Interactomics: Protein Arrays and Label-Free Biosensors.
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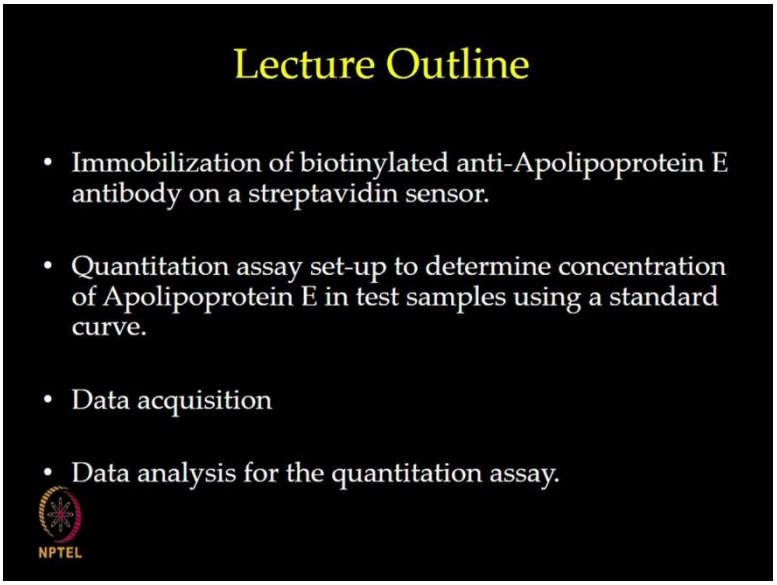
Lecture-17.
Label-Free Quantification of Proteins Using BLI

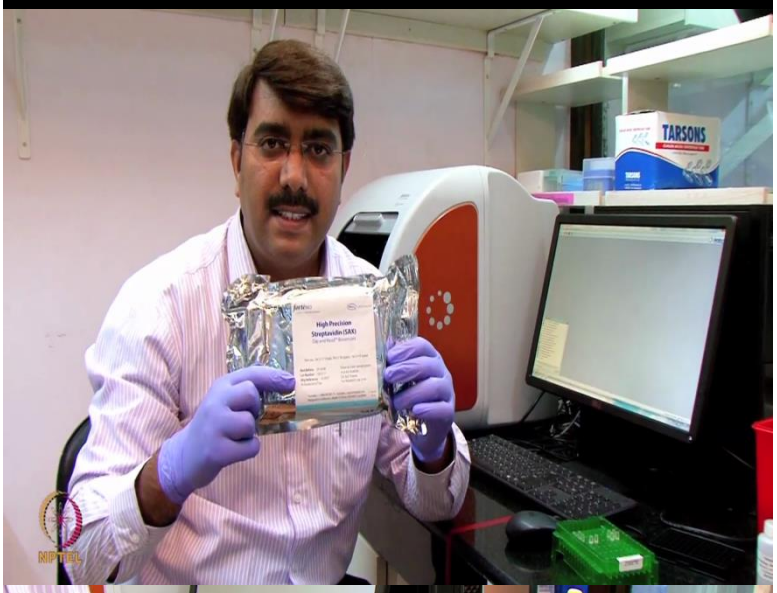
Welcome to the MOOC intractomics course, today we will be focusing on another application of biosensors, biomolecular concentration analysis. The task of measuring the concentration of specific protein in a complex solution is not simple and a reliable analysis method for the same desired which is which is able to analyze samples with different sample matrix composition.

Today we will be studying the concentration analysis of a protein, Apolipoprotein E using a standard curve through bio-layer interferometry. In this concentration assay different concentrations of the analytes are injected and the response from these different concentrations is plotted against the concentration of each sample. From this, a calibration curve is calculated and used to determine the concentration in a sample with unknown concentration.

(Refer Slide Time: 1:37)



Immobilization of the antibody on the sensor surface

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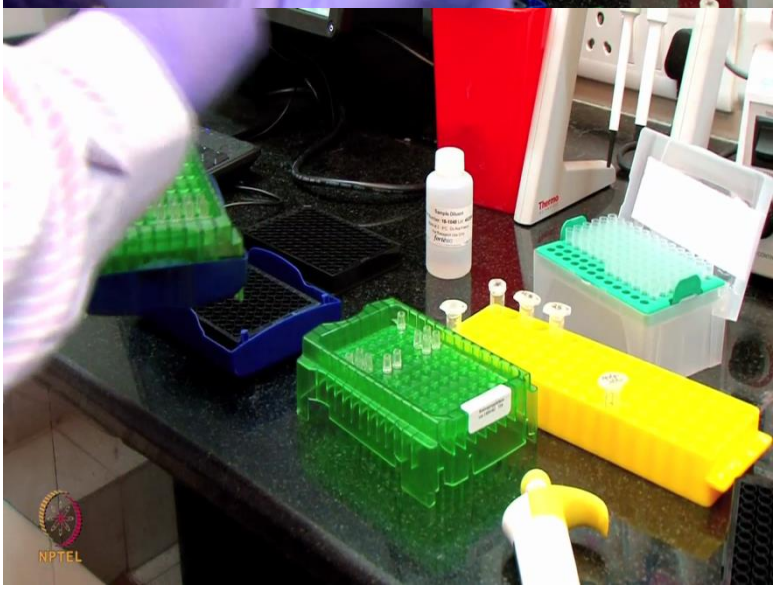
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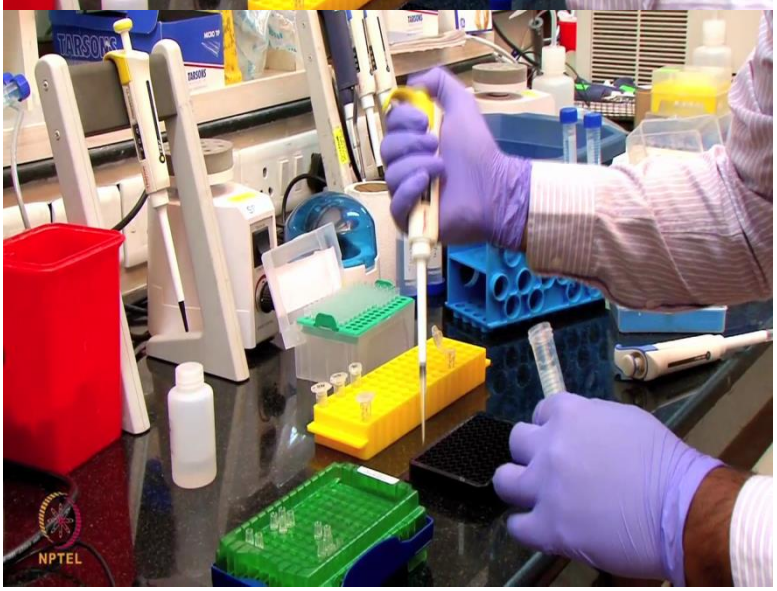
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TARSONS
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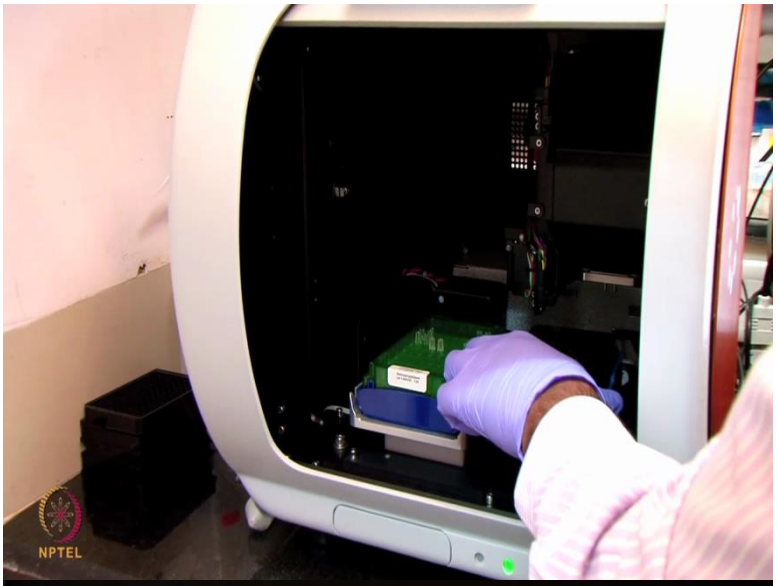


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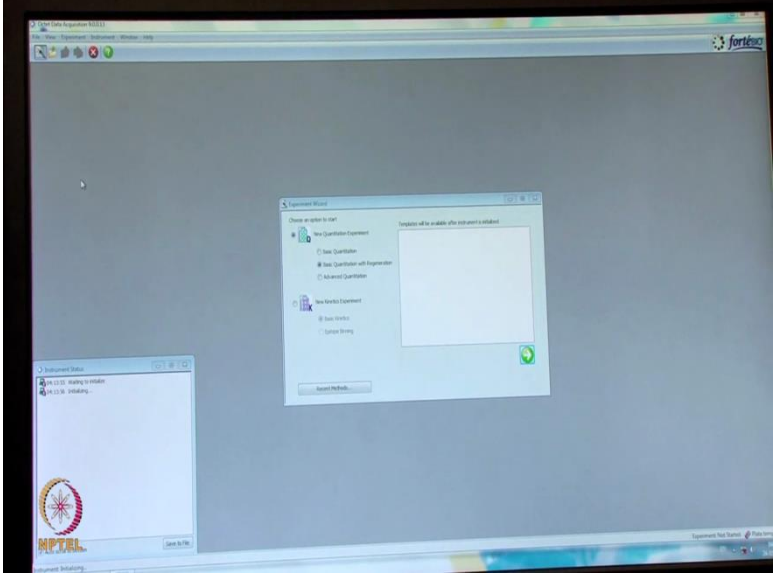
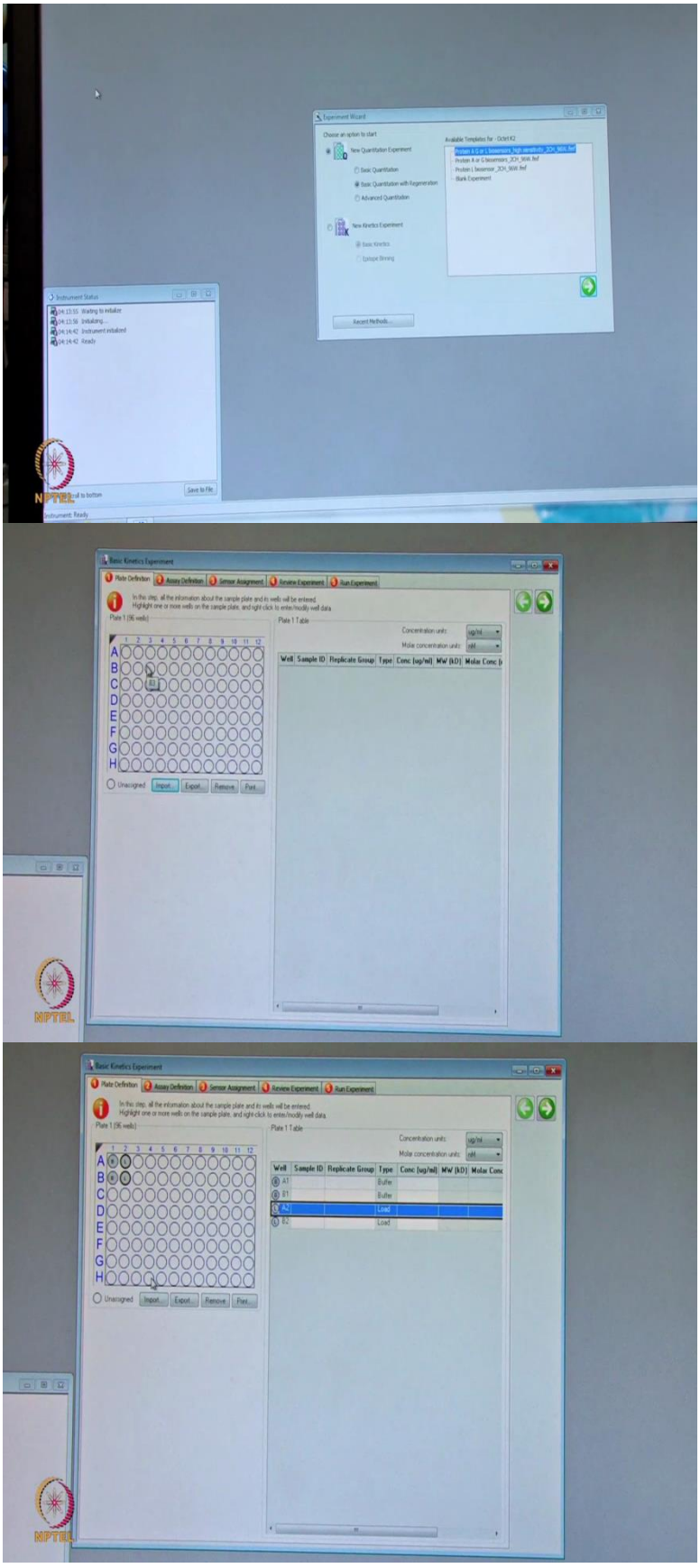
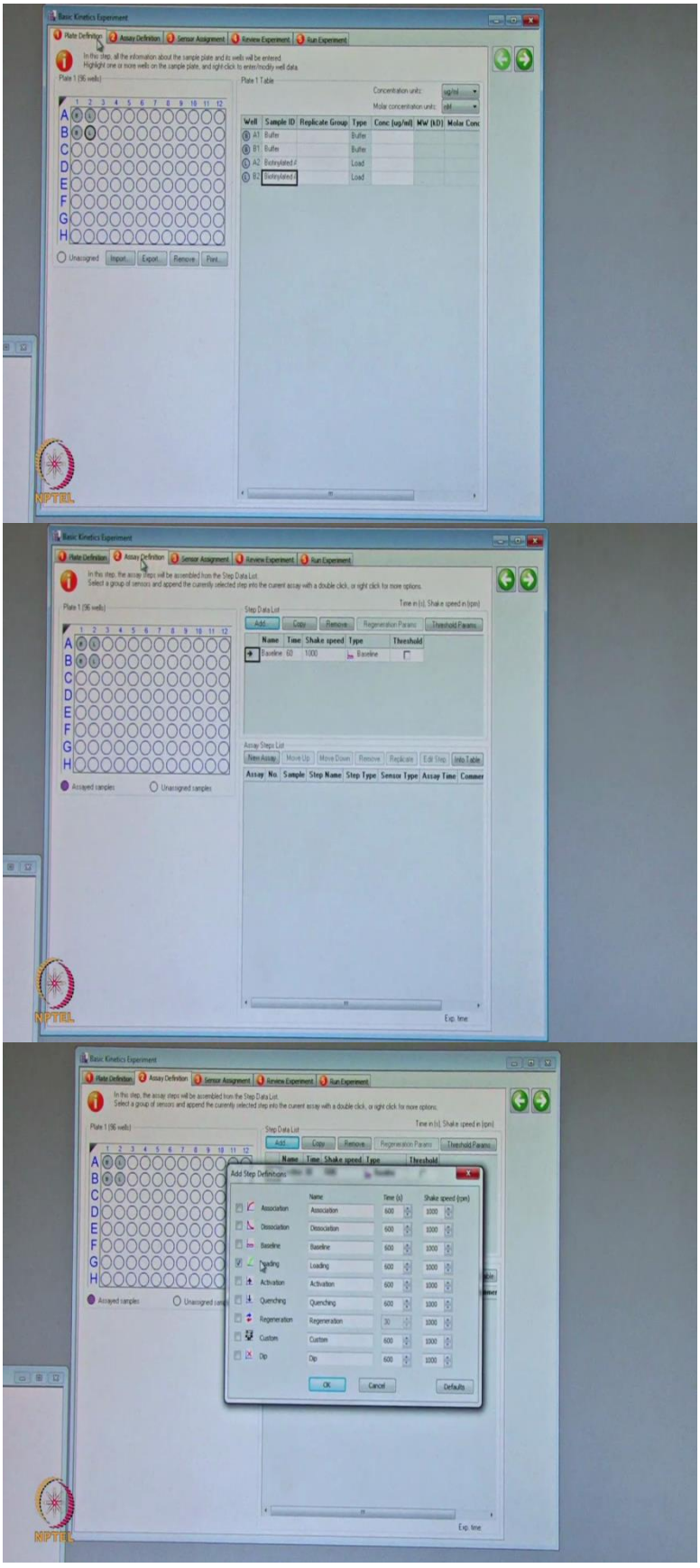
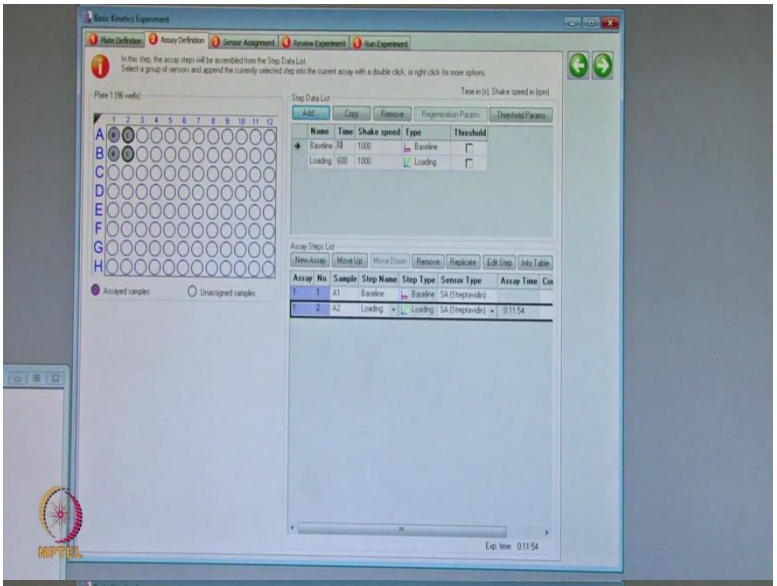
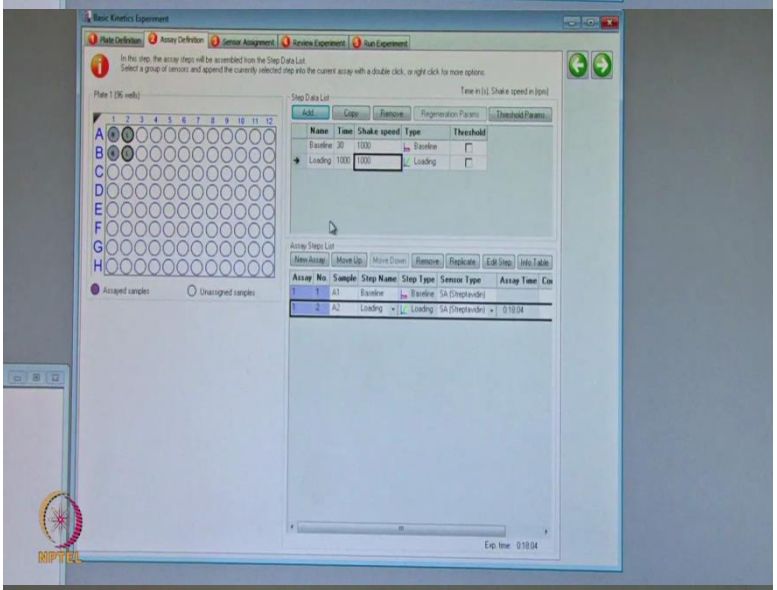
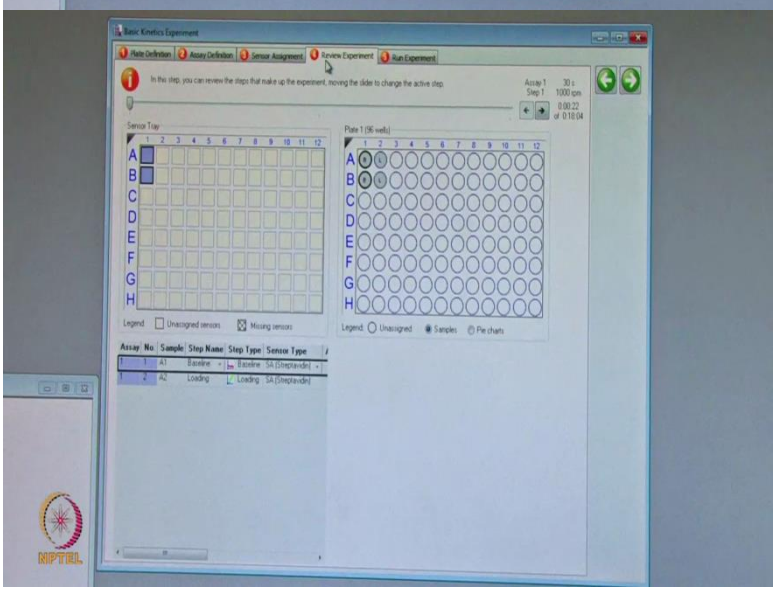


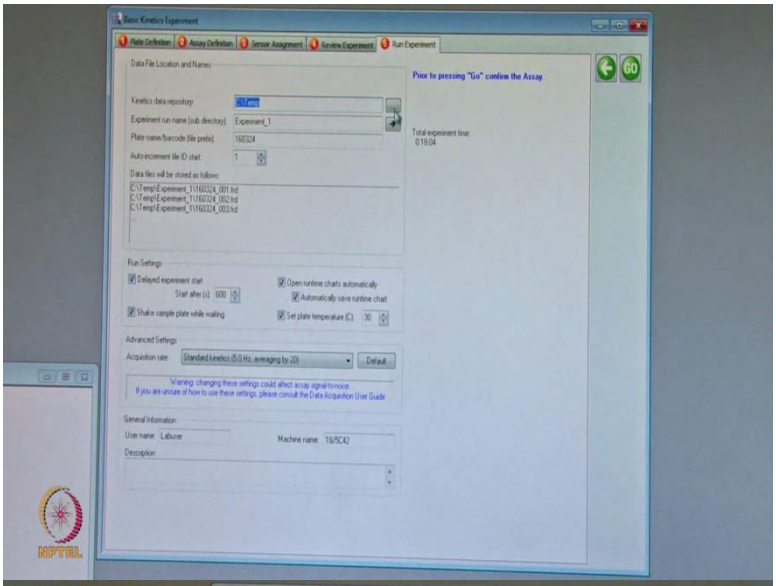

Experiment Wizard
Instrument Status
Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table
Well
Sample ID
Replicate Group
Type
Conc (ug/ml)
MW (kD)
Buffer
Load
NPTEL

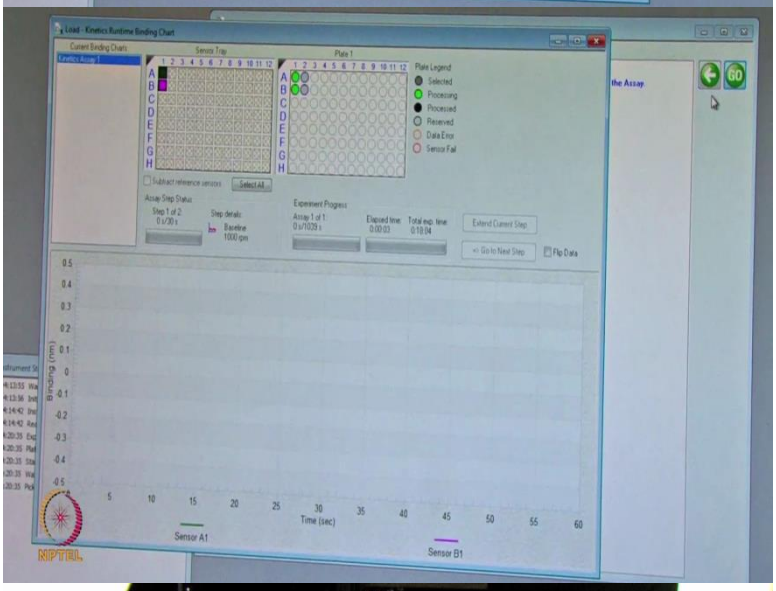

Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table
Add Step Definitions
OK
Cancel
Defaults


Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
In this step, the assay steps will be assembled from the Step Data List.
Select a group of sensors and append the currently selected step into the current assay with a double click, or right click for more options.
Plate 1 (96 wells)
Step Data List
Add
Copy
Remove
Regeneration Params
Threshold Params
Name
Time
Shake speed
Type
Threshold
Baseline 30 1000 Baseline
Loading 600 1000 Loading
Assay Steps List
New Assay
Move Up
Move Down
Remove
Replicate
Edit Step
Info Table
Assay No.
Sample
Step Name
Step Type
Sensor Type
Assay Time
1 1 A1 Baseline Baseline SA (Streptavidin)
1 2 A2 Loading Loading SA (Streptavidin) 0:11:54
Assayed samples
Unassigned samples
Exp. time: 0:11:54

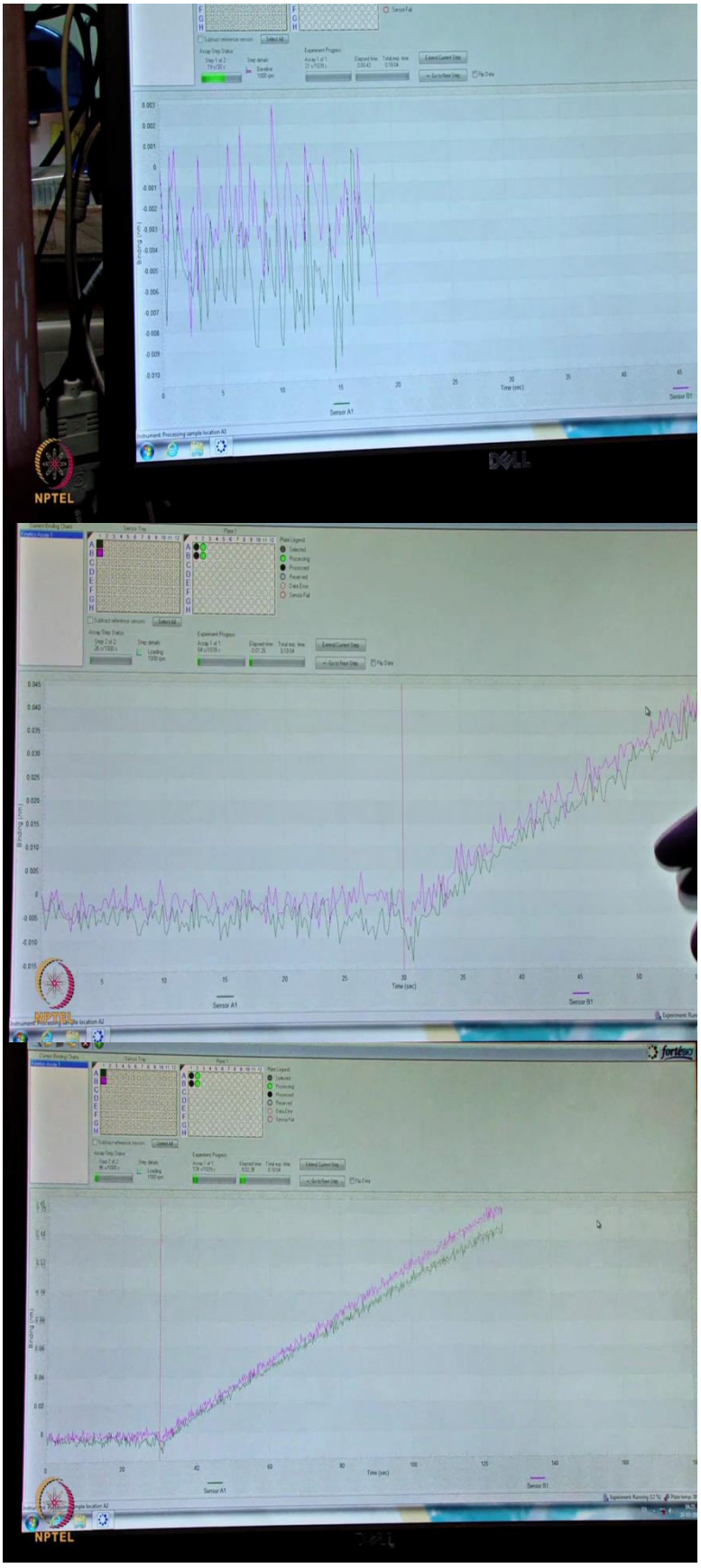

Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
In this step, the assay steps will be assembled from the Step Data List.
Select a group of sensors and append the currently selected step into the current assay with a double click, or right click for more options.
Plate 1 (96 wells)
Step Data List
Add
Copy
Remove
Regeneration Params
Threshold Params
Name
Time
Shake speed
Type
Threshold
Baseline 30 1000 Baseline
Loading 1000 1000 Loading
Assay Steps List
New Assay
Move Up
Move Down
Remove
Replicate
Edit Step
Info Table
Assay No.
Sample
Step Name
Step Type
Sensor Type
Assay Time
1 1 A1 Baseline Baseline SA (Streptavidin)
1 2 A2 Loading Loading SA (Streptavidin) 0:18:04
Assayed samples
Unassigned samples
Exp. time: 0:18:04

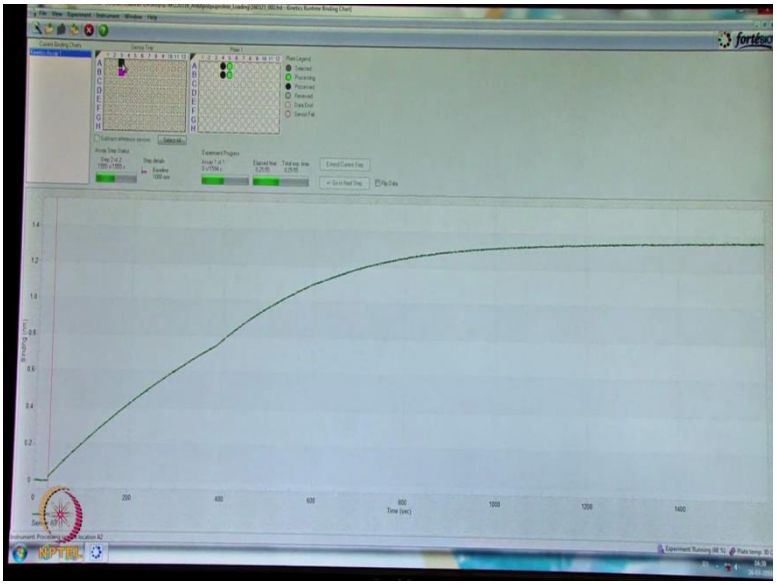

Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
In this step, you can review the steps that make up the experiment, moving the slide to change the active step.
Assay:1 30 s
Step:1 1000 rpm
0:00:22
of 0:18:04
Sensor Tray
Plate 1 (96 wells)
Legend: Unassigned sensors Missing sensors
Legend: Unassigned Samples Pie charts
Assay No.
Sample
Step Name
Step Type
Sensor Type
1 1 A1 Baseline Baseline SA (Streptavidin)
1 2 A2 Loading Loading SA (Streptavidin)


Basic Kinetics Experiment
Plate Definition
Assay Definition
Sensor Assignment
Review Experiment
Run Experiment
Data File Location and Names
Kinetics data repository:
Experiment run name (sub directory):
Experiment_1
Plate name/barcode (file prefix):
160324
Auto-increment file ID start:
1
Data files will be stored as follows:
C:\Temp\Experiment_1\160324_001.hrd
C:\Temp\Experiment_1\160324_002.hrd
C:\Temp\Experiment_1\160324_003.hrd
Prior to pressing "Go" confirm the Assay
Total experiment time:
0:18:04
Run Settings
Delayed experiment start
Start after (s):
600
Open runtime charts automatically
Automatically save runtime chart
Shake sample plate while waiting
Set plate temperature (C):
30
Advanced Settings
Acquisition rate:
Standard kinetics (5.0 Hz, averaging by 20)
Default
Warning: changing these settings could affect assay signal/noise.
If you are unsure of how to use these settings, please consult the Data Acquisition User Guide
General Information
User name:
Laburer
Machine name:
16/SC42
Description:
GO
NPTEL


Load - Kinetics Runtime Binding Chart
Current Binding Charts
Kinetics Assay 1
Sensor Tray
Plate 1
Plate Legend
Selected
Processing
Processed
Reserved
Data Error
Sensor Fail
Subtract reference sensors
Select All
Assay Step Status
Step 1 of 2
0 s / 30 s
Step details:
Baseline
1000 rpm
Experiment Progress
Assay 1 of 1
0 s / 1029 s
Elapsed time:
0:00:03
Total exp. time:
0:18:04
Extend Current Step
=> Go to Next Step
Flip Data
Binding (nm)
Time (sec)
Sensor A1
Sensor B1
GO
NPTEL


NPTEL


NPTEL
Time (sec)
Sensor A1
Sensor B1
NPTEL
Time (sec)
Sensor A1
Sensor B1
NPTEL
Time (sec)
Sensor A1
Sensor B1



Let us now understand the experimental workflow from the demonstration session. Good morning one and all, myself Susheelendra Vaidya from pall Corporation, I am going to give a demonstration on how this BLI technology we can use in the quantitation. Now, I am going to demonstrate the experiment here is like that quantitation of the Apolipoprotein using a antibody against the apolipoprotein.

The biotinylated antibody I am going to use immobilization of the antibody on to a streptavidin sensor. This is the streptavidin sensor I am using for the immobilization step. So, here in this experiment we are using a streptavidin sensor. The streptavidin sensor has to be hydrate prior for the immobilizations. Now, I am going to do this hydration of the streptavidin sensors. So, what I am doing this I am adding the 1x PBS into the (())(2:43) this experiment 200 microliter of the 1x PBS. So, I am (())(2:54).

Now, what I am doing is next step I am taking the biosensors which is the streptavidin sensors, these are the sensors looks like this, I am going to put on to the sensor chip , this is the sensor holder tray , I am putting these sensors like this. Now, this sensor tray I am keeping for the hydration for the 10 minutes. So, this is I am going to keep like this. So, now I will set aside these sensors for the 10 minutes.

Now, I am going to demonstrate you that how we are going to immobilize the apolipoprotein, anti-apolipoprotein on to the streptavidin sensor. Taking by 200 microliter of the 1x PBS buffer, this is prepared, I am going to add 200 microliter in each to a well 96well. Now, I am putting the, this is the biotinylated anti-apolipoprotein. So, now I am going to add 200 microliter of each biotinylated anti-apolipoprotein to a 96 well plate.

Now, the sensors are hydrated I am going to place in the instrument, the sensors holder I am placing here like this, it should be firmly fixed in the groove and now, I am going to put the to the sample plate in which contains the biotinylated antibody, I am going to place like this in the sample tube. This is we call it as this particular part we call it as a sample compartment, this part we call it as a sensor compartment.

Now, I am going to click on to the data acquisition mode. So, when you click on the data acquisition, the instrument, now we can see this how the moment of the optical head spectrophotometer itslef is initializing the instrument. In the monitor, during the initiliasation we can observe this initialization status, once it is comes the signal as a ready then we can use for the immobilization step.

So, now the we can able to see here the instrument status it is ready, when we click on the experimental wizard setup, you can see that there is an new quantitation experiment as well as the kinetics experiment. Here I am doing the immobilization step so that is why I am choosing the basic kinetics with the experiment, I am choosing this blank experiment then I will say this go.

Now, we can see there is a page , in this page it is on your left hand side is showing the 96 well plate, what things you have put in the 96 well plate. Here, I had put in the A1 and the B1 here I have put a buffer red we called as a buffer right click it and say it as a buffer, this indicated by AB, then we have to select these two. And now, we have to mention this step as load.Load is nothing but the immobilization of the biotinylated antibody against the apolipoprotein that we had placed in the setting A2 and the B2.

So, we have to mention here in the sample ready just it is a buffer. Okay, then (())(6:47). Now, it is biotinylated Apo and I have mentioned biotinylated Apo and I can copy it, paste it here. In the next step, in the assay definition we have to mention what is the steps the instrument has to be performed. The first step is baseline and the second step on clicking on to the add here we have to load, the next step is loading step, that is nothing but your immobilization step and that is say. Ok.

The first step is base line I had take arrow mark here, right click here and the next step is the immobilization we have to use a loading. This is the time, here it is mentioned it is 30 seconds (())(7:39) around around 1000 seconds for the immobilization of the antibody. I am using a streptavidin sensor by default it is coming as streptavidin. This is we had assign the

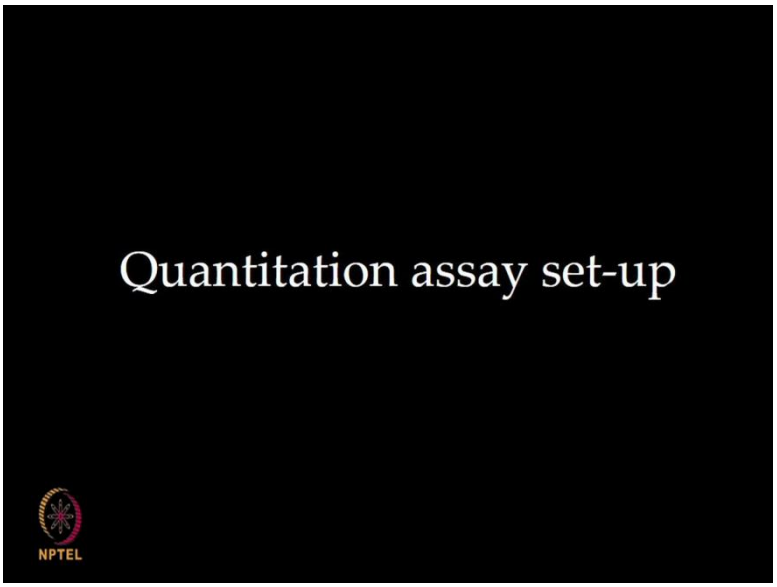
assay definition step this sensor assignment where you have kept the sensor, in the sensor compartment we had kept the sensors it says, A1 and the B1.

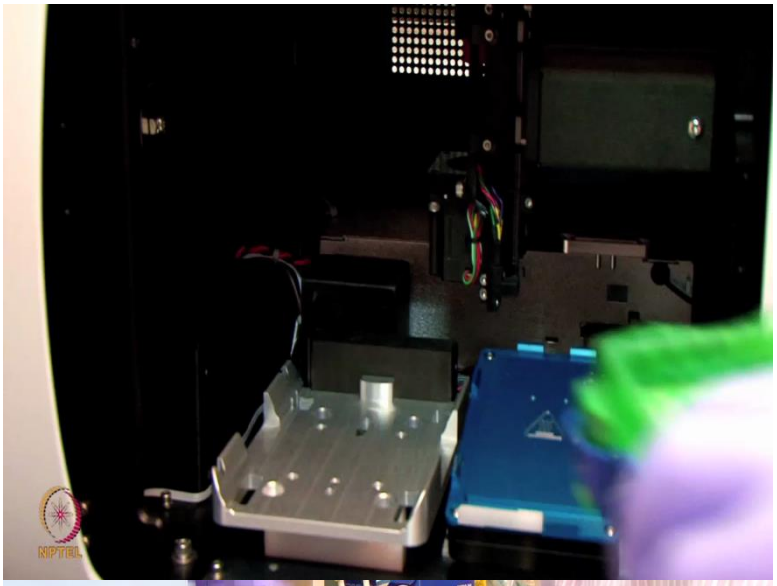
That is by default it is a showing, if it is somewhere, it is in the tray we have to assign. In the review experiment whether my methods are fine or not you can test it. The first step is the sensor has to be picked from the A1 and the B1, it is going to be use the baseline that is nothing but your buffer. It is highlighting by the black color on the surface.

So, next step is your loading step. So, it is moved, in the run experiment where you want to save the data, I will select this and the experiment I can queue as a load. Okay, then I can uncheck this these two, say go here. Thus now the sensor is picking from the sensor tray here the. If you look at there is a shaker here, we are working at 1000 rpm speed, it is moving to the baseline, it is acquiring for the 30 seconds the baseline.

Now, we can see the immobilization step, the biotinylated antibodies going to immobilize onto the streptavidin sensors. Generally, we will load it has to reach to the equilibrium, roughly around it should be a more than 0.7 nanometer is (())(9:36) for immobilization level, we can go for the quantitations. Now, you can see that desired level of the biotinylated antibody immobilized onto the streptavidin sensors. Now, we can able to see there is a 1.4 nanometer loaded on to the sensor.

(Refer Slide Time: 10:00)

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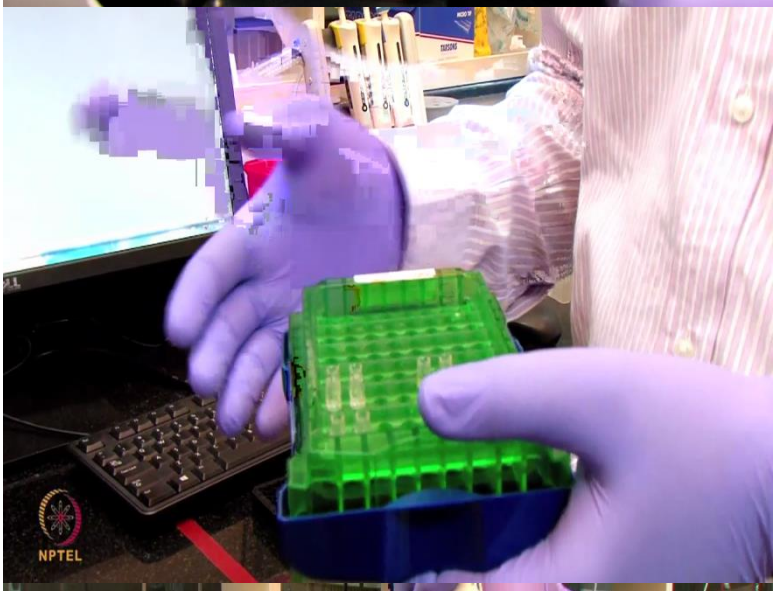
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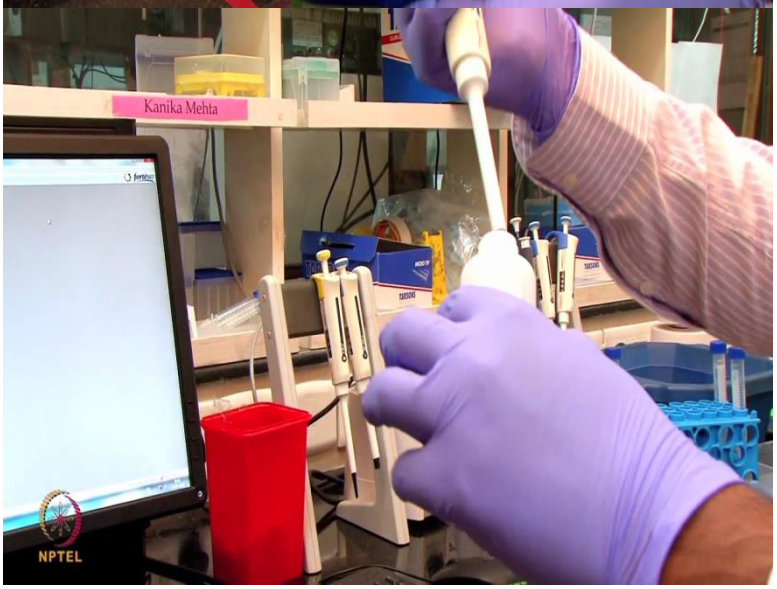
Kanika Mehta
NPTEL


TARSONS
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TARSONS
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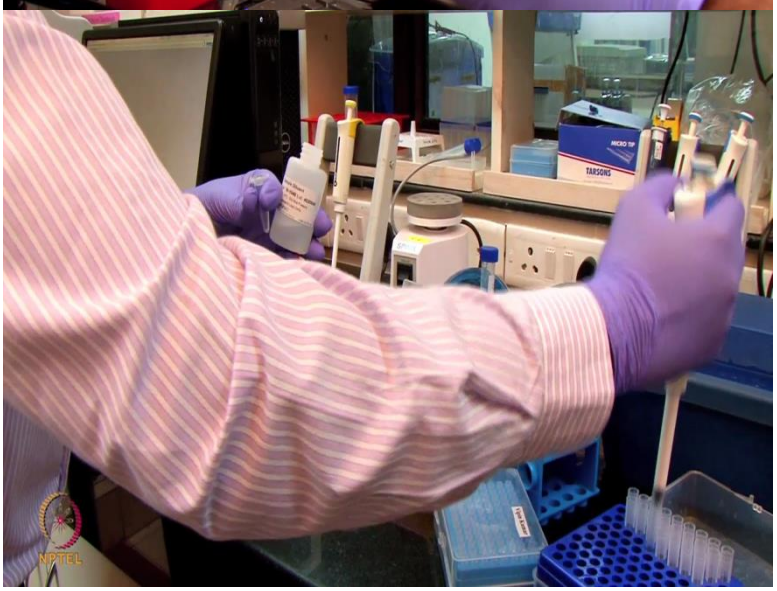
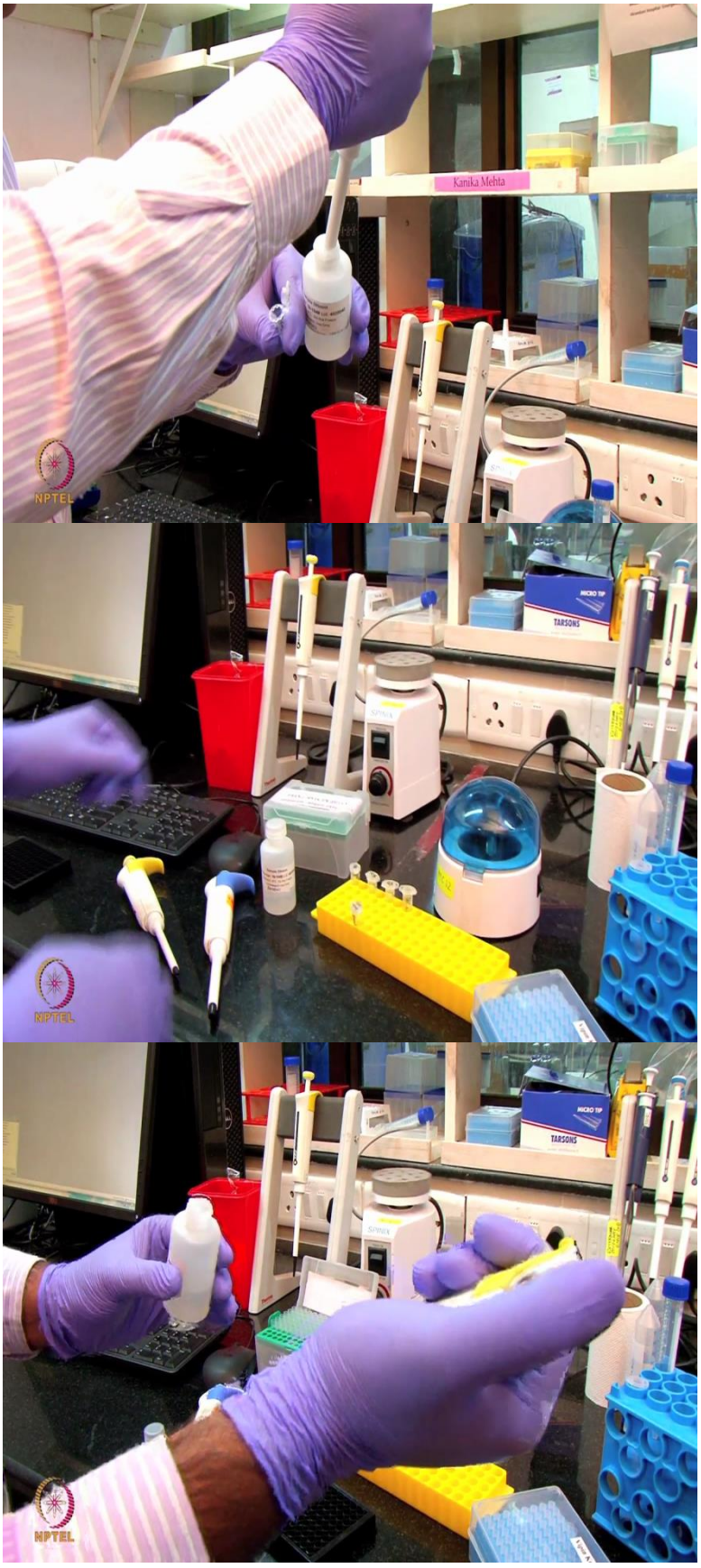

MICRO TIP
TARSONS
NPTEL


Kanika Mehta
NPTEL
MICRO TIP
TARSONS
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MICRO TIP
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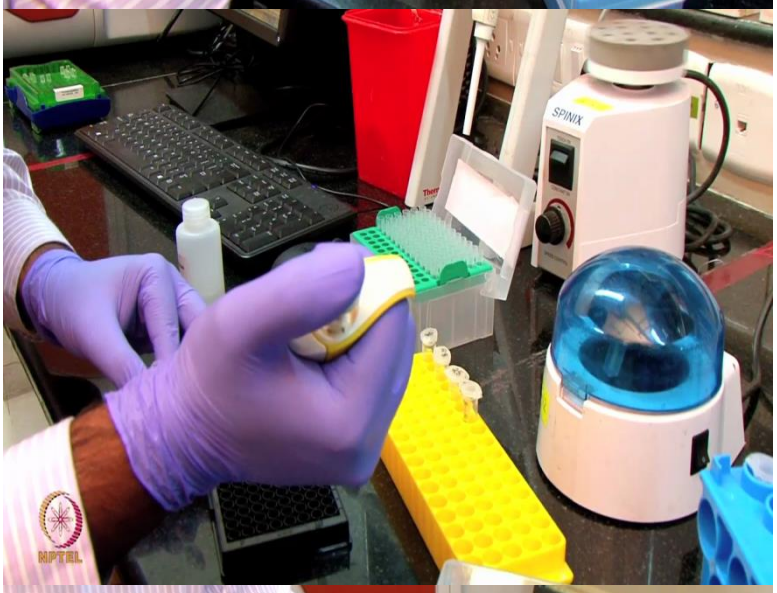
NPTEL


TARSONS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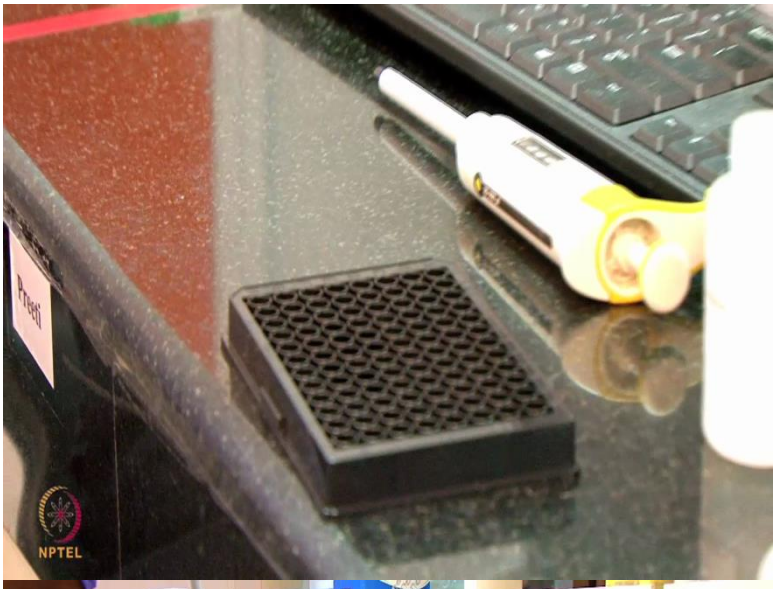
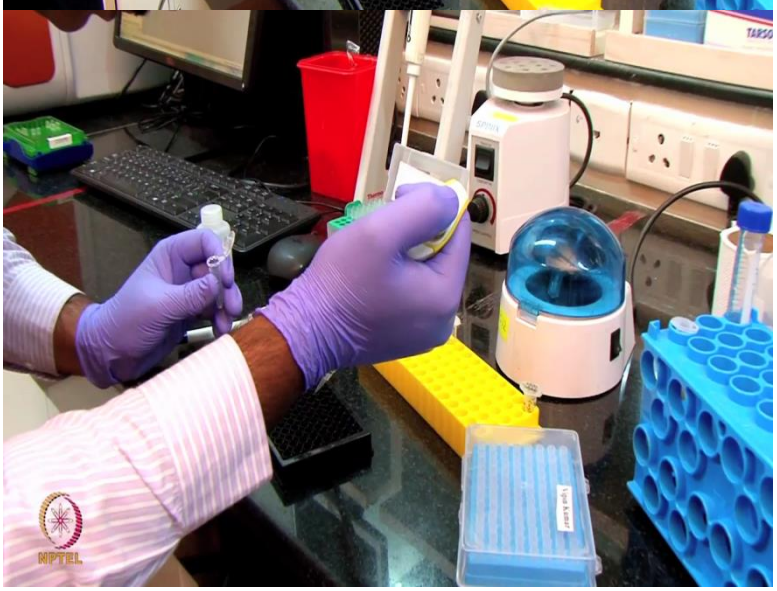

Preeti
NPTEL


Kanika Mehta
NPTEL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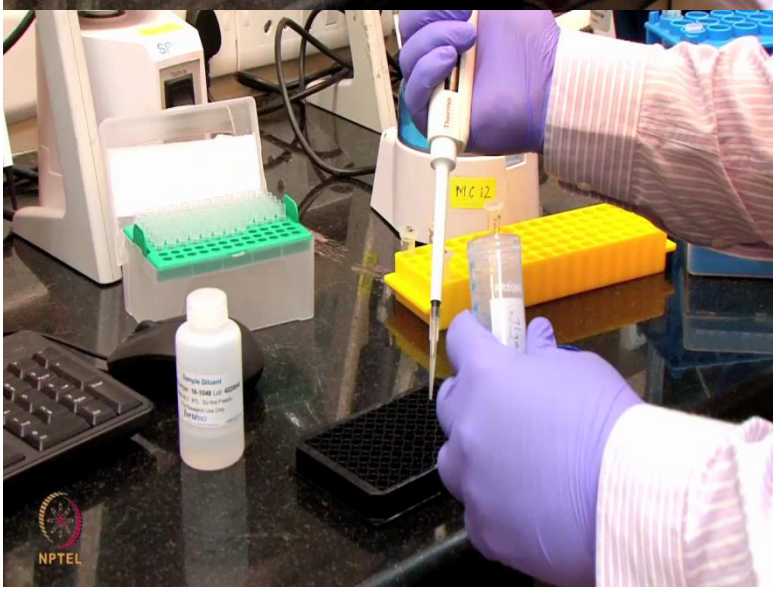
MC 12
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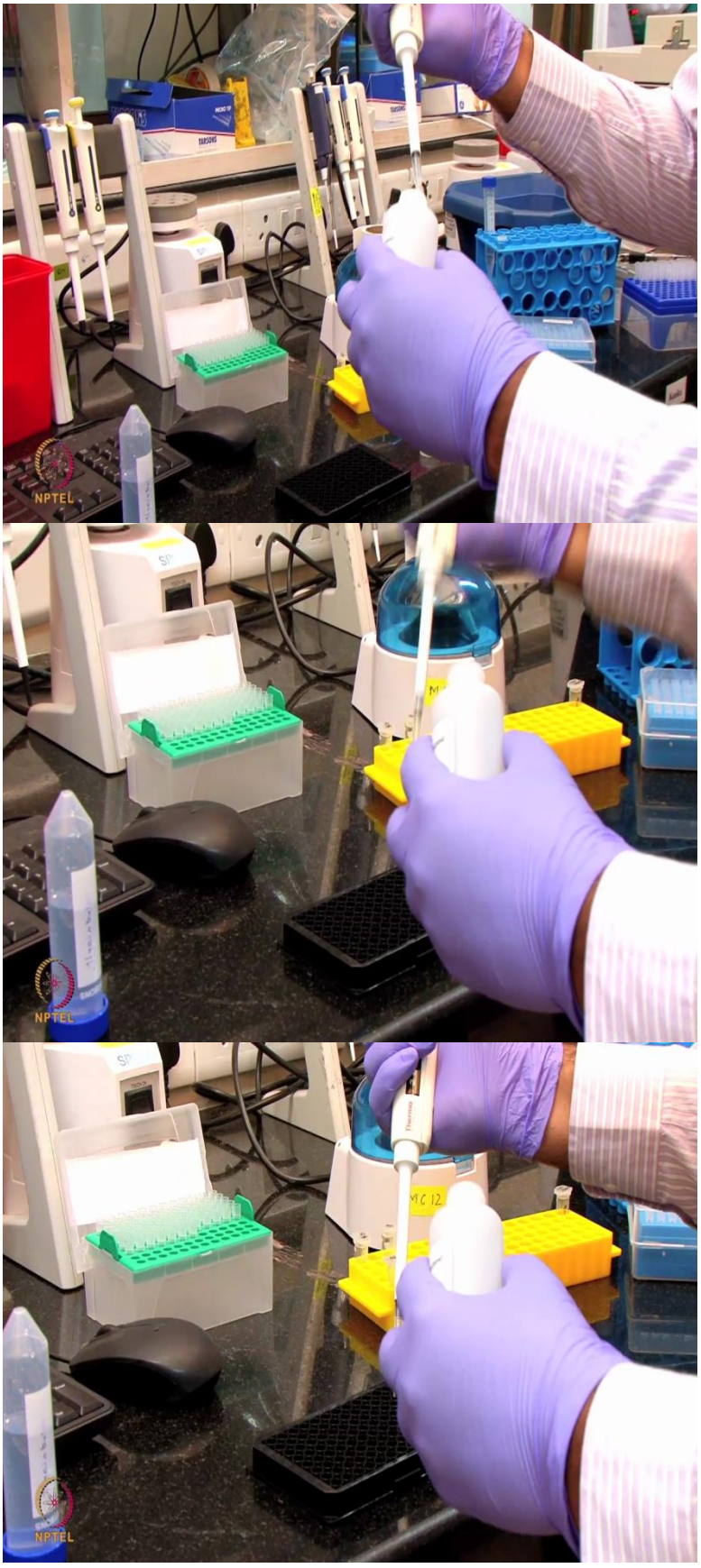
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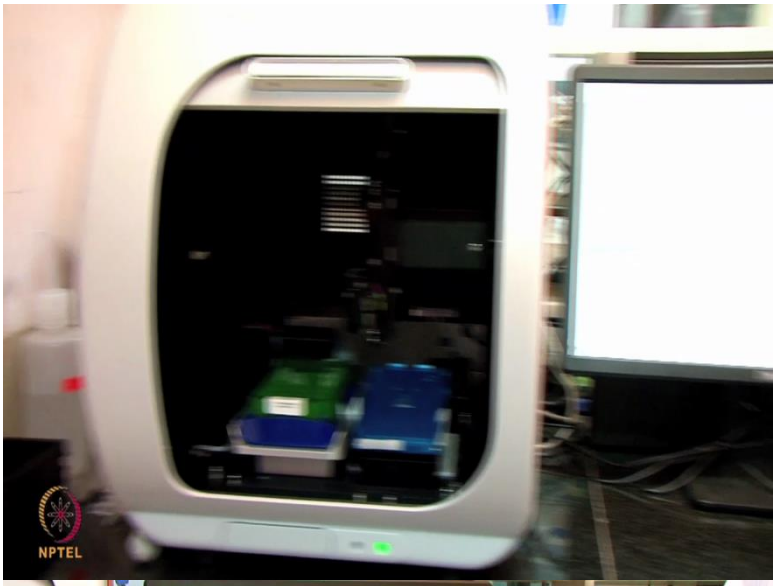
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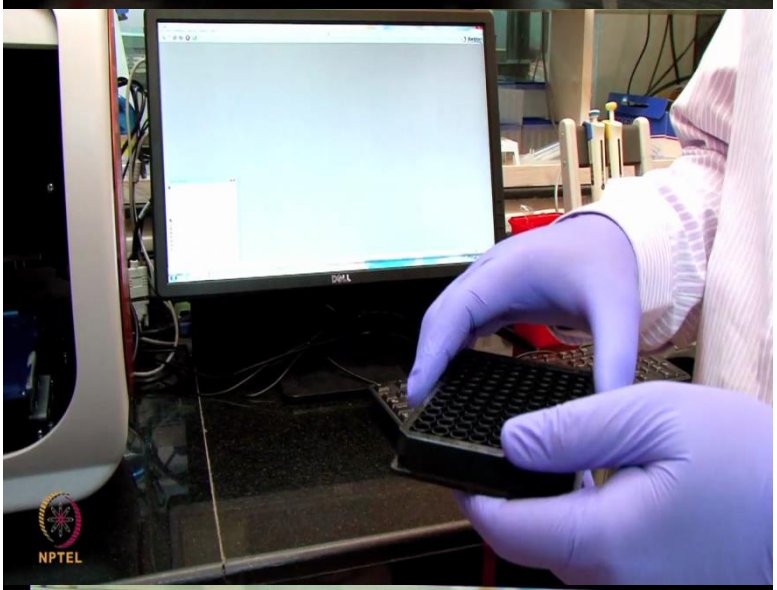
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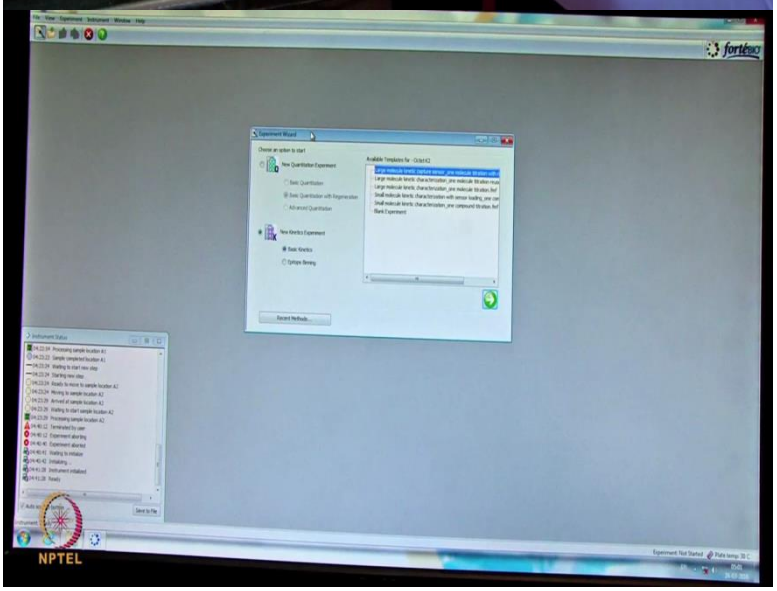
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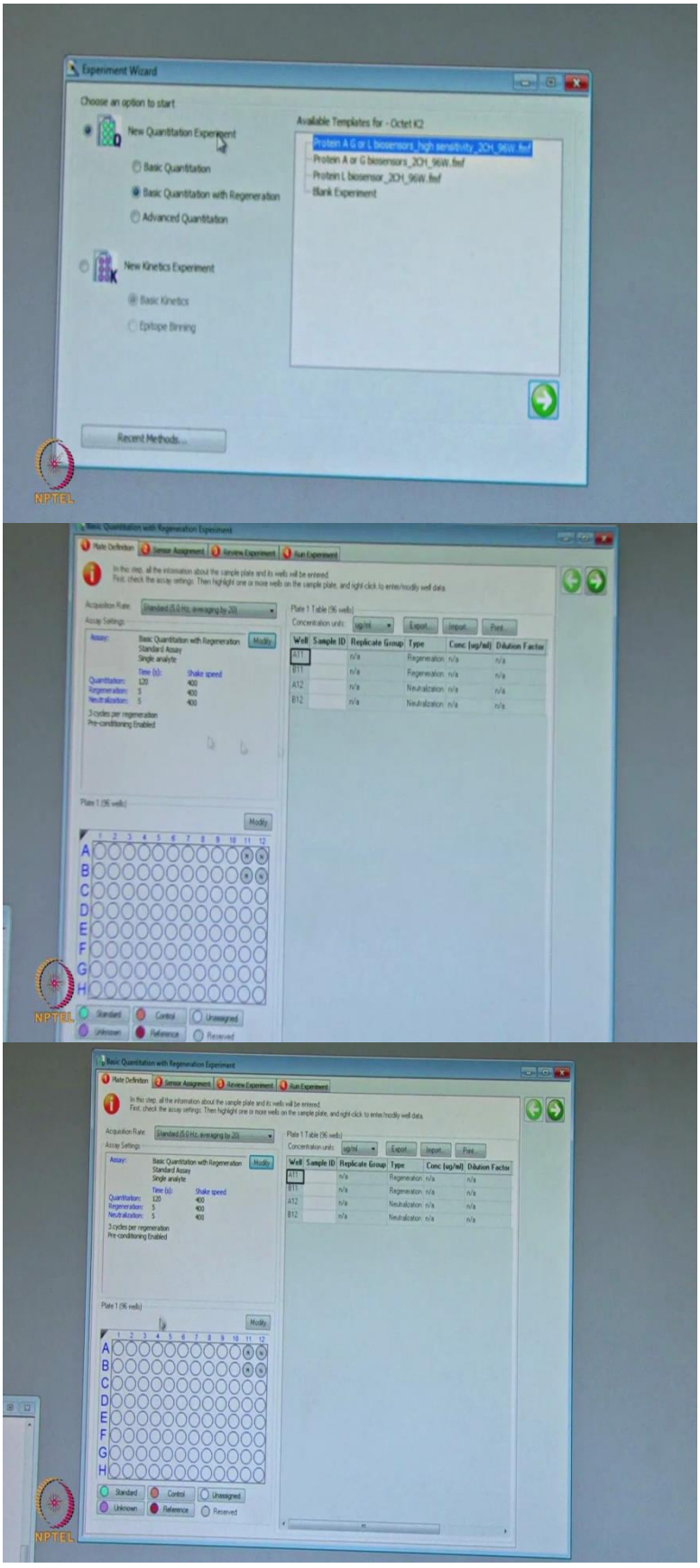
Experiment Wizard
Choose an option to start
New Quantitation Experiment
Basic Quantitation
Basic Quantitation with Regeneration
Advanced Quantitation
New Kinetics Experiment
Basic Kinetics
Epitope Binning
Available Templates for - Octet K2
Protein A G or L biosensors_high sensitivity_2CH_96W.tmf
Protein A or G biosensors_2CH_96W.tmf
Protein L biosensor_2CH_96W.tmf
Blank Experiment
Recent Methods...
NPTEL

Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table (96 wells)
Well
Sample ID
Replicate Group
Type
Conc (ug/ml)
Dilution Factor
Standard
Control
Unassigned
Unknown
Reference
Reserved
NPTEL

Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table (96 wells)
Well
Sample ID
Replicate Group
Type
Conc (ug/ml)
Dilution Factor
Standard
Control
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Reference
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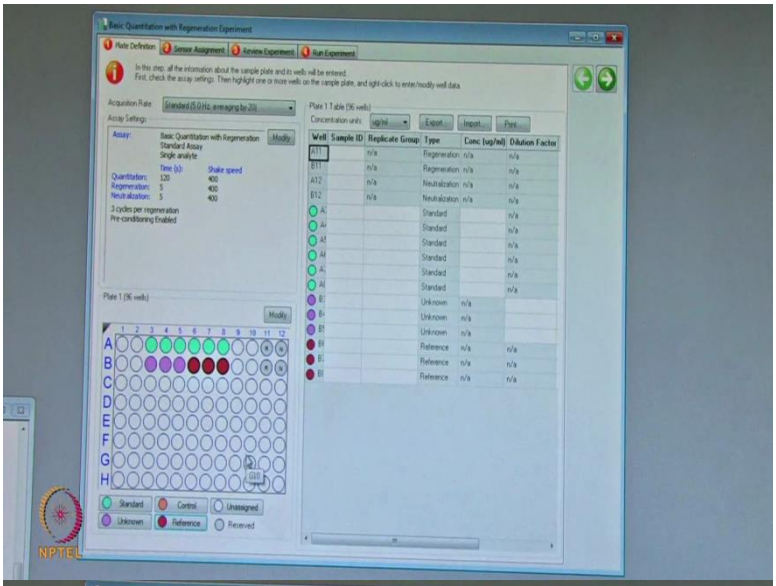
Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table (96 wells)
Well
Sample ID
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Type
Conc (ug/ml)
Dilution Factor
Standard
Control
Unassigned
Unknown
Reference
Reserved
NPTEL


Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Plate 1 Table (96 wells)
Well
Sample ID
Replicate Group
Type
Conc (ug/ml)
Dilution Fac
Standard
Control
Unassigned
Unknown
Reference
Reserved
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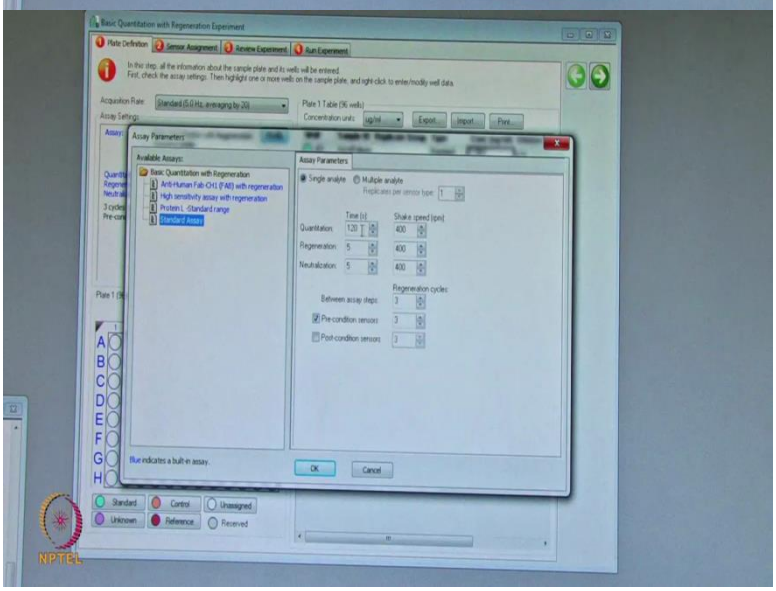
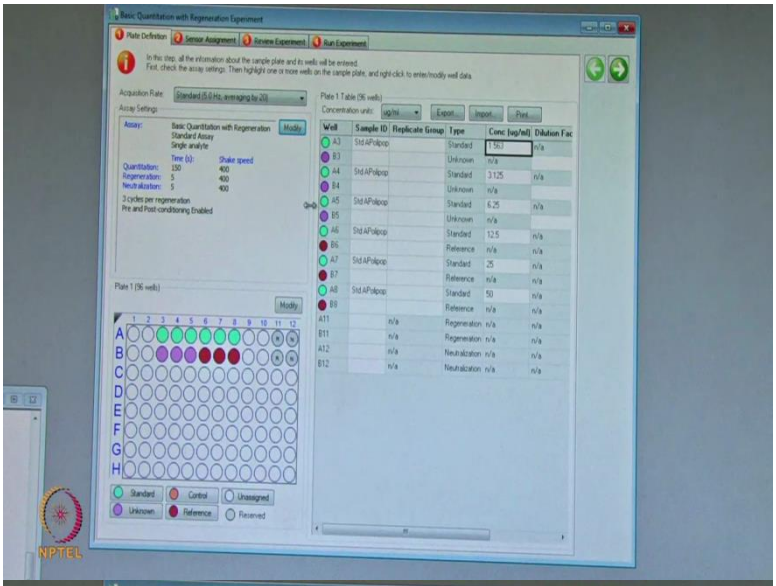
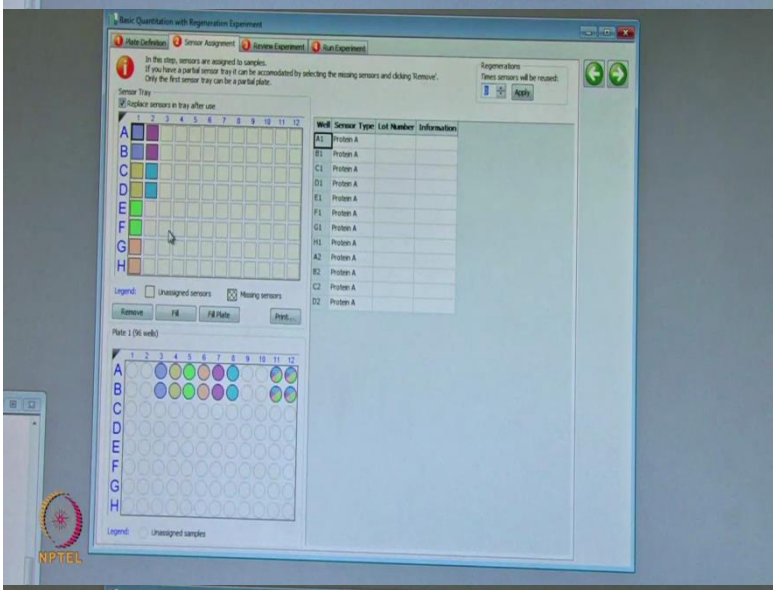
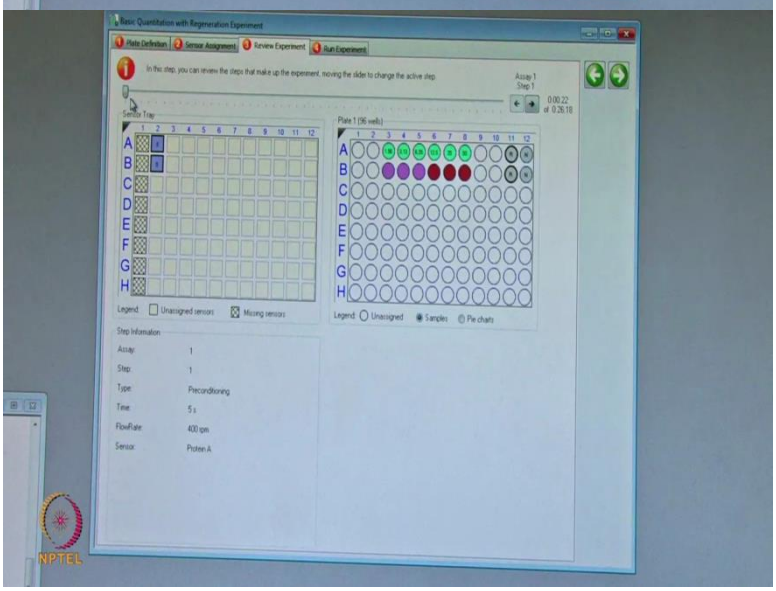
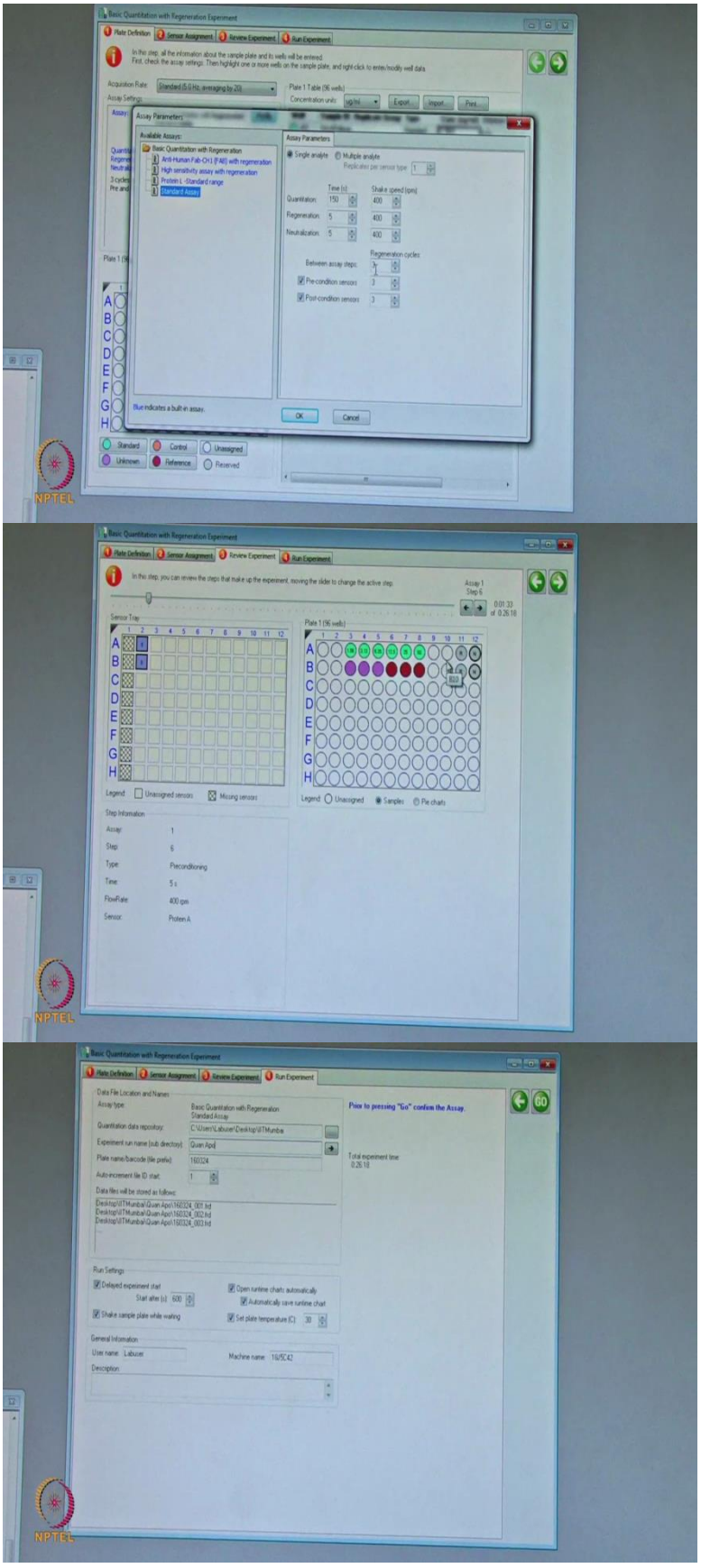

Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Assay Parameters
Available Assays:
Basic Quantitation with Regeneration
Anti-Human Fab-CH1 (FAB) with regeneration
High sensitivity assay with regeneration
Protein L -Standard range
Standard Assay
Assay Parameters
Single analyte
Multiple analyte
Time (s)
Shake speed (rpm)
Quantitation
Regeneration
Neutralization
Regeneration cycles
Between assay steps
Pre-condition sensors
Post-condition sensors
Blue indicates a built-in assay.
OK
Cancel
Standard
Control
Unassigned
Unknown
Reference
Reserved
NPTEL


Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Standard
Control
Unassigned
Unknown
Reference
Reserved
NPTEL


Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Unassigned sensors
Missing sensors
Remove
Fill
Fill Plate
Print...
Unassigned samples
NPTEL


Basic Quantitation with Regeneration Experiment
Plate Definition
Sensor Assignment
Review Experiment
Run Experiment
Step Information
Assay: 1
Step: 1
Type: Preconditioning
Time: 5 s
FlowRate: 400 rpm
Sensor: Protein A
NPTEL



Now, I am going to take back this sample plate and also, sensor tray. Now, the sensor actually which is hydrated in the PBS buffer and also, our biotinylated antibody also it is in

the PBS buffer. Now, I am going to equilibrate these sensors with the PBST buffer the PBS buffer having the twin in that. Then this is the PBST buffer we call it as a sample relevant buffer A, I am going to take a 200 microliter of this PBST buffer I will add up here in the tube A2 and the B2.

So, I will now the sensor position is I am changing from A1 B1 as like that the A2 B2. So, now I will start the equilibration of sensors with a this particular buffer, we are going to do a quantitation of the apolipoprotein. So, the in the previous step what we had done is we immobilize the biotinylated antibody on to the streptavidin sensor. Now, we can do the quantitation using that sensors is pre-immobilized. So, what I am going to do is I am making a apolipoprotein standard with a concentration is 50 microgram per ml. So, (I am) here I am having a apolipoprotein standard I am going to dilute to a 50 microgram per ml using the sample deviant.

So, from this I am going to do a, this standard, I am going to do a serially dilution, like two-fold dilution I am going to do this a buffer. Prior to that, what I am doing is, I am taking this 96 well plate, I am adding the buffer into the wells. Here, I will add I am going to use a around the 6 concentrations the two-fold dilutions. So, I will add here, buffers I am going to do a dilution in the plate itself.

So, 200 microliter of each of the sample relevant (())(12:28) buffer I am placing in the 6 wells of the 96 well plate (())(12:31). So, in the at (())(12:33) I am going to use here pre as a references this plane buffer uses as a reference here, this is the 50 microgram per ml protein, I am serially diluting doing a two-fold dilution in the plate.

So, now we had made a 6 concentrations of the standard apolipoprotein. So, starting from the concentration range 1.56 to 50 microgram per ml, I had put these standards, the 1.56 in from A3 to A till A8 that is the last 50 microgram per ml as well as I had put a reference buffer, the buffer which we are using for the reference subtraction here. So, that I had put it as B6, B7 and the B8.

Now, I am going to add up unknown samples, we have the three unknown samples here. So, that I am going to put it in the B3, B4 and the B5. So, these three unknown proteins we are going to determine on the concentration of the unknown apolipoprotein in the samples using the standard.

Now, I am going to add the 3 unknown samples here, I am going to place at the B3 this is a sample number one, which is sample number 8 B4, the sample number 3 at B5 to these three unknown samples. So, I am using the glycine buffer that is a pH 2, 10 milimolar glycine buffer, this is used for the regeneration of the the sensor. So this the regeneration buffer I am going to place at the A11 and the B11. So, this will the bound apolipoprotein will be washed then the sensor detect the this acidic buffer.

So, also we have to place one more which we call it as a neutral buffer that is what is PBST buffer, I am going to place this one at the A12 and the B12. Now, I am placing this sensor tray into the instrument at the sensor compartment and also, we had prepared the sample tray here, we had put the unknowns as well as the standards of lipoprotein E in the sample plate.

Now, our aim is like here we are performing a quantitation experiments. So, now we have to choose the quantitation mode here, new quantitation experiment. Here, in this we are using a basic quantitation with a regeneration, because two sensors we have immobilized and that same sensor will be regenerates and reacquired the different concentration data. So, we have to choose the blank experiment and save basic quantitation regeneration blank experiment then we have to go here.

In the experiment, when you open this experiment we will see the plate definition. In the plate definitions more and more samples we had put it here. So, here from A3 we had put a around standards A3 to A8, 6 concentrations we may choose. These 6 concentration need to choose it this as a standard.

Okay, Now, we can see the this (())(16:22) (())(16:23) as a the green color . And also, here one more we have chosen these three arrow unknown samples and these three are your reference you can choose the reference or later also, you can in you can take it as the reference into like even unknowns, later in the data with sensor that can be modified as the reference.

Here, in the table on your right hand side we have to put the information. In the A3, what you had put and A8 what you had put. In the method modify section here, single analyte we have to choose 120 seconds we have to use 400, 400 the regeneration is 5 seconds, it will dip in the acidic buffer 5 seconds, it will dip in the neutral buffer. Thus, that cycle has to be performed at least for 3 three cycles. So, then pre-conditions means sensor first will regenerate and then

goes to sample, post-condition is nothing but once sensor is put it into the sample after that we go for the regeneration.

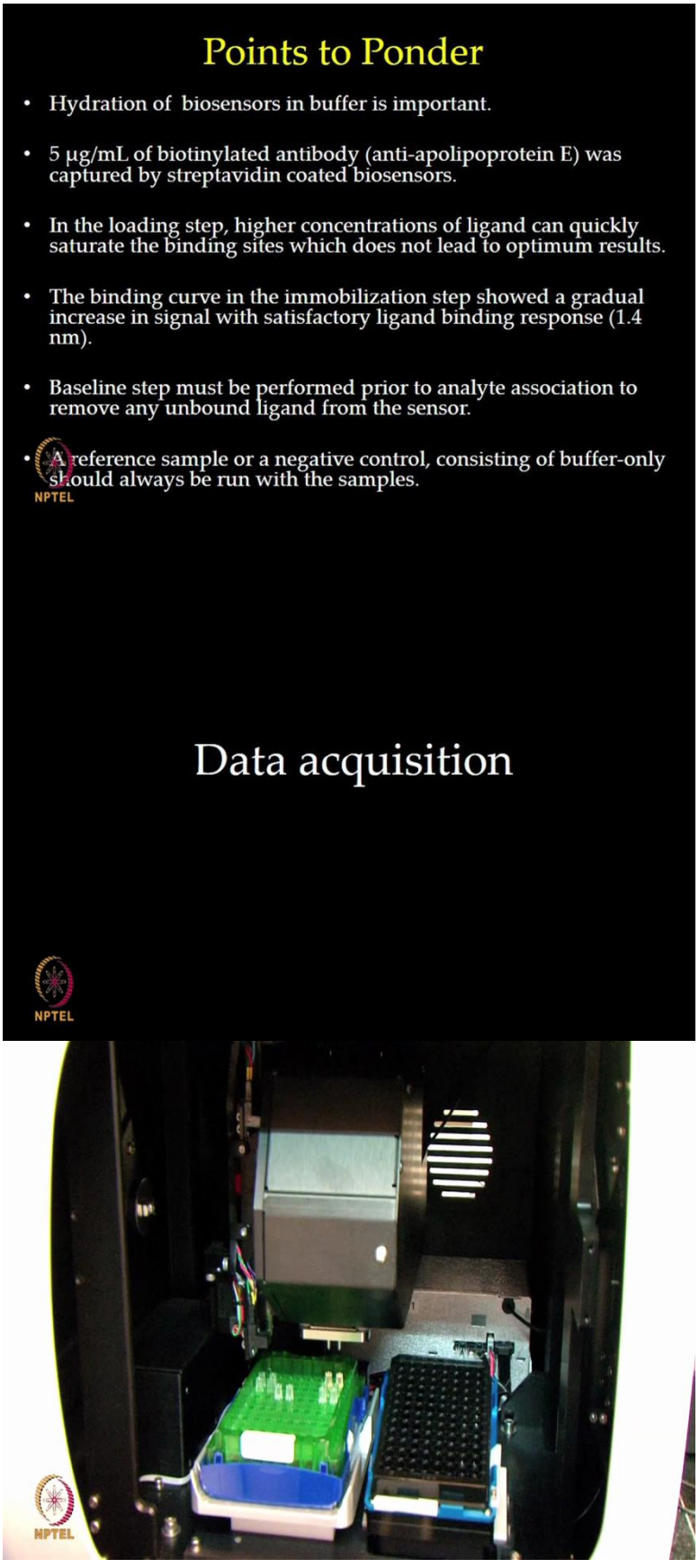
So, we have to choose both pre-condition and post-conditions sensors, 120 seconds is the acquisition time, I can still extend to a 150 seconds here then say ok. 400 is the shaker speed I will say, Ok, here. And then in the sensor assignment, it is showing so many sensors here but we are using a two set of sensors for the acquiring the all the samples. So, now we have kept the sensor at the position one. So, I will say (())(18:50) automatically your sensor is placed at the second we had kept sensor for the equilibration using the the PBST buffer.

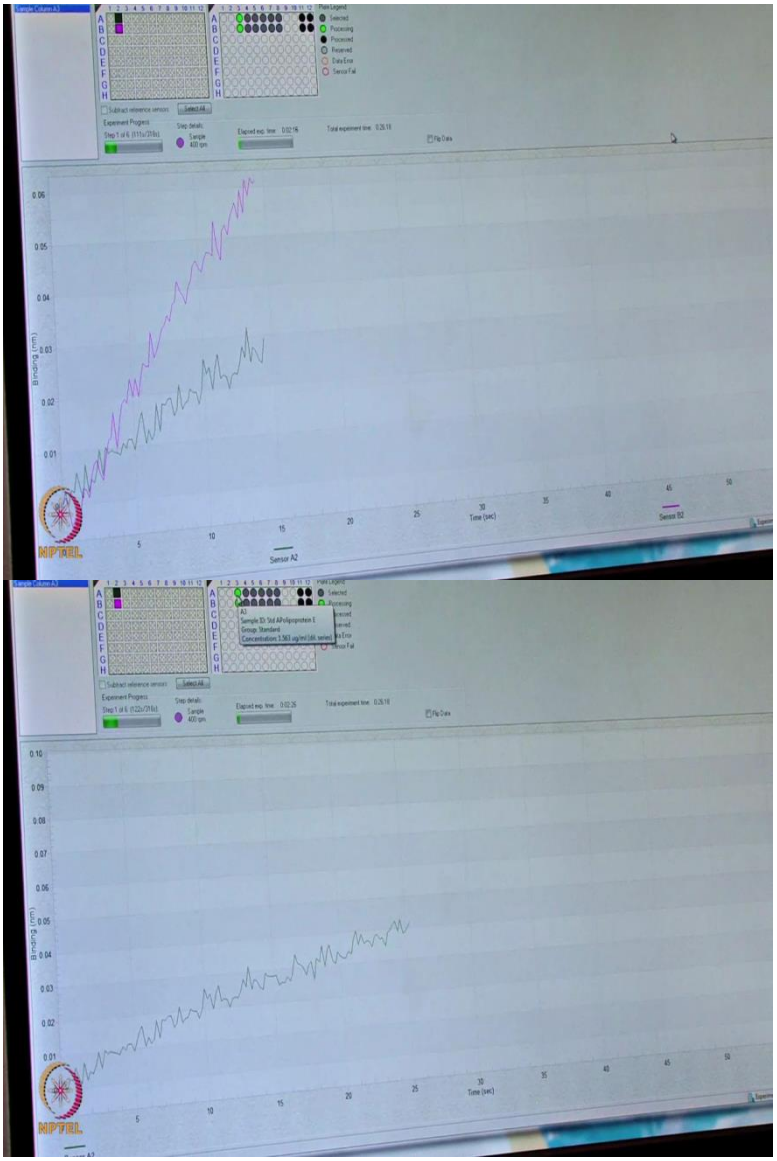
In the review, we can see how exactly the instrument will perform. Sensor is picking from the second position it is going to a regeneration. Then if you click on this arrow; it is a neutralization regeneration, neutralization regeneration neutralization. In the method we had said that it has to in the plate acquisition we had mentioned that, it has to perform for a 3 cycles.

So, that is what is indicated by in the experiment, it is acquiring for a 3 cycles then later it goes to a sample then once again regeneration-neutralization, regeneration-neutralization, regeneration-neutralization then goes to here second step and regeneration-neutralization, regeneration-neutralization, regeneration-neutralization and the third same the process will continue in the last.

In the running experiment, where you want to save the data I will mention this file as a Quan apolipoprotein. Okay, sensors already (())(19:35) I will uncheck this then I can say go here.

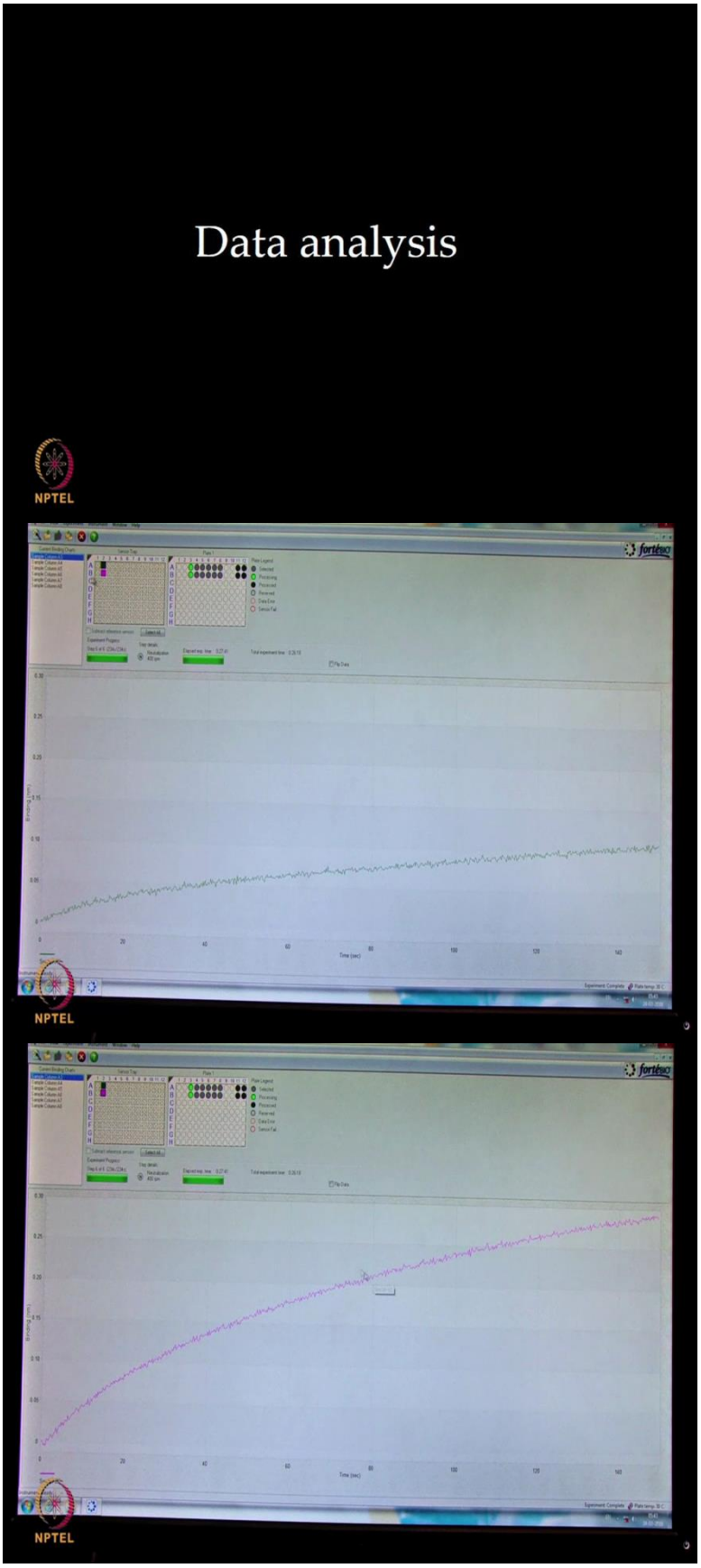
(Refer Slide Time: 19:41)

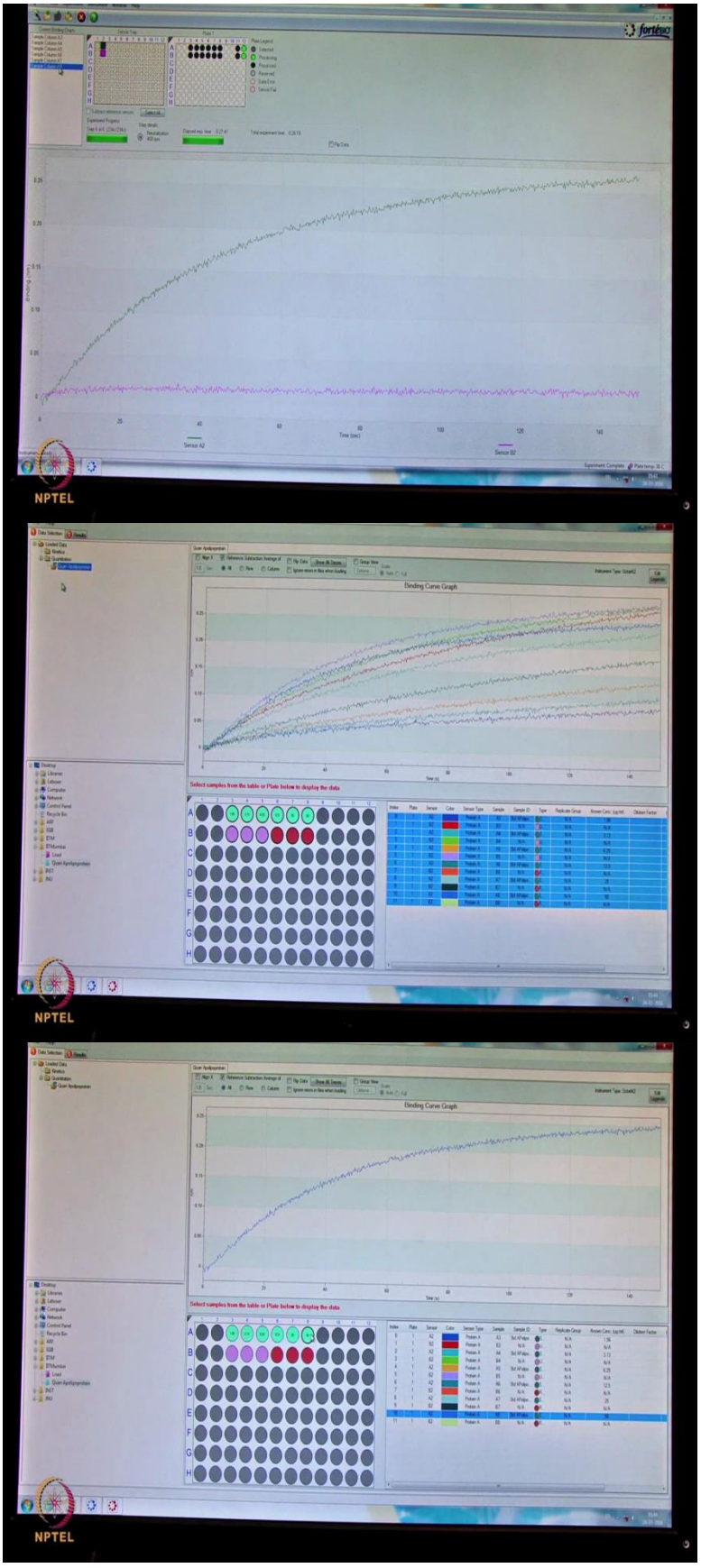




Now, the instrument is picking the sensor in the sensor rack and it is going through a regeneration. See the pink one is the one which is unknown sample here and the green the sensor, it is indicating the lowest concentration which around 1.5 microgram.

(Refer Slide Time: 20:52)




fortéBio
Binding Curve Graph
Select samples from the table or Plate below to display the data
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Binding Curve Graph
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Binding Curve Graph
Select samples from the table or Plate below to display the data
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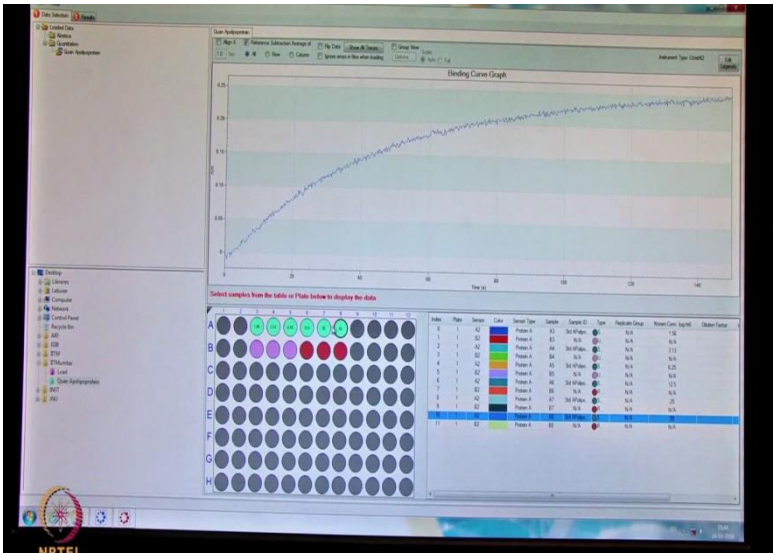
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Binding Curve Graph
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Binding Curve Graph
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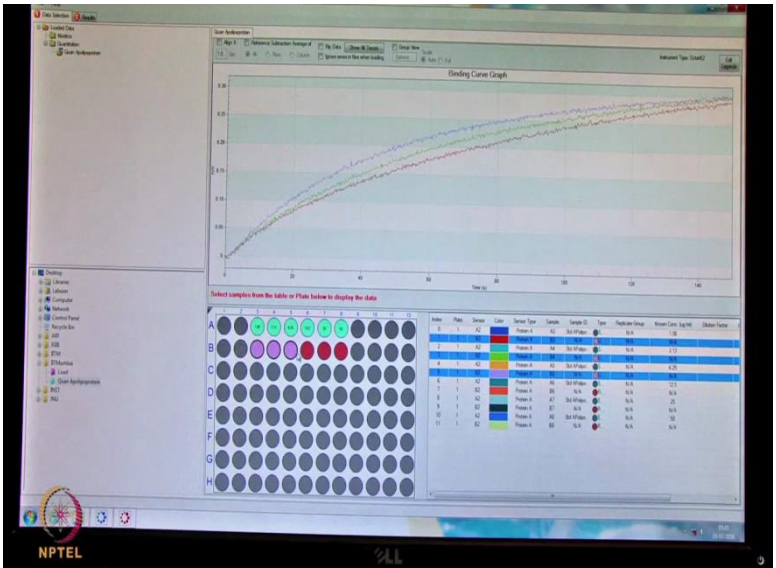
Binding Curve Graph
Select samples from the table or Plate below to display the data
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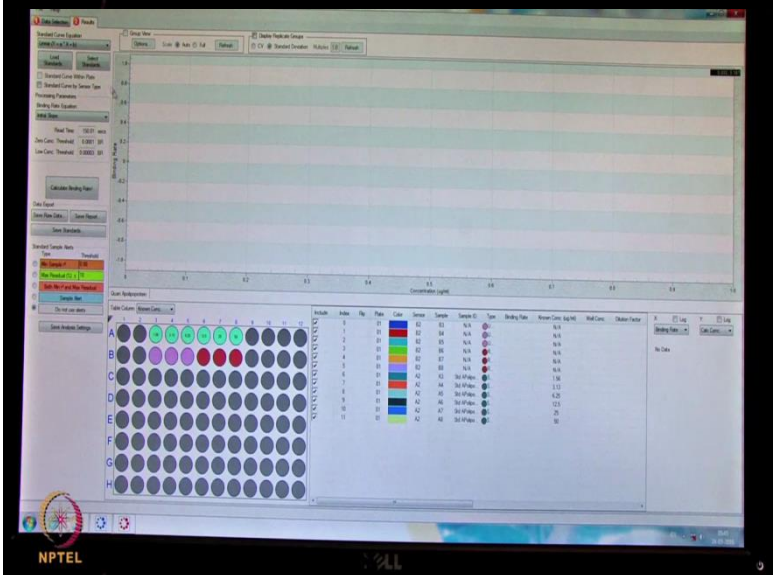
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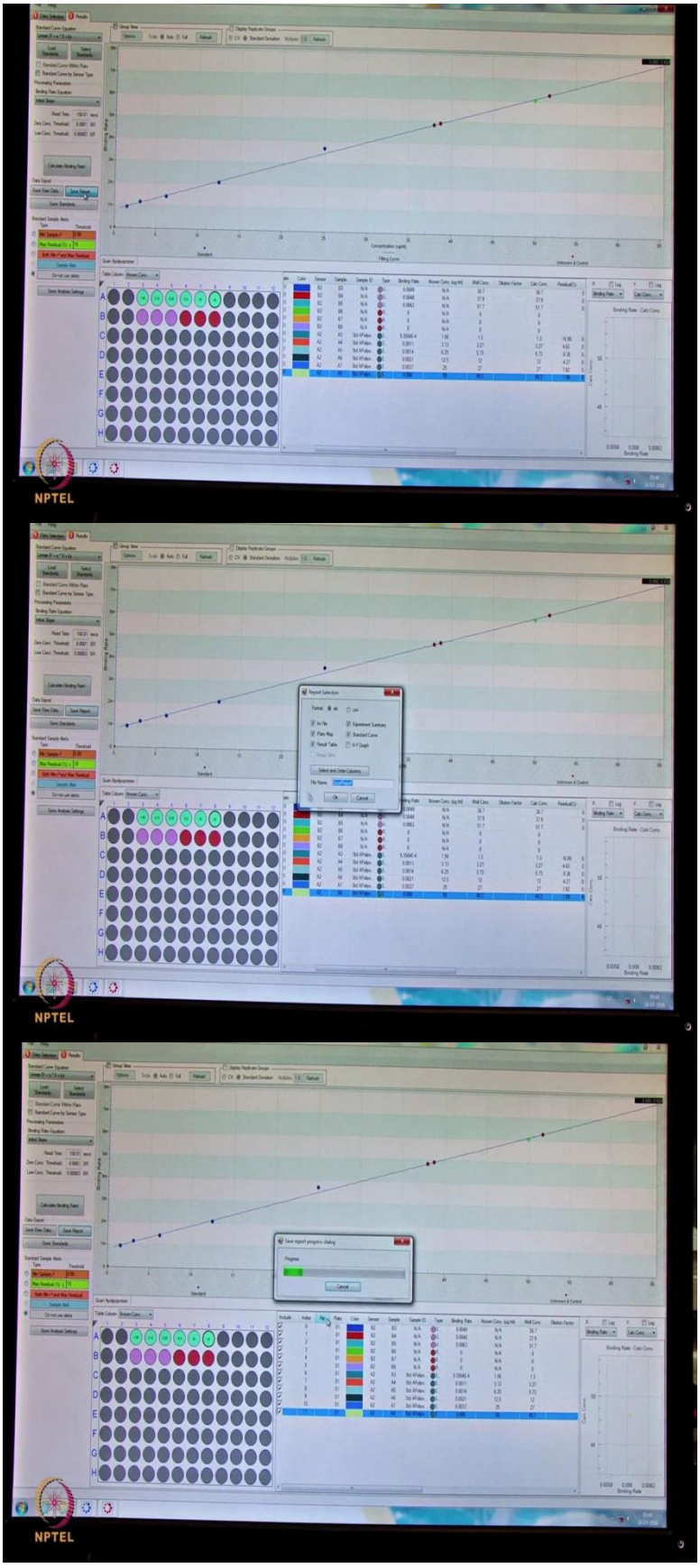
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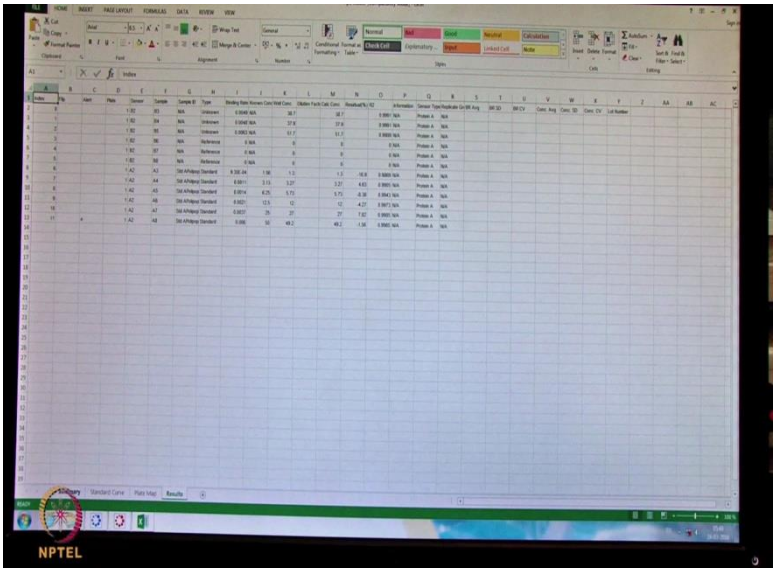

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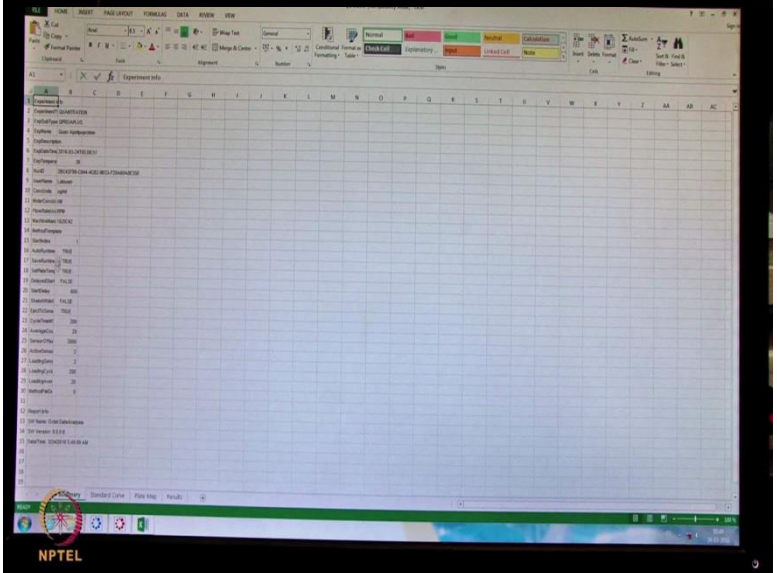

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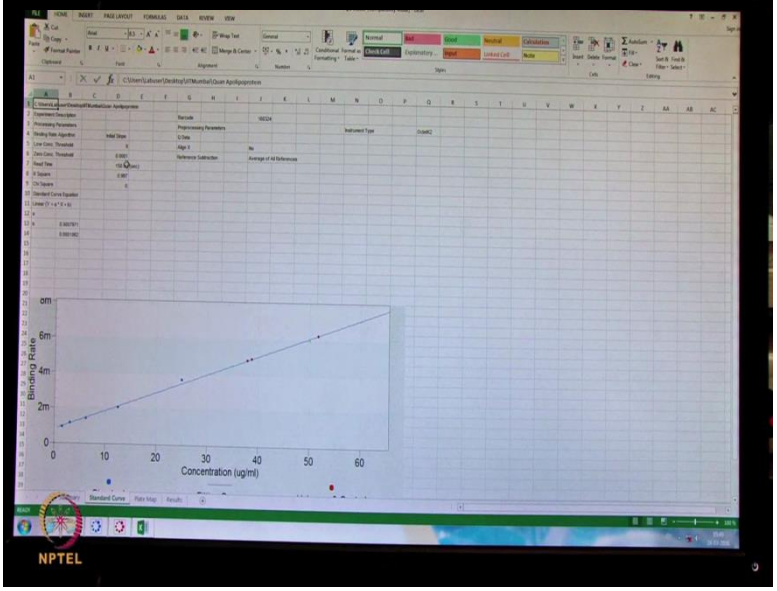

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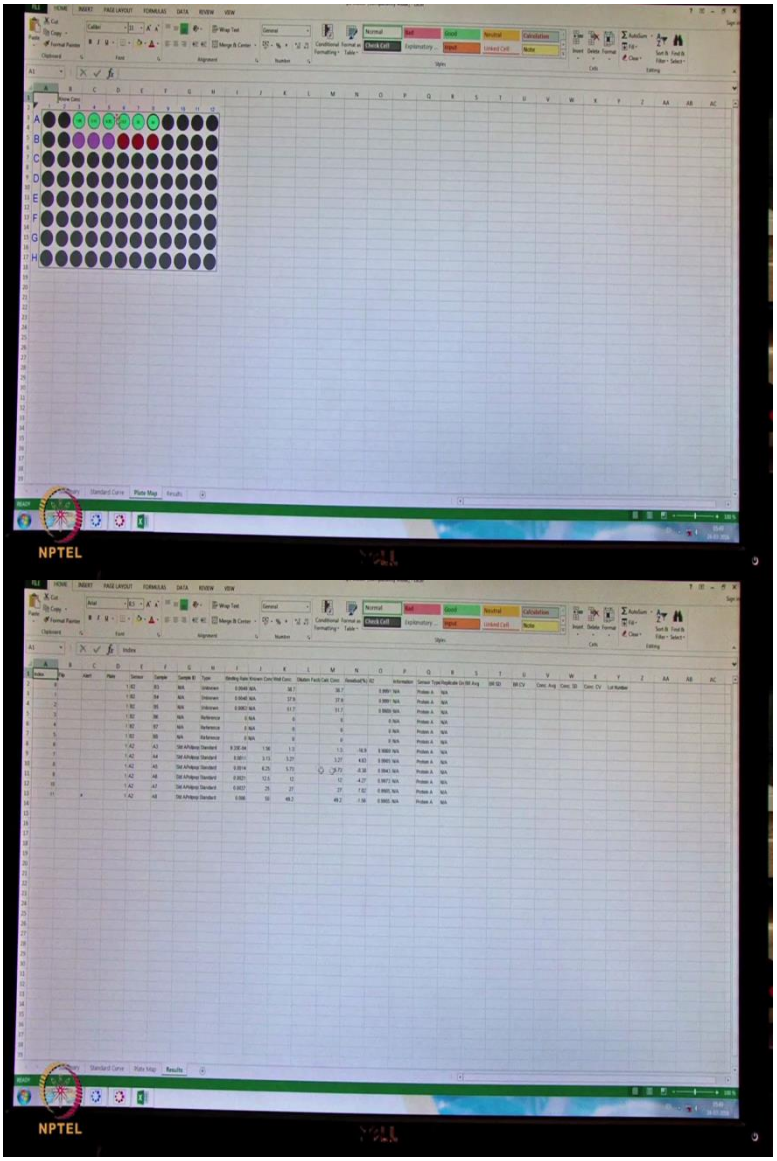

Once, this data is acquired the green indicates this is the standard and as well as the pink indicates this, as unknown sample. Now, the acquision is done we will go for the data analysis. This is the file, this is the acquision file indicated by a green color here. So, now we can see this, the graph which is indicating the all in the standard.

This your standard graph, the one which is the 50 microgram is the highest the binding curve is showing in a (())(21:31) then the second, then the third, then the fourth and the the fifth concentration and the sixth. So, highest will be the 50 and the lowest will be the 1.56 microgram per ml.

Here, there are the three unknown samples also we had kept in the B3, B4 and B5 and the three reference samples. These reference samples are nothing but your buffers. Buffers also sometimes gives the artifacts, we subtract the artifacts from the from the signal here, () you

have to use a method called as a reference subtraction method. Here, we are going to take this reference subtraction average because these three are (())(22:50) the buffers.

So, it will get subtracted from the all the acquisitions call as different concentrations as well as the unknown from that. So, now we have to go here in the result section. In the result sections, we have different equations here, in the standard curve equations. Which one you want to choose like linear point to point, we have 4 peer methods, 5 peer methods as well as linear y is equal to a mx plus c kind of equation, A into x plus B.

So, we will go with this equation to linear equation here and also, we had put standard in the plate itself as well as the the unknowns. So, we can easily do a data analysis we can quantitate the unknown samples. Then we say calculate binding rate. When you say calculate binding rate we will see the straight line.

The blue dots indicates the different concentrations of your standards and the red ones which indicates your unknown sample. There are the three unknown samples here. So, in the unknown sample we can see standards if you look at the standards 50 microgram the back calculated instrument back calculated is recovered as a 49.2 percent. If you see the percent residue is, that is what we are seeing it should be (())(23:35) to (())(23:36) and for the (())(23:37).

So, this is we are getting around 49.2, 25 we are getting as a 27, 12.5 we are getting as a 12, 6.25 we are getting as a 5.73, 3.13 we are getting around 3.27 and 1.56 we are getting 1.3 but somewhat this value should be a more than 10, even we can explore also in the binding rate calculations (24:01) now required. I think here in the unknown concentration if you look at all the three samples which are required, the well concentration is calculated to the thirty point 38.7, 37.9, it is nothing but the 38 microgram and 51, it is somewhat, 51 is something like a very high is showing but our standard curve is 50, but still the instrument extrapolates and calculates the concentrations.

So, now we can save this data, we can save this as a save report If you save report, it will convert this into a excel sheet, like you can give a Apo standard curve then save ok. It will create as a excel sheet, the report will be created as a excel sheet. In the summary, you will see the what about the data we will (())(25:00). And the standard curve if you look at this as a standard curve we had, the standard curve also Chi square this 0.997 r square and Chi square is a zero.

This is a very good actually, if you look at all data points almost on (())(25:17) (())(25:19) and in the plate (())(25:21), where you had put the samples and the unknown samples in the result section, this is your standard along with your percent residuals and also for the unknown samples here, it is assigned unknowns that indicated to your calculated concentrations.

If it is any you like (())(25:39) sample is dilution, dilution diluted, we have to put a dilution factor often calculates is the calculated concentration with respect to a dilution factor. If you look at the r square values of the all the unknown samples as well as the standards it is more than 0.9. So, the data is accepted.

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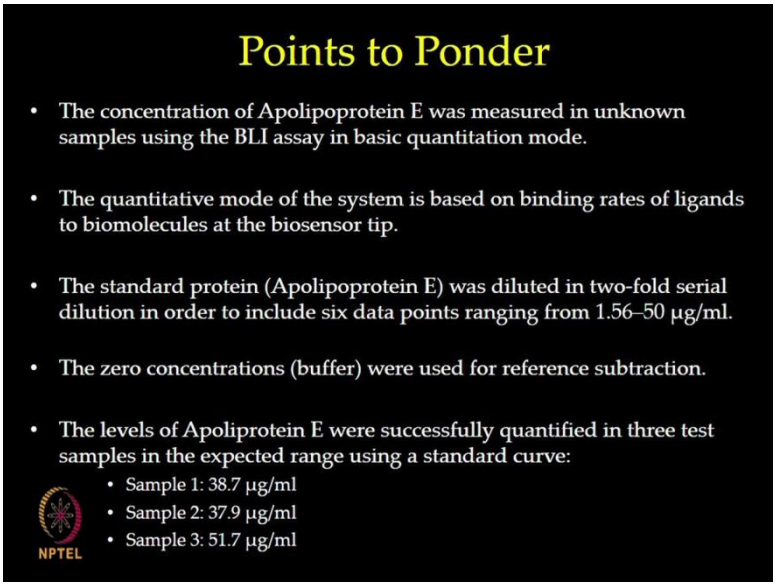

So, with this I am going to finish the quantitation experiments. Thank you. As observed in the demonstration session for apolipoprotein E the standard curves were linear and had good dynamic range with high response levels. We could measure the concentration of the protein in unknown sample by using standard curve; such methods can further be optimized and utilized to measure the concentration of specific protein even in complex solutions without the need to purify the proteins. Thank you.

(Refer Slide Time: 27:07)