Interactomics Basics and Applications

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Lecture – 16
Applications of Protein Microarrays in Malaria Research-I

We have discussed there are many ways of printing features on the microarray based platforms; especially cell free expression based systems could be very powerful to generate proteins on the chip. In this slide (Refer Time: 00:32) one of the powerful technologies which we discussed with Dr. Joshua Labaer. Additionally did advent of many innovative ways of producing proteins on the chip without need to express in purify them.

And in collaboration with University of Washington with Dr. Pradeep Rathod and his group, we have tried to utilize one of the cell free expression based platform which is based on the wheat germ extracts. And one of the PhD student in the lab Apoorva Venkatesh, she perform a malaria based project using this novel protein microarray based technology.

So, today we are going to talk about Applications of Protein Microarrays; especially how it can be used for research on malaria, using a high throughput microarray based platform which is also based on the cell free expression. These experiments were conducted in our proteomics lab and Apoorva was going to give you some insight about how to perform such experiment and interpret data to make it much more meaningful insights from these kind of high throughput experiments. So, let us welcome Apoorva for this lecture on microarray technologies and it is application in malaria research.

I am Apoorva Venkatesh a TA for this course, and in today's lecture and in the next lecture we are going to speak about microarray technology and one of it is applications. Before we can we go on to what we can do we are going to see how to perform the particular experiment.

And in today's class I am going to take you to the lab and I am going to show you stepwise how the experiment is performed in a laboratory. So, let us move on to the proteomics laboratory at IIT Bombay.

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So, in today's lecture, I will be showing you a microarray experiment to study antibody levels of malaria positive patients to parasite antigens.

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For this particular experiment today, first I will show you how to setup the experiment, how to assemble the slides, and then I will walk you through the various steps one by one. We will first begin by assembling the slides in the slide cassette.

So, before which I would first like to show you how a slide looks.

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So, it is very important to know that all the slides have to be stored in a light proof box. Normally slides are stored in a desiccators. I have a light proof box here and I have my slides in this box. I am going to first show you one of the slides and I am going to close the box.

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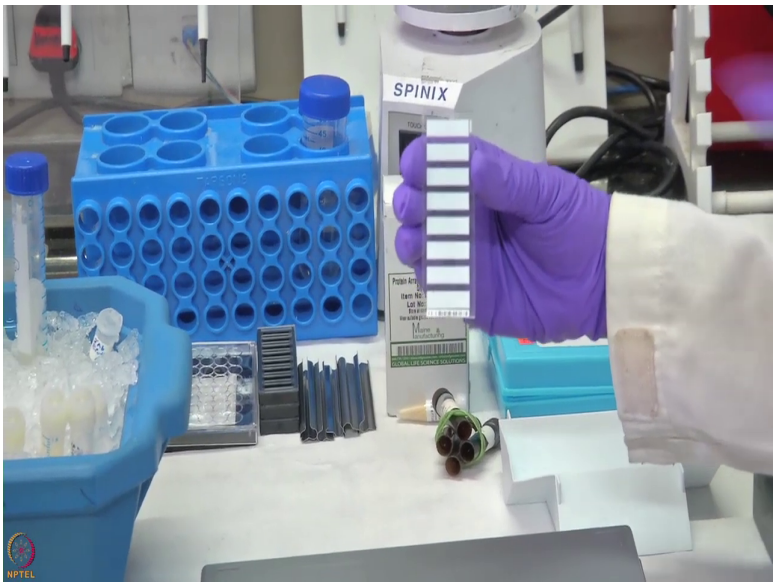

So, this is basically how one microarray slide looks. If you notice this slide there are 8 sub arrays in this slides. So, let us call this sub array 1 and there are 8 sub arrays like this in this particular slide. What is important to know here is that the proteins printed on this slide are basically parasite proteins. So, plasmodium falciparum and plasmodium vivax are two malaria parasites that cause malaria in humans; of course, there are others as well.

But these are the two most dominant parasites. So, in this particular chip, we have both plasmodium falciparum and plasmodium vivax proteins which are printed on this chip. Now how these proteins are printed will I will talk to you in the next lecture. Today we will only speak about how this particular experiment is done in the lab.

So, what is important here to know is that, each of these sub arrays can probe one patient serum; which means that, at the same time I can study the responses of 8 patients using one

slide. So, that is the advantage of this particular setup. So, the most important thing in this particular experiment is a slide set up; because if this slide is not set properly, then there could be leakage and any small error in a microarray experiment can actually cause erroneous results at the end which should be very difficult for us to interpret.

The first thing I am going to do is to show you how to set this slide in the slide holder.

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What I have here is, basically the slide separator which is going to separate each and every sub array which I am going to now call pad. I am going to call one sub array as one pads; this slide holder has to be placed really carefully on the slide edge to edge.

It is important to not apply too much pressure in the center or in the corners; you have to apply equal pressure throughout. This is now attached this slide; make sure that this is set properly and it is firm and tight.

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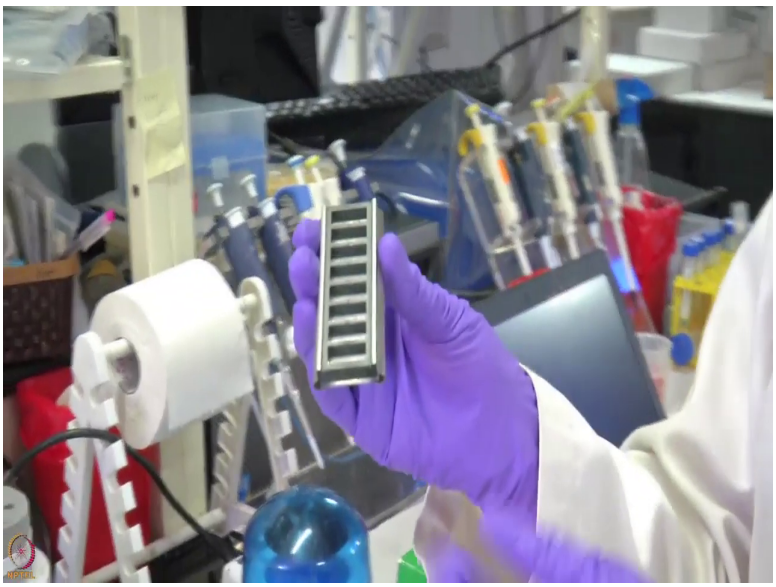

So, once this is done, we will now clamp this up using these clamps. We have now successfully clamped this slide.

Now, what we need to do is place it in a slide holder. So, this is the slide holder and you will see here that, this slide holder can now take three slides; which means that if one slide can probe 8 patient serum and this slide holder can take three slides, which means that one shot 24 patient serum can be screened. For today I am going to take only one of these slides and I am going to place it now in the holder.

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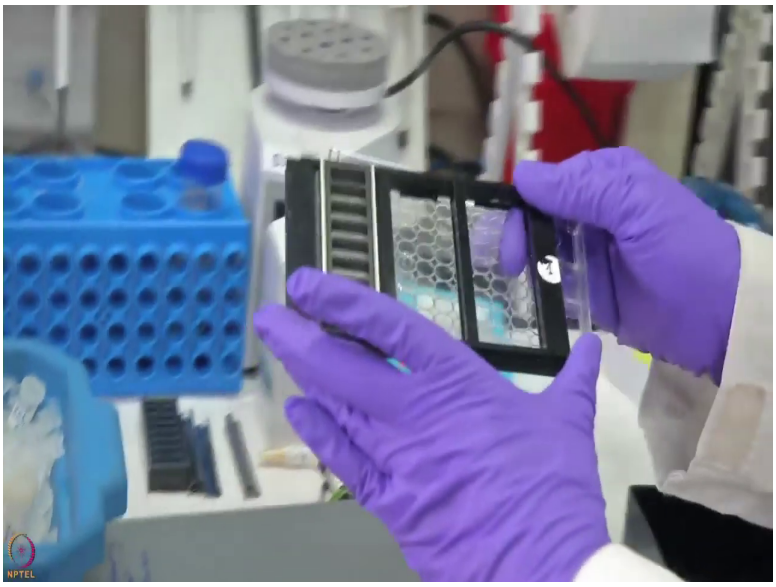

So, this is how we place it in the holder and I will close the holder. So, this completes our slide setup.

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The next thing we need to do is to set our reagents. So, first of all what all do we need for the microarray experiment?

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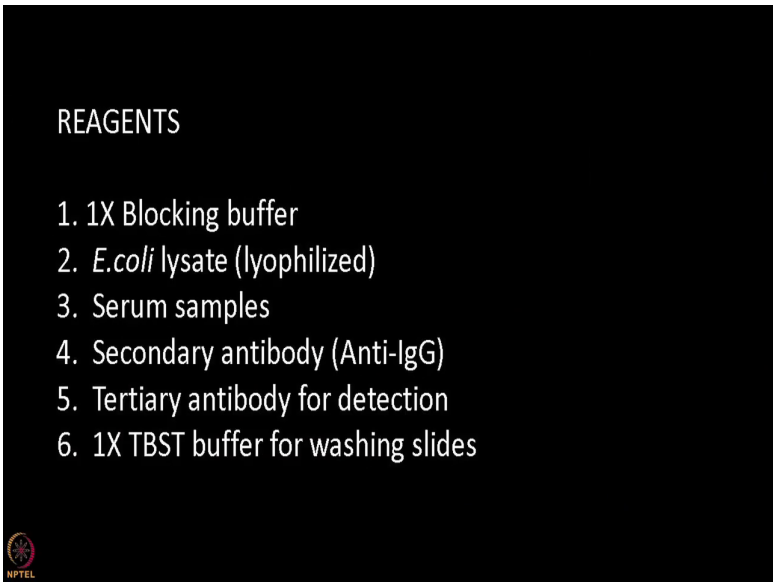

First thing we need is a blocking buffer and the second thing we need is an E coli lysate.

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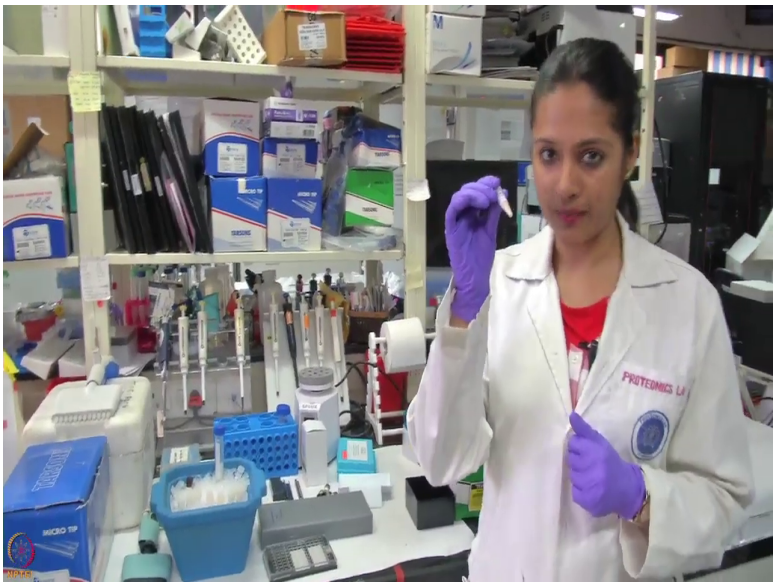

So, this is an E coli lysate which is in the powder form.

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And then we have blocking buffer here, which I will use throughout the experiment. So, I need to make 10 percent of this E coli lysate in blocking buffer. So, what I will do first is I am going to take 1 ml of blocking buffer and added to my E coli which is in the powder form.

So, I am adding 1 ml of the blocking buffer into the E coli lysate and what I am going to do is I am going to mix it really well. This E coli lysate which is 1 ml, I am going to add it to 9 ml of blocking buffer. So, this is going to give me 10 percent E coli lysate in blocking buffer.

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So, this step is basically required for incubating our serum samples, before we start the hybridization, to remove any anti E coli non specific antibodies from the serum. So, now this is my 10 percent E coli lysate, and I am going to place it back in ice.

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So, once you position the chamber onto the slide and slide into the slide holder, we are going to take 1 X blocking buffer and we are going to add this onto the chip and we are going to incubate this chip for 30 minutes. Preferably it is better to carry out this using a multi channel pipette; but now that we are going to have only one slide, I am going to use a normal pipette to add 200 micro liter of blocking buffer into the slide.

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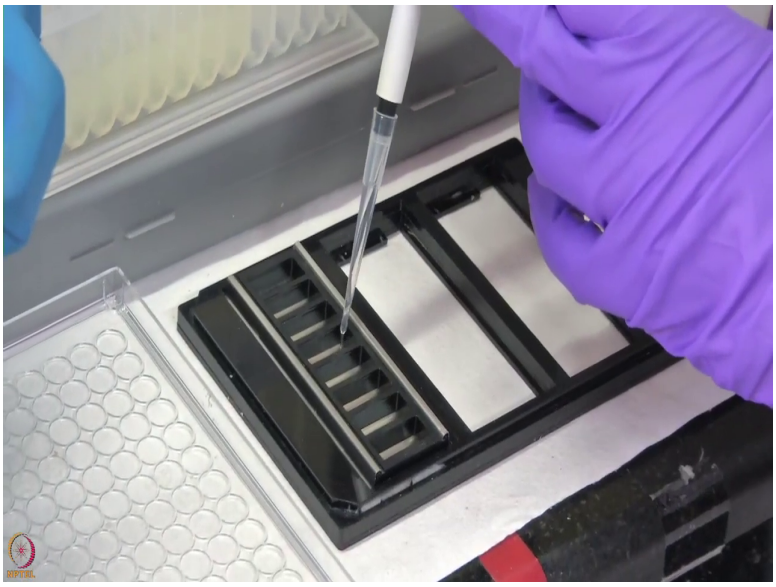

So, what I normally do is, I add blocking buffer for an extra 30 seconds in alternate pads. This is basically to check if the setup is fine and if there is any sort of leakage and I am going to place it on the rocker.

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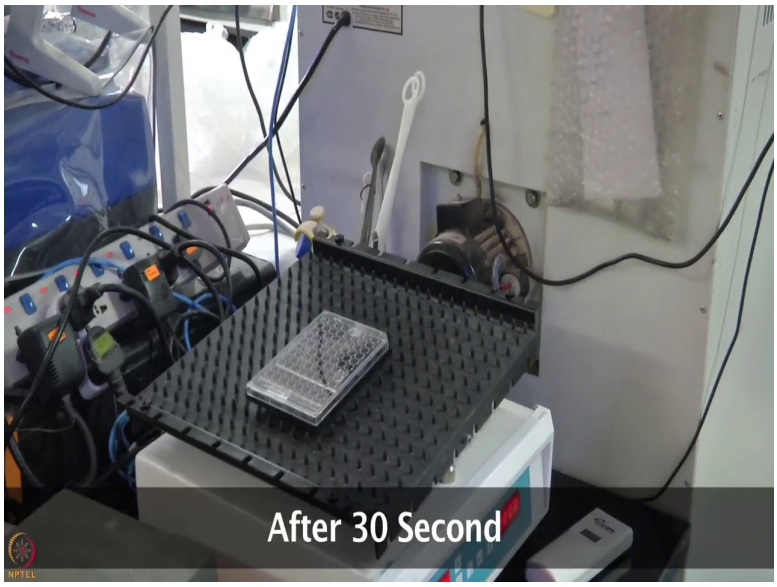

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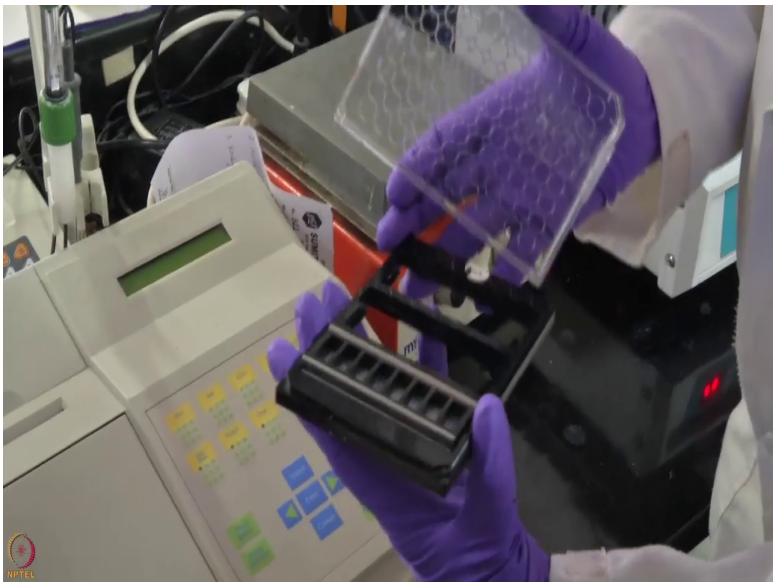

So, when I check this, I see that there is no leakage. So, now, what I will do is I will add the blocking buffer in the other pads. It is important to make sure that, there are no bubbles in this process.

So, once this is done, we will now carefully place this back on the rocker for 30 minutes. So, while we are rehydrating our slide with the blocking buffer; meanwhile what we will do? We will incubate a serum samples with the 10 percent E coli which we had prepared previously.

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So, these are my serum samples which are in cryovials. We need to make a dilution of 1 is to 100; which means that, I will take 99 micro liter of the E coli lysate in blocking buffer and I am going to take 1 micro liter of serum.

So, now because each pad can take 200 micro liter volume what we will do is we will take 2 micro liter serum in 198 micro liter of 10 percent E coli lysate.

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I have already allocated the 10 percent E coli lysate; now what I will do is add 2 micro liter of serum samples into this.

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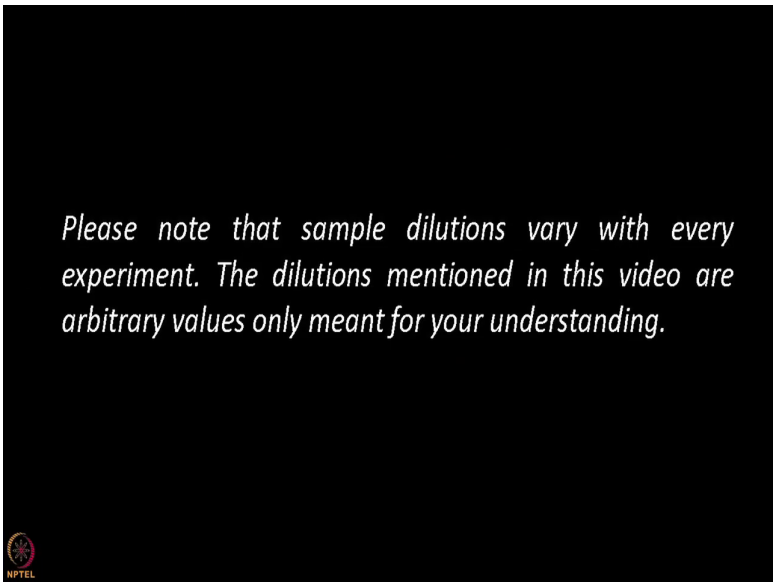

So, usually this is also done using a multi channel pipette or sometimes is there are also automated ways to do this; but since we are again dealing with only one chip, I am using a normal pipette.

So, that way we have now incubated 2 micro liter of 8 serum samples in E coli lysate and we will incubate this for 30 minutes in the same platform rocker..

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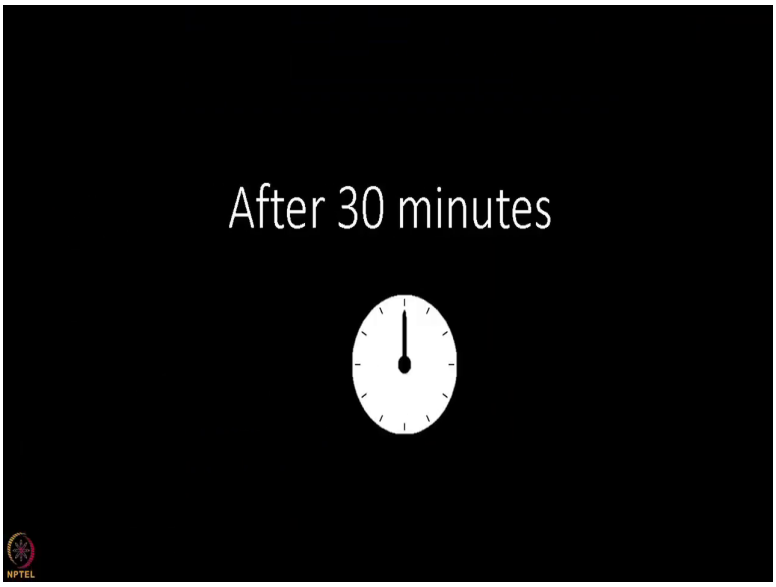

So, now, it is time to add our serum samples to the slide. So, the first thing we need to do is to aspirate the blocking buffer which is already present on our slide.

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For this I will take the multichannel pipette.

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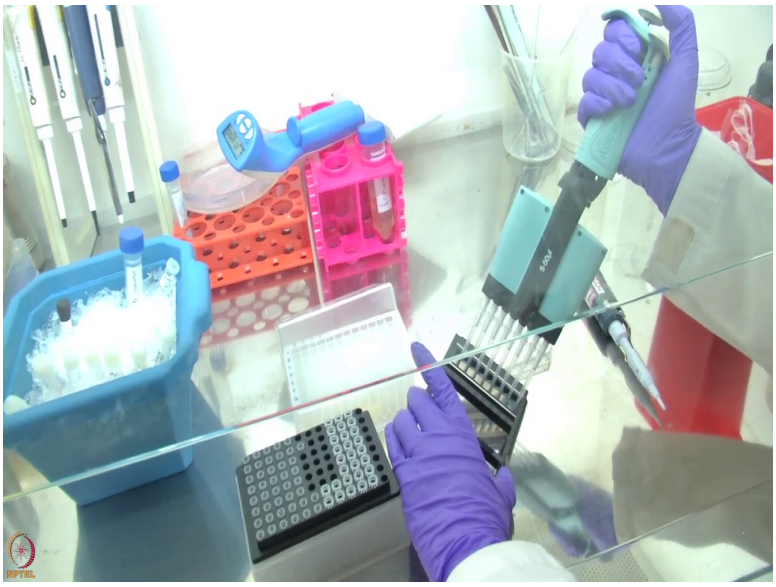

So, this step has to be performed really carefully. As you see these are really fine tips which have to be used to avoid any scratches. It is important to keep the pipette at the very end of the slide, this way and then you aspirate really slowly. The other important thing is that the slide should never dry up. So, it is always good to keep a little blocking buffer behind.

As you see the blocking buffer has been completely removed and now immediately we will add our samples. So, this is the 50 micro liter pipette.

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I am going to mix this serum sample once before adding into the slide. So, once this is done, we will add the serum samples to the slide.

So, we have totally added 200 micro liter of serum samples which have been diluted in 10 percent of E coli lysate.

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So, to avoid any kind of evaporation; what will do is we will place a foil over the lid and then we place this whole slide on a moist towel.

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We will then place this slide overnight in a cold room which is at 4 degree.

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So, after overnight incubation, the next thing we need to do is aspirate the solution just like we did previously.

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And we are going to wash this pad with tween buffer; I am not going to show the washing steps now, because it is similar to how we aspirated. The same way you aspirate and then you wash it and then you aspirate again, this has to be done thrice.

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This is our secondary antibody; what we will do is we will dilute this 200 times. I am going to take 1 micro liter of the secondary antibody in 199 micro liter of buffer and I will calculate this for 8 pads.

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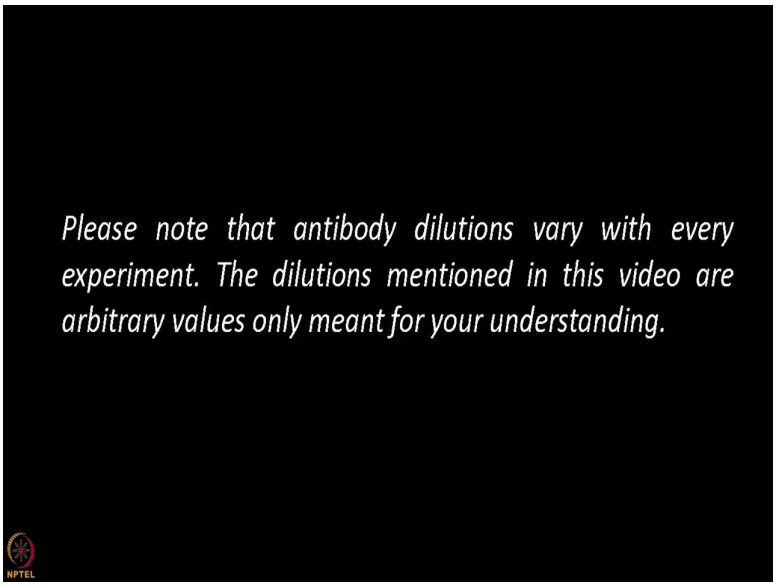

So, basically I have already calculated the volume required for 8 pads.

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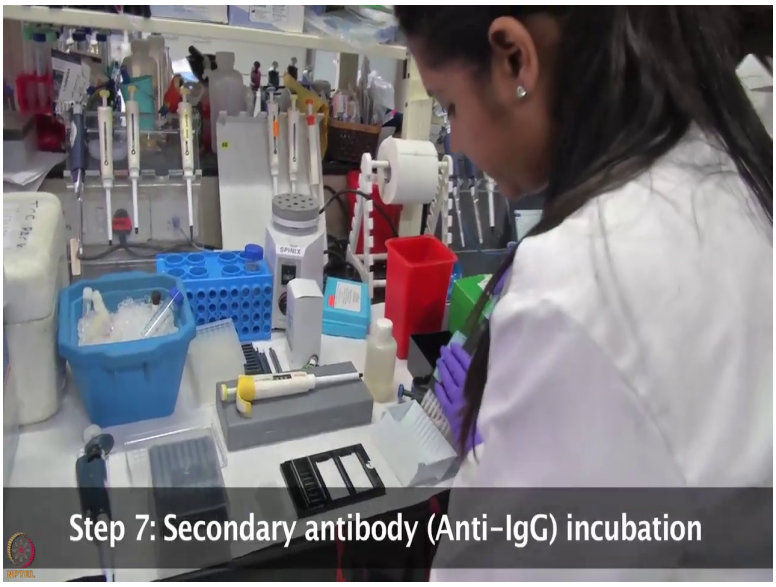

And I am going to simply add 200 micro liter of secondary antibody to each pad.

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Is it important to not scratch the pad and also I have as I have mentioned earlier; the pad should never dry. So, once this step is done, we will again incubate this slide on a platform rocker, ok.

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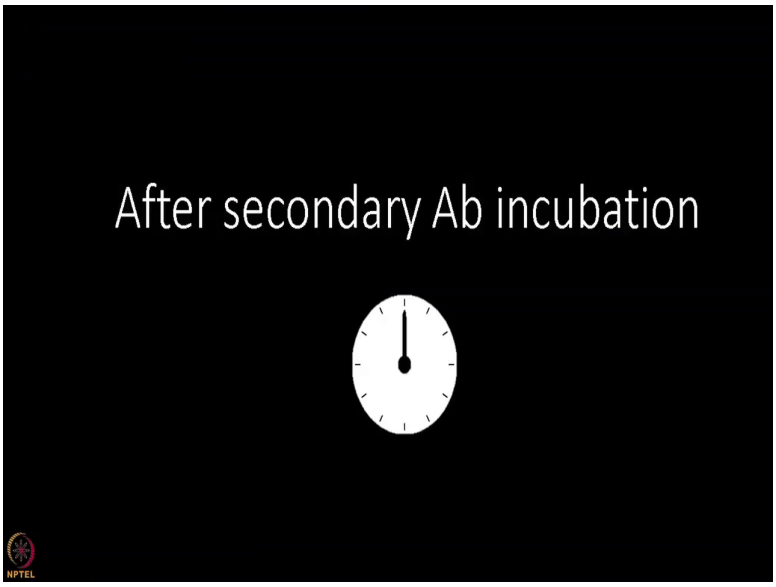

So, now, what we have done is we have incubated the slides with the secondary antibody and now we will incubated with the tertiary antibody; this process is again done after washing the slide thrice with tween buffer.

The dilution for this antibody is similar to the previous one; 1 and 200 and I have again calculated accordingly.

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So, now we will add 200 micro liter of tertiary antibody which is a streptavidin conjugate to the slide.

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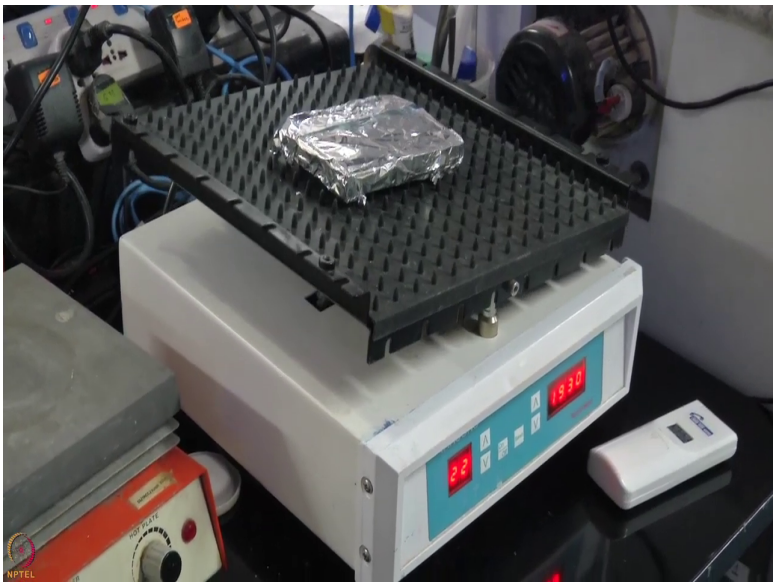

it is very important to close the slide and wrap with foil immediately as this tertiary antibody is light sensitive.

So, once this is completely wrapped, we will again place this on a platform rocker. I would like to mention that after this step, every other step has to be performed very carefully and we should try to avoid light as much as possible.

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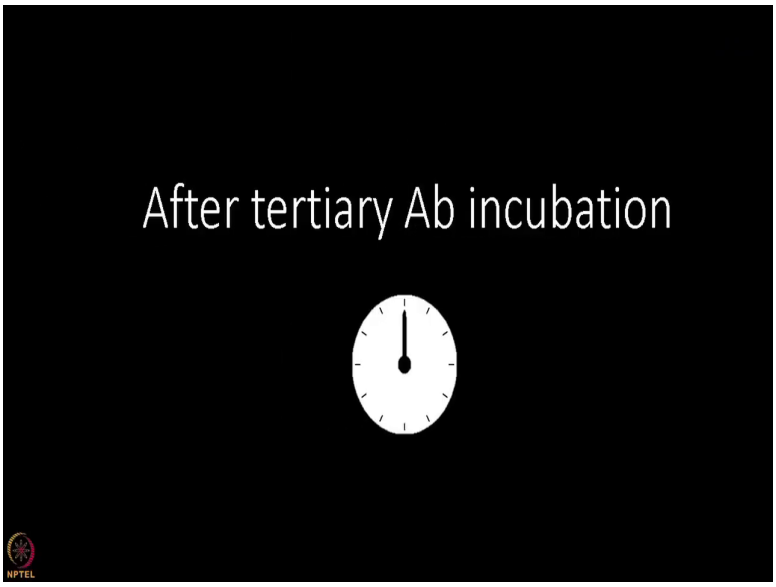

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So, after incubation with tertiary antibody, we are going to now watch this slides just like previously with tween buffer.

So, every time you add tween buffer, we placed on a high speed rocker for 5 minutes and then you wash it again. So, this is this process is done 6 times.

Now, it is time to remove the slide from the slide chamber for scanning. So, I will carefully remove the slide from the chamber.

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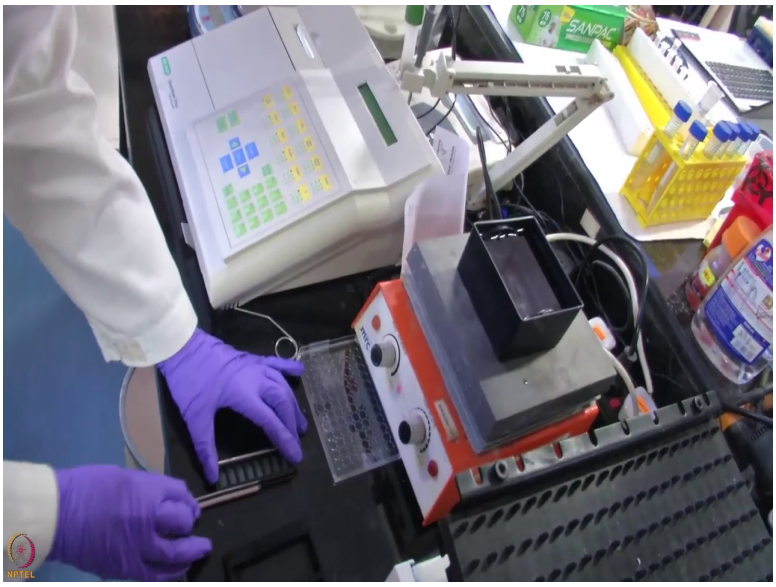

I am removing the clamps; you have to be careful not to scratch the pad surface.

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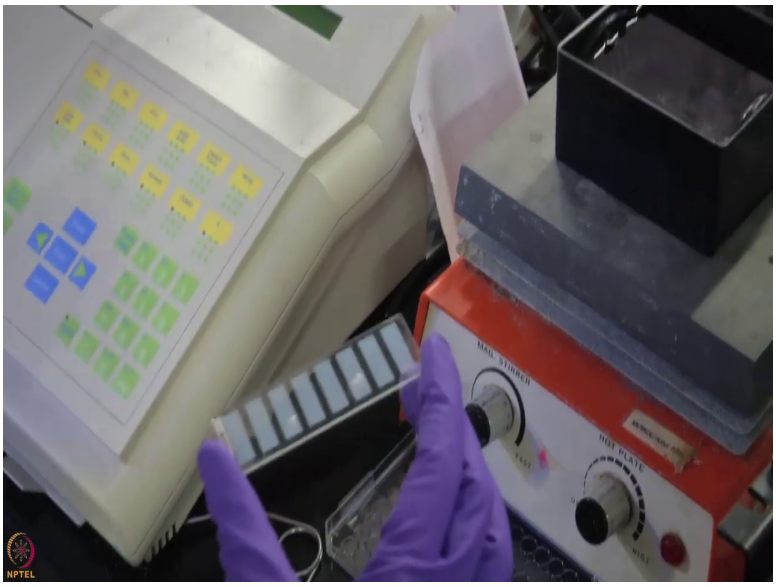

So, we slowly remove the chamber from the slide. And then we will immediately transfer the slide into the slide holder.

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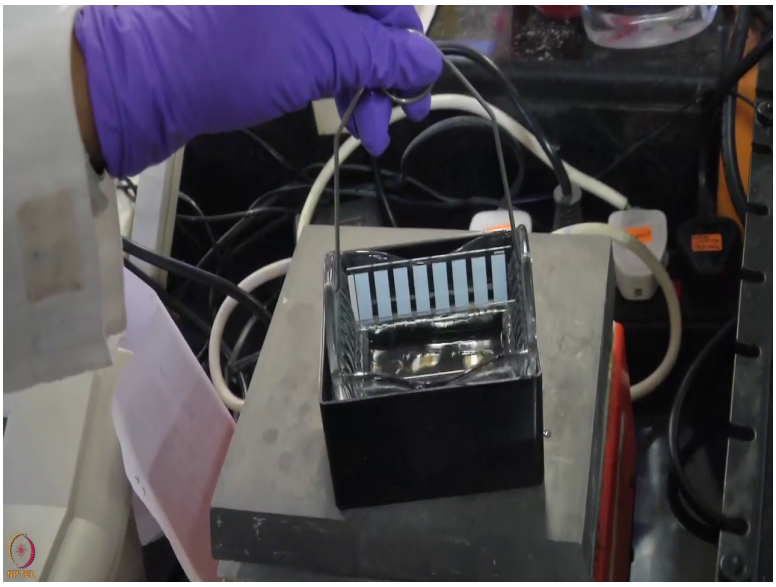

So, this is basically distilled water to wash this slide before we scan it. So, we have a slide holder with this slide and a balance, which we will now centrifuge at 2000 rpm for 5 minutes.

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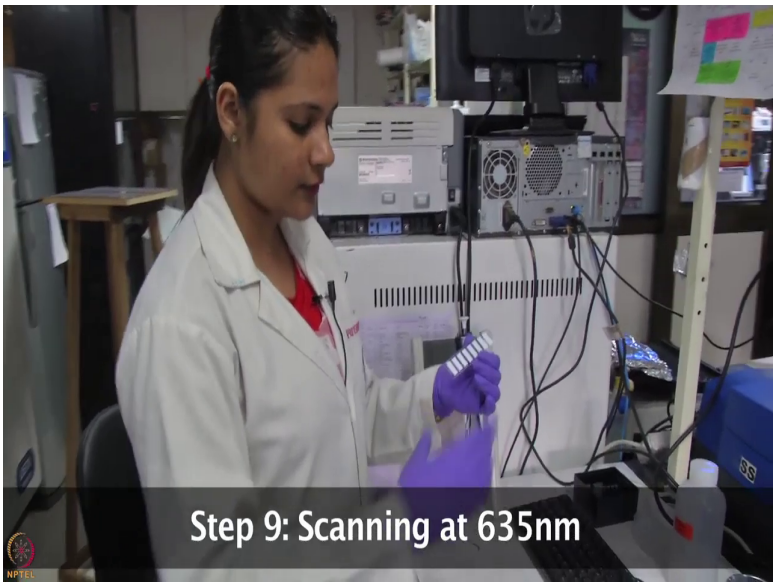

So, we are going to now scan the slide; as you will see this slide is dry after centrifugation.

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We will place the slide carefully in the scanner with the barcode side facing downward.

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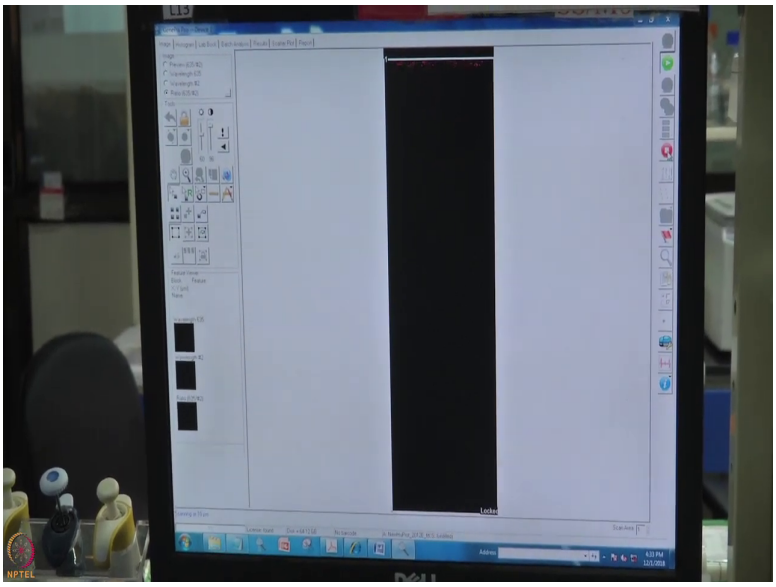

So, now we scan the slide.

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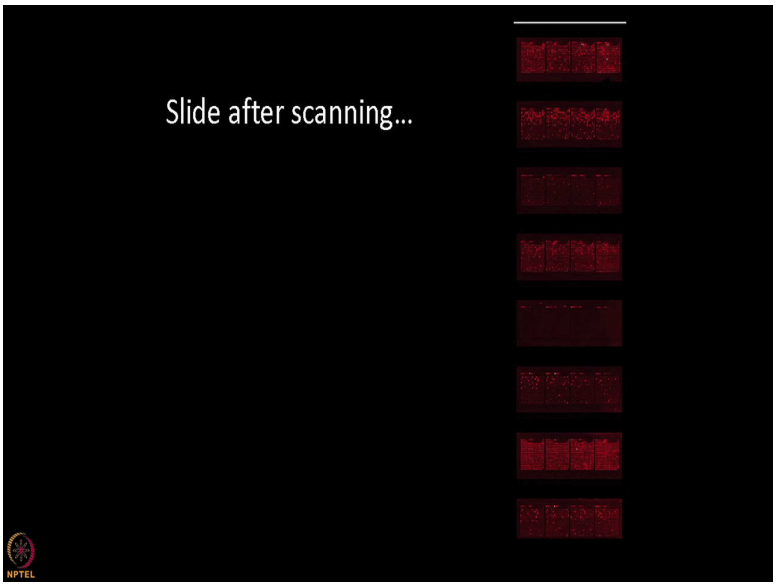

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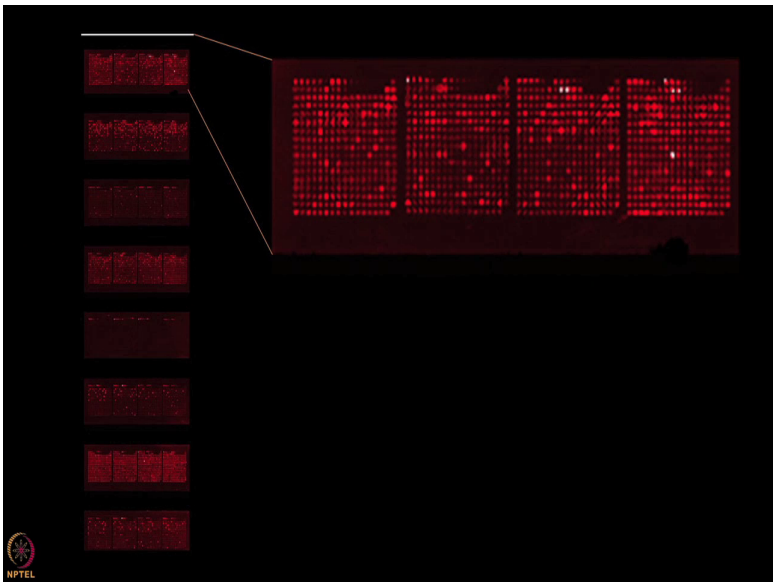

So, hope you familiar with how to perform the microarray experiment. So, it is not difficult, it is just little TDS and there are certain precautions which you need to take while performing this particular experiment.

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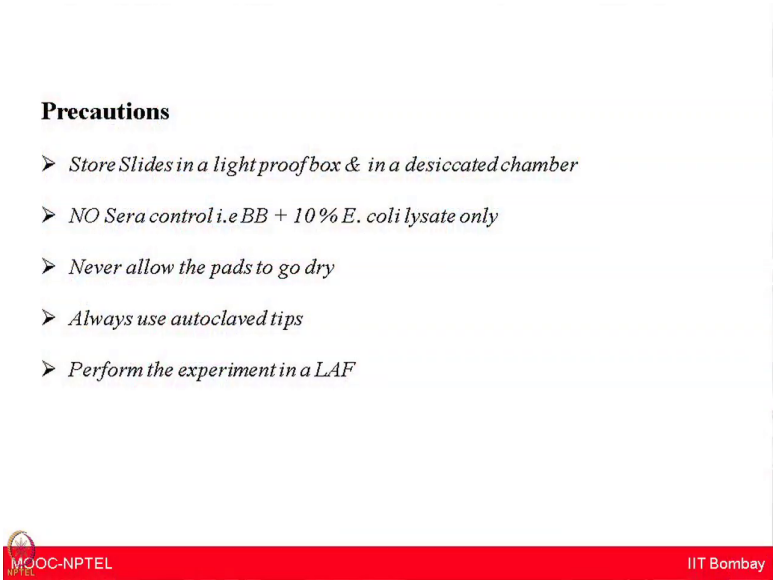

So, basically it is very important to store the slides in a light proof box and in a desiccated cabinet if you have it. QC of each sample is already been done I am sure; but it is important to ensure that it is being done, because these are all IVTT spots on the on the chip and they have to be check once before you receive them. And it is important to probe one no sera control in every batch. I have already shown you that, but is very important in probably in every slide if you can probe one its great; if not, at least in every batch there has to be one no sera control, which is nothing but your blocking buffer plus 10 percent E coli lysate. The pads must never ever go dry; others you will actually see huge background noise, which is very difficult to then later on eliminate.

Also you have to use autoclaved tips all the time and the last thing is to label this the very carefully; otherwise towards the end of the experiment, you will never know which slide was

used for which proving which samples. And especially when you have a large number of samples like 200 and 300, it becomes very confusing at the end.

In the next class I will teach you how to analyze data using excel; I will show you how to what you do after you export data from your microarray scanner. This will be very helpful for a few of you who are just starting microarray experiment in the laboratory. See you next week, thank you.

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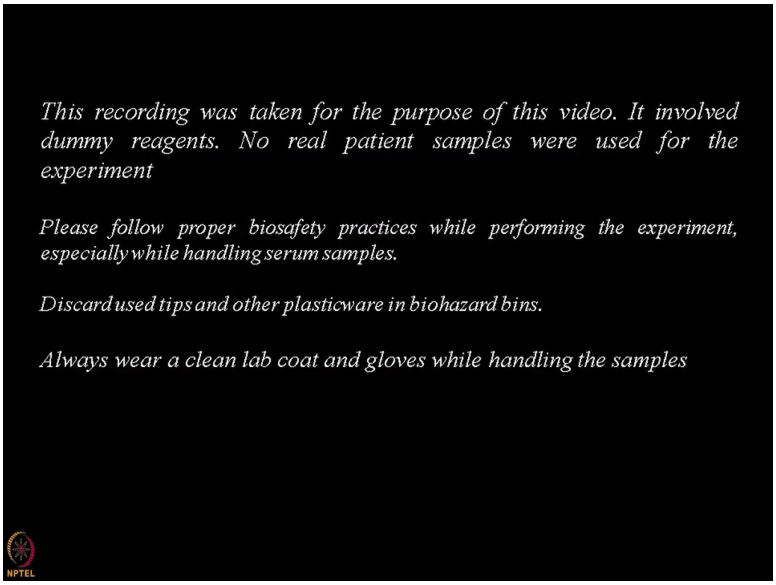

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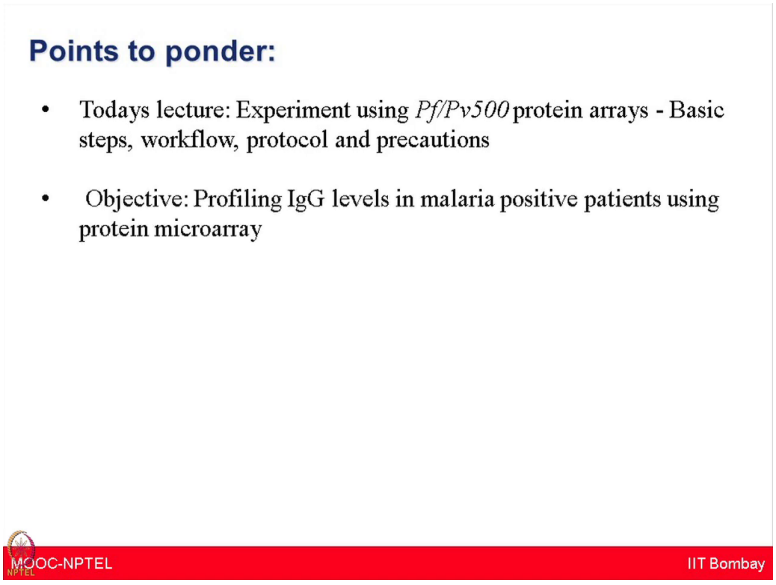

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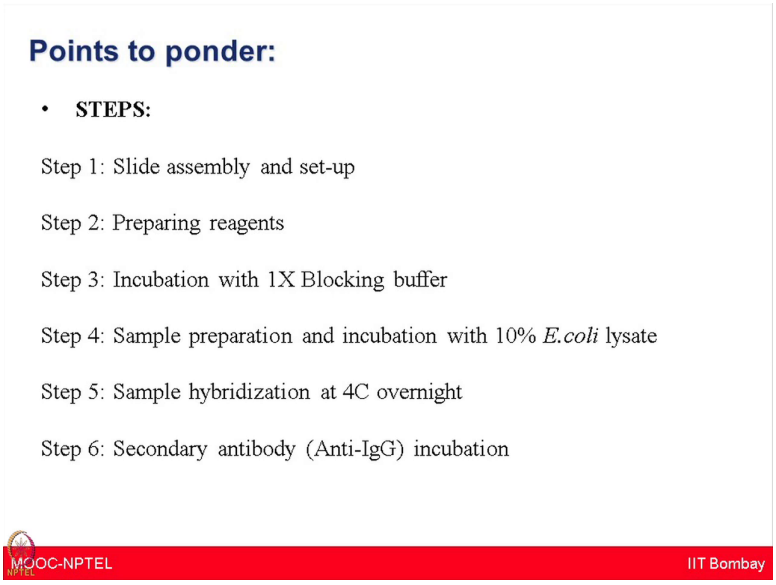

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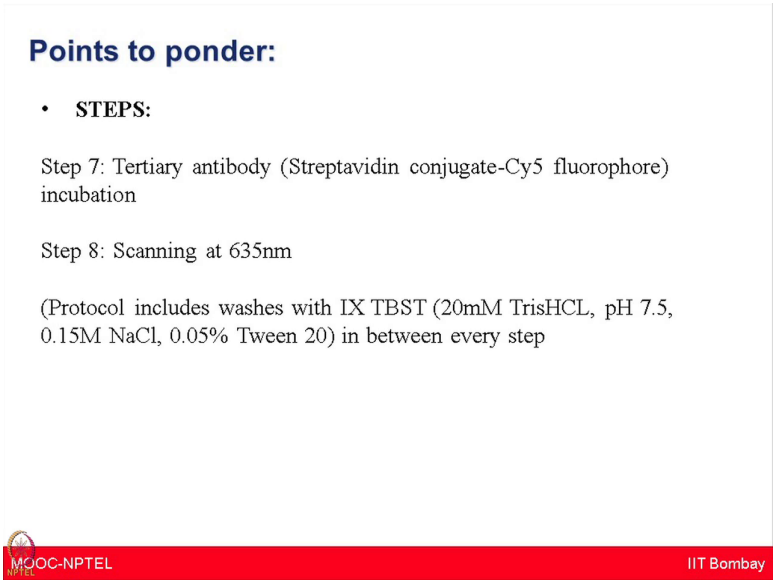

I am sure now you are convinced there are many ways of producing the contents on the chip in different ways; both cell based or cell free expression based manner. Cell free expression provides lot more flexibility that you can generate large number of proteins of interest without need to purify them. And especially in this case when we want to do a research on malaria, we had different pathogens, different parasites from which these genes were that the clothes are made from falciparum and vivax; and they were printed on the chip and thought was can we express them using in vitro transcription translation mix and made the protein on the chip and then use those to screen the patients biological sample.

I hope you got some understanding about how to perform a microarray experiment in our proteomics lab, which was demonstrated today. You also understood every step is so crucial in high throughput biology. If you are looking at every step meticulously; your protocols, your

SOPs, your quality control checks are in place, then only you can obtain a result which could be reproducible and meaningful.

In next lecture we will continue our discussion about using such approach and application and how to then analyze the data and make more meaningful insights from this kind of experiments.

Thank you very much.