**Oral Biology**
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**Lecture - 31**
**Immunoassay**

(Refer Slide Time: 00:16)


Hi, this is Doctor Rajesh Kumar Chief Scientist and Associate Professor from Nanobiomedicine Lab and Aquatic Nanotoxicology Unit. This is Cellular and Molecular Research Centre and I am in the Department of Pharmacology Saveetha Dental college and hospitals, SIMATS Chennai. And in lab we are preparing all types of nanoparticles, nanocomposites and novel nanoformations and all used in the biomedical applications as well as in the dental applications.

In dental applications we are preparing herbal and nano based products like toothpaste, mouthwash, dental varnishes and nano gels apart from your nanoparticles we are coating with different types of implants also.

And our nanobiomedicine lab is associated with all the different divisions of dentistry like prosthodontics, endodontics, orthodontics, publical dentistry and then periodontics. We have developed a lot of products in our lab itself and we have more than 15 patents.

Apart from in our lab we are offering some lab facilities like for the activities such as antimicrobial activity, anti inflammatory activity, antioxidant activity and then anticancer activities. And in our lab we have one unit for aquatic nanotoxicology unit in that we are doing zebrafish based studies.

In India we are the only dental college which associated with nanobiomedicine lab including aquatic nanotoxicology unit by using zebra fishes we are doing many biomedical applications like hepatoprotective activity, anticancer activities and anti diabetic activity, wound healing activity.

In wound healing our major specialization and we have developed a new novel products for a healing of wound by using nanocomposites and nanoformations and that is all. And now we are a move on to practical, what is a practical? We are going to do which is related to immunology; immunology based studies we are going to do now.

Now, we are going to lab for doing the practical that is related to immunology. This is Blue Lab Molecular and Cellular Research Centre, in that we have different types of disciplines.

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Such as Marine Biomedical and Research Laboratory headed by Doctor Sivaperumal.

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This is Clinical Genetics Lab headed by Doctor Jayashree Priyadarshini.

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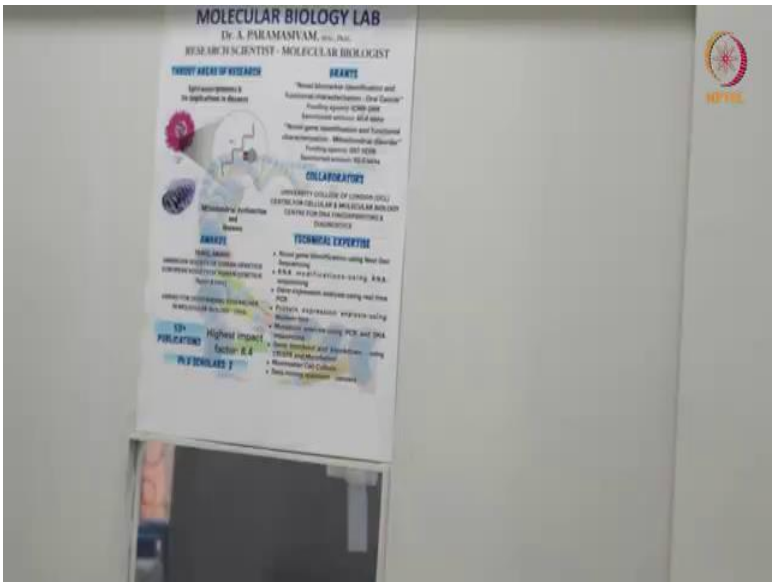


This is Molecular Biology Lab headed by Doctor Paramasivam.

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And we have one of the best sophisticated facility of a room of refrigeration and it is called as a Cryolabs.

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Biochemistry and Molecular Biology Lab headed by Doctor Selvaraj Jayaraman.

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This is Aquatic Nanotoxicology Unit.

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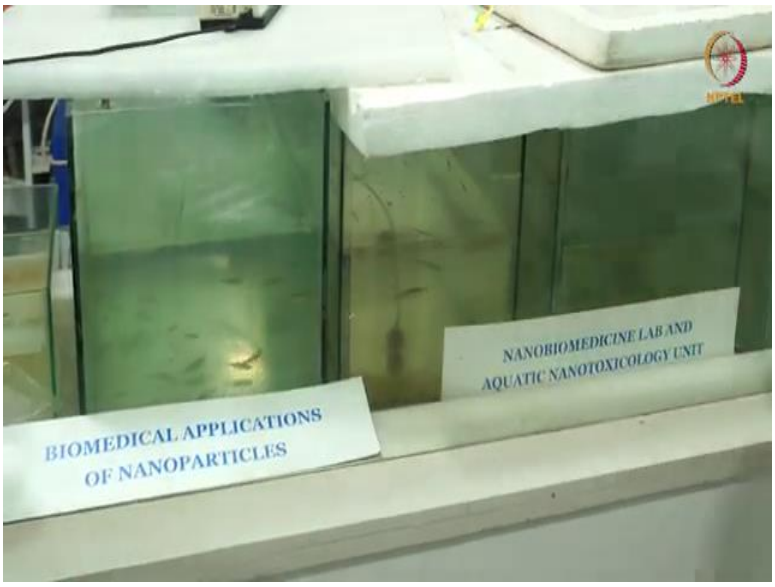


See we are doing research with the zebrafishes; one of the important animal models and it is very sensitive research, we are going to do for the biomedical application.

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This is RNA Biology Lab headed by Doctor Durairaj Sekar.

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This is Nanobiomedicine Lab and Aquatic Nanotoxicology Unit. See already I told these are all some of my publications and some of my book chapters published in these books.

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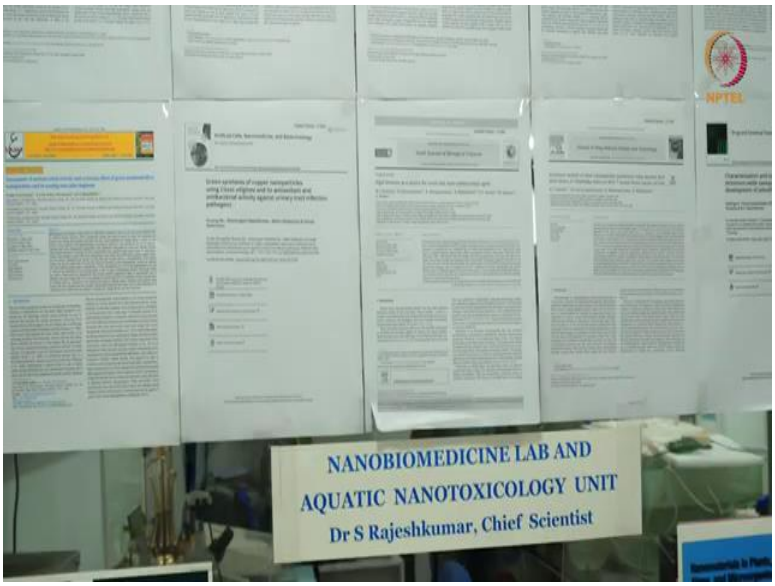


This is Nanobiomedicine Lab and Aquatic Nanotoxicology Unit.

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Here only we are going to do our practicals for a immunological assays. Today we are going to see cortisol immunoassay the quantitative diagnostic analysis of cortisol by using kits.

We know that nowadays lot of kits are developed and within one or two hour we will analyze COVID. Today we are going to see how to analyze the cortisol by using

immunoassay for that we have a kit and then the instrument we are going to use it is ELISA reader.

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Principle of the test is the DRG cortisol ELISA kit is solid phase enzyme linked immunosorbent assay. The microtiter wells are coated with monoclonal antibody directed towards an antigenic site on the cortisol molecule, endogenous cortisol of a patient sample completes with the cortisol horseradish peroxidase conjugate for binding to the coated antibody.

After incubation the unbound conjugate is washed off, the amount of bound peroxidase conjugate is inversely proportional to the concentration of the cortisol in sample. After the addition of the substrate solution the intensity of the color developed is inversely proportional to the concentration of the cortisol in the patient sample.

Today we are going to check cortisol analysis. The novel thing of this immunoassay is actually peoples will do cortisol analysis by using a serum samples only. today we are going to see the salivary cortisol level in the samples. The materials available in the kit is the reagents and the materials provided in the kits are; first one microtiter wells.

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This is a 96 well plate in that 7 wells are there; first one 0, 20, 50, 100, 200, 400 and 800, the concentration is this and all the nano grams per ml. And third one is enzyme conjugate this is a cortisol conjugated to horseradish peroxidases contains a non mercury preservative and the fourth one is substrate solution.

A substrate solution contains tetramethylbenzidine and the fifth one is stop solution; stop solution is 0.5 molar of S2SO4 and the sixth one is wash solution; wash solution is actually 40 X concentrated that we will dilute and use it for our study. Final one is a wash solution; wash solution is a 30 X concentration solution. So, we will dilute it for 1 X concentration and use it for our study, then we will move on to analysis of corticosteroid by saliva samples. Now, we will start the cortisol analysis this is a 96 well plate.

Always before starting the procedures you have to make the protocol, you have to read the instructions carefully and you have to write it in your notebook, then only the procedures will always be in your mind.

And then now we will going to add standard control and a samples today we will take three samples for our study and the sample and patient details and all we have it in our notebook.

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First this is a 96 well plate you can see this, so many people's know that what is a 96 well plate totally 96 wells will be there at that time we will do more than 20 or 30 samples if we will do it as a triplicate. First we will add standard.

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This is a 20 mule of standard we are going to add already we told in standard itself 7 different concentrations are there first one 0.

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First one 0. So, this is a 20 mule we are going to add the samples see this.

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A 1 is 0, then AB 1 is 20.

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Third one is 50, fourth one is 100.

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Fifth one is 200.

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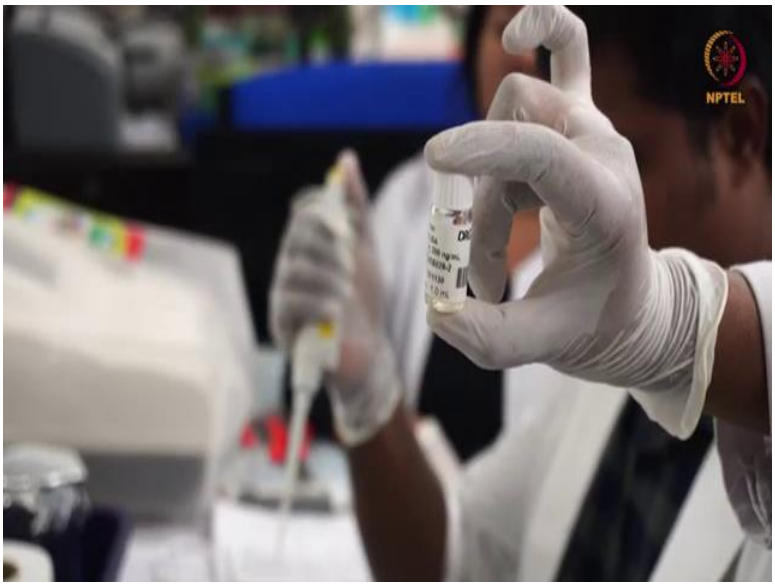

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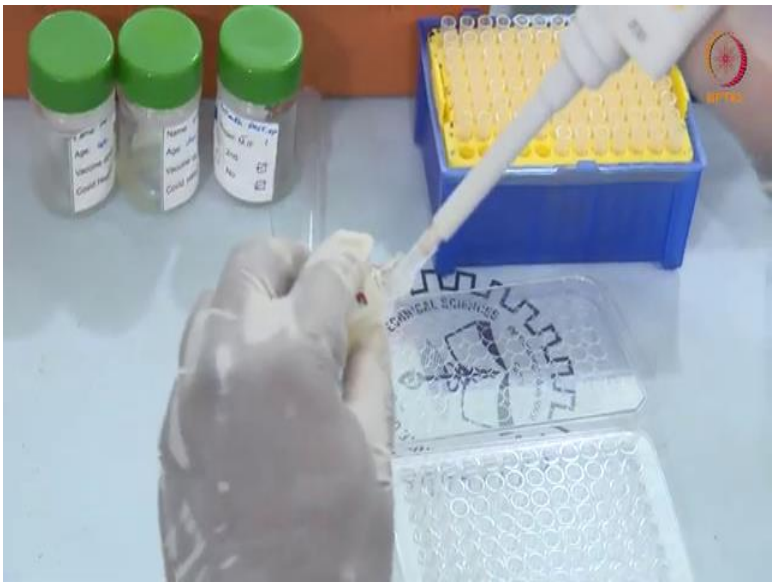

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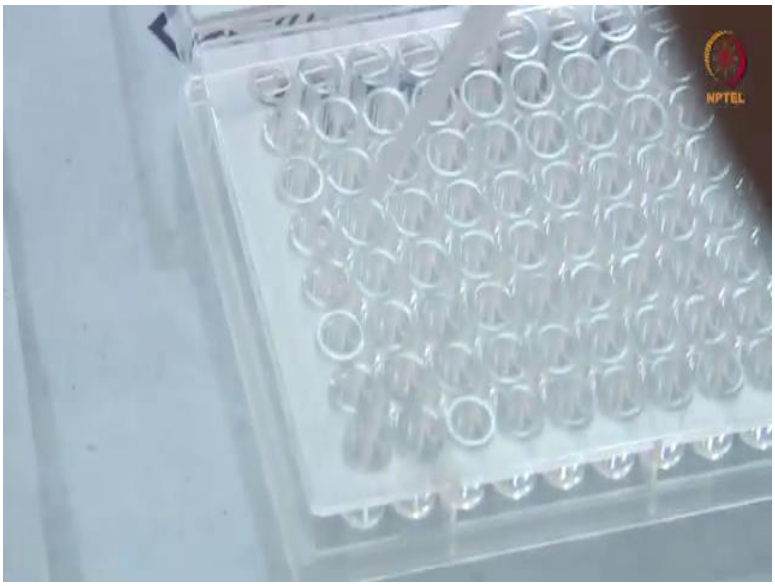

The fifth one is 200 mule.

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The sixth one is 400 mule.

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The final  last one of our standard is 800 mule, we are going to add. The next group is control we are going to use, control also the same 20 mule only we are going to add let us take the control.

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Yeah in the ELISA well plates we will add the samples continuously, but for the demonstration purpose especially you have to understand what we are doing. So, we are doing it as a control as a second well, like this we will plan and do.

As it is easy to understand the color changes and all for understanding purpose only I will add this in a second row, the second column we will add this is control. Control we will add 2 times also just to understand the color changes, we will add 2 times; 2 times or we will do 3 times also.

Student: Yes sir.

So, just like triplicate we will do. Control in the column 2 I have added the same sample with the three times in the samples ok.

Student: Yes sir.

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Yeah today we are going to check 3 samples with 3 different concentration not the concentration triplicate we are going to do.

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So, first one is standard, second one is control and third one is patient sample

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Patient sample also the same 200 mule only we are going to use it and this is third column 1, 2, 3.

So, and the next sample is Sai Ganesh and we have the list of patients name. So, it will be considered for the fourth column we will add, fourth column 1, 2, 3, over close it and the third patient the patient name is Hari Prasad this one we will add it in the fifth column; fifth column 1, 2, 3.

So, triplicate we are going to do. Now, we are going to add enzyme conjugate in the each wells 200 mule of enzyme conjugate is we are going to add up with standard control and 3 different samples ok, we will add the enzyme conjugate.

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Give me that yeah 200 mule of enzyme conjugate is we are going to add it in the all the samples including standard, control and then 3 different patient sample 200 mule.

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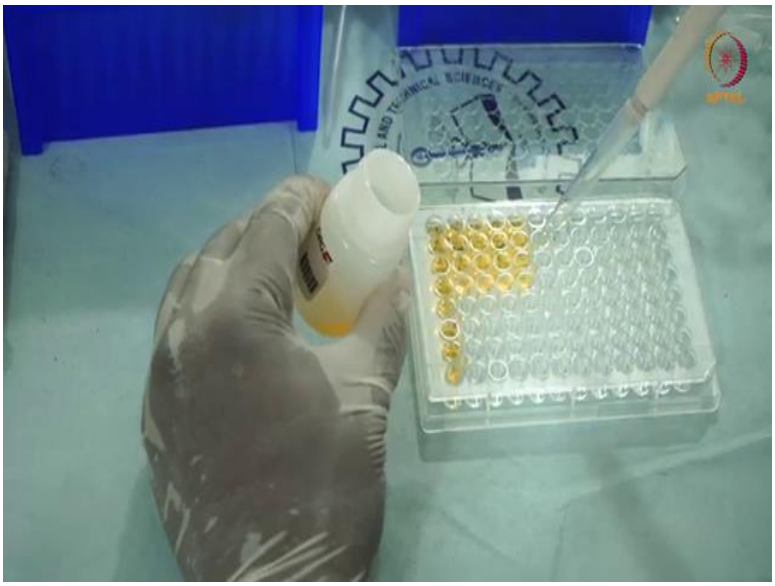

Now, you can see first one standard we finished next is control. Control already I told you know that control also we are going to do triplicate, control is over and from the third one that is a sample one. And you already note that you know the information of the patient name is very important.

First we have to write the name and second one is gender male or female and now we are going to check cortisol nowadays we are going to check COVID positive or negative and they did the first dose or second dose that information and all you have to write it in your notebooks. And the both\7b u bx the first and second patients are did their first dose of injections for COVID because to make the graph and all the information is very must.

Student: Right sir.

In that second sample we are adding.

Student:

Enzyme conjugate.

Student:

And now we are going to add third sample ok. Now, we have added a enzyme conjugate in the all the samples. See close it this procedures and all adding of enzyme conjugate we

have to finish it in 10 to 20 seconds we have to finish after that we will added all the samples in the wells. You know that already I told what is that first one? First one is standard, second one is control, third one is patient 1, patient 2 patient 3.

Now, we have added enzyme conjugate in the all the samples I told you know that first one is standard; standard of 7 different concentration of standard and second one is control, third one is patient 1, this one is patient 2, patient 3. We have added and just we will keep it here for 1 hour 60 minutes we are going to keep it without disturbing this plates just we keep it just we have to close it and without disturbance it will be kept for 60 minutes.

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Already we told the next step is washing. So, in that first slide itself I told we are going to prepare wash solution, this is a highly concentrated wash solution if you will add directly means what will happen? That enzyme bond proteins will get erased.

So, we will use this diluted form of this wash solution and that it was clearly mentioned that how much this is a 30 ml of wash solution they have provided and in that book itself they have mentioned this was concentrated. So, we will make it as diluted 40 X it if it is a 40 X solution means how we will dilute it? 1 ml in 39 ml of distilled water it will mix we will get the wash solution.

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Now, we are going to prepare the wash solution in the beaker.

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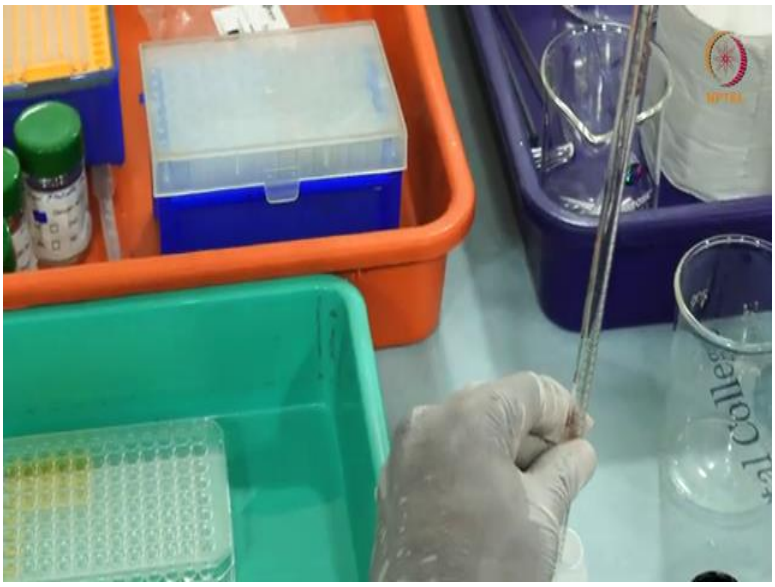

See this is a 5 ml prepared in that we are going to prepare the wash solution. See I took 5 ml in that we will add 5 ml of the concentrated solution, 5 ml and anyway for the studies and all we need a 400 ml of wash solution.

So, I am going to prepare 400 ml. So, I told in that 1 ml if you will mix it with 39 ml then the 40 X concentration will become 1X concentration. So, if you will add 10 ml of wash solution means how much distill water we are going to add? We are going to add

390 ml of distill water in this and that 390 ml of distill water will make and it will become 400 that is a diluted 1 X concentration of wash solution we are going to use it for the stud.

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See handling of measuring cylinder, handling of adding of solutions and all this art and the chemistry labs.

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See you can see always you can look this how much concentration if you want 50 mule ml of concentration means always we will keep the solution like this, then only we will

we are going to add 390 ml. So, this is 100 ml measuring cylinder always the measurement is this is 100 exactly 100 I am going to add it in the solution always we will add the solution mixing of solution is in that corner only always we like to add.

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100, 200. See we want 400 ml already I told you know.

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So, we will change it into another one glass. See this will be useful for the mixing also and third one how much this is third one, then 300 ml then remaining is?

Student: 90 ml

90 ml yes, 90 ml we are going to add it in the using with the in this. See 90 ml I am going to add it.

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This already I told 10 ml of concentrated wash solution plus 390 ml of distill water we have to mix it gently for the preparation of this 1 X wash solution.

See in that chemistry labs the calculation is very important, each and every chemical we have to calculate it addition mixing and all we have to do it properly and neatly we have to mix it always we will make gentle mixing or else we will use magnetic stirrer also for the mixing of the solutions mix it.

See that incubation of the solution is going on within 5 minutes it is going to finish after 5 minutes we are going to add wash solution, then the wash solution is ready this we will write.

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Yeah the next step of our study is washing.

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Washing by using wash solution we are going to use. So, in that 60 minutes gap we have prepared our washing solution that in each wells we are going to add 400 mule of wash solution, 3 times we are going to wash.

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This one is 400 mule we are going to take the wash solution.

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This is a prepared wash solution we are going to add it in this wash it.

Student: (Refer Time: 25:16).

See in the each wells we are going to add wash solution each of wells.

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3 times we have finished the washing and the next we are going to add 100 mule of substrate solution.

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See this is the substrate solution and we are going to add 100 mule in each wells we are going to add it ok. Then give me 100 mule this is 100 mule we are going to add in this.

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Student: Yes.

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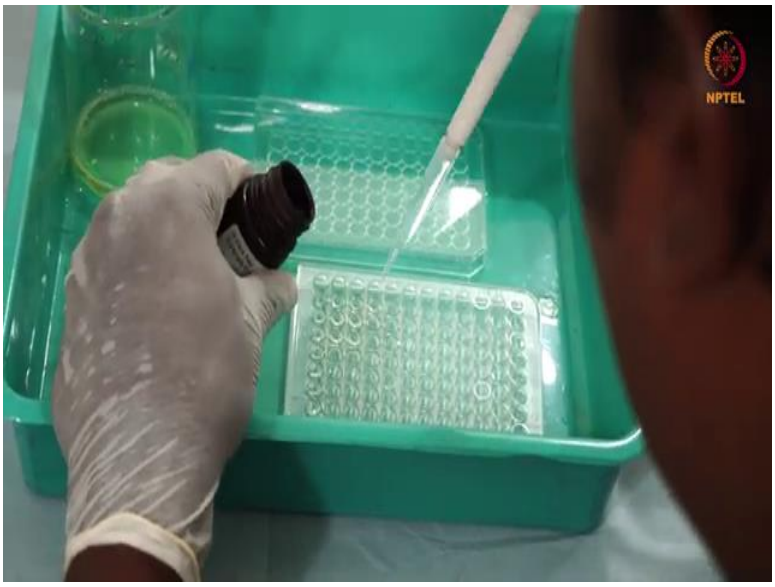


Already I told that first one is a 7 different concentration of standard and the second one is control and third one is patient 1, fourth one is patient 2 and fifth one is patient 3 ok. After the addition of a substrate solution in this we are going to keep this 15 minutes in a room temperature just we have to close it, we will keep this for a 15 minutes incubation and after 15 minutes we will stop the next step of our study.

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This is the ELISA reader and the final results we are going to analyze by using this ELISA reader.

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And this is specially used for the 96 well plate readers. On it cortisol we are going to analyze, cortisol and today's date we also we will mention it here like 8 ok, save it and we are going to analyze at 450 nanometer this is the exact absorbents we are going to analyze by using this; this one and already I told in that we took 4 wells ok 4 columns 1, 2, 3, 4, 5.

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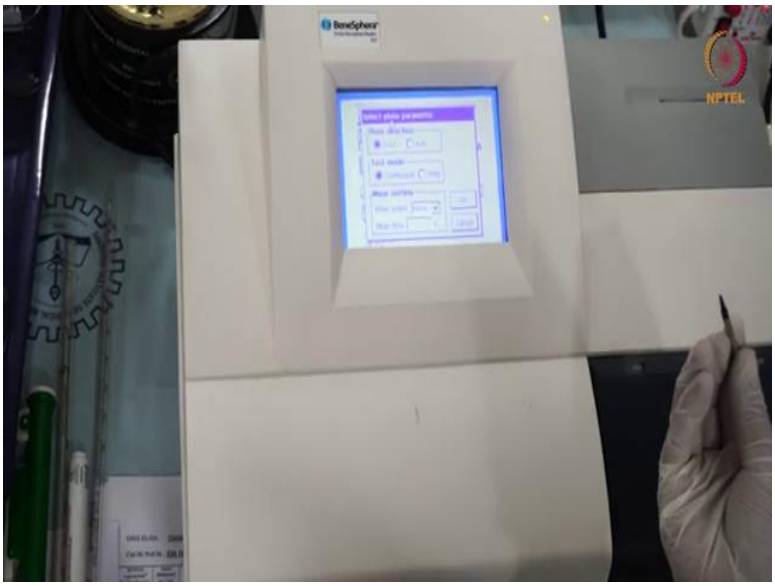

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Student: (Refer Time: 33:37).

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Yeah we are going to analyze already I told that 1, 2, 3, 4, 5; five columns we are going to use the column is 1, 2, 3, 4, 5 and A, B, C, D, E, F, G, H up to H it will be there and the first one is standard and first in that first one we are going to analyze 1, 2, 3, 4, 5, 6, 7 and 8 ok.

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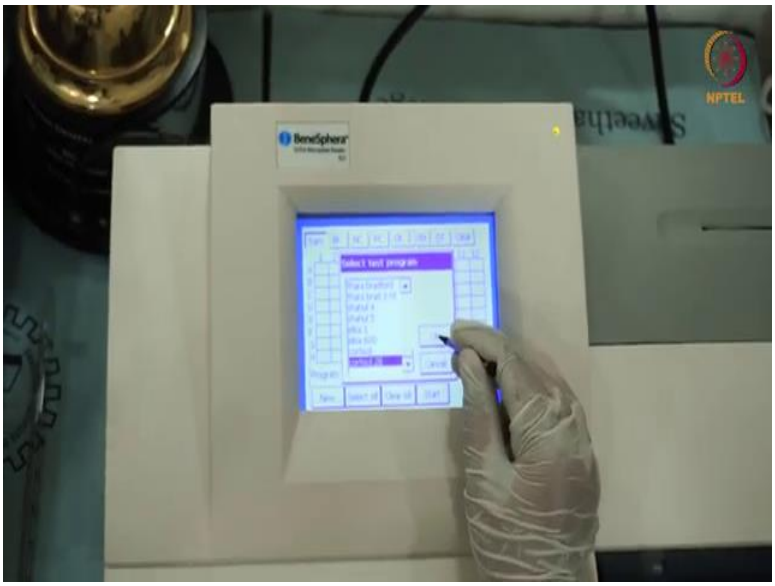

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Student: Save it.

They wanted only in general only.

Student: (Refer Time: 34:11).

And our analysis is 32, 58 ok and what are the things we are going to analyze? 1, 2, 3, 4, 5, 6, 7 ok. And the next one is control 1, 2, 3, 1 ok 3 for another one and yes and each one we have to analyze 3 3.

Student: 1 1,

1, 1 one and one more. So, totally first one is standard, second one is control, third one is patient 1 patient 2, patient 3 we are going to analyze ok. Our incubation time is over.

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And the final thing we are going to add it here see this color change was happened in this; this blue color solutions. So, it indicates some reaction is happened and that our sample as well as the control standard is mixed with that substrate solution and made some color changes.

So, the reaction has happened. So, what to do for next step all? We would like to stop the reaction. If you stop the reaction what we will do? We will add stop solution. So, 100 mule of stop solution is we are going to add it with all the wells up to this is the stop solution we are going to add a this reaction ok.

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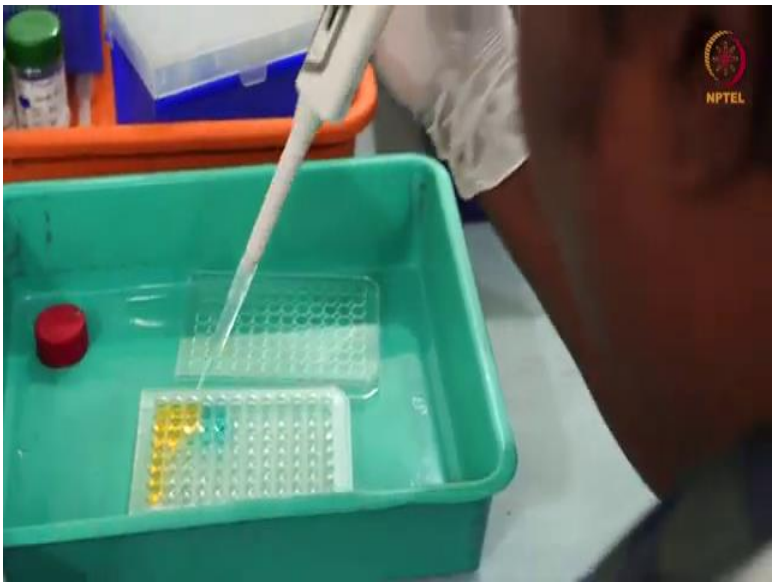


Open it. See this while you are adding the stop solution the color change how much color change will be there in this. 100 mule 10 seconds, third one the blue color turned into yellow color which indicates that stop solution working properly and it stopped that reactions ok

And the third one is patient 1, patient 2 finished and third one is patient 3 the triplicate values.

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So, just before adding this just you can see this is a substrate added one that remaining and all that stop solution added see while we are adding this the color turned into yellow color.

The stop solution did its activity properly and just you will mix it and then close and this all the practical procedures will be over ok we have started with, what we have started with? Standard control and all added and then we have added the enzyme conjugate, then we have washed and third one we have added substrate solution the substrate solution closely that reacts with that samples and all and then we have added stop solution up to this the all the reaction will be over.

And what is the next point? Next we are going to analyze this values we want exact absorbent values we are going to analyze by using this ELISA reader you keep it.

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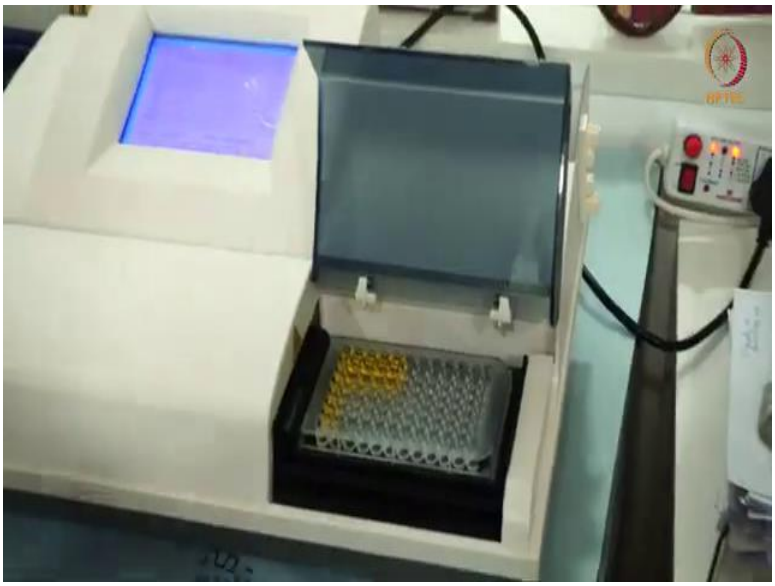

In that the reaction mixture the 96 well plate we are keep it in that ELISA reader and you know that already we have mentioned that 450 nanometer we are going to analyze it ok that reaction is finished and we kept it that 96 well plate in the ELISA reader and already we fix the reading we will take the reading at 450 nanometer we are going to take and then the reaction is finished.

So, we will close start the ELISA read extract. In that ELISA reader at a time already I told up to 96 samples we will analyze by using this instrument, see we have received this values,

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Ok 388, 0.237, 255, 0.242, 0.574 this is the readings and we have the printing option also. So, we do not want to waste the paper and it will be useful for our environment just we will take the photo of this values and then we will enter it in excel file we will make it the values, we will enter and we will give the results to the sample patients or whatever

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Today we did the invitro diagnostic study of cortisol by using samples what we have used is saliva and what are all the steps we did this you observed that and based on that only in ELISA kits nowadays a lot of kits are developed to analyze the all the

biochemical parameters such as already I told that COVID from COVID to and biochemical parameters like enzymes and all we analyze by using ELISA reader.

And then for all those things the procedure is same just, we will add the substrate we will add the solution and then we will add the stop solution and we will take the absorbents in the equipments.

These are all the simple procedures we have to read the procedures carefully you can read it and then you have to write your own handwritten you can write your protocols then you can proceed for your studies. That is all today's cortisol diagnostic analysis is over. Thank you so much for this opportunity.

Thank you all.