the pressure and from the other water kind of you know create the vapor. So, in a high pressure and in a vaporized environment you sterilize the stuff. So, whenever you are keeping anything for a sterilization or autoclaving you put them in a autoclave pouches, their pouches which are available. So, it is kind of a you must have all have seen envelopes, where you keep our send our letters or something is almost similar to envelop and you can seal them.

So, they come like this something like the envelopes like this you know, and this is the sealing when you seal them. So, there is a spot you will see after a certain point of heat and moisture the color of that thing changes, which indicates that this stuff has been autoclaved. And if you visit websites of autoclave bags autoclave bags, you can see these kind of autoclave bags. They are really very interesting stuff explore it online go online and check it out, you will you will really enjoy seeing them there are whole different range of autoclave bags which are available, will have this autoclave bag. So, whatever you want to sterilize you keep them inside the and they come in different size and shape mostly different size and shapes.

So, you can have bunch of them already purchased for your facility and you keep your stuff in them and you bring it out. Till you reach on the laminar flow hood or just close to the laminar flow hood, you do not open it. So, you get these instruments autoclaved and bring it inside your facility like. So, you are out here. So, now, as I told you that either you can keep the autoclave outside or you can keep a table top autoclave somewhere out here, especially for this kind of facility which I personally prefer a table top autoclave. Which is in your control it is not outside, it is not bothering anybody, you have a small table top autoclave and these are all available they are of kind of you know like one feet size or something like this, and they are very cool stuff to you know work with this does not create much problem.

So, close to the sink because you have to fill water and everything and they need a power supply and that is all you need it. So, you get this instrument inside, and while you are dissecting or automatically you may feel like you know, if I could restore lies because from one animal to second animal from one embryo to next embryo. So, for that you need dry sterilization which is a table top sterilizer. So, these have these are sterilizer something like this they are small like water feet stuff like this and there are lot of glass

bead kind of a stuff in them and you keep the instruments in it and you have a panel like this, and it is connected to the power supply and this dry sterilization takes around 90 seconds to a minute.

So, while you are working you can re sterilize, but there is a difference this is where you have a wet sterilization. Wet sterilization which is far more long term stuff and here inside the hood you have dry sterilization, where you are only utilizing the heat you are not utilizing the moisture or the pressure into the game right. So, you have the dry sterilizer sitting out there; see you keep your dry sterilizer there. So, while you are dissecting you can always you know re sterilize it in 90 seconds and it still you can use.

So, these are some of and of course, you wanted to have a bunch of pipettes which most of these labs used has dedicated ones for this kind of dissecting hoods, generally I personally used to prefer on those glass walls on both sides to have the pipettes can be kept something somewhere like this. You know you can have system where you are not exposing them out and you have a sterilized pipette tips, which are kept inside something like this. If the pipette tip sitting there on the bench which time to time are sterilized and every time you use somebody comes with a fresh pipette box, pipette tip box and kept it there and remove it and again replace it some new pipette tip box.

So, these are some of the basic things, what you prefer to have inside the dissecting microscope or dissecting laminar hood. So, let us summarize what all you need to have you need to have a microscope first a dissecting microscope of course, if possible connected to the camera and a viewer with a computer or some CCD device, where you can monitor. Second you have to prefer to have a dry sterilizer, third a dedicated pipette and pipette tips sitting out there. So, now, we added one more component into it the laminar flow hood in outer facility ok.

So and where you wanted to put the autoclave. So, my preference will be have an autoclave inside a small table top, you can have a big autoclave outside maybe on the side of the corridor or somewhere, but a small one out here may not be a bad idea, and you have the water supply we talked about it. So, from here in our next class, we will further build up the story, how this whole facility will eventually look. Because we have just started on the outer layer and there are few other things, what I am going to highlight

here before I move into the inner chamber or the inner culture room, where other things will be taking care.

So, thanks for your patient listening, once again we will continue in the next week for the development on these layout design and instrumentation.

Thanks.