NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING (NPTEL)

Applications of Interactomics using Genomics and Proteomics technologies

Course Introduction by Prof. Sanjeeva Srivastava

MOOC-NPTEL

Applications of Interactomics using Genomics and Proteomics Technologies

Lecture-12
Applications of Protein microarrays in Malaria Research-1

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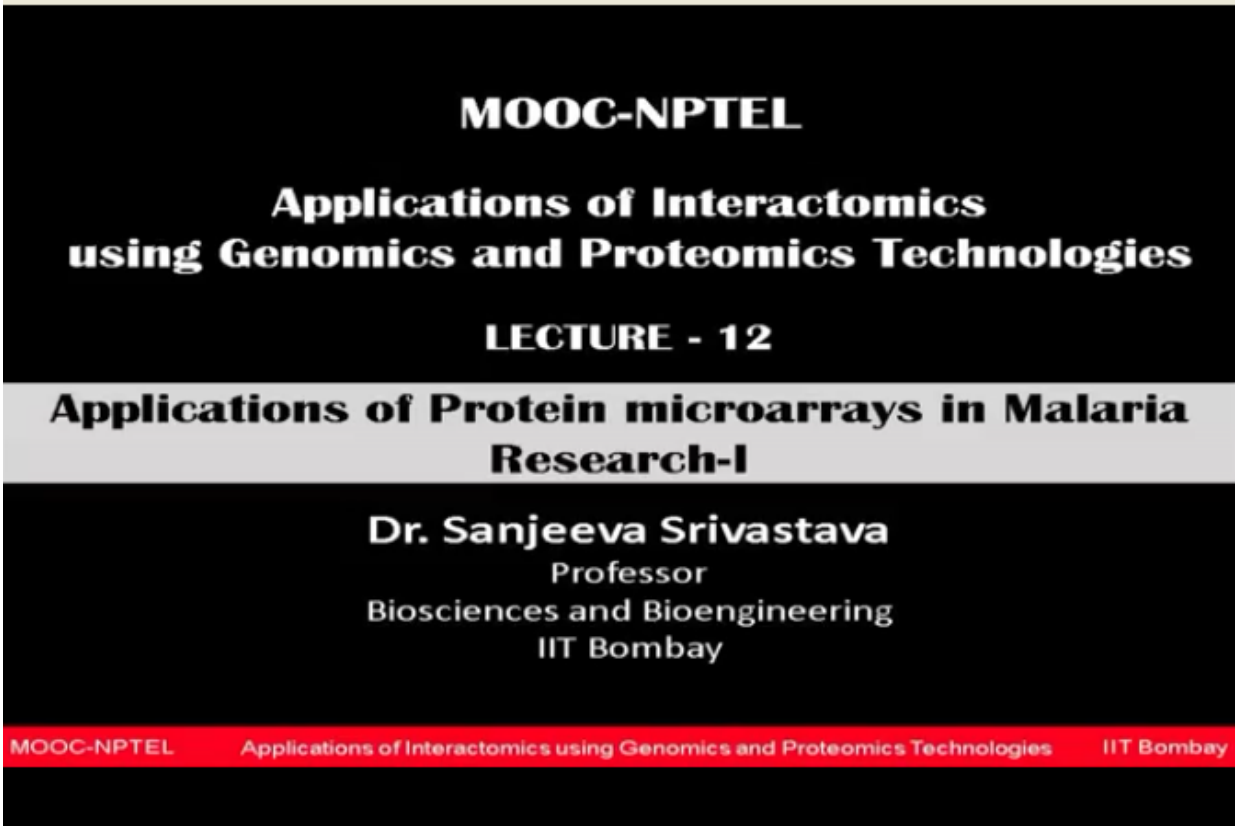

Welcome to MOOC course on applications of interactomics using genomics and proteomics technologies. We have discussed there are many ways of printing features on the microarray based platforms especially self-array expression based systems could be very powerful to generate proteins on the chip.

In this slide one of the powerful technologies which we discussed with Dr. George Lubber, additionally that the advent of many innovative ways of producing proteins on the chip without need to express and purify them, and in collaboration with university of Washington with Dr. Pradeep Rathod and his group we have try to utilize one of the self-array expression based platform which is based on the weed geological extracts. And one of the PHD student in the lab Apoorva Venkatesh, she performed 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 and malaria using a high throughput microarray based platform which is also based on the self-array 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 insight from these kind of high throughput experiments.

So let's welcome Apoorva for this lecture on microarray technologies and its application in malaria research [music].
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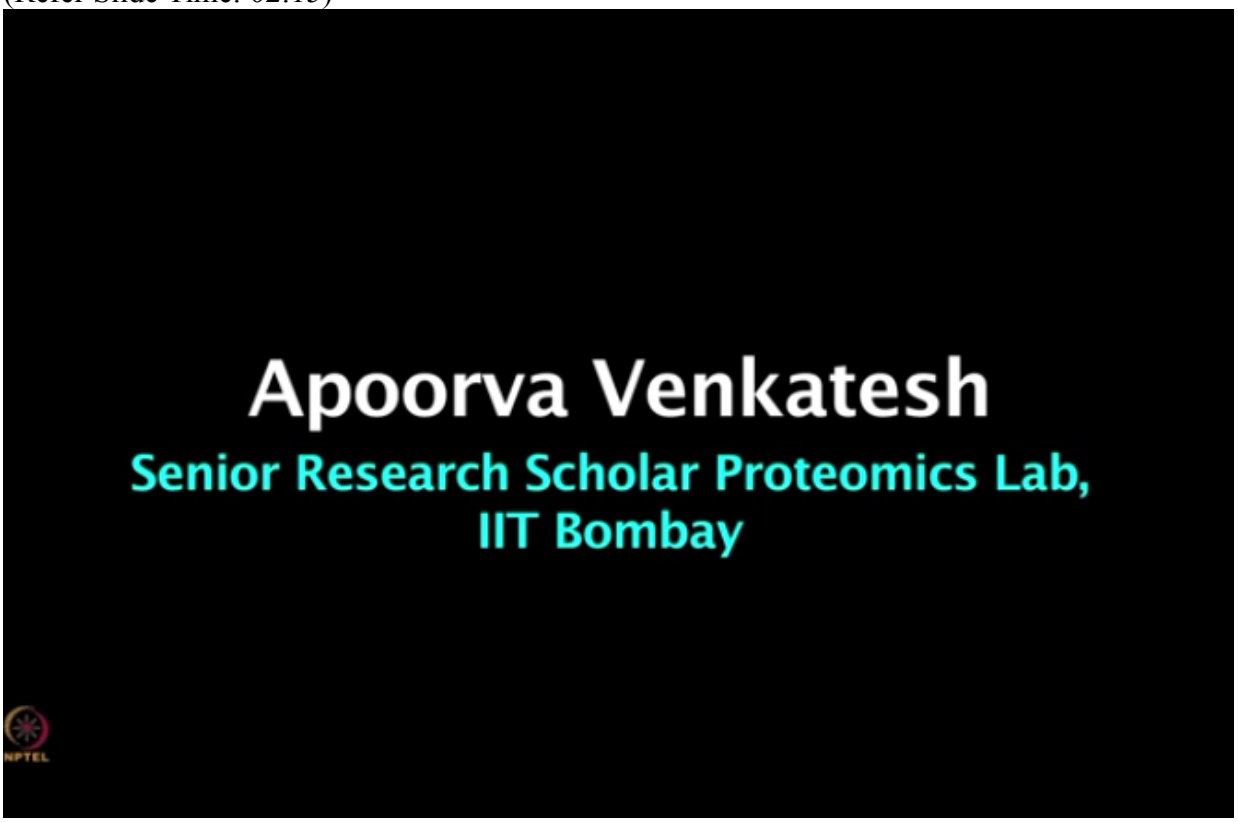

I'm Apoorva Venkatesh TE for this course, and in today's lecture and in the next lecture we are going to speak about microarray technology and one of its applications, before we go on to what we can do, we are going to see how to perform the particular experiment.

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

So in today's lecture I'll 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'll show you how to set up the experiment,
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how to assemble the slides and then I'll walk you through the various steps one by one.

We'll first begin by assembling the slides in the slide cassette, so before which I'll first like to show you how a slide looks, so it's very important to know that all the slides have to be stored in a light proof box, normally slides are stored in a desiccator, I have a light proof box here and I have my slides in this box, I'm going to first show you one of the slides and I'm going to close the box, so this is basically how one microarray slide looks.
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If you notice this slide there are sub-arrays in the slides, so let's 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 I'll talk to you in the next lecture, today we'll 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's the advantage of this particular setup.

So the most important thing in this particular experiment is the slides setup, because if the 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 will be very difficult for us to interpret.

The first thing I'm going to do is to show you how to set the slide in a, in the slide holder, what I have here is basically the slide separator which is going to separate each and every sub-array which I'm going to now call pad, I'm 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's important to not apply too much pressure in the centre or in the corners, you have to apply equal pressure throughout, this is now attached to the slide, make sure that this is set properly and it is permanent tight, so once this is done we will now clamp this up using these clamps.

We have now successfully clamped this slide,
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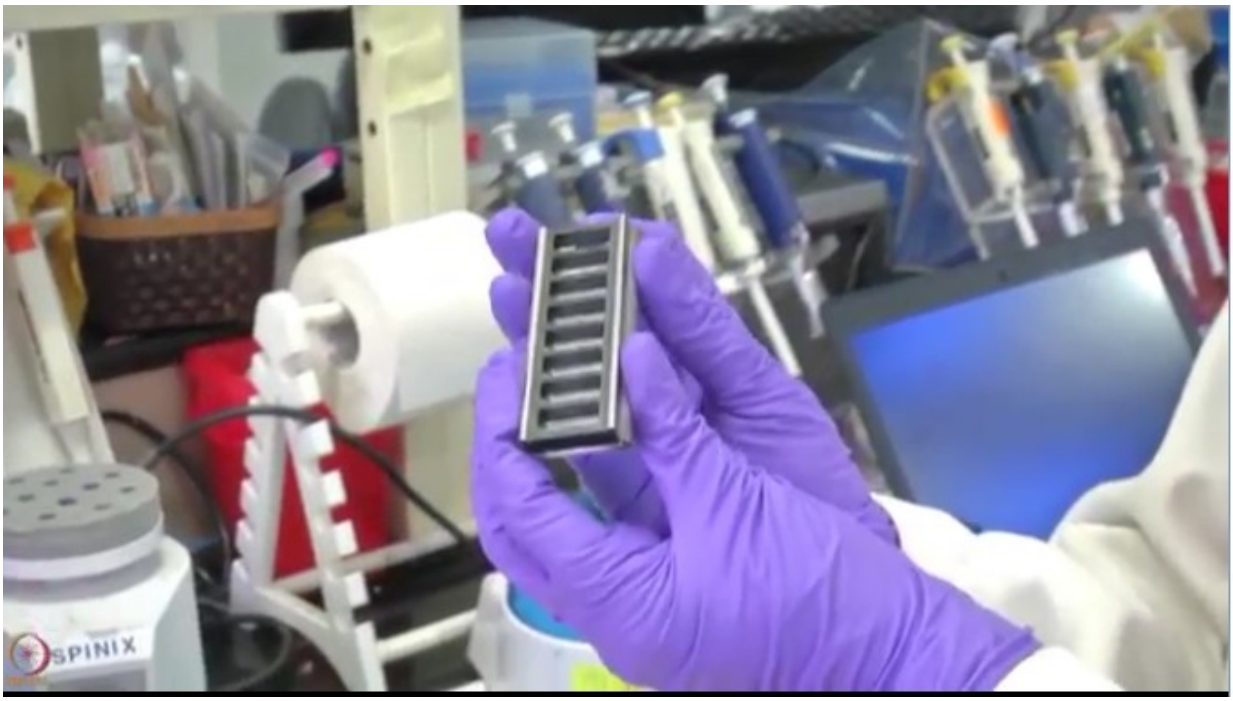

now what we need to do is place it in the slide holder, so this is the slide holder and you will see here that this slide holder can now take 3 slides, which means that if one slide can probe 8 patient sera and this slide holder can take 3 slides which means at 1 shot, 24 patients sera can be screened.

For today I'm going to take only one of the slides and I'm going to place it now in the holder, so this is how we place it in the holder and I'll close the holder,

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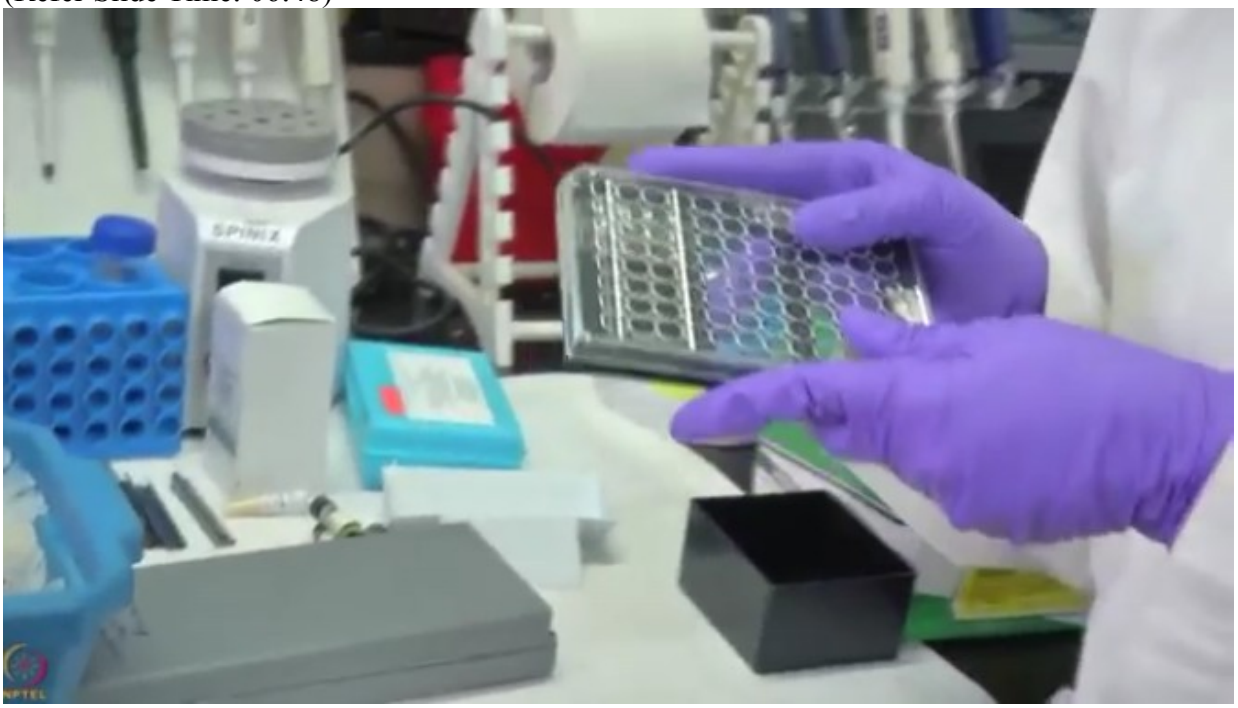

so this completes our slide setup.

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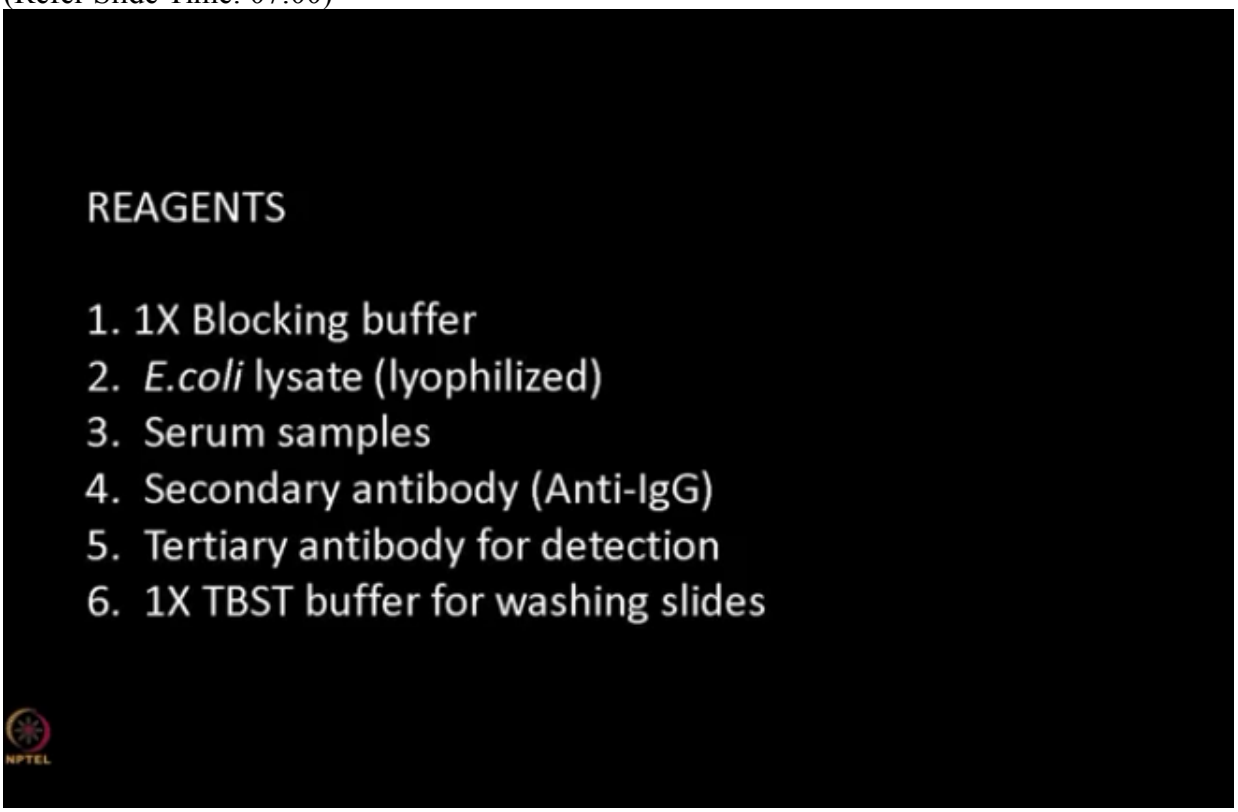

The next thing we need to do is to set our reagents, so first of all what all do we need for the microarray experiment. First thing we need is a blocking buffer, and the second thing we need is E. coli lysate, so this is an E. coli lysate which is in the powder form, and then we have

blocking buffer here which I will use throughout the experiment, so I need to make 10% of this E. coli lysate in blocking buffer, so what I'll do first is I'm going to take 1ml of blocking buffer and add it to my E. coli which is in the powder form, so I'm adding 1ml of the blocking buffer into the E. coli lysate and what I'm going to do is I'm going to mix it really well, this E. coli lysate which is 1ml, I'm going to add it to 9ml of blocking buffer, so this is going to give me 10% E. coli lysate in blocking buffer.

So this step is basically required for incubating our serum samples, before we start the hybridization to remove any anti E coli nonspecific antibodies from the serum, so now this is my 10% E. coli lysate and I'm going to place it back in ice, so once your position the chamber on to the slide, and the slide in to the slide holder you are going to take 1X blocking buffer and

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you are going to add this on to the chip and you are going incubate this chip for 30 minutes.

Preferably it is better to carry out this using a multichannel pipette, but now that we are going to have only one slide, I'm going to use a normal pipette to add 200 micro litre of blocking buffer into the slide, so what I normally do is I add blocking buffer for an extra 30 seconds in alternate pads, this is basically to check if this setup is fine and if there is any sort of leakage, and I'm going to place it on the rocker, 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, is important to make sure that there are no bubbles in this process, so once this is done we'll now carefully place this back on the rocker for 30 minutes, so while we are rehydrating this a slide with the blocking buffer, meanwhile what we'll do we will incubate a serum samples with the 10% 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 1:100 which means that I will take 99 micro litre of the E. coli lysate in blocking buffer, and I'm going to take 1 micro litre of serum, so now because each pad can take 200 micro litre volume, what we'll do is we'll take two micro litre serum in 198 micro litre of 10% E. coli lysate, I've already allocated the 10% E. coli lysate, now what I will do is add 2 micro litre of serum samples into this.

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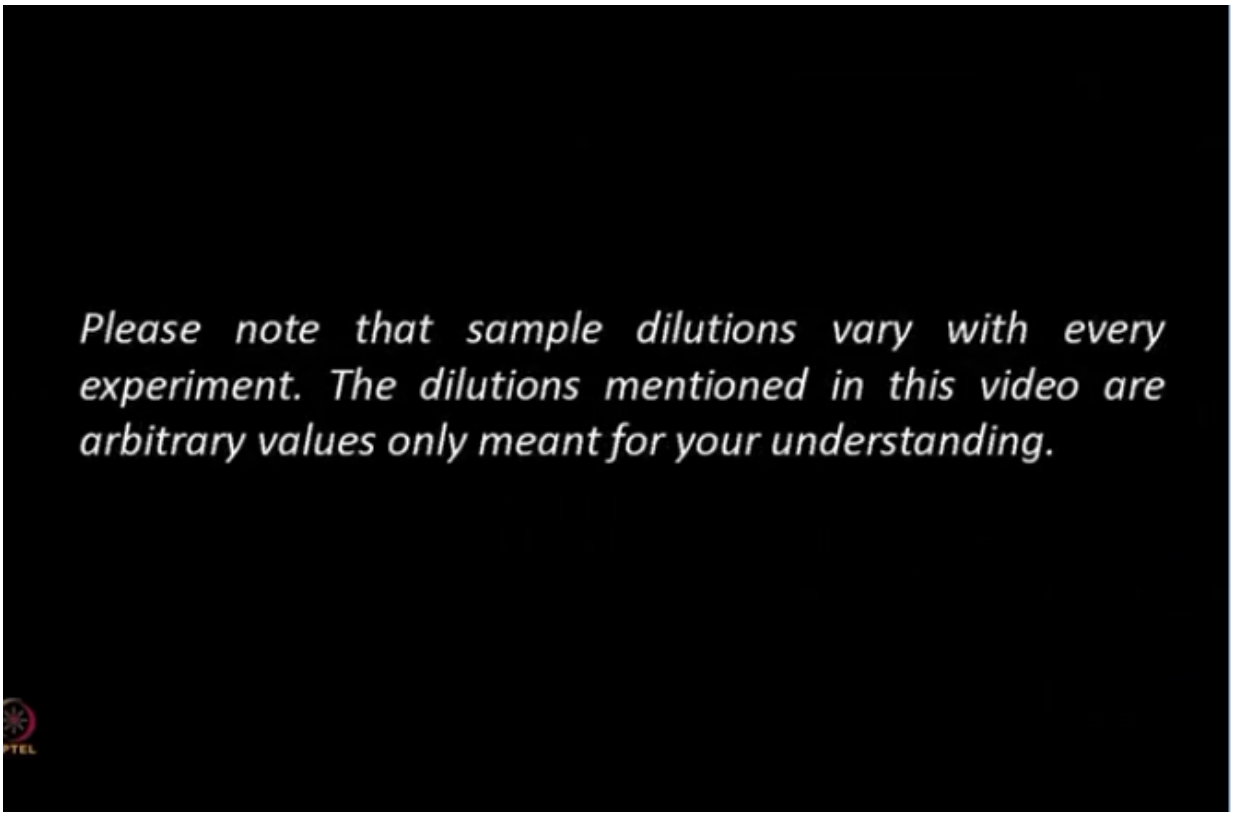

So usually this is also done using a multichannel pipette or sometimes there are also automated base to do this, but since we again dealing with only one chip, I'm using a normal pipette, so that way we have now incubated 2 micro litre of 8 serum samples in E. coli lysate and we'll incubate this for 30 minutes in the same platform rocker.

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So now it's 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 in our slide, for this I will take in multichannel pipette, so this step has to be performed really carefully as you will see these are really fine tips which have to be used to avoid any scratches, it's 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's always good to keep a little blocking buffer behind, as you will see the blocking buffer has been completely removed and now immediately we laid our samples, so this is a 50 micro litre pipette, I'm going to mix the serum sample once before adding it to the slide, so once this is done we'll add the serum samples to the slide, so we've totally added 200 micro litre of serum samples which have been diluted in 10% E. coli lysate, so to avoid any kind of evaporation what we'll do is we'll place the foil over the lid, and then we place this whole slide on the moist towel, we'll then place the slide overnight in a cold room which is at 4 degree.

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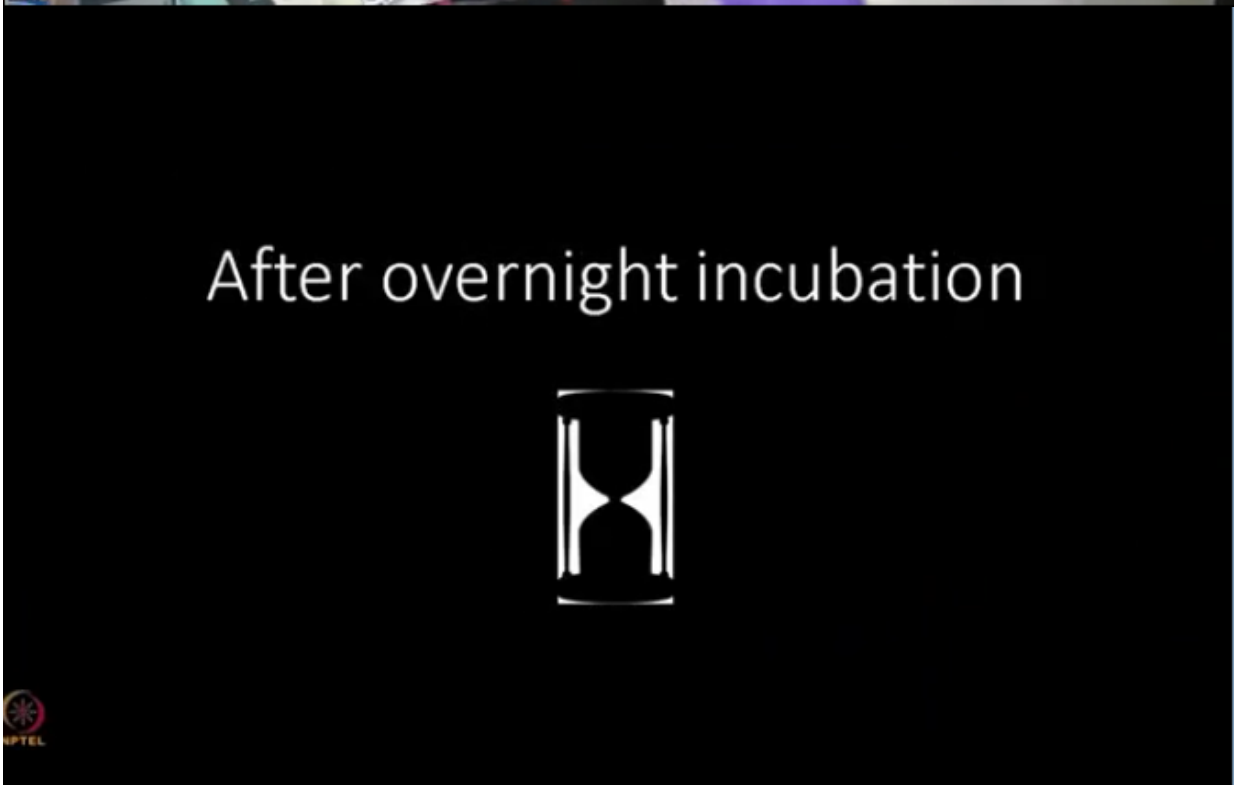

So after overnight incubation the next thing we need to do is aspirate the solution just like we did previously and we are going to wash this pad with twin buffer, I'm not going to show the washing steps now,

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because that is similar to how we aspirate it, the same way you aspirate and then you wash it on your 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 one micro litre of the secondary antibody in 199 micro litre of buffer, and I'll calculate this for 8 pads.

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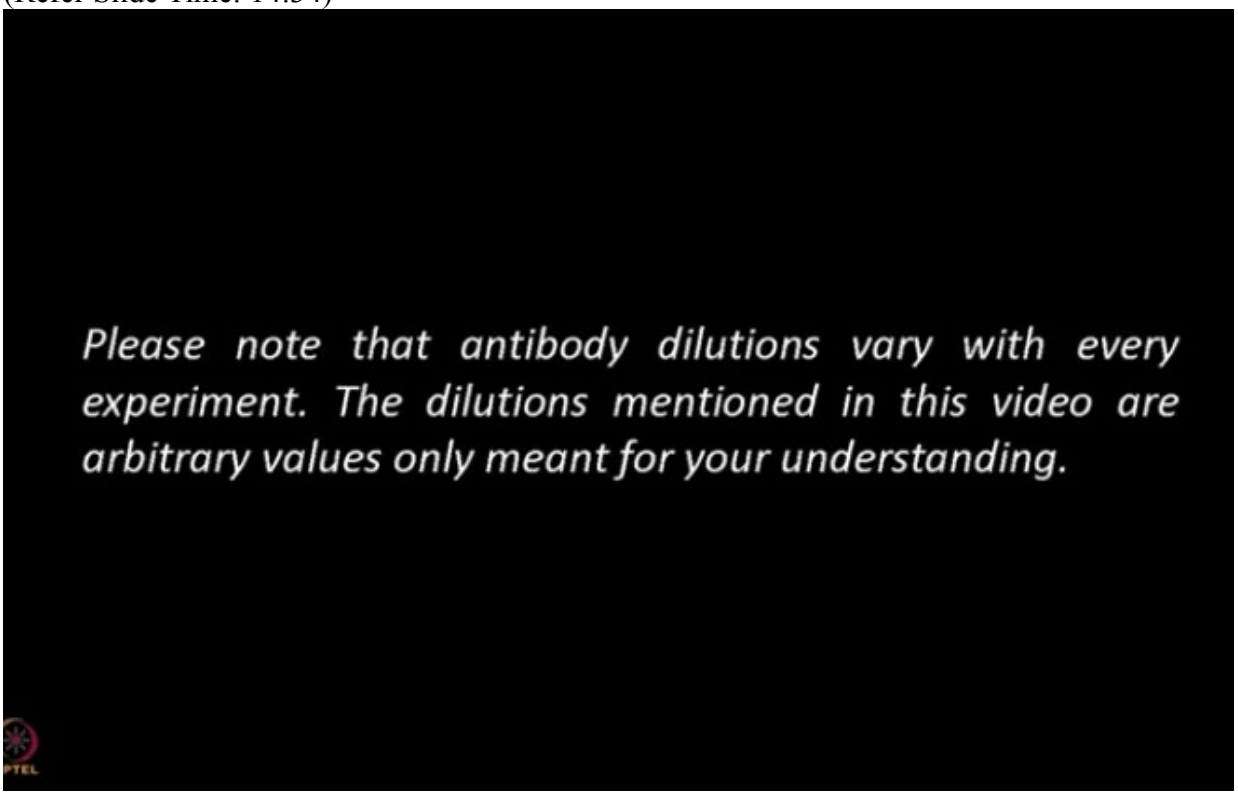

So basically I have already calculated the volume required for 8 pads, and I'm going to simply add 200 micro litre of secondary antibody to each pad,
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it's important to not scratch the pad, and also as I have mentioned earlier the pad should never dry, so once the step is done we will again incubate the slide on a platform rocker,

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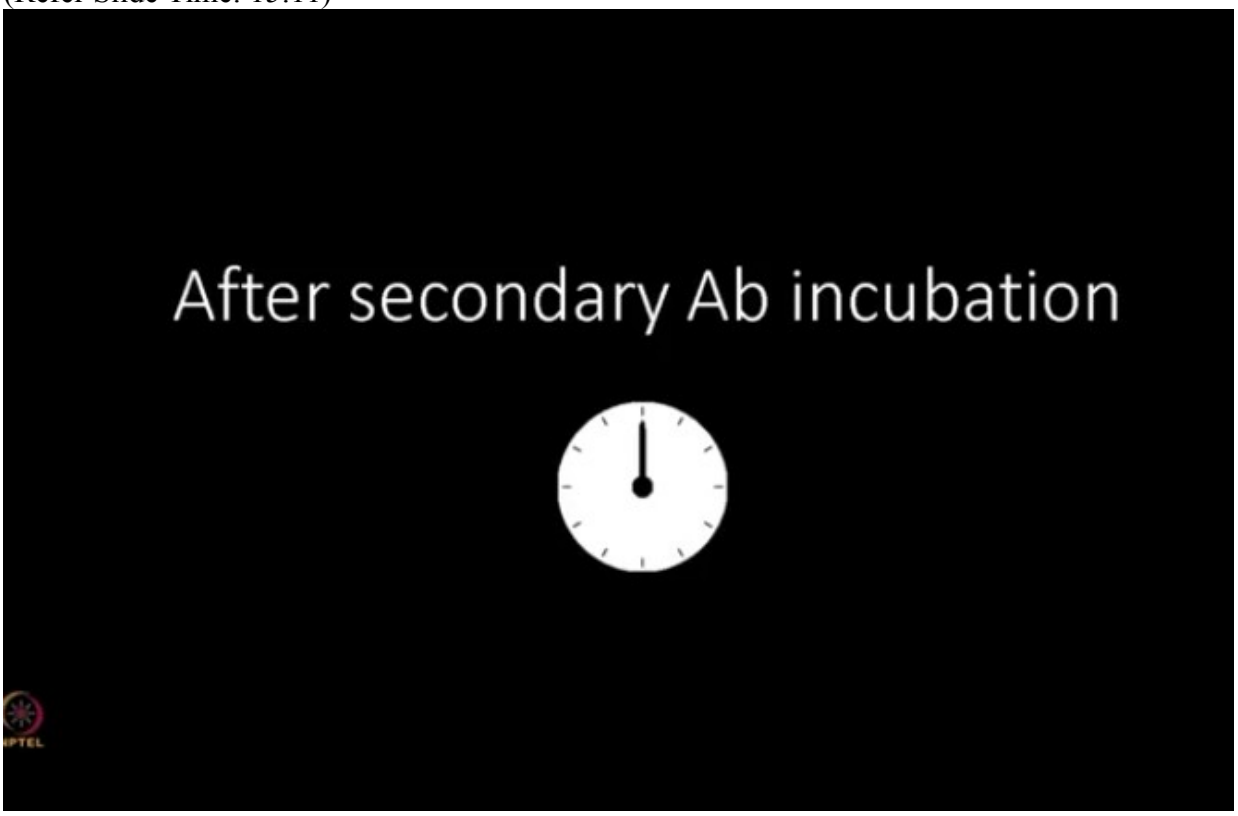

okay, so now what we have done is we have incubated the slide with the secondary antibody, and now we'll incubate it with a tertiary antibody, this process is again done after washing the

slide thrice with twin buffer, the dilution for this antibody is similar to the previous one, 1 and 200, and I've again calculated accordingly,
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so now we'll add 200 micro litre of tertiary antibody which is a scripta with hidden conjugate to the slide.

It's very important to close the slide and wrap it with foil immediately as this tertiary antibody is light sensitive, so once this is completely wrapped, we'll again place this on a platform rocker,
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I'd like to mention that after this step every other step has to be perform very carefully and we should try to avoid light as much as possible,

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so after incubation with tertiary antibody we're going to now wash the slides just like previously with twin buffer, so every time you add twin buffer you place it on a high speed rocker for 5 minutes, and then you wash it again, so this is, this process is done 6 times.

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Now it's time to remove the slide from slide chamber for scanning, so I'll carefully remove the slide from the chamber, I'm removing the clamps, you have to be careful not to scratch the pad surface, so we slowly remove the chamber from the slide and then we'll immediately transfer the slide into the slide holder, so this is basically distilled water to wash the slide before we scan it, so we have a slide holder with the slide and the balance which we will now centrifuge at 2000 RPM for 5 minutes.

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So we’re going to now scan the slide as you will see the slide is dry after centrifugation, we will place the slide carefully in the scanner with the barcode side facing downward, so now we’ll scan the slide.

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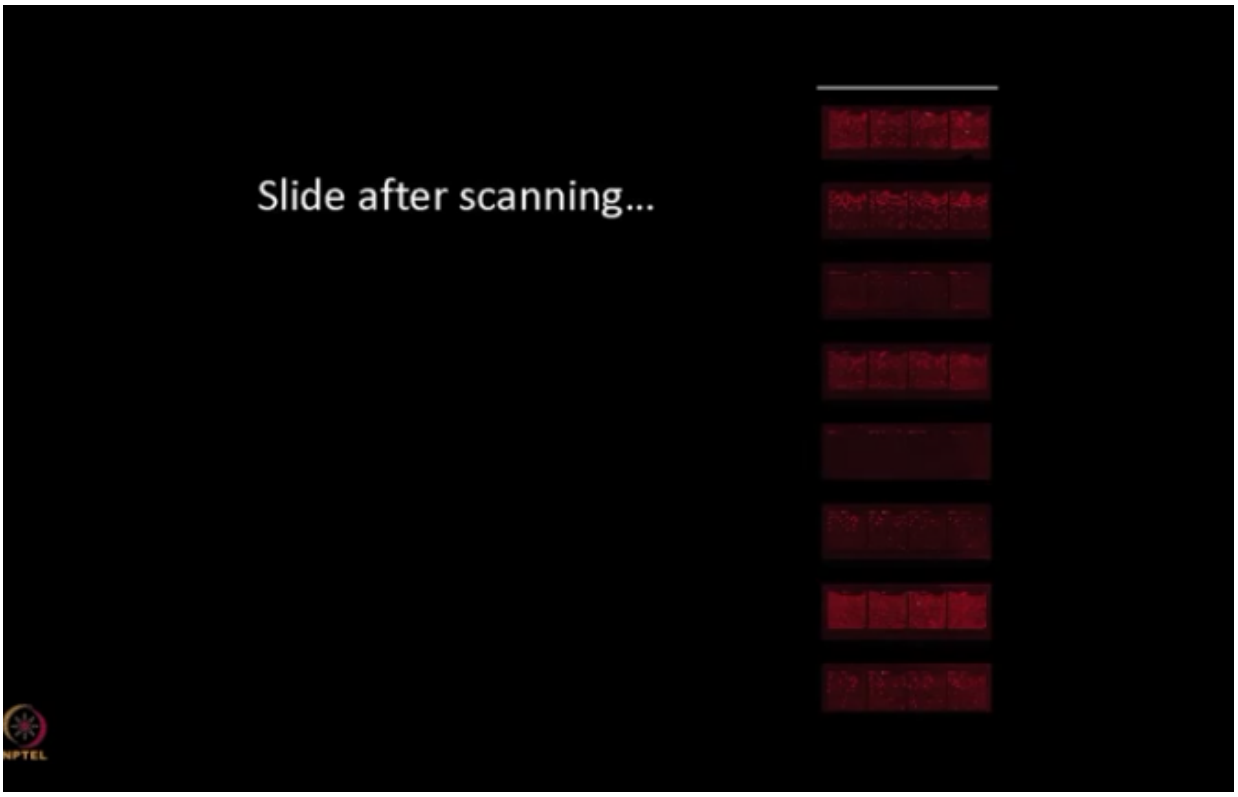

So hope you are familiar with how to perform the microarray experiment, so it is not difficult, it is just little tedious and there are certain precautions which you need to take while performing this particular experiment,

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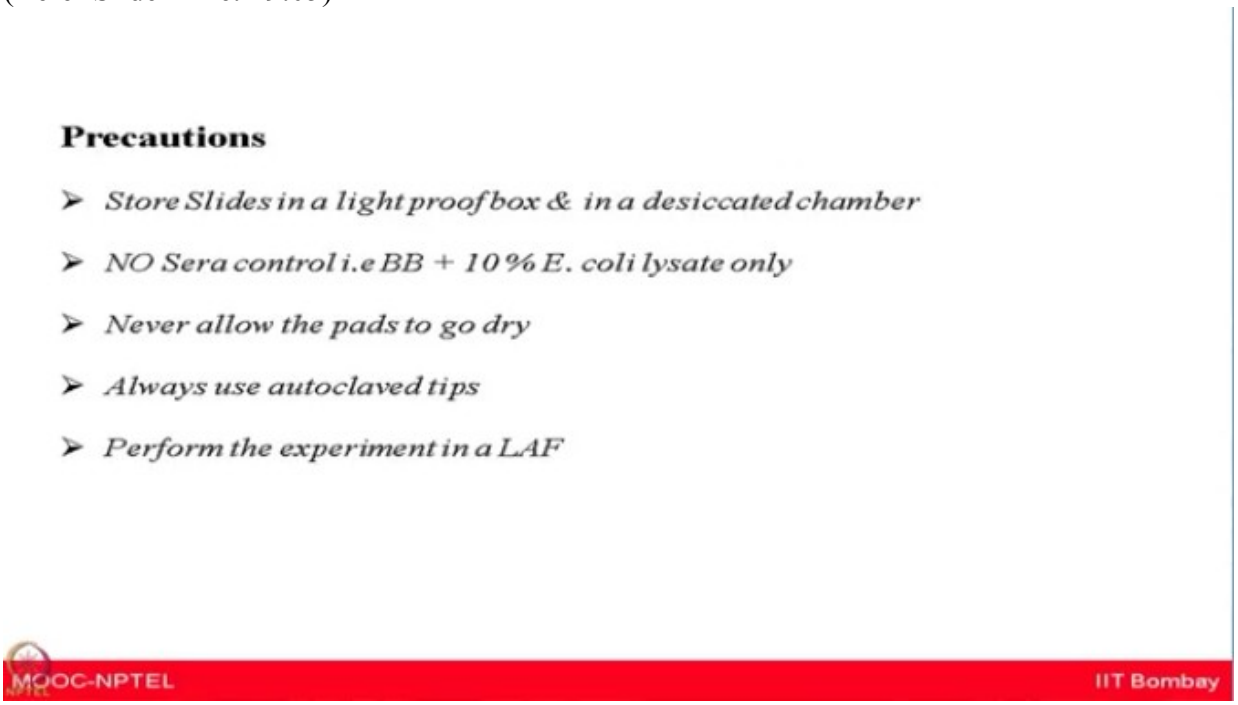

so basically it's very important to store the slides in a light proof box and in a desiccated cabinet if you have it, QC of each sample is already been done I'm sure but it's important to ensure that

it has been done, because these are all IVTT spots on the chip and they have to be checked once before you received them.

And it's important to probe one no sera control in every batch, I've already shown you that but it's very important in, probably in every slide if you can probe one it's great, if not at least in every batch there has to be one no sera control which is nothing but your blocking buffer + 10% 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 the slides very carefully, otherwise towards the end of the experiment you will never know which slide was use for which proving which samples, and especially when you have 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 is just starting microarray experiment and the laboratory, see you next week. Thank you.

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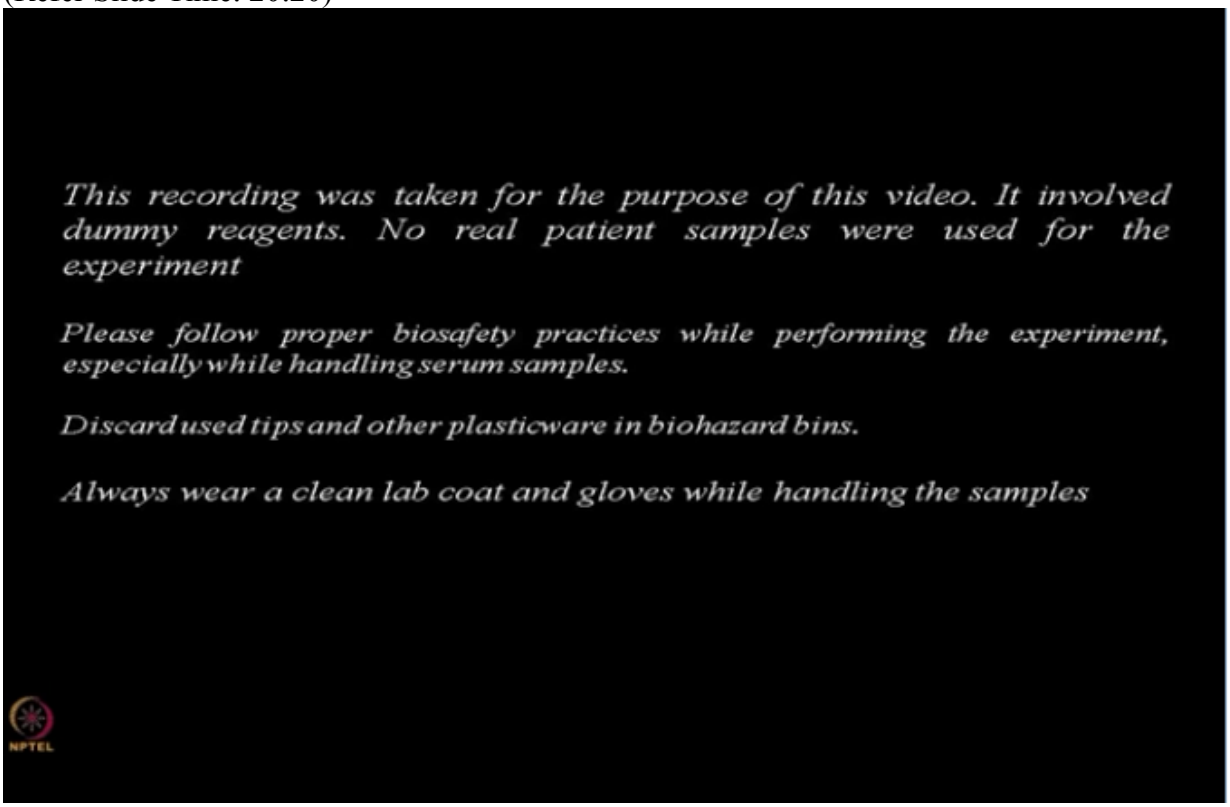


Points to ponder:

- Todays lecture: Experiment using *Pf/Pv500* protein arrays - Basic steps, workflow, protocol and precautions
- Objective: Profiling IgG levels in malaria positive patients using protein microarray

Points to ponder:

- **STEPS:**

Step 1: Slide assembly and set-up

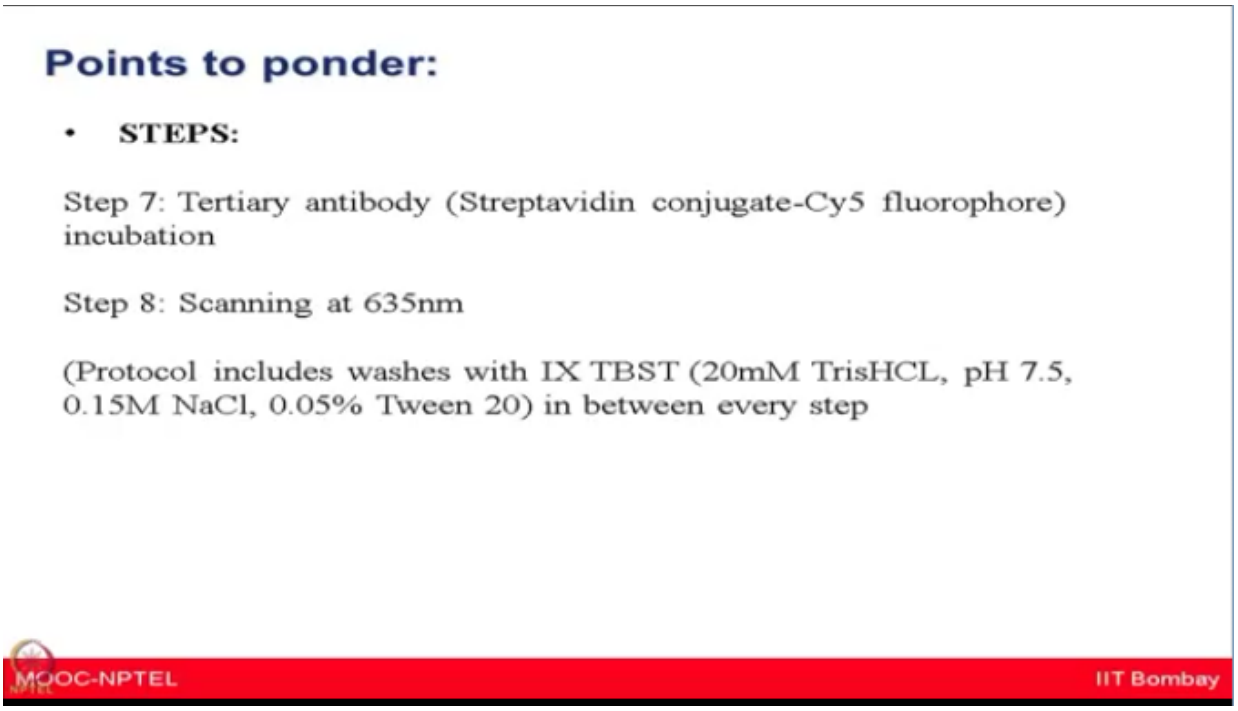
Step 2: Preparing reagents

Step 3: Incubation with 1X Blocking buffer

Step 4: Sample preparation and incubation with 10% *E.coli* lysate

Step 5: Sample hybridization at 4C overnight

Step 6: Secondary antibody (Anti-IgG) incubation



Dr. Sanjeeva Srivastava: I'm sure now you are curious, there is a many ways of producing the contents on the chip in different ways both self-based or self-array expression based manner.

Self-array expression provides lot more flexibility that you can generate large number of proteins of interest without need to purify them, in phrase especially in this case when we want to do a research on malaria we had different pathogens, different parasites from which these jeans were, because the clothes are made from falciparum or vivax, and they were printed on the chip and thought was can we express them using in your transcription, translation mix and make 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 have also understood every step is so crucial in the high throughput biology, if you are looking at every step meticulously your protocols, your showpiece, your quality control chips are in place, then only you can obtain, result which could be reproducible and meaningful.

In next lecture, we'll 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.

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