Experimental Biotechnology
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Module-I
Basics of Laboratory Research

Lecture-04
Laboratory Instruments Operation (Part-2)

(Video Starts: 00:23) (Video Ends: 00:57) Hello everybody this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati.

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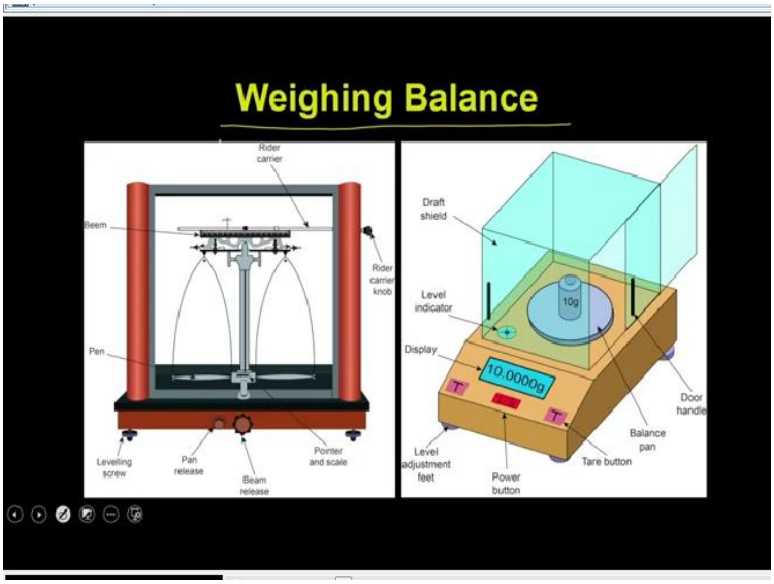

Now in today's lecture, we talk about weighing balance. So, we have 2 different types of weighing balance, we have the analytical balance or we have the digital balance, the working principle remains the same as far as the whether it is the analytical balance or the digital balance, what happened is that in analytical balance the balance is being protected within a wooden chamber where on the both side you have the doors, through which you can be able to open the door and keep use these pans.

And then you have the 2 pans, one which are going being connected with the rod and then these rods are being kept on to a middle pillar and with the axis here. So, you can imagine that if I have to measure the 10 milligrams of compound. So, what I will do is I will keep the 10

milligrams of weight on to this pan and then I will start putting the powder into this pan. So, what happened is when I put the 10 milligrams of pan that actually is going to exert pressure onto this pan and because of that the central rod is going to be tilted.

And then you are going to add the powder into this side and you will keep adding until this rod is again going to be the straight. So, in the beginning the rod is going to be like this. And when you add the rod will come down and it will go into, you know, equalize and by doing so you can be able to measure or you can be able to weigh the amount of that particular compound. You might have seen these kind of analytical instruments in some of the jewelry shops.

And other places which are now eventually been replaced by some of these analytical instruments. And whereas in the case of the analytical instruments or whereas in this case of digital instruments, what you have is you have a digital display, you have a button for tearing, so that it actually makes the 0. And then you have the balance pan and then on this pan, you can actually keep your object.

And then you can measure, you have the 2 doors on both the sides that can be open and close and this whole chamber is been enclosed in a glass chamber. So, let us see how the digital balance works.

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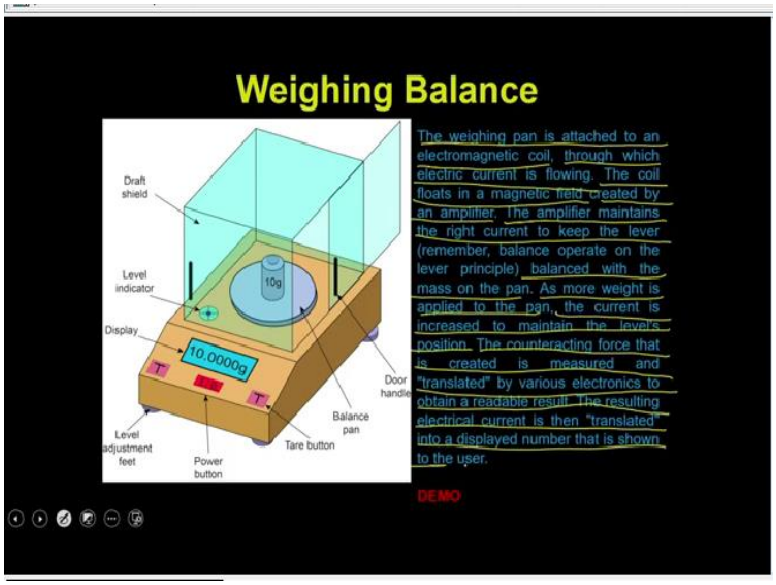

So, the weighing pan is so in this case, the mechanism is little different that the weighing pan the place where you are going to keep your substance to measure. It is actually attached to a electromagnetic coil. So, it is actually attached to a coil and through which the electric current is flowing, the coil floated in a magnetic field created by the amplifier, the amplifier maintains the right currents to keep the level balanced with the mass on the pan.

As more weight is applied to the pan the current is increased to maintain the levels of the positions, the counteracting force that is created is measured and translated into the various electronic obtain to readable result. The readable result current is then translated into a display number that is shown into the user. So, what happened is this pan is being connected to the coil. And that coil is being supplied with the electric current and because of that the coil will remain into the central positions.

And when you are actually applying a pan or you are keeping anything that is actually displacing the position of that particular coil. So, the position of the coil is being changed, you actually apply the current, so that the magnetic field around that coil is going to be increased. And because of that, the coil will again come back to its central positions. So, the amount of current what you have applied is actually been resulted into a particular type of force.

So, if you are actually applying the electromotive force to bring this coil to a central positions that can be converted into the amount of the weight what you have put, because the weight what you have put is actually pressing the coil into the horizontal side and then you are applying a particular electromotive force to bring the coil into the normal position. So, this actually can be calibrated, and then it actually can be displayed in the form of the milligram or grams, like that.

So, that is a working principle of the digital display weighing balance and let us see how you are going to use that. And what are the precautions you should take when you are actually using a weighing balance. **(Video Starts: 06:01)** In a good lab practices we are now going to show you the demonstration of the weighing scale. And so there are different types of weighing scale which are available in different labs.

For example in the chemistry lab, you have the weighing scale because of a larger capacity where you have the pan weighing scales, where you have the, you know, 2 pans and you can use the weight on one side. And you can use the powder on the other side and that is how you can be able to weigh the solid amount. And in the other places, you also have the different types of weighing scales because depending on the type of work, what they are doing.

So, in most of the biotechnology or the molecular biology labs, you will have the 2 different types of weighing balance, one is called as the core balance or the larger scale balance which actually goes from the milligrams to gram weight. And then you have the fine balance, which actually goes from the sub milligram range, which means you can actually be able to measure the milligram, the weight from you can be able to weight the substances from the 0.1 milligrams to 100 milligram range.

So, this is the instrument which you can use for the fine balance, which actually goes from the 0.1 milligrams or 0.01 milligram to 100 milligrams. Whereas, this is the course or the bigger balance, which you can go from the 1 milligram to 100 mg 1 milligram to 220 gram. The both of the balance the weighing scheme or the variety is almost the same in both of these things, what you have is you have a pan on which is actually being kept on to a spring and depending on the weight what you are going to put. So, once you put the weight onto this pan get pressed inside.

And by pressing of the pan, it actually causes tension to the underlining springs. And because of that it actually and that how much the spring is going to be compressed. That is a weight it is actually going to give you that weight because that is the already means calibrated in the system. That is why when you start the machine you have to ensure that 2 parameters. When number 1 weighing balanced because it is depends on the how much the pan is going to go inside the weighing balance has to be kept on to a plain surface.

The weighing balance has to be kept in a way, so that it should not have any kind of misbalance, for example this side or this side down. So, to ensure that the main balance is on plain surface the main scales are always been having a bubble in the machine and these bubbles have to be present

in the center. So, as you can see, the bubble is there and that bubble is present in the center of the ring.

So, that should be the case. So, when you start the machine in the beginning what you have to do is you have to ensure that bubble is in the center of the machine and then you can start weighing, so you can see we have a glass cage on top of this machine. And this glass cage is only to ensure that there will be no injury of air because the air is also going to exert pressure onto the pan. And that is how it may actually give you the wrong readings.

So, suppose I would like to measure the glucose and would like to prepare the 10 mg per ml glucose solutions. So, what I will do is I will first going to measure the eppendorf the where we are going to weigh the glucose and then subsequently we are going to add the glucose. So, what you have to do is without disturbing the weighing balance first you have to open one side of the glass door, very carefully, you have to put your eppendorf.

And what you can see is that the weighing balance is giving me a reading of the eppendorf. Now, I have to nullify this weight so that I will be able to know the weight of the glucose. So, there is a tare button. So, there is a tare button is just to make the weighing balance or the weight of the eppendorf. So, what I do is I press the tab button. And what you see is that the, it has now showing me a weight in the gram.

Now what I will do is I will remove this eppendorf I will open the tube button. So, when you want to measure any solvent or any when you want to weigh any powder and while you are opening the cap what do you have to ensure that the rim of the cap or rim of the bottle is very clean, should not have any salt or anything deposited. So, you can actually clean this. So, that it should not get into your bottle.

And that is how it may should contaminated your compound. So, what you can do is you can take the small amount of tissue paper and what you can do is you can just clean your rim of this tube. So, that it whatever is compounded or dust is present on the rim should not get into your

bottle. Now, you take out the salt or the substances we have the different amount, different types of spatulas.

So, this is a biggest spatula, if you want to measure in the gram range, which is a middle spatula or middle range spatula, if you want to do it in the sub gram range and this is the thing which you want to measure if you want to measure. If you want to weigh the very small amount, so in this case we want to measure only the 10 million. So, what I will do is I will just take the small amount of spatula and put it into the eppendorf okay.

And as soon as you are done, just close the bottle first and then you are going to measure and as I said very carefully, you have to put your things so that you should not press or you should not disturb the pan forcefully, then what you do is you close the door and see the reading. So, this is the reading of the 83 milligrams what you have. So, now what I will do is I take out a small amount of powder from here.

And because we have to make the thing at the 10 mg per ml. So, accordingly, I will take out, and then pour it again. And then, again, I will measure and that you continue so this time is now I have removed a small amount. So, it is going to now showing me the 80 mg. So, what I will do is I will just make the solution according to the 80 mg and then I will take up the things, because it is always desirable, as long as salt the salt is not very costly.

You should not take the salt from the eppendorf and put it back into your main stack, in case the salt is very costly, even then you can just take out and put it into a separate vessel. So, that you can use the subsequent usage. And once you are done with your weighing, it is always important that you should clean your spatula first with the tissue paper. And then, with the help of the water. So, that there will be no leftover salt being deposited onto this.

Also when you are actually measuring the corrosive substances such as the sodium hydroxide or other kinds of corrosive powder, you have to ensure that it should not be remade onto the spatula that time you have to clean the spatula with the water, followed by that you remove the water or

the moisture using the tissue paper. So, the same procedure you have to follow for even for the fine balance as well. **(Video Ends: 14:59)**

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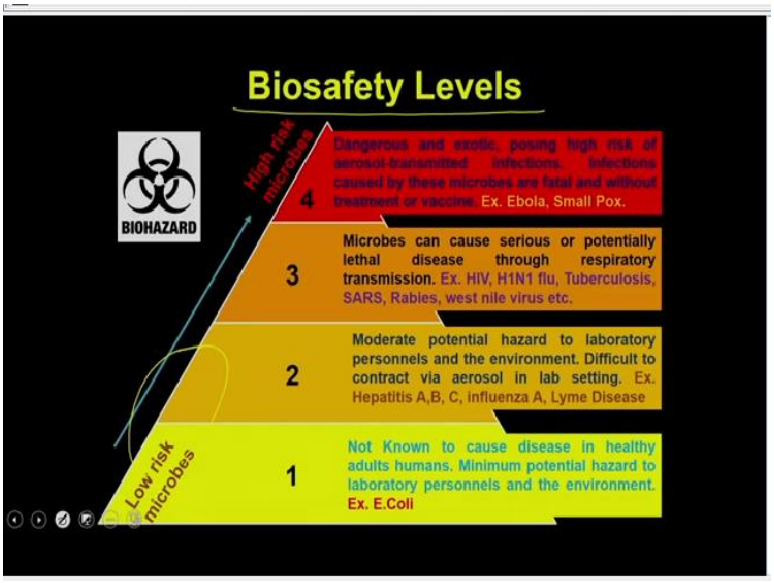

Apart from that, if you remember, a in the previous lecture we have discussed about the different types of the biosafety levels. We talked about that there are 4 biosafety levels, biosafety level 1, biosafety level 2, 3 and 4 and all these biosafety levels are being classified based on the amount of risk. It has been associated. So, the biosafety level 1 and 2 are associated with the very low or the moderate risk.

Whereas the biosafety level number 3 and 4 are associated with the high risk. So, all these biosafety levels microbes are being handled in a clean benches, or the benches which are being meant for the handling these kinds of microbes. So, we are going to discuss about the laminar hoods, what is being used to handle the low risk microbes like, which are falling into the biosafety level 1 and 2.

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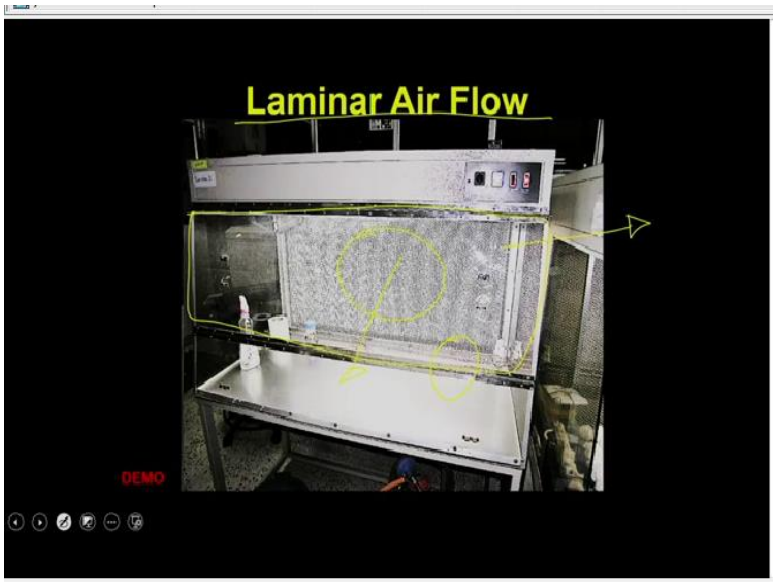

So, this is our typical laminar air flow and in a typical laminar air flow, what you have is you have a filter through which the air is coming into towards you and then it is having a glass panel, which is actually been protected from the UV and then you have a place for turning this whole chamber into the UV. So, that you can be able to sterilize this chamber. And then you also have the other kinds of things, you have the lamp.

So, that you can actually do the heater sterilizations. So, let us see how you can be able to use a laminar flow and what are the precautions you should take while you are using the laminar flow and what are the different types of cleaning procedures, you have to follow while you are using the laminar flow. **(Video Starts: 16:52)** So, in this video I am going to show you the equipment laminar air flow chamber.

So, when using the laminar flow chamber what are all the precautions we have to take and how to use it. So, basically this equipment is used for the hope of maintaining the aseptic condition. If we are working on some biological samples, some plant tissue, some animal tissues. So, we need to make the aseptic conditions. So, if you want to remove all the contaminants. So, for that purpose we use this laminar airflow chambers.

So, basically the equipment is containing 4 switch here, one is the main making it on and off, another is maintaining the flow that is low and high. The third one is the UV light and this one is

the impulsive light clean white tube light. So, this equipment is basically works on the principle. First of all has the filter bed, then blow up and then inter filters. It contains the filter bed, just to filter the air or the dust particle from the air.

And then it has the blower to pass the air and another filter that is HIPAA filter. HIPAA stands for the high efficiency particulate air filter. It is HIPAA filter. So, HIPAA filter is basically it can filter out all the microorganisms, including bacteria, fungus and all. So, let us understand how to use this. So, first of all, when you do this we have to make it on by this switch main right, we have to lift the lid. Simultaneously, we have to switch on the tube light.

Before using this laminar air flow chamber we should sterile all the area using the 70% ethanol, with the help of some tissue paper. After wipe over with the tissue paper we need to sterile all the facets and blades, we are going to use for your work by using a 70% ethanol. We have to use the beaker sterilization also, after switching on the lamp we need to sterilize blades and facets up to air hot condition.

After sterilizing the floor of the laminar and the faucets and blades we need to close the ethanol and that flame also. After closing this we have to make this UV on, now when the UV light is on stay away from the laminar, because UV light can be dangerous to the operator also. And you will like has to be switched on for 15 to 20 minutes for sterilizing all the areas. And we can also keep if we want to some media and something to get stabilized by the UV light we can also keep that inside.

As you can see the flow of the air from the inside to outside that the filter air from the inside is coming to outside because we have to maintain the aseptic condition inside. So, the air inside is completely sterile without any contamination or without any dust particles. Now after making it all up to 20 to 30 minutes now we can use it for any type of our biological work like transferring of any plant or animal tissue.

So, this is our media in which we are going to transfer our sample. So, let make this flame again, sometimes we can use it like half closed or fully open up to your convenience. We have to make

sure we have to sterilize our hands properly with 70% ethanol. And after sterilizing we have to keep our hand inside the lamina area. We should not take them out while using. Now this is our media in which we are going to transfer our sample.

Now, let me tell you how to handle the media, how to re-sterilize, re-sterilize this opening up. So, we have to take out the cotton plug in this fashion. Now after taking out we need to re-sterilize the opening area. After this re-sterilize using any faucet, we can transfer our sample inside the media. And then after transferring we have to again re-sterilize the opening and then we can close it by using that cotton plug.

After finish your working we need to clean it again the floor, first we need to switch it off the flame. Thank you. I hope you understand how to operate this and what and all the precautions we are doing and what are the principle on this it works. **(Video Ends: 25:11)**

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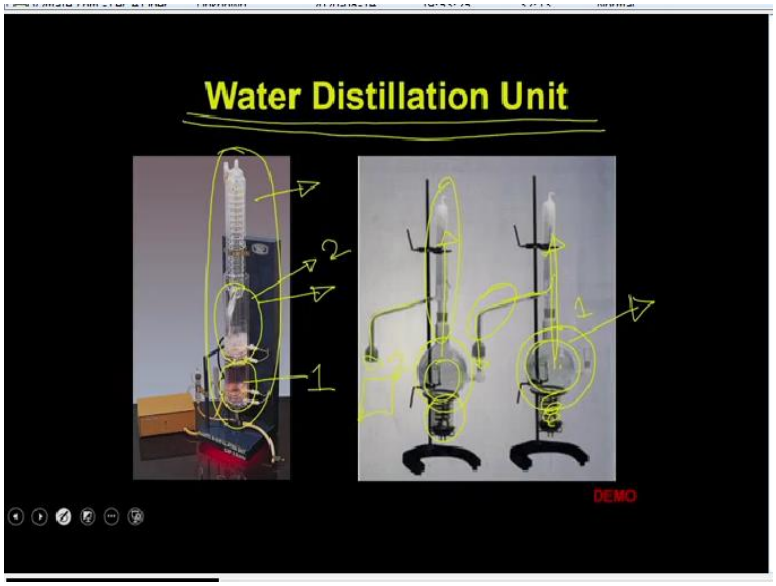

And let us move on to the next system, the next system is the water distillation units. So, the water distillation units are being used mostly in the chemistry lab, as well as some of the biology lab and these are been mostly been used to do the distillation of the water so that the water what you are getting from the tap is been get distilled twice or you know, single distillation units. And that is how you are actually going to remove the particulate impurities from that particular water.

So, in a typical water distillation unit like for example this is a double distilled water distillation unit , you can have the integrated system where you can have the both the chamber together or you can have the different system where you can have the 2 chambers separated and connected to a condenser. So, whether this is the integrated system or a separate system, what happens is that when you turn on the coil into the first chamber.

The first chamber the liquid goes into the, it starts boiling okay. And then it goes into the condenser, from condenser the water get condensed and then it goes into the second chamber. So, this is the chamber number 1 and this is the chamber number 2. And that is how that condensed water is comes into this chamber and then this in this chamber it again been boiled with the help of the another heater.

And that is how it goes into the condenser and then from condenser it gets condensed and then you can be able to collect this particular liquid into a vessel. So, this is called as the double distillation units. What is the advantage of double distillation unit is that the water is been more purified compared to the single distillation unit. Similarly, the same thing can be done in an integrated unit where you have the 2 chambers.

This is the lower chamber which is being used, which is almost equivalent to the chamber number 1 and this is the top chamber which is equivalent to the chamber number 2 and both the chief chambers are connected to the single distillation operators. And we are going to take you to my lab and I will show you how to use this distillation units and what are the precautions you should take. **(Video Starts: 27:47)**

Today we are going to give you a small demo about how to utilize or how to use the water distillation unit. So, in a double distillation water distillation unit, you have the 2 chambers, what you have is a small chamber, which is a lower chamber and a top chamber when you turn on the water, the water comes into the first chamber and then when you turn on the heater is actually going to boil from here, it is going to be distilled by this condenser.

And then the water will not come outside but instead, the water is going to fall into the second chamber. And once the second chamber will be almost more than 60% full, then you can actually be able to turn on the second chapter and then from second chamber, when the vapor will evaporate they will be get cooled by the condenser and then the water will come out from this particular nozzle.

So, let us see how to operate this water distillation unit. So, before you start the water distillation unit you have to ensure that you have the adequate water into the lower chamber and as well as the water is well connected to the condenser because that does not happen, then the water will start boiling from the lower chamber and then we just start eating the whole unit and ultimately it may actually cause the damage to the unit.

So, first what you have to do is you first have to open the tap, so that it will start filling the water into the lower chamber. If you see the tubing, it is actually been connected to the condenser, so the same water source it is actually circulating the water into the chamber, as well as the same tubing is also giving the water to the lower chamber and then the excess water is actually been coming out from the this waste tubing.

There is a sensor also being kept here, which actually monitor the level of the water. So, if the level goes down below to this level, suppose someday you the water is not flowing and you turn on the machine, then the water level we go down to this unit into the sensor**,** the machine will stop working. So, let us start with this. So, first what you have to do is, first you have to turn on the first chamber that enter in the first chamber.

So, what you see is this is the controlling unit, so first you have to press this button and that actually will going to give the power into the lower chamber. And then what you see now is the heater is heating and then it is actually boiling the water into the lower chamber and then slowly you will see that this water will evaporate and condense from the condenser and then the water will fall into the upper chamber.

And I will show you if there will be any disconnection or if you suppose the stop the supply of the water then it is actually going to give you an alarm and it will stop the connection. For example, if I remove the sensor. It is actually going to stop the machine. So, that is kind of a control present in this particular machine. So, that there will be no damage because if the machine is running without the water it needs.

Then it is actually can cause if it get damage and it may also affect your because this is made up of a glass. So, if the glass got broken it may actually cause the injury to the students as well. So, with this I would like to conclude my demo here, what you can see now is that the water is boiling into the lower chamber and then slowly you will see this water will go into a double chamber. **(Video Ends: 31:39)** So, with this I would like to conclude my lecture here. Thank you.