Experimental Biotechnology
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Module-I
Basics of Laboratory Research

Lecture-03
Laboratory Instruments Operation (Part-1)

(Video Starts: 00:23) (Video Ends: 01:00) Hello everybody this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. Now in today's lecture, we are going to discuss about the different types of instruments, what you are going to operate into a common biology, as well as the chemistry laboratories and what are the precautions you should take while you are operating these instruments. And how you can be able to maintain these instruments.

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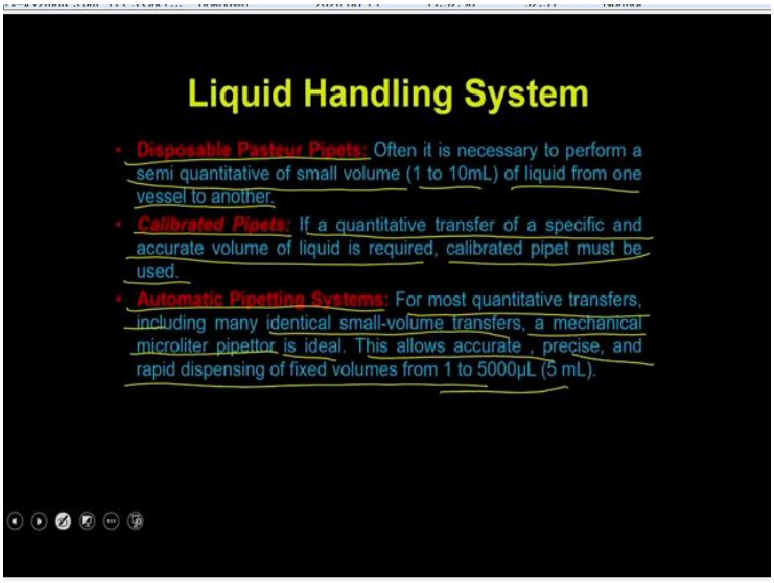

So, the first thing what we are going to discuss about is the liquid handling system. So, when in any biology laboratory or even in the chemistry laboratories, you will have to use the different types of the different types of liquid handling systems like the these are the systems which you are going to use to dispense the different amounts of the liquids and let us see what are the different liquid handling system you have.

You have a disposable pasteur pipette and disposable pasteur pipettes are mainly been used to dispense the semi quantitative small volume liquids like from 1 to 10 ml. And it is normally been used to transfer the liquid from one vessel to another vessel instead of adding the, you know very quantitative or accurate amount of liquid. Then you have the calibrated pipettes. So, if a quantitative transfer of a specific and accurate volume of liquid is required you can use the calibrated class pipettes.

Apart from the calibrated pipette, you also have the automatic pipetting system. So, for most to quantitative transfers, including many identical same volume, transfers a mechanical microliter pipettor is ideal. This allows the accurate, precise and rapid dispensing of fixed volume from 1 to 5 ml. So, these are the multiple options what you have when you are talking about a liquid handling systems.

And let us understand how these liquid handling systems are actually withdrawing the liquids and the dispensing the liquids.

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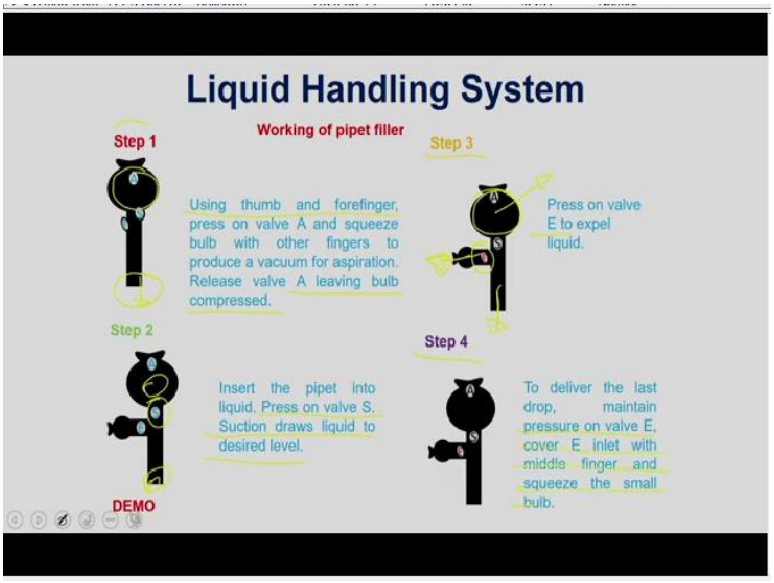

So, in a typical system or suppose I have taken an example of the bulb. The rubber bulb, what you have is a actually rubber bulb where you this end you are going to connect the pipettes and how the rubber bulb is going to work in the step 1, you are going to use your thumb and the

forefinger to press the wall A which means the bulb actually you are going to press. And that actually is going to squeeze the air from this pipette filler.

So, that actually is going to make the bulb compressed and in the step 2, you are going to connect the pipette to this and then you are going to insert the pipette into the liquid and then you press the wall S, as soon as you press the wall S you are actually going to allow this particular wall to draw the liquid from the liquid from the liquid vessel with the help of the suction. And then in the step 3, you are going to press the wall E.

So, once you press the wall E, you are actually going to expel the liquids from the pipette what you have withdrawn because when you press the bulb A or press wall A, you are actually going to expel the air and when you connect the pipette and dip it into the liquid, it is actually going to draw the liquid. And then if you press the wall vacuum. And as a result, the liquid is going to be dispensed from your pipette and then step 4, because the last drop of the material will remain into the pipette, you have to press the wall E.

And cover the E inlet with the middle finger and squeeze the small amount of the bulb. These are the 4 steps, in the 4 steps what I have tried to explain you is that irrespective of the different liquid handling systems, whether it is the calibrated pipette or pasture pipette or the automatic pipetting system, you are actually simply using the different ways to generate the vacuum.

And then with the help of these vacuum you are actually drawing the liquid and then ultimately you are releasing the vacuum and once you release the vacuum, the liquid will dispense into another vessel. So, with this I would like to show you some of the different types of liquid handling system and how to maintain that pipettes. **(Video Starts: 05:50)** Hello everybody this is Dr. Vishal Trivedi from department of biosciences and bioengineering and today I am going to introduce you to liquid handling systems.

So, in this series first we are going to show you the automatic pipettes. So, the automatic pipettes are being used to handle very liquous, small amount of liquid to very large quantity of liquids and that is why the these automatic micropipettes are coming into the different volumes. For

example, you have micropipette which is from the 0.2 to 2 microliter. So, this pipette is always been used in the range of 0.2 to 2 microliter and the may know what you can dispense from this pipette is the 0.2 microliter and 2 microliter.

For the practical purposes, as well as for considering that the handling might have some errors. This pipette is never been used less than the 0.5 microliter. Similarly, we have the 2 to 20 microliters pipettes, so in 2 to 20 microliter pipette you have the minimum, what you can dispense using this is 2 microliters and the maximum is 20 microliter. And in the same, same way you have the 20 to 200 microliter pipettes and 300 to 1000 microliter pipette and as well as for larger volume, sometimes you can also use the 0.5 to 5 ml pipette.

Now, most of these micro pipettes what you could see is that you have to have plungers through which actually goes in and out. And that actually creates a vacuum within this tube. And by creating the vacuum into this tube, it actually allows a sucking up the liquid. So, the, if you see the dial, the dial is always saying the same number. So, what you have to do is you have to say for example this is right now it is showing the 200 as the number which means it is actually at set as the 200 microliters, so suppose I want to be I want to dispense the 100 microliters.

Then what I can do is I can use this top knobs and I can just set it to the 100 microliter by rotating it the clockwise. And once it reaches to the 100 marks, then you are set that you have set the suction pressure of this plunger to the 100 microliters, which means when you press this plunger, it is actually going to suck the 100 microliters air into the tube and because the pipette is connected to a tip.

The tip is also going to suck the 100 microliters of the liquid. So, let us see how you are going to use this pipette to dispense the liquids and what are the different precautions, you have to take. So, depending on these pipettes, you have the different types of tips. For example, for 0.2 2 to 2 microliter you have the micro tips. Then you have the microchips, which are in the range of the 2 to 20 micrometer or 2 to 200 microliter.

And then you have the bigger tips which can go up to 100 micrometer to 1 ml. So, now I am going to demonstrate how you can be able to dispense or how you can be able to withdraw the liquid utilizing these micro pipettes and what are the precautions you have to take. So, when you want to connect these tips what you have to do is that you bring to the pipette to the tip. Then what you have to do is you have to push it really hard and then you have to ensure that it is properly been connected because ultimately you have to generate a vacuum into this tube.

So, if there will be anything which is leaky or if this tip is not properly connected, it is actually going to suck up the liquid or suck up the air from the outside and that is how it is actually going to give you the error prone liquid handling. So, once you are sure that it is properly been connected what you can do is you can take your liquid okay. And you have to ensure that you should not submerge this whole tip into the liquid.

What you have to do is you take your bottle, dip the end of this tip and then you have to suck, while you are doing this sucking as well as the dispensing of the liquid what you have to do is, what you see is this plunger actually goes up to the end of this tube okay. And then it stuck. So, this is the place where you have to stop. If you push forcefully, you could be able to generate, even the higher vacuum than the what is being set.

So, for example if I am setting at 100 microliter. If I press the little hard I could be able to generate the vacuum which is equivalent to the 120 or 130 microliter which means the liquid what you are going to suck by doing the forced push you are going to suck the higher amount of liquid. So, that is why you have to be very careful when you push this plunger, it should stop at that point and then only you should start sucking.

So, what you have to do is you first push it up to the lock period, then you bring it to the tip. The fine tip of the end of the tip and then bring it into the liquid and then slowly, you have to release the plunger, you should not release the plunger in a very, very fast mode, because if you do so. Then also, the amount of liquid what you are going to withdraw is going to be lesser than the your desire amount okay.

So, you have to be very careful that you should push the plunger up to the lock position, then you have to suck it very slowly, without submerging your tip end okay. Once you have sucked the liquid into the tip, then you have to dispense this liquid. And when you dispense this liquid you have to ensure that your suck goes up to the lock period, and then you have to push little hard because.

By doing so you will be able to dispense the last drop of the liquid from the tip. Also one more precaution what you have to take while you are taking the liquid out from the bottle or some reservoir is that if you see there are some water particles which are going to be attached outside the surface of this tip. So, that you have to wipe up so that you should not have any liquid which outside the tip.

And then you dispense as I said you dispense and then you see the last drop is still there, which means that you are actually going to do the under dispensing. So, to dispense the last drop, you have to push a little harder beyond the lock period. Apart from these precautions because most of these micropipettes are using the spring and the spring is present into the body of the pipette, you also have to take a lot of precautions when you are actually withdrawing the corrosive liquids.

For example, if you are withdrawing the strong acid, strong base or any solvents. So, most of these pipettes are having a rubber gasket at the end of this tube and that rubber gasket is very sensitive for the organic solvents, as well as when you are taking other media or other kinds of microbiology stuff. You also have to be ensured that you should not suck these liquids or the paper inside these tubes.

So, that it should not get contaminated. So, that bacteria will start growing into this tube. Apart from that when you are withdrawing the corrosive liquids, for example the strong acid or the strong base you have to ensure that you are going to use the tips with the filters, as well as you have to ensure that you should not suck this liquid inside because if you suck the very strong acid or the weak strong base, you are going to destroy these spring, at the end of this tube.

And that is how it is actually going to stop working or it will start showing the malfunctioning. Apart from this, it is a good practice that is supposed currently I am using a pipette of 20 to 200 microliter. But suppose I am withdrawing the liquid which is around 100 microliter and I am doing all this for the whole day. But, it is always desirable and recommended that by the end of the day, when you will leave the lab you should put this pipette back to its maximum position.

For example in this case the pipette is from 30 to 200 micrometer, but I have right now dispensing the liquid which is equivalent to 100 microliter. So, what I will do is by the end of this today or by the end of the day when you are going to leave the lab, you are going to dispense or you are going to rotate the, you know the tube turning knob and bring it to the 200 microliters. So, that by doing so when you turn it to the 200 microliter, you are actually going to reduce the tension which is there on the spring.

Because when you are bringing it get to the 100 microliter, you are bringing to tension to this spring and that is all it is actually pushing little less okay. So, at 200 microliters the spring is going to be in a relaxed position. So, when if you are not using it, it should be at a relaxed position, so that that spring what is being used to work as a sucking device in this pipette should last for a longer period of time.

So, with these precautions if you use this pipette, you will be able to dispense the liquid more accurately and you will be able to perform the experiments more smoothly, what we have discussed, we have discussed so far about the different types of automatic pipettes which you can use to deliver the liquids, but the pipettes are actually been used only up to the level of, like few mls.

But if you are interested, to dispense the liquids of the higher volumes you can use a even the glass pipette connected to the pipettor or the pipettors, but before getting into the details of a glass pipettes I would also like to show you a another pipette which people are very oftenly used for performing the cell culture based assays. So, this pipette is called as the multi channel pipette. So, what you can see is that this pipette has the 8 different nozzles.

So, that you can be able to withdraw the same amount of the liquid for 8 different tips and that is how it actually expedites the process. So, in most of the pharma companies or the biotechnology company where the scientists are performing the cell based assays to screen the compounds or even the other kinds of applications, you are using this kind of multichannel pipettes. The basic principle, or the working principle is remained the same that you have to set the volume which you would like to withdraw and then you have to press the plungers.

And then you have to deposit, all the tips into the media or reagent which you are interested to add to the cell culture dishes. And then you have to dispense it according to the same way and then it has a ejector, which actually can be used to reject all 8 tips at the same time. And then you can actually, you know, actually you know attach to a few more new tips and can be able to dispense the liquid and perform the cell based assay.

So, that this kind of multichannel pipettes actually reduces your efforts to finish the work and also actually save the time and the effort from the human being. So, apart from this, if you are interested to dispense the large quantity of the liquid to, even suppose you want to feed the cells with a large quantity of the liquid. Like liquid media like 5 ml, 10 ml 20 ml and 25 ml, then you can use the class pipettes.

So, this is a typical glass paper, which is a 10 ml glass pipette. And the only thing what you have to do is when you are basically trying to use the pipette for dispensing the large volume liquids, you cannot do the mouth sucking, because if you do the mouth sucking the most of the corrosive liquids or other kinds of liquids can actually directly get into the mouth. So, that is why it is not advisable to use the glass pipette with a mouth sucking, instead of mouth sucking you can use the pipettors.

So, which actually can do the same job and it can actually used more, you know, with more safety and it can be more it gives more, you know, useful or reliable. So, you have the 2 different types of pipettors, one is called as the manual pipettor, where you have the body. And you have a plunger which, if you run this wheel, what will happen is this plunger goes into upward

directions and that is how it actually can suck the liquid and once you are done with this, you can actually press this plunger and that is how it actually going to withdraw the liquids.

So, let us see how to use this manual pipette. So, first you have to do is you have to connect this glass pipette to your pipettor. And make sure that there is a no leakage of the air because the working principle remain the same whether it is an automatic pipette or the manual pipettor for sucking the liquid, because**,** there will be a gap between the glass and the pipette, then it is going to suck the air instead of sucking the liquid.

So, once it is connected tightly, then what you can do is suppose we have to be withdraw the liquid from a bottle. And so, you can dispense or you can submerge this tip into the liquid and then you will slowly turn this knob, so that this rod will go in the upward direction. And then, what you see is that it is actually sucking the liquid into this pipette and since we have maintained the vacuum the liquid will not fall.

So, then what you can do is you can bring the liquid to the 0 position, simply by turning this knob in a reverse direction. And what you can see now is that there is a level of the liquid up to the 0. And when you are seeing this kind of liquid what you have to see is the lower end of the meniscus because what you see there is a meniscus or there is a bubble, half bubble and the half bubble has the side corner and the middle depression.

So, ideally, people accept that you should use, you should be able to see the lower end of the meniscus. And that is the accurate position of knowing that it is reached to the 0 point or not, once it is reached to the 0 point and suppose I have to dispense the 4 ml. What I can do is I can just simply press the button. And that actually will start dispensing the liquid. And that is how when it reaches the 4 you can just stop it.

What you have to do is always bring the glass to next to your see, eye level. So, that you will be able to see the meniscus very accurately. So, now once you are done all the pipetting, then you can just leave the vacuum and that is how actually it is going to dispense a liquid to, but you can

see is now we have a very last drop of the liquid left in the pipette, so to dispense that what you can do is you can just simply turn this rod down.

And that is how it is actually going to dispense, even the last drop of the liquid from the pipette. So, this is the manual pipettor what you can use to dispense a liquid, but in many cases when the manual pipettor is actually very, very, you know tiring and it is very taking a lot of time because you have to every time you have to use this wheel to, you know, suck the liquid and then you dispense a liquid to avoid that people are also using the automatic pipettor, the working principle remains the same, that in a automatic pipettor you have the 2 buttons.

One is to suck the liquid in the upward direction, which is this button okay. And the second button which is actually going to release the vacuum. So, that the, the liquid is going to be formed from the pipette. So, the mechanism remain the same you have to first connect your pipette very tightly to the tubing of this pipettor and then you have to cut the submerge the lower end of the pipette into the glass into the liquid, and then you pres this button.

And it is actually going to withdraw the liquid from the bottle and then what you have to do the exactly the same, you have to use the other end and make sure that the liquid reaches to the 0 point and then you can just press this button the lower button and that actually is going to release the liquid into your reagent or your cell culture vial or something okay.

And that is how you can be able to do the pipetting, in both in this pipetting, you have a filter which is connected to this body and that filter is connected to a vacuum pump. So, when you press this knob, it actually runs the vacuum pump. So, if it runs the vacuum pump in a clockwise direction, it actually sucked the liquid. If you run the vacuum in a reverse direction then it actually releases the vacuum.

And that is how it actually suck the liquid from your bottle with the help of the glass pipette. Since this pipettor is always been or mostly being used in the cell culture facilities where you are actually dispensing the liquid into the cell culture with pipettor also has the 0.2 to 2 micron filter

attached to this. So, that you should not when you are actually pouring the liquid into the cell culture dishes.

You should not pour the air or what should not pour the bacteria. Because of that, you should not stop the liquid beyond this. Otherwise, if the liquid will go into the filter the filter is going to be trough and then you will not be able to suck the liquid anymore. So, to avoid any kind of clogging you have to ensure that you should suck the liquid only up to this point. And because that is good enough for you to maintain you know the wide at 10 0 point okay.

And therefore you can be able to avoid the blocking of the filter. So, this is all about the different types of liquid handling system what we use in the laboratory to handle there are different types of solvents whether it is a cell culture solvent or corrosive acids. In both cases, you have the different options to use the different types of liquid handling system. **Video Ends: 27:29)**

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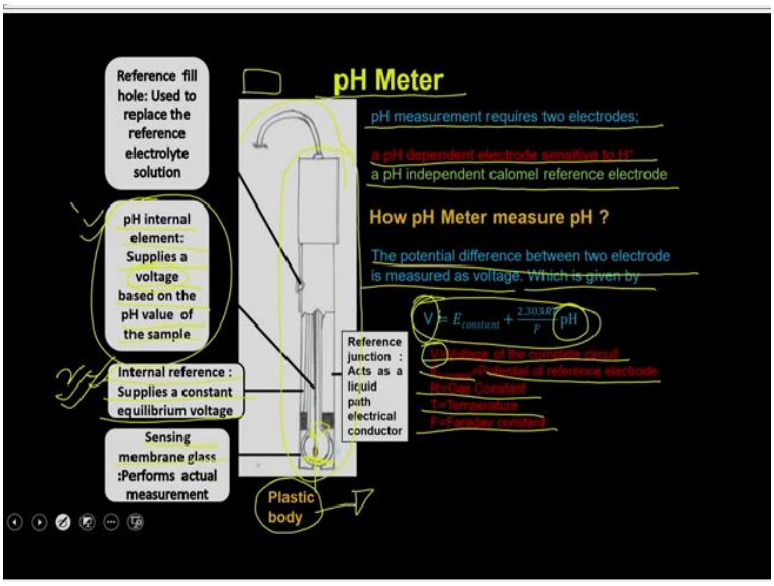

Now let us move on to the next system and next system is called as the pH meter. So, this is a typical pH meter what you are probably might have used or you might be seen in your laboratory. So, in a typical pH meter, what you have is pH meters are either been made up of the plastic body or to the glass body, depending on the different types of pH meter what we have. And in a pH meter, what you have is you have a body or processing units, of which is actually just been used for the display purpose.

And then you have the electrode, which actually is you used to measure the pH, in a pH electrode what you have is, you have the 2 different electrodes, you have an internal electrode which actually supplies a voltage based on the pH value of the sample. So, you have a pH internal elements which actually going to respond to the number of hydrogen ions present within the solution and then you have the internal reference electrode which actually going to supply at a constant equilibrium voltage.

Apart from that, you are also going to have the sensing membrane glass, so you are going to have a sensing membrane which actually allows the selective movement of the hydrogen ion into the pH probe. So, that it would be able to generate the voltage and that voltage can be used to measure the pH of that particular pH electrode. So, how the pH is actually measuring the pH of a solution is that pH measurement required the 2 electrode.

A pH independent electrode sensitive to the H + ions. So, this electrode. And then you have a pH independent calomel reference electrode which is this one. And what happened is when you dip the pH probe into a solution, it actually causes a potential difference between this electrode and between this electrode. So, this potential difference between the 2 electrodes can be measured in the form of voltage.

And this voltage is always been given in the form of an equation which is called as, V is equal to E constant + 2.03RT by F and the pH. So, where the V is the voltage of the complete circuit which means the voltage of the full circle, then E constant is the potential of the reference electrode which means the voltage of this particular reference electrode, R is the gas constant, T is the temperature and F is the faraday constant.

So, the voltage difference is what you are actually going to measure and that can be converted into the pH of that respective solution and that is going to be displayed by the display unit, but the pH meter has to be operated with a multiple steps. And let us discuss that.

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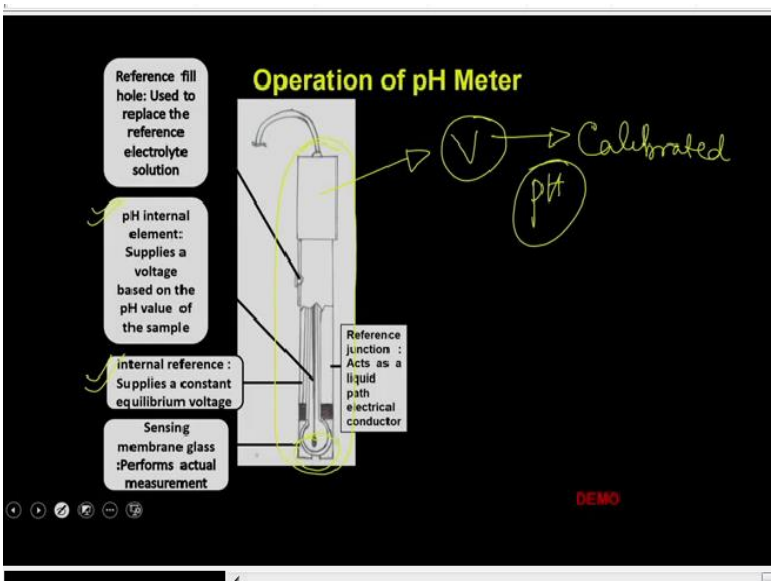

So, in a pH meter as I said you know you have a pH electrode and this pH electrode is going to be dipped into the solution where you are actually interested to measure the pH, but before doing that because the this pH electrode only knows that what is the voltage difference between the internal electrode versus the reference electrode. So, this V has to be calibrated with the help of the different pH solutions.

And then only the pH electrode will know that what pH, they are actually measuring. So, for that purpose the force what you have to do is you have to turn on the machine, you have to dip it into the different standard pH solutions. And then you have to measure. So, the pH standard what you are going to use is the 4, 7 and 9.2 and these are the 3 pH solutions you can use the standard pH solution you can use to calibrate the pH.

So, if you are working in the acidic region, you can simply use 4 and 7 and that should be good enough. But if you are working in the basic range, then you can use the 4 and 9.2 and by using this you are actually going to calibrate the pH meter and then you can dip the pH probe into your buffer, or the solution where you are interested to measure the pH and then it has to be completely submerged into the liquid.

And then it will actually going to give you the pH of that particular solution. So, it looks very simple that you have to do all this, but when you are going to operate a pH meter, you have to

take multiple precautions, as well as you have to be very careful when you are handling the pH probe because it is very susceptible for multiple types of damages, because the body is made up of glass and as well as this membrane is very sensitive for different types of reactions or different types of treatment what you are going to do within the solutions. So let us see how to operate a pH meter. **(Video Starts: 32:47)**

So, when you go with the pH meter, the pH meter has a central unit which actually is a kind of a display unit as well as the controlling unit. So, this display as well as the controlling unit is going to show you 2 informations 1, which the top what you see is the pH of the solution where the pH probe is being kept and the lower side what you see is actually the temperature of the solution where you are using this. So, as you can see these 2 information are important.

That is why in all modern pH meters, you always have 2 probes, one is the probe which is actually the pH probe, which you are going to use for measuring the pH. So, what you can see is, it is a glass tube. And at the bottom of the glass tube, you have a membrane and because the glass tube is being protected by plastic covering. So, that you might even if by accidentally, it is going to hit the magnetic bars or some other kind of the liquids.

It should not get broken down because the semipermeable membrane is present on to this bulb what you see, and that bulb is being protected by the plastic cap. So, that bulb should not hit the solutions. So, before you start the measuring the pH meter and suppose you have turned on the pH meter in the morning, what the first thing what you have to do is you have to calibrate the pH meter.

In case you have turn off the pH meter, the day before. And so first thing what you have to do is you have to measure the pH meter you have to calibrate the pH meter utilizing the different solutions and by how to do that, actually, so you can easily buy the standard pH solutions from the companies, so what the companies are doing they are giving you the standard solutions of different pH.

For example, in our lab, we are using the, the pH capsules. So, what you can see is it is a pH capsule of pH 4. So, what you have to do is you have to take this 1 capsule and dissolve it into 100 ml of the water. And once you dissolve it completely it is actually going to give you the pH 4 at the temperature 25. Similarly, we have the capsule for pH 7 and you have also have the capsule for the pH 10.

Because what you are supposed to do is you have to calibrate the pH meter in a range where you are more oftenly using the buffers, for example, if you are using the buffers of pH 5 or 6, you can use the pH 4 and 7 or sometime you even you can use the pH 10 as well so that you are going to do the 3 point calibrations. So, when you want to calibrate the pH meter what you are supposed to do is you are going to make the solution of the individual standard solutions.

And then you incubate or you put your probe into that standard solutions. And then what you do is, there is a there is a button for the calibrating the pH meter so you can just press this button. And once you press this button, it actually will going to ask you, what kind of pH solution you are or what kind of standard pH solution you are immersing. So, you can say that okay I have put the pH 4 standard solutions.

So, it will take up the that particular solution as a pH 4 solution, then once the pH 4 is over, then you can take up the pH 7 and then subsequently you can take up the pH 10. So, by doing all the 3 point calibrations, it is actually going to draw the calibration curve of utilizing the standard pH and at the end it is actually going to give you the correlation curve or it is going to give you the correlation factors.

In most of the calibrations when you are doing the 3 point calibrations, you should get a reading of more than 95% which means the pH what you are going to measure utilizing this pH meter, after the calibration is going to be 95 to 99% accurate. And that is very important to do so and that has to be done the at a particular temperature, in which your buffer solution is also present.

So, if suppose you are going to withdraw this liquid from the fridge or suppose you have prepared a buffer and it has been kept in fridge, it is always desirable that or it is always

recommended that you first take out the solution bring it to the room temperature and then only you measure the pH. So, and then only you maintain the pH also. So, all the solution has to be at 25 degrees Celsius.

Because you have calibrated the pH meter at that particular temperature, so it is that curve or the calibration curve what you have drawn utilizing the 3 different pH solutions of 4, 7 and 10 is only holding at T temperature, 35. Now once your calibration is over, then what you can do is you can take your probe and immerse it into the liquid in which you are interested to measure the pH.

And then you can use that solution to then you can use the, depending on the pH for example if it is showing a pH 5 and you are interested to make a buffer of pH 7.4 or 7.9. Suppose you are maintaining a trace pH or trace buffer and you are it is showing a pH of 5, then you can add the corresponding base to maintain the pH, in some cases you can add the NaOH. In some cases you can add the acid to either decrease or increase the pH.

And that is how you can be able to maintain the pH, why you are doing so you have to be very cautious that the magnetic bead, what you are going to use should not be very close to the amenity to the probe. So, suppose for example and I am trying to measure the pH of this particular solution. So, what I will do is I will put the beads into the solution, I will start the matrix terror, so that the solution is going to be mixed up.

But what you are supposed to do is suppose I measured the pH okay, so it is showing a pH of 8.5, which means and suppose I would like to maintain the pH of 7.4. So, what I will do is I will add the same amount of Hcl or the acid, so that the pH will come down to 7.4. So, to maintain the pH at 7.4 what I will do is I add the small amount of acid. So, the first thing that you have to do is, while you are adding the solution for maintaining the pH.

What you have to do is you have to add the acid or whatever the liquid you are adding very far away from the probe, which means it should not be added next to the probe. Because if you add the acid or the base next to the probe, first of all, it is going to affect the membrane, what is being

present into the probe, which is actually being responsible for exchange of protons and that is the way it is actually measuring the pH.

The other point is that if you are adding a very corrosive acid or the base very close to the probe, it is actually going to change the pH very drastically. And that is how it is actually going to give you the wrong readings. But, and also, you should not measure the pH instantly okay, you should wait for the magnetic bead to run for an additional 1 or 2 minutes. So, that all the acid what you have added is going to be mixed up completely.

And it is going to make the homogeneous solution, because if the hydrogen ions are not evenly distributed throughout the solutions. It is actually not going to give you the accurate pH of the solutions, it is actually going to give you the pH of that particular instant moment, next to the probe and that actually is going to give you the inaccurate rating, then you can keep adding the acid or the base, as long as you are not going to get the desired results.

Once you have got the desirable results, and you are done with the pH meter, what you are supposed to do is you are going to remove your probe okay. And you have to wash the probe before you going to do anything else. And you have to wash it very thoroughly so that all the acid or base what you have used in this pH maintaining process should be removed from the probe, all the buffers salt everything should be removed.

And once you are done with that, what you have to do is you have to take a tissue paper and you have to wipe the tube and the lower bulb you are supposed to remove the whole liquid but you should not touch it with the very harshly or very strongly, you have to just touch the tissue paper to the cover. And that actually is going to withdraw all the liquid and that is how it is going to be very, very protected from the any kind of damages.

Because this bulb what you will see in the probe is very, very sensitive. And once you are done with that, you just put it into the cover. And this is the 3 molar KCL solutions and as soon as you are done by the end of the day, you have to pour this probe into the 3 molar KCL solutions and

you have to install the probe into these 3 molar KCL solutions. So, that the probe should not experience, or should not be get dried up.

So, you have to just use that, like that and then it should be ready for the storage. So, apart from this pH probe, you also have the, this temperature probe. So, this is the temperature probe but you have to also immerse into the liquid for measuring the pH at very accurately at a particular temperature. So, if you work very nicely with a pH meter. It is actually going to give you the accurate pH.

And if you maintain or if you take all the precautions you do not let the probe to be get dried up. And apart from that, since these books are only meant for measuring the pH of the solutions, you should never use these pH probes for measuring the pH or any kind**.** For example you cannot use it for maintaining the pH of a microbiology media or the cell culture media or the animal cell culture media.

Because of the only reason that the microbiology media or the animal cell culture media contains very large quantity of proteins or the amino acids and the protein or the amino acids are going to choke the membrane which is responsible for the proton exchange from the flow electrode. So, once the membrane is going to be blocked. It is not going to perform the prone exchange and so that is the way you are going to destroy the probe and then you will have no other option but to change the probe with a new probe.

So, with all these precautions if you use the pH meter, you will be able to perform the research, very nicely in your laboratory. **(Video Ends: 45:40)**

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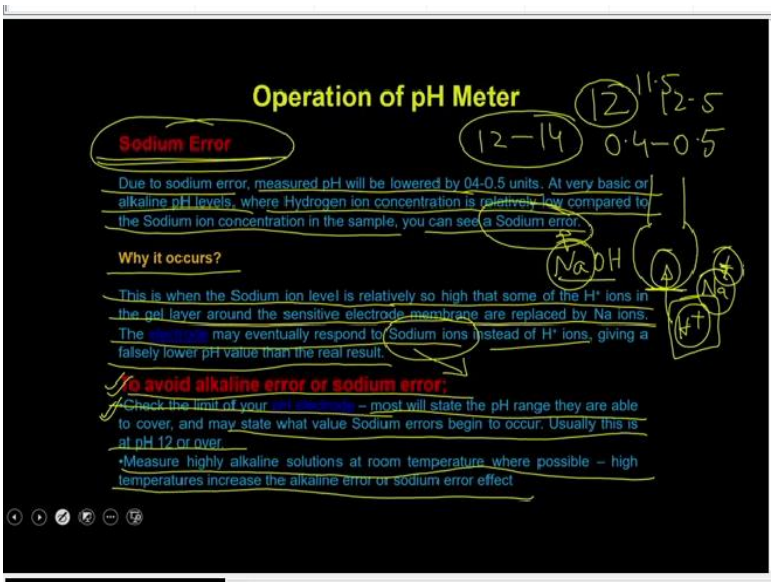

Now, once you have understand the operation of a pH meter. You could understand that there are multiple precautions that you have to take and there are multiple ways in which you have to maintain the pH probe, otherwise it is going to give you the erroneous pH results. Apart from, even if you take these precautions the pH meter is still can be able to give you the pH of the, you know, it can give you the wrong pH.

And there are multiple reasons of giving you, one of the major reason is that it is actually going to show you a sodium error. So, sodium error is actually happening because you are measuring the pH in a range of the 12 to 14, so if you measuring a pH in the alkaline region, it is actually going to cause you a sodium error and because of the sodium error, the pH what you are going to measure is going to have a deficiency of 0.4 to 0.5 units which means if I am measuring a pH of 12.

It could be 12.5 or it could be 11.5. So, this means that it is actually a very, very big error. And it happens at a very basic or alkaline pH level, where the hydrogen ion concentration is relatively low compared to the sodium ion consideration in the sample. And that is what it is going to show you the sodium error. Why that there is a sodium error, why it actually causes into the solution. It happens, because the sodium level is relatively so high that the H + ion in the gel layer around the sensitive electrode membranes are replaced by the sodium ions.

So, it happens is because you have a pH probe and to this membrane it actually is only allowing H + ions, but because you have such a high level of sodium into the solution because you know that at pH 13 or 14, you are going if you suppose I am taking a solution of NaOH. The concentration of the Na + is going to be so high, that the H + ions are going to be so little that the pH probe is actually going to give me a very you know erroneous pH.

The electrode may eventually respond to sodium ions, instead of the hydrogen ion, because there are so many high concentration of sodium, the electrode is actually started responding to the sodium ions and instead of giving the pH relative to the hydrogen ion concentrations. And that is why it is actually giving the falsely lower pH value than the real results, how to avoid that, you can avoid that simply by either checking the limit of your pH electrode.

Most will state the pH range they are able to cover and may state with eventually this is at pH 12 or over. Then you measure the alkaline solution at room temperature, where possible, because if you have the high temperature that actually is going to increase the sodium effects. Because if you have high temperature that eventually going to change the sufficient constant of that particular solution and that actually is going to increase, even the sodium level further up.

Apart from that you always should avoid using the solution of very high pH solutions and in some cases what people do, is people are actually trying to optimize the or calibrate the pH electrodes, even at a high values or high in an alkaline range. And then they are actually trying to calculate the error percentage and that actually they are considering while they are making a pH or while they are measuring the pH of the buffer solutions where the pH is above 12.

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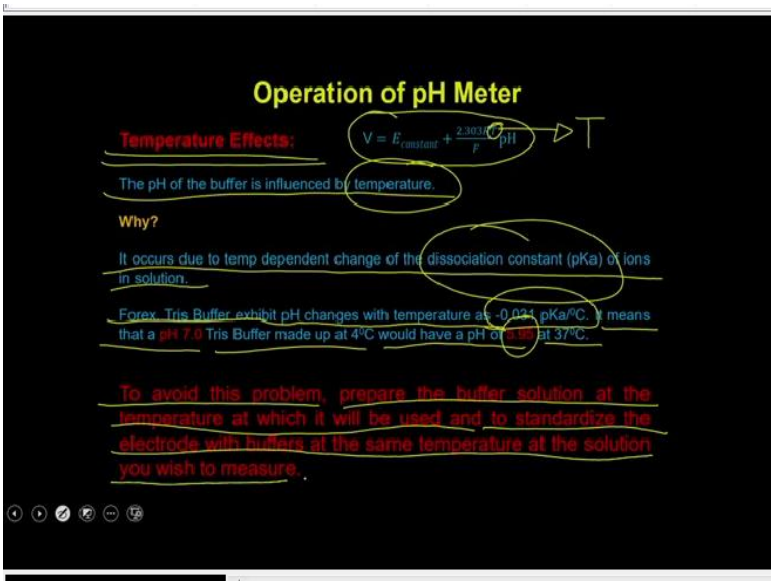

Apart from that you also have the temperature effects, which means the temperature is going to change the buffer. So, why the pH of the buffer is influenced by the temperature, because of this equation that you have already discussed that the V is E constant + 2.3RT by F, 1 parameter, what you see is actually the temperature. So, if you change the temperature, it actually going to change 2 things.

One, it is actually going to change the dissociation of the solution. So, it is actually going to change the dissociation constant of that particular compound, what is being dissolved into the buffer and as a result, it actually going to increase the dissociation of that particular compound. So, instead of having the normal dissociation it is going to depreciate more. And because of that, the resultant amount of ions are going to be more.

And you know that the pH is directly proportional to the number of ions what is present in the solutions. So, it occurs, why the temperature because it changes the dissociation constant or the pKa of the ions in the solutions, you can take an example. For example if you, prefer a trace buffer and so, you are going to see a change in the 0.03 pKa per degree Celsius, it means, if you prepare a pH of 7, the trace buffer made up of at 4 degree would have a pH of 5.95 at 37 degrees Celsius, which means there is a huge change in pH values.

If you are changing the temperature of that particular solution and that is why it is important that you should prepare the buffers at the same temperature, where you have actually kept the electrodes. So, how to avoid that, to avoid this problem prepare the buffer solution at a temperature at which it was going to be used and to standardize the electrode with the buffer at the same temperature at the solution you wish to measure, which means if you are interested to measure the pH at 4 degree.

Then you have to calibrate the electrode with the 4 degree solutions of 4, 7 and 9.2 and that actually is going to give you the more accurate results or I will say it is actually going to decrease the percentage error in the pH electrodes. So, with this I would like to conclude my lecture here. Thank you.