INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec - 16
Nanotechnology in Point of Care Diagnostics

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Hello everyone I welcome you all to the 16th lecture of this course this 16th lecture is on Nanotechnology in Point of Care Diagnostics.

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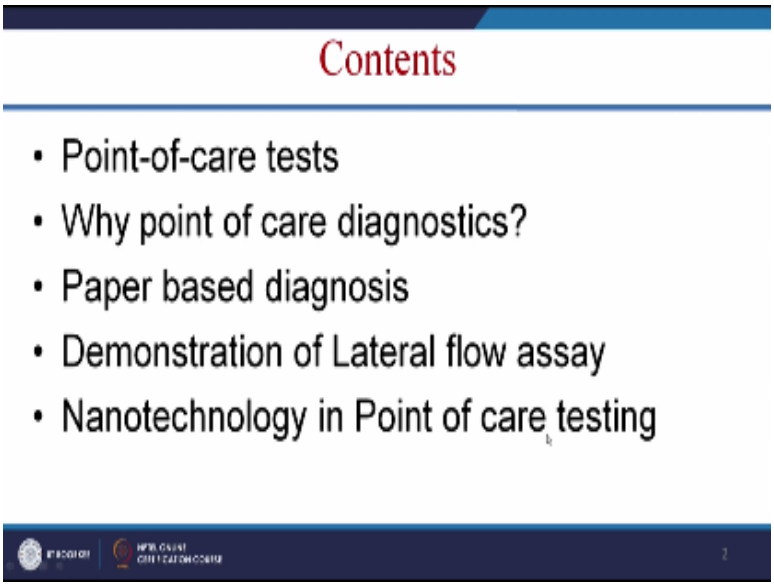

So in this lecture we will learning point of care test and why point of care diagnostics and also we are learn various paper based diagnosis and I will demonstrate a simple experiment to understand the lateral flow assay and we are also going to learn the role of Nanotechnology in Point of Care testing.
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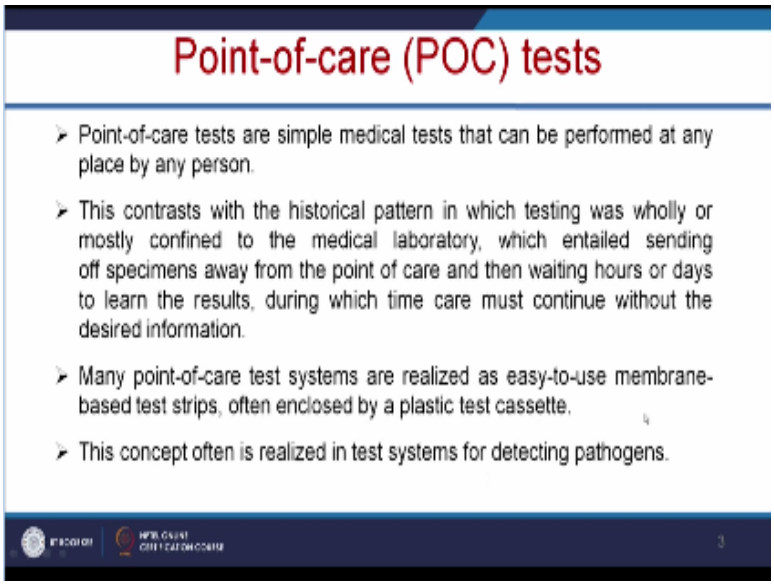

So let us see what is point of care test so it is simple medical test that can be performed at any place by nay person without the help of any medical technician and a this is in contrast with the historical pattern where you will be sending the sample to the medical lab and waiting for hours to days to get the results and during which time care must be continue without the descried information.

So most of the point of care test systems are based on the principle of easy to use membrane based test strips and these are mainly useful for a repaid deduction of pathogens.

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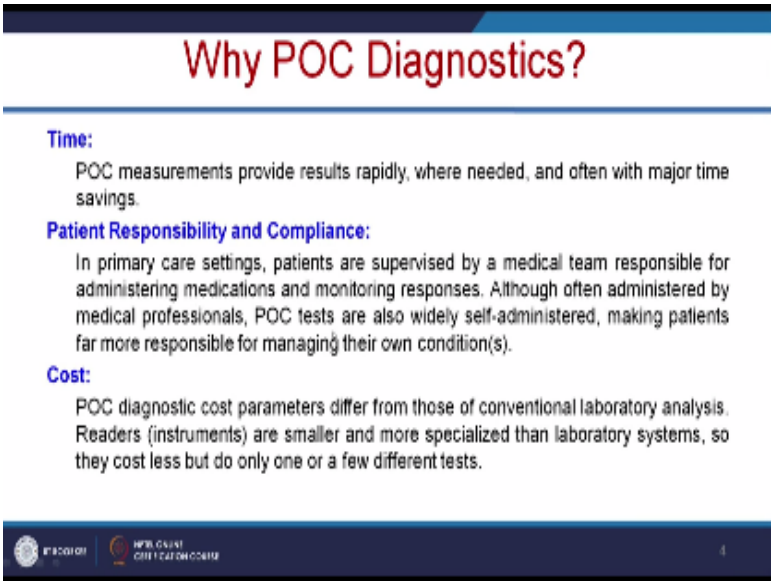

So why we need point of care diagnostics so here the main important property is like it give the results rapidly okay and it would save lot of time and then next one is patient responsibly and compliant so her the point of test are widely self administer so it is making the patients for more responsible for managing their own condition and here the cost is less when compare to the conversional laboratory analysis's and but it can do only one or few different test.

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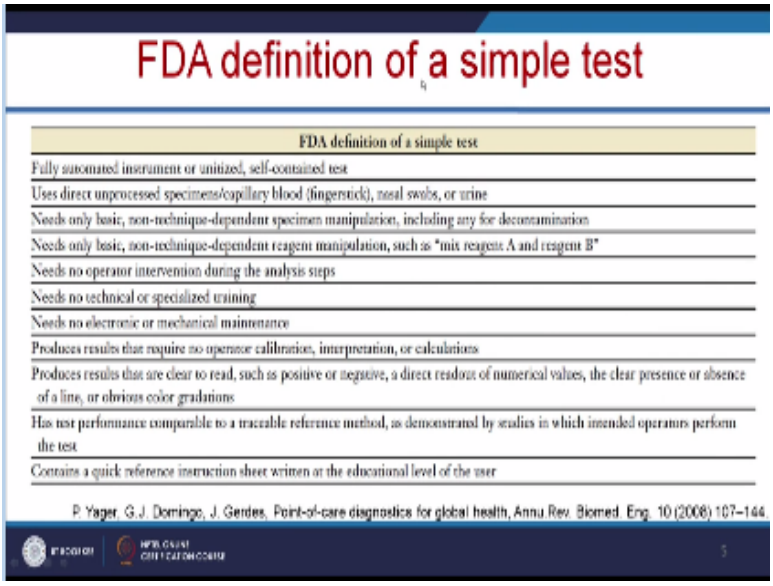

So let see the FDA definition of a simple test so according to FDA so the simple test should be fully automated instrument or self contained test and it could allow the use of direct unprocessed test sample for example can you use the blood or nasal swabs or urine sample directly and it needs a only basic non technique depended reagent manipulation for example mix reagent A and mix reagent B we will get the result.

And here it should not recover any technical or specialized training and no need for electronic mechanical maintenance okay and also it should produce the results that are clear to read such as positive and negative and also it should contain a quick reference sheet written at the educational level of the user it should be in a simple language so that the any one nay conman man can understand and he can use the particular test kit.

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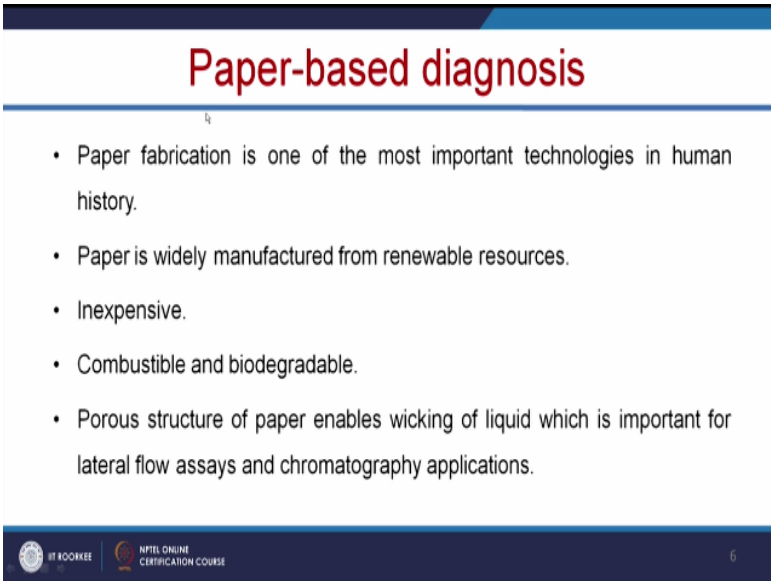

Let us see what is a paper based diagnosis so the paper fabrication is one of the most important technologies in human history because this paper is widely manufacture and from the renewable resources and it is inexpensive and also it is combustible and biodegradable for example if you're making a paper based diagnostic kid for deleting the pathogens you can after delivering the pathogens we can discuss the kid by simple insulation you can burn it okay and again the porous structure of paper enables wicking of liquid okay.

So which is important for lateral flow assays and chromatography applications and this paper based diagnosis is suitable for biological applications because the cellulose is compatible with the biological samples and this paper surface can be easily manipulated through printing coating and impregnation and can be fabricated in large quantities and the another important property is this paper can be easily altered to suit different application.

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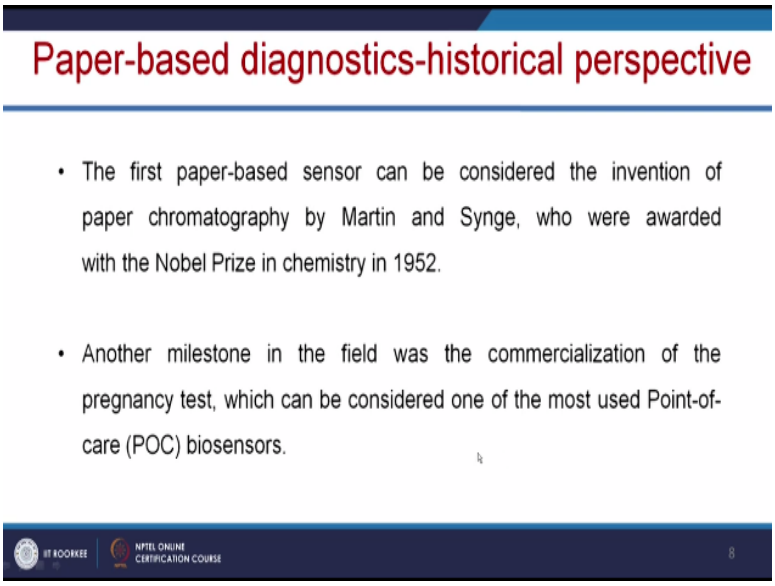

So let us see the historical perspective so this first paper based sensor can be considered the invention of paper chromatography by Martin and Synge so they were awarded with the noble prize in on 1952 and another miles stone in this field was the commercialization of pregnancy test kit okay so which can be considered one of the most used point of care diagnostics.

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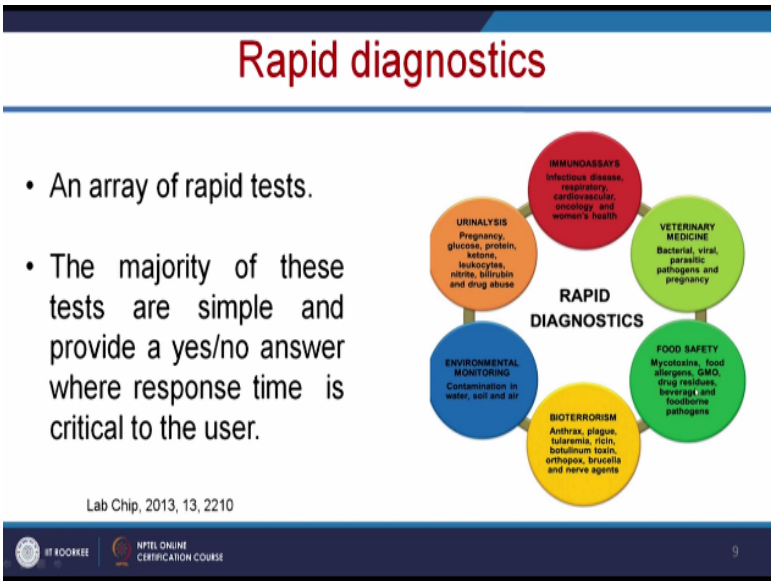

So this paper based kits are mainly useful for rapid diagnostics okay so the majority of these testes are simple and provide a yes or no answer where response time is critical to the user. For example in case of food safety so if you want to know whether your food is contaminated with micro toxins to add a drop of food sample to the paper based diagnostic kit and it will tell you the presence of or absence micro toxins.

And in case of conventional technique you have to take to the sample to the lab and you have to wait for few hours but in case of paper diagnostic kit you can get the result within a few seconds okay simpler it could be useful for various application like environmental monitoring for example if you want to check the weather the water is contaminated with the or some other micros so it can be useful for monitoring those things with the simple paper based diagnostic kit.

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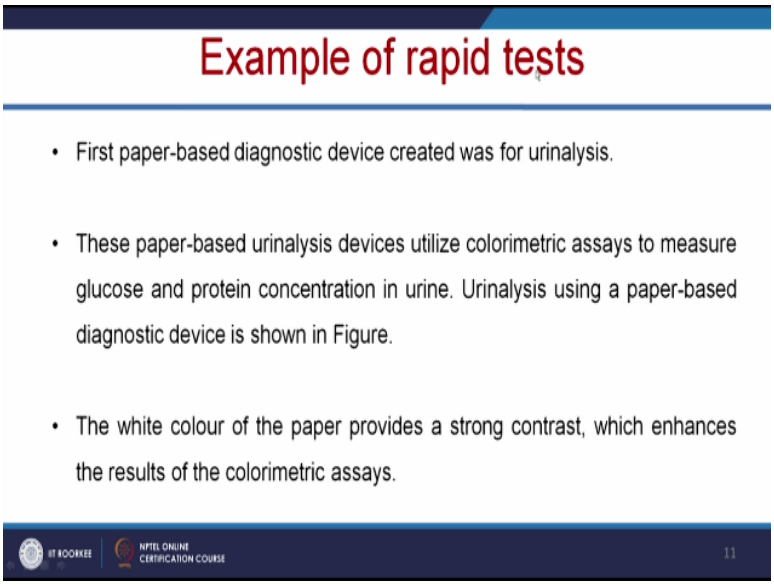

So these are some of the examples of commercial rapid test kits so for example we have the kits for HIV, TB influence and malaria so these are the various paper based test kits which is a commercial available in the market so let us see of the examples of rapid paper based test kits so first paper based diagnostic device was created for urinalysis okay so these paper based urinalysis device utilize colorimetric assays to measure the glucose and protein concentration in the urine. So here the white color of the paper provides a strong contrasts so which enhances the result of the colorimetric assays.

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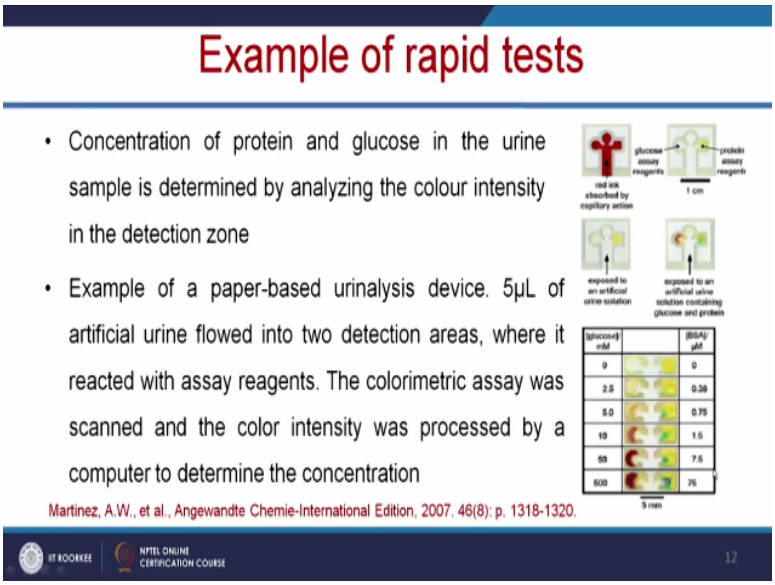

So you can see her this is your simple paper based rapid kit for meaning the glucose protein concentration in a urine sample and when we add the sample her so if get the colour in this side it will indicate the glucose presence of glucose and if you get the color in side it indicate the presence of protein, so you can see here so with respect to the concentration the intensity of the color is getting increased okay.

So this is for the glucose and this is for the protein and based on the strip reader we can easily read the instance color intensity and we can estimate the concentration of glucose and BSI in the particular urine sample.

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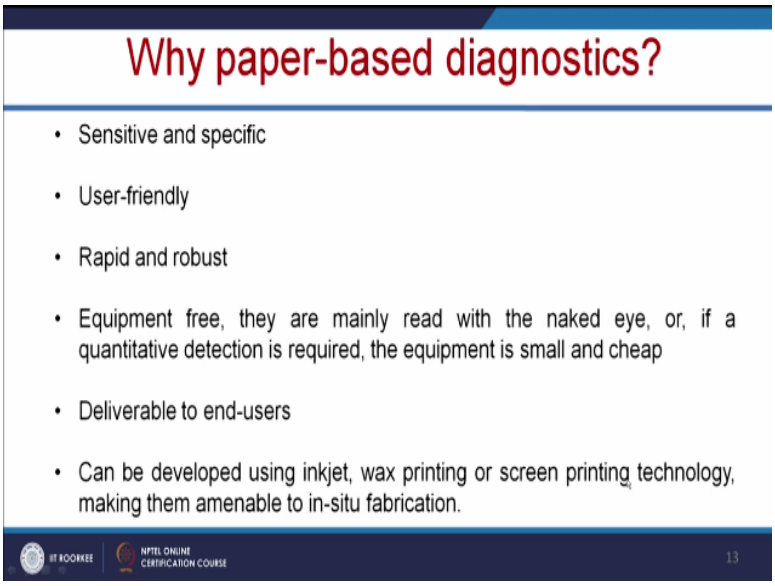

So let us see why we need paper based diagnostics so as I told you earlier these are sensitive and specific and user friendly and also it is rapid and robust and it is equpoiemnts free we do not need any sophisticated equipment for using this paper based diagnostic kit okay so they are mainly read with naked eye or if a quantitative detection is required the equipment is small or cheap okay.

And it is deliverable to end users and can be developed using inkjet or wax printing or screen printing technology.

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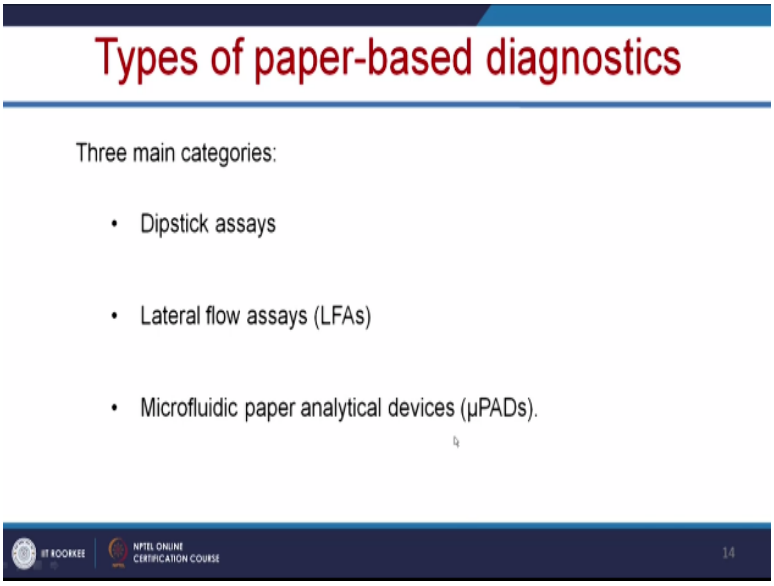

So there are 3 types pf paper based diagnostics dipstick assays lateral flow assays and micro fluidic paper analytical devices so let us see one by one in detail.

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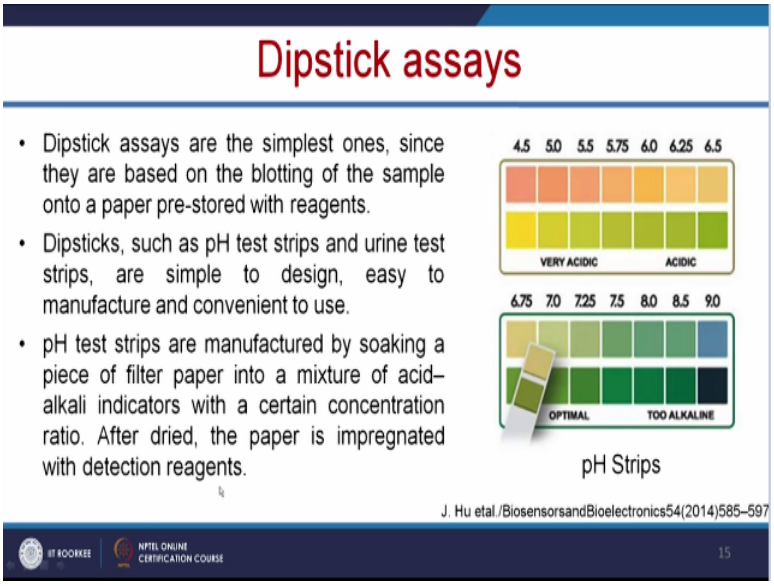

The first one is dipstick assays so these simplest once because these are based on blotting of the sample onto a paper pre stored with reagents. Some of the examples are pH test strips are urine test strips, so these are simple to design and easy to manufacture and it is convenient to use okay so how they make the pH test strips so the pH test strips are manufactured by soaking a piece if filter paper into a mixture of acid alkali indicators with a certain concentration ratio. So after it is dried the paper is impregnated with the detection reagents.

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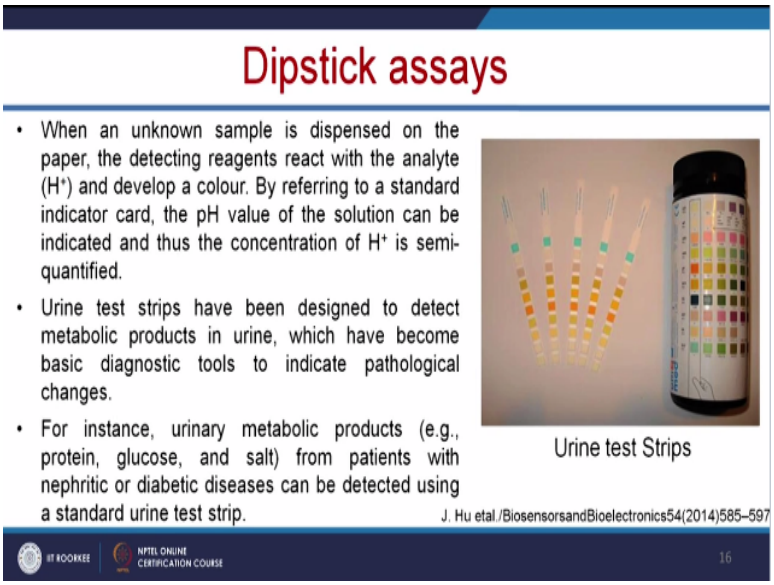

So when an unknown sample is dispensed on the paper the detecting reagents with the analyte okay and it will develop the color so by referring to a standard indictors card the pH value of the solution can be indicated and thus the concentration of H+ is semi quantified, so might have used the pH paper in your lab so just you put a pH paper in a any unknown solution it will tell you the pH of the particular solution okay.

So it is based on the principle of dipstick assays and similarly the another thing is urine tests stripes okay so it have been designed to detect the metabolic products in the urine so which have become basic diagnostic tools to indicate the pathological changes for example urinary metabolic products like glucose, protein and salt can from the patients with nepotistic or diabetic diseases can be easily detected using a standard urine test strip. So we can use this kind of urine test strips and we can repeat the urine sample of the patient and we can easily analysis the presence of glucose or protein or the salt.

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So next one is the lateral flow assays okay so this lateral flow assay have all the reagents pre stored in the strip so it is also simpler to the dipstick but it will also integrate the flow of the sample so here the flow passes through the different zones of the strip which have different reagents for different function so usually this lateral flow as they have 4 different parts the first one is simple pad the rest one is conjugation pad and 3rd one is reduction pad and 4th one is absorbent pad.

So the sample pad is mainly made up of cellulose and it will filter the sample from imputes and it stores the dried assay buffer, next one is a conjugation pad so it is made up of glass fibers and it is used as dry regents storage for the labels and in this pad the binding reaction between the labels and analyte starts okay so in the detection pad it is made up of nitrocellulous the capture reagents are fixed and the signal is developed in this detection pad and 4th one is absorbed pad it is made up of cellulose filters so the function of the absorbent pad is to wick the fluid through the membrane okay so in this way the amount of sample can be increased resulting in an increased sensitivity.

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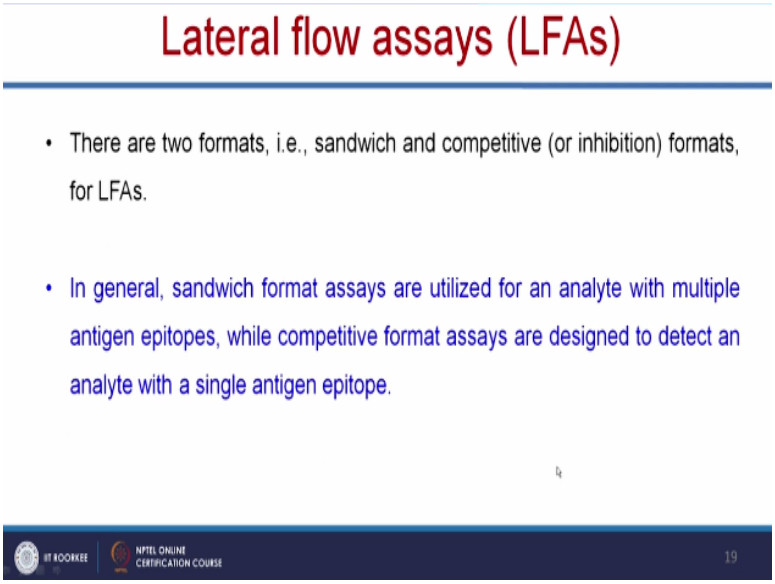

And this lateral flow as a is available in two formats sandwich and completive so even in the sandwich format assays are mainly utilized for analyte with multiple antigen epitopes and this completive format assays are designed to detect an analyte with a single antigen eptiope.

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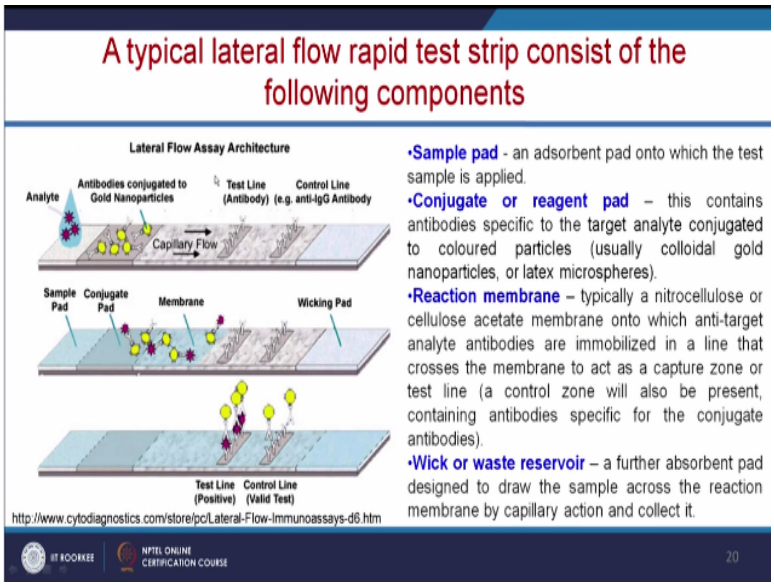

So let us see the lateral flow as in detail okay so this lateral flow as it contains 4 different pads sample pad conjugate pad and membrane and wicking pad so in this sample pad we will be adding this sample and in the cogitate pad it contains antibodies conjugate of the gold nano particles or lattice micro spills and here the sample and antibody everything move in this direction you think the capillary force.

And in the test line you will be having the antibody which is specific for your antigen and in the control line will be having the anti IgG antibody okay so which is specific for you gold nano particles conjugated antibody. So when you add the sample so the antigen present in the sample will bind with the antibody in the conjugate pair, okay. So it will move towards these test line so in the test line so this antigen will be sandwich between two antibodies.

All the unbound antibody will move to the control end where antibody, antibody reaction will happen, okay. So let me explain the lateral flow as a one more time in detail.

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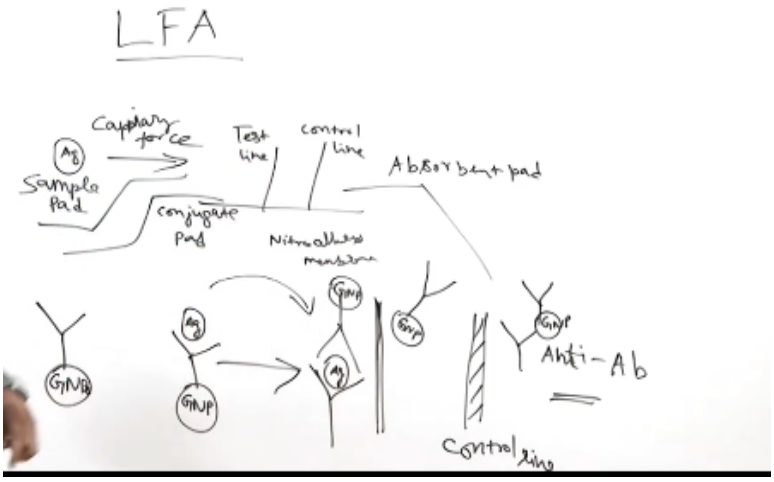

There is a most of the commercial kit is based on later flow, so here in the lateral flow air will be having sample pad and you will be having conjugate pad and below that you will be having nitro cellulose membrane and here will be having absorbent pad having the nitro cellulose membrane you will be having test line and control line, so when we add the sample here so the sample contains the antigen.

So the sample will move in this direction through capillary force and when it reach the conjugate pad in the conjugate pad it will be having antibody conjugated with gold nano particles, so here the conjugate pad so this antigen will come and bind with the antibody which is conjugated with gold nano particles and this antigen antibody complex will move in this direction, so when it reach the test line.

So in the test line you will be having antibody specific for your antigen, so this antigen antibody complex will come and bind here, so antigen antibody complex will come and bind and your antigen will be sandwich between the two antibodies okay. So here you will get a color line and this unbound antibody will move to the next line that is your control line, so this unbound free antibody conjugated antibody which is having this gold nano particle.

It will move to the control line so in the control line will be having anti antibody, so this antibody is specific for your this antibody conjugated antibody, so this conjugated antibody will come and bind here, so it form a color line here. So a positive sample will get two lines one is your test line next one is a control line so this control line conforms your kit is working properly and the

sample floor is in the proper direction, so here in the test line you will be having antigen antibody reaction and in the control line you will be having antibody, antibody reaction, okay. So this is a basic principle of lateral flow assay, I hope you understood the principle of lateral flow assay.

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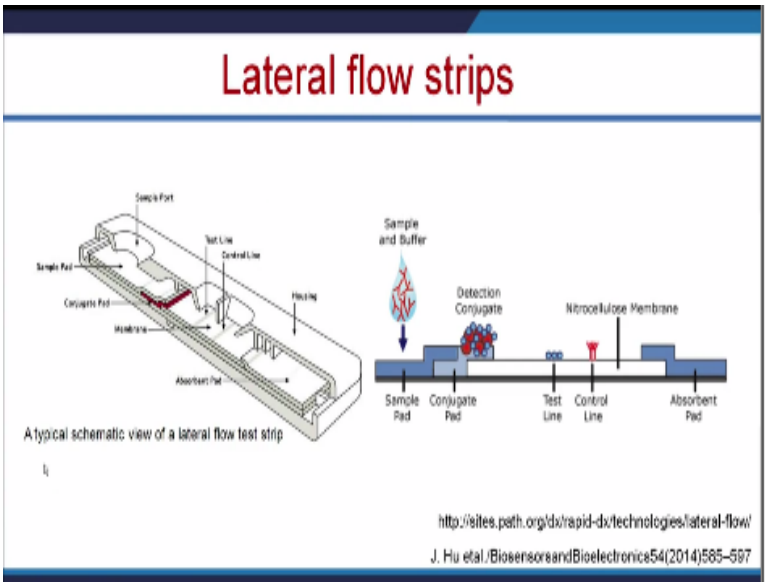

So this is a typical schematic view of lateral flow tested okay so it is having this kind of arrangement if you open the kit you will have this kind of arrangement I will open and show you how it look like, so the sample pad you can see the arrangement is like a step like arrangement and below the sample pad you are having the conjugate pad and followed by you are having the negative cellulose membrane and this negative cellulose membrane is having a test line which is having the antibody specific for your antigen.

And in control end you are having a antibody so that antibody is specific for your antibody that is the conjugated antibody, okay. And this one is absorbent pad it will absorb the excess sample.

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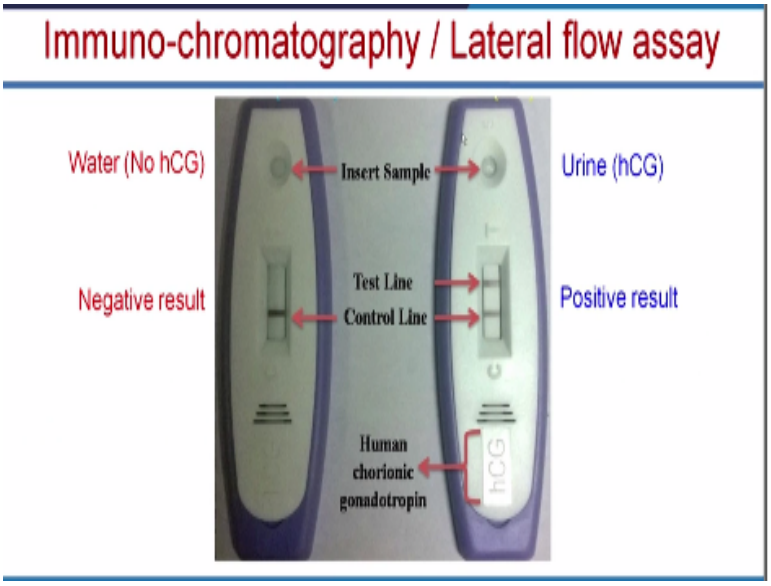

So let us see the simple experiment to understand the lateral flow assay using the pregnancy test kit, so here we will be using this urine sample of pregnant women so the urine sample of pregnant women contain hCG that is Human chorionic gonadotropin, okay. So when we add the sample here you will get two lines so the two lines indicate the positive result and in the other one, we will be adding this simple water where there is no antigen and we will get only formation of line in the control line.

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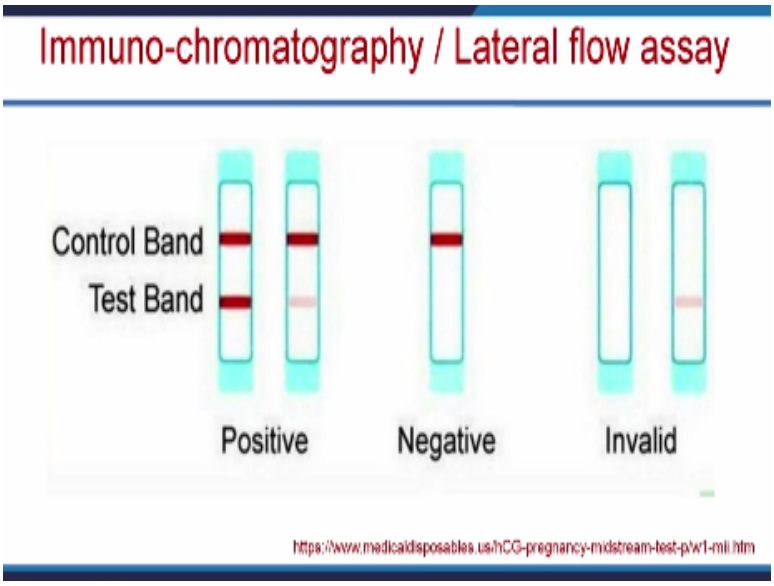

For the past two sample you will be having the two bands control band and test band okay and in case of negative you will get the band only in the control line okay, and if there is no line the test is invalid, so I will show you the demonstration of lateral flow assay using the pregnancy test kit so you can easily understand the concept of lateral flow assay.

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So let me explain the principle of lateral flow assay using a simple pregnancy test kit, so first I will open this pregnancy test kit I will show you the arrangement of various pads in these kit, so as I told you earlier this lateral flow assay contains various pads the first one is your sample pad, so where your sample will be loaded and this one this color one if your conjugate pad where your antibodies conjugate are gold nano particle or some color beads.

And the below one is our membrane nitro cellulose membrane so where you are having the test line as well as the control line okay so the test line is containing antibody specific for your antigen and this control end it is having antibody specific for the conjugated antibody and this is your absorbent pad so this will observe the excess sample okay. So in this two kit in the first one I will add the only the water okay. So this is a sample port so where I will add the simple water.

So when I add the water the sample will flow using the capillary force and in water there is no antigen so you will get only one line so we will get line only in the control you can see here this is the C is the control and T is the test line, so you will get the lien only in the control. You can see here the formation of color line that is only in the control you are getting this line okay; in the other kit I will add the urine sample.

So in the kit where I added the urine sample you will be able to see two lines so one is for test line and other one is for control. We have two lines that mean the particular person is pregnant. So you can see here you are able to see two lines right so one is for the control and one is for the testy line you are able to see the two lines in case of urine sample and in case of water you are

able to see only one line, okay so you are getting one line in the control and in case of urine sample you are able to see two lines.

So by the using experiment you are able to understand the concept of lateral flow assay, I hope you understand the concept of lateral flow assay now we move on to this micro fluidic based paper analytical devises, okay.

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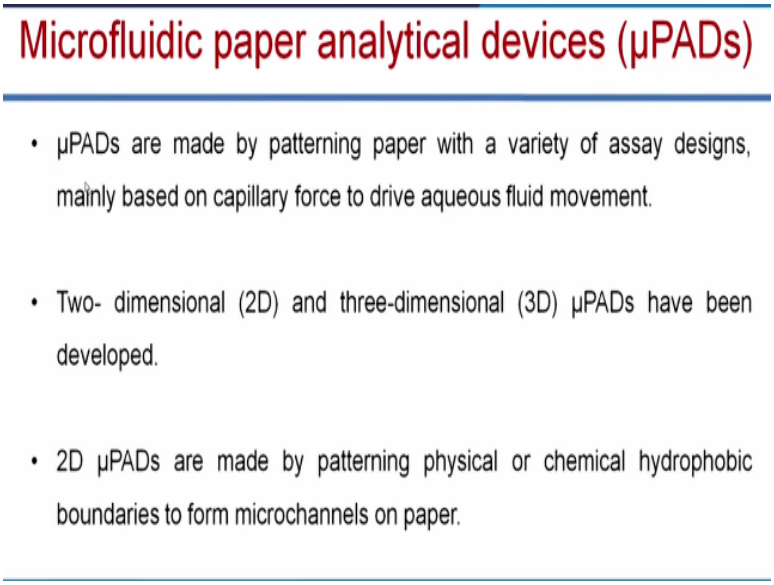

So this micro pads are made by pattering paper with variety of assay designs so it is mainly based on capillary force to drive the aqueous fluid movement, so we can make the two dimensional or 3 dimensional micro pads and this two dimensional micro pads are made by pattering physical or chemical hydrophobic boundaries to form the micro channels on paper.

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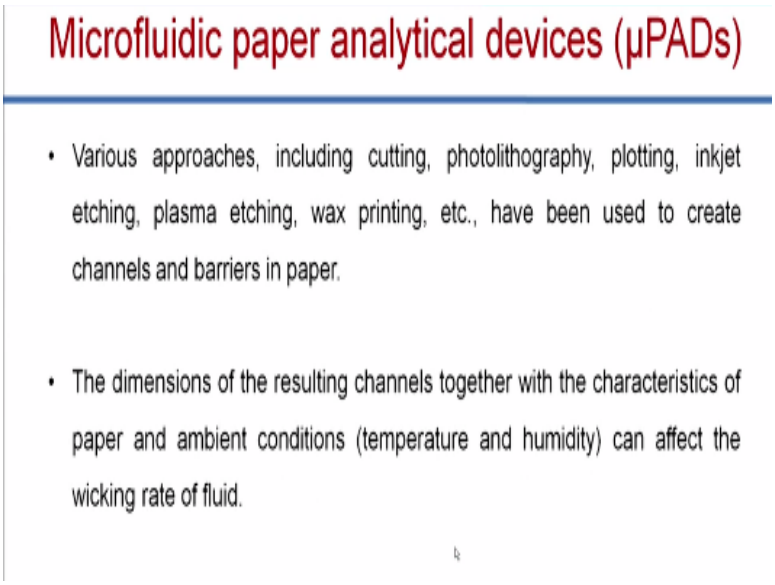

And various approaches like including cutting or wax printing can be used to create the channels and barriers in the paper.

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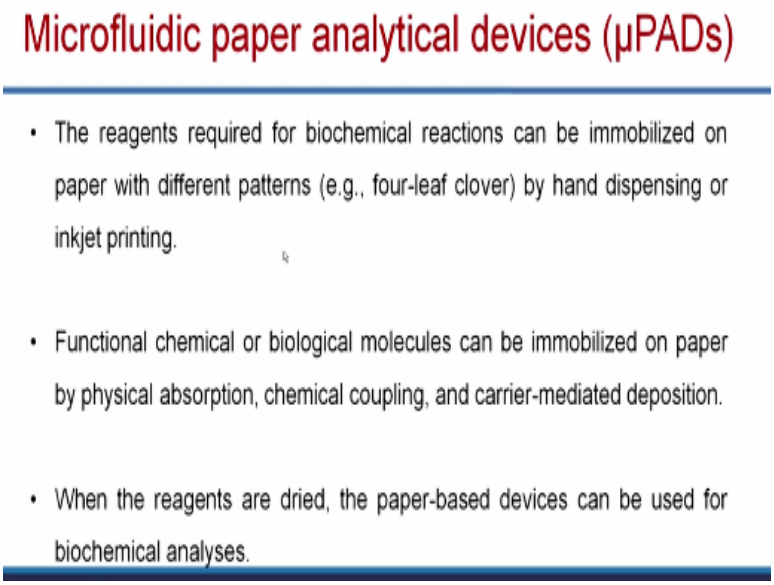

And here the reagent required for bio chemical reactions can be immobilized on the paper with different patterns for example we can make a four leaf clover by hand dispensing or inkjet printing technology and when the reagents are dried the paper based devices can be useful for bio chemical analysis.

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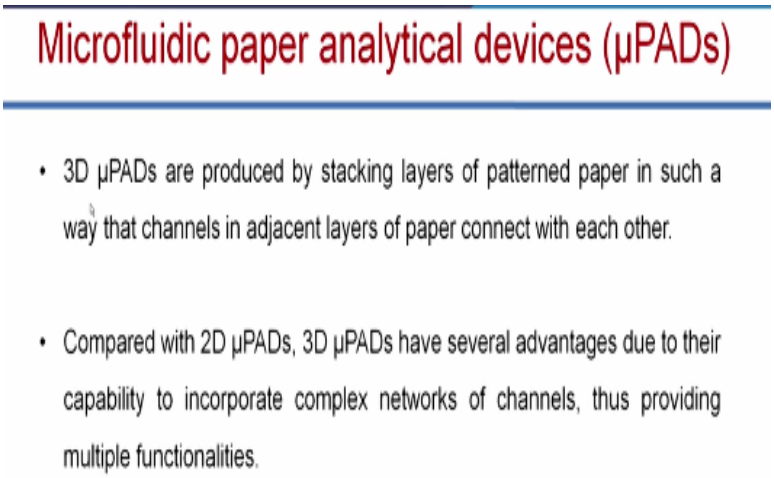

So this 3 dimensional micro pads are produced by stacking layers of patterned paper in such a way that channels in adjacent layers of paper connect with each other and compared to the two dimensional micro pads this 3 dimensional micro pads have several advantages so due to their capability to incorporate complex networks of channels and thus providing multiple functionalities.

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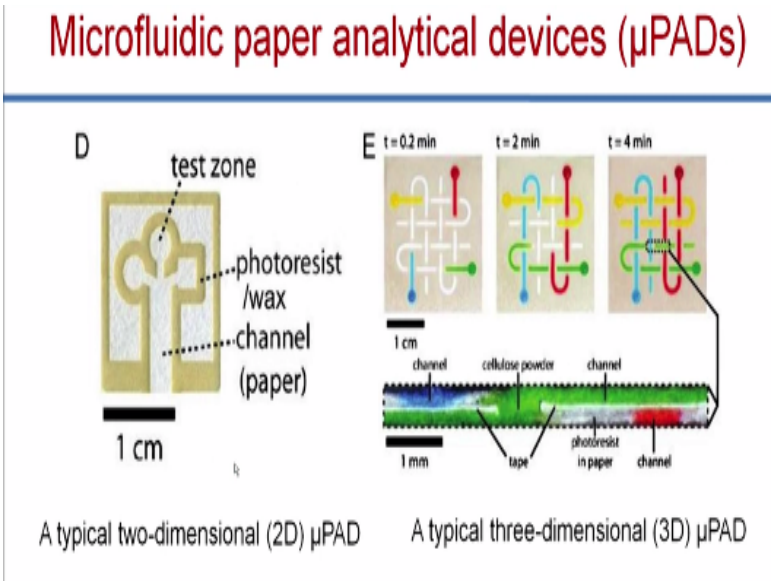

So this is a typical example of two dimensional micro pad so this is a channel okay and this channel can be created using photo resist or wax and this is the test shown and when you add the sample so it can detect the glucose of the protein in the urine sample or any other sample and this is a three dimensional micro pads okay, so here it is having multiple channels and which could be useful for detecting multiple pathogens.

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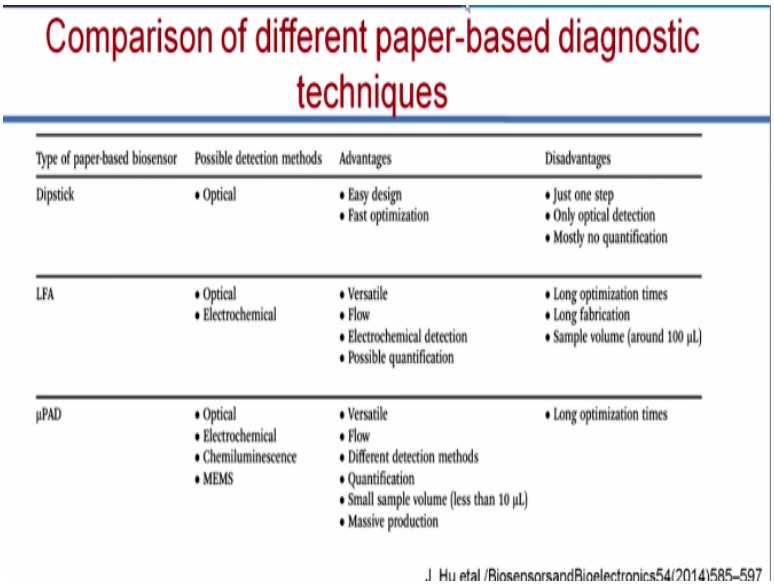

So let us see the comparison of different paper based diagnose techniques so each has its own advantages as well as disadvantages so the dipstick method is easy and fast optimization but the problem is it is a only optical detection so there is no quantification and this LFA it is a versatile and it is a based on a flow and we can also do that electrochemical detection and the problem is long optimization times and also the long fabrication.

And many use this micro facts so it is a versatile as well as it is having this different method of deduction methods and main advantage is sample volume is here it is less we can use only 10 micro drop sample but the drawback is it lead to long optimization times.

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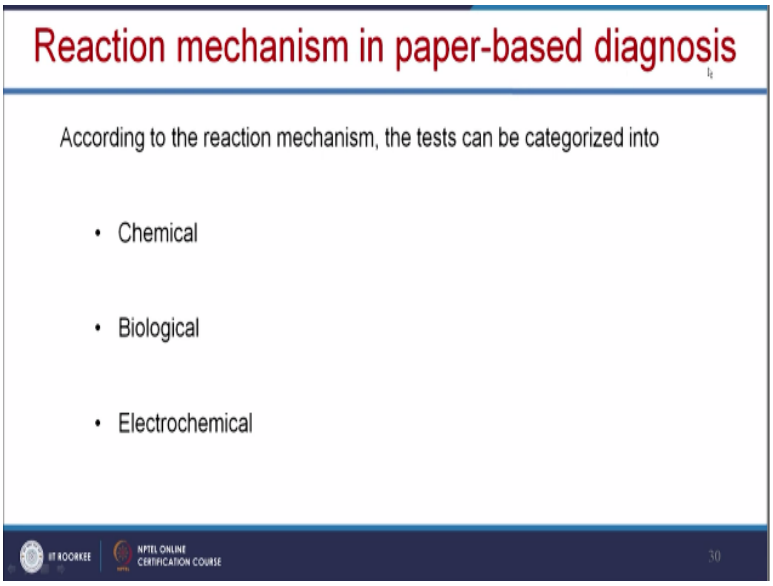

And this paper based diagnosis also categorized based on the reactions it can be categorized into chemical biological and electro chemical

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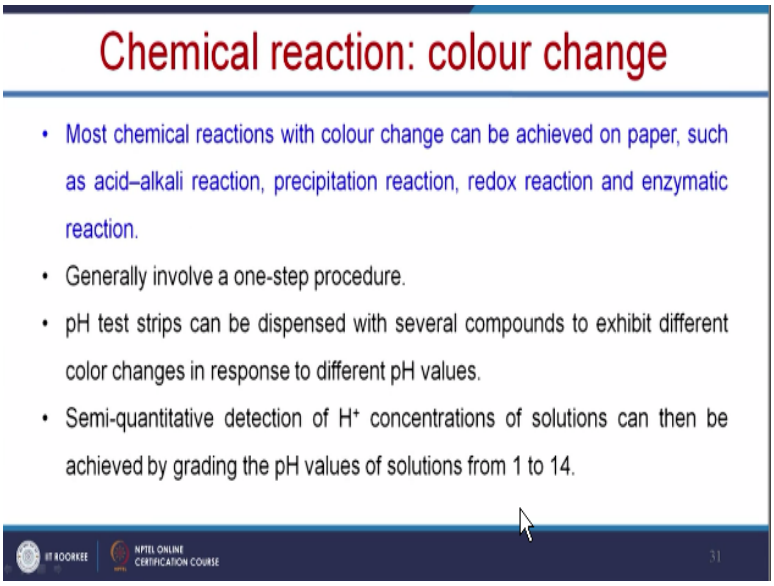

So the most chemical reactions with colour change can be achievedon apper such as acid-alkali reaction or precipitation rection and redox reaction so which I already explained you the example of pH tests.

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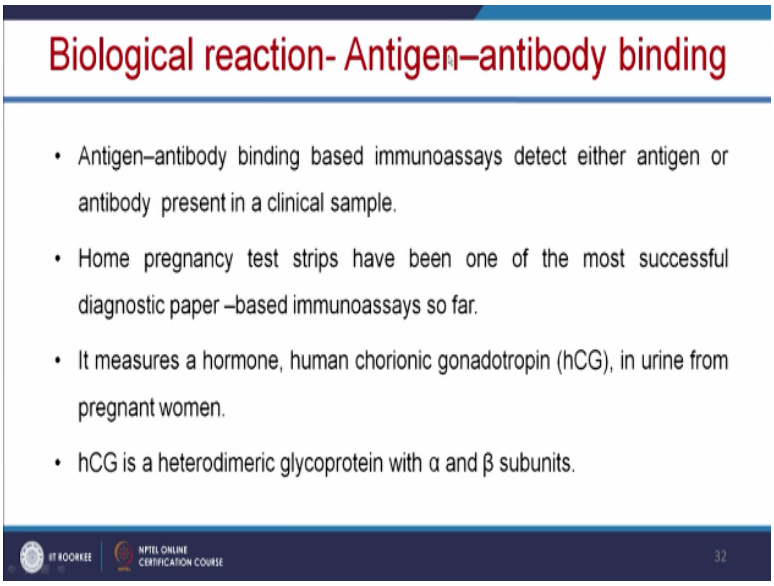

So next one is biological reaction antigen antibody reaction so the example is home pregnancy test strip where the antigen antibody reactions happens okay.

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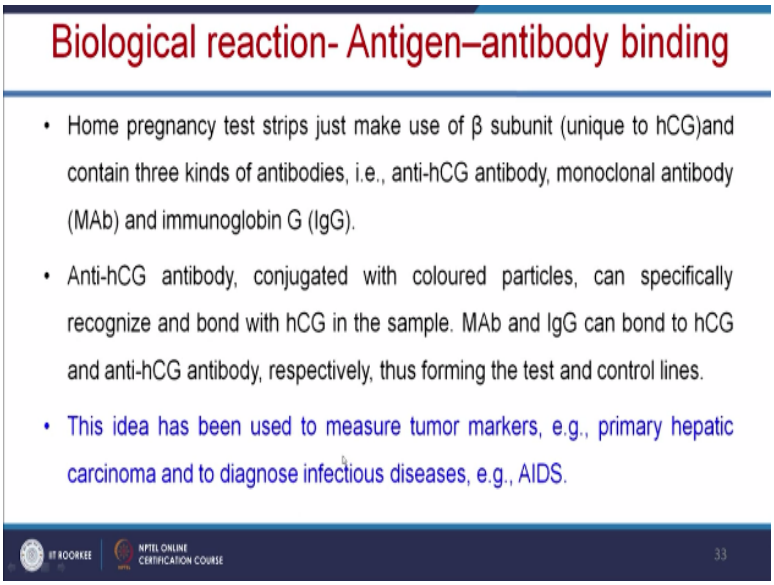

So the same idea could be useful for the measuring the tumor markets as well as for diagnose for various infectious diseases

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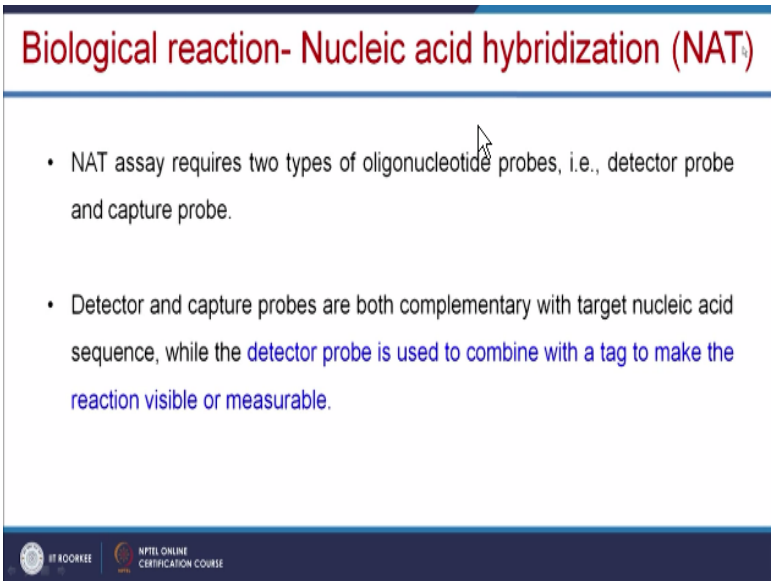

So next one is biological reaction by nucleic acid hybridization that is NAT here there is NAT assay requires two types of oligonucleotide probes first one is detector probe and next one is capture probe and this detector and capture probe are both complementary with target nucleic acid sequence and here the detector probe is used to combine with a tag to make the reaction visible or measurable.

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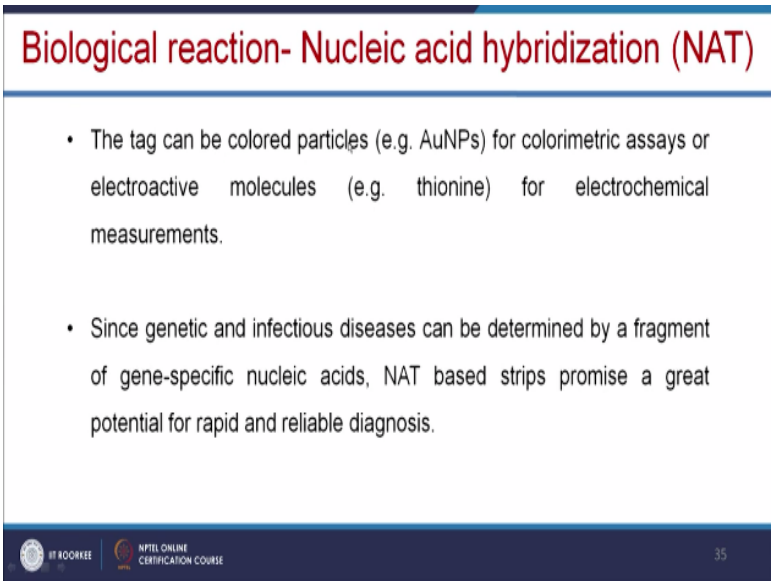

And here that tag can be colored particles or it can be colorimetric assays and it can be electro active molecules for example thionine for electrochemical measurements so you think that NAT has a easily identify the genetic as well as infectious diseases so let us see what is the electro chemical reactions.

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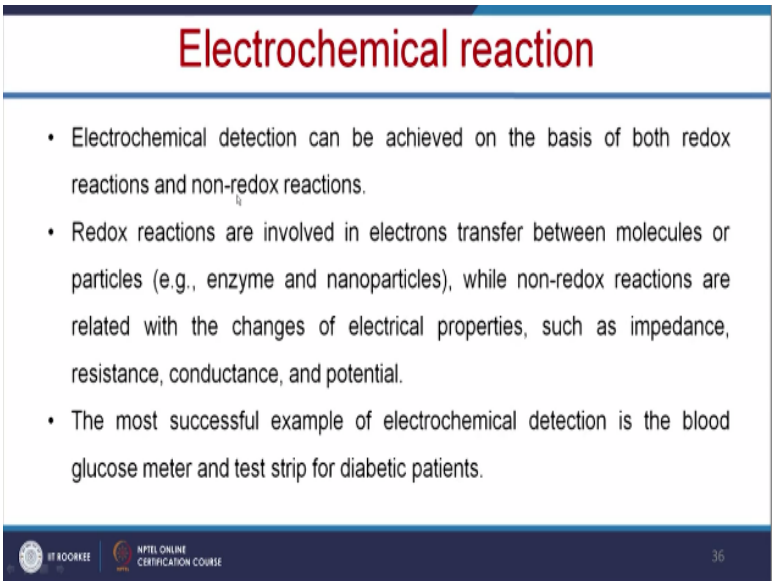

So here this electro chemical reactions can be achieved on the basis of both redox reactions nad non-redox reactions so the redox reactions are involved in electrons transfer between the molecules or particles and this non-redox reactions are realted with the changes of electrical properties for example impedance or resistance will get changed so the most successful example of electro chemical detection is the blood glucose meter.

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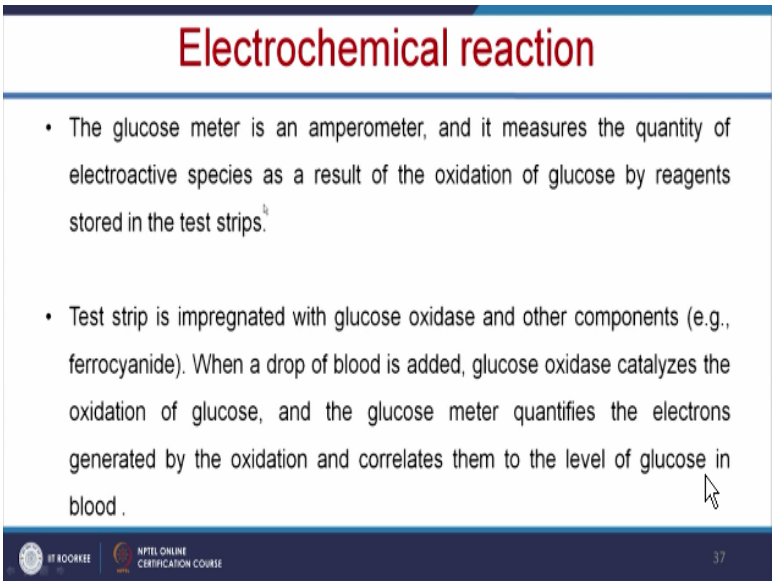

So this glucose meter is an amperometer okay and it measure that the quantity of electroactive species as a result of oxidation of glucose by reagents stored in the test strips so this test strip is impregnated with glucose oxidase and other components so when you add a drop of blood the glucose oxidase catalyzes the oxidation of glucose and the glucose meter quantifies the electrons generated by the oxidation and correlates them to the level of glucose in the blood.

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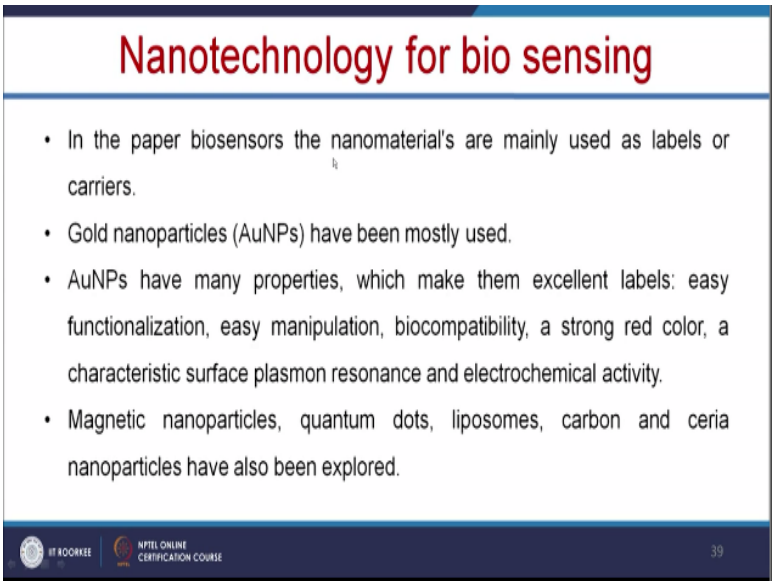

So let us see the role of nanotechnology in point of care testing so in the paper based biosensors so the nanometer are mainly used as a labels or carriers so the gold nanoparticles have been mostly used and because this gold nanoparticles have been excellent labeling properties and easy functionalization easy manipulation and also it is biocompatibility and it produce the strong red color it also characteristic surface Plasmon resonance and electrochemical activity and some of the other nanoparticles like magnetic nanoparticles like quantum dots liposomes carbon and ceria nanoparticles have also been explored for point of care diagnostics.

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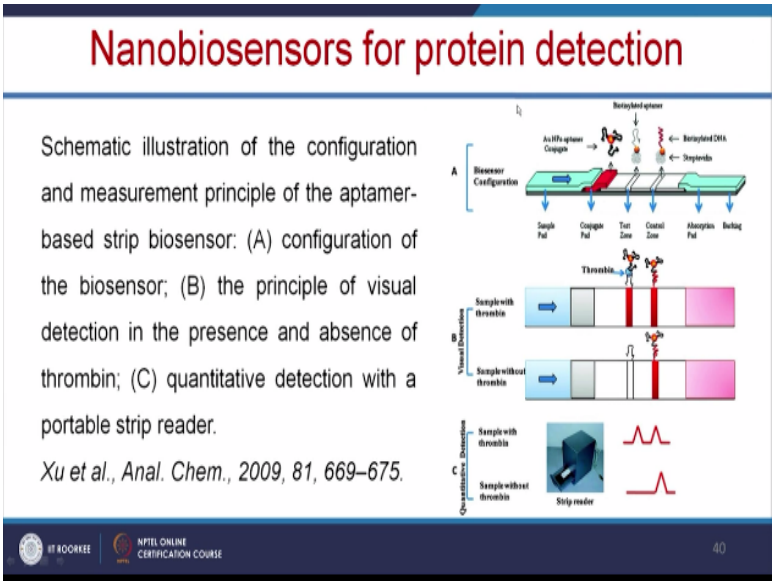

So let us say example of nanobiosensors for protein detection and here we can detect the thrombine okay so this is also similar to the your pregnancy test only and let us flow sink you can see a sample pad and conjucted pad okay and if you are interrupt antibody you will using this aptamer so aptamer are only for nuclear oligopeptades so which is fescue for the particular protein okay.

You can see here when you add this sample thrombine you are getting two lines okay that is known as well as control zone you are getting this line and here this sample without thrombine there is no line in the zone so using this strip reader we can easily quantitated the amount of thrombine present in the particular sample.

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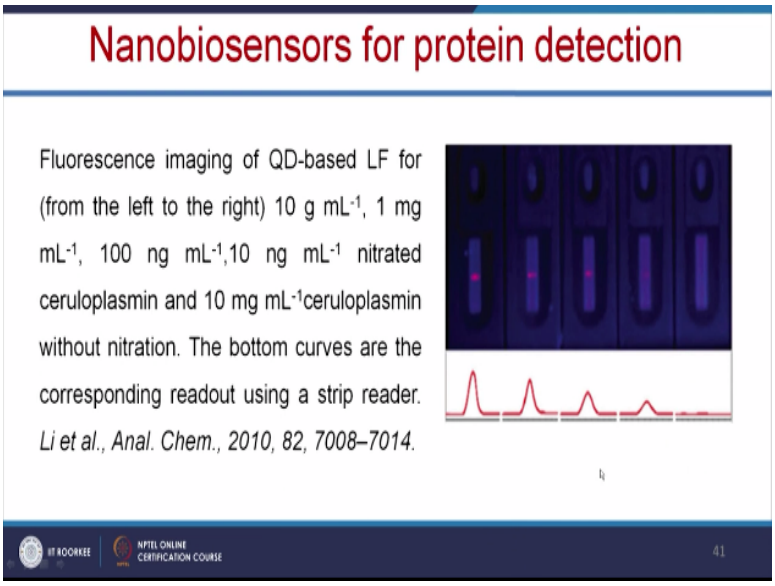

So the next example is we can use the quantum directs we can use the quantum directs also for a diagnostic application and we can measure the flow of sensitivity using the strip reader.

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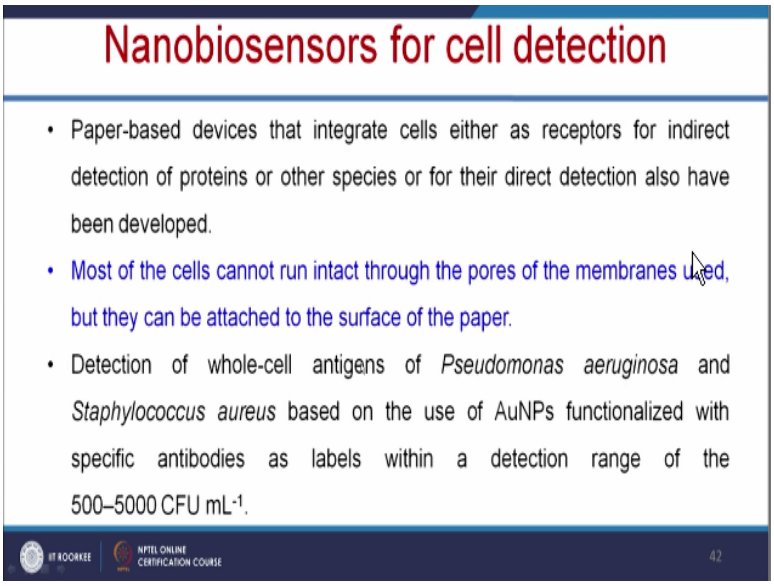

So next example is nanobiosensors for cell detection so here the cells cannot run intact through the pores of the membranes so but they can be attached to the surface of the paper so using this we can detect the pathogens like pseudomonas staphylococcus bacteria and where will be using this gold nanoparticles functionalized with specific antibodies and it can be deduct 500 to 5000 CFU.

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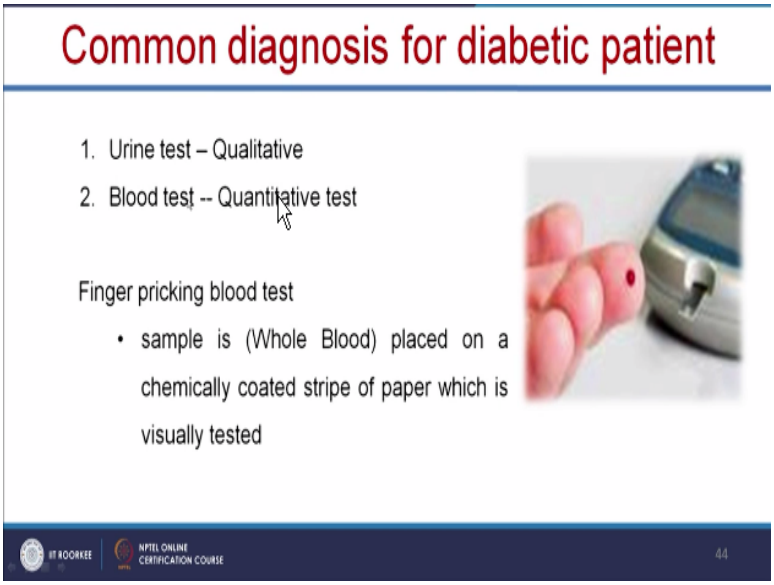

So common diagnosis for diabetic patient is urine test or blood test okay so the urine test will give the qualitative test and blood test will give the quantitative test and this blood test can be done by finger pricking method so here the sample is whole blood it placed on the chemically coated stripe of paper and which can be visually tested so but the major drawback is you have to prick your fingers everyday to measure the glucose level so it is a little bit painful situation for patient with diabetics.

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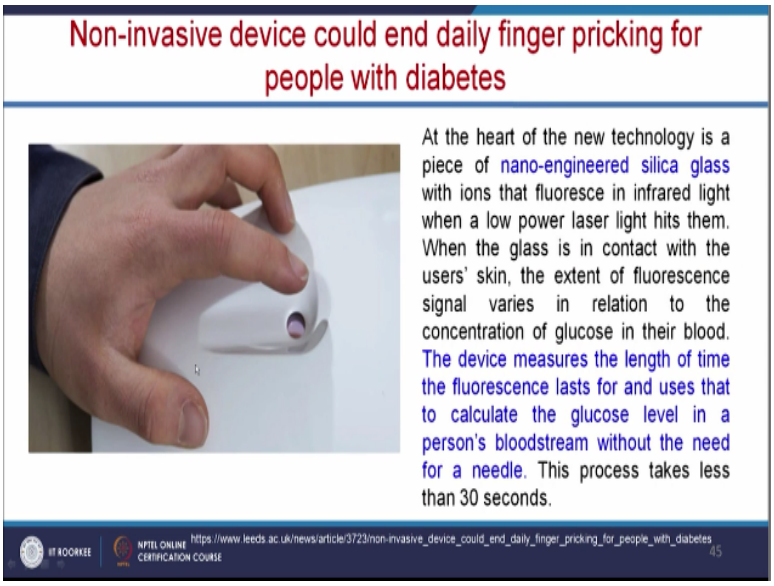

So to overcome this invasive techniques so scientist from least university they will develop their non invasive device okay that could end your daily finger pricking for people with diabetes and they were used the engineered nano-engineered silica glass with ions that fluoresce in infrared light when low power laser hits them and when the glass is in contact with the users skin the extent of fluorescence signal varies in relation to the concentration of glucose in their blood.

So here the device measures the length of time the fluorescence lasts for and uses that to calculate the glucose level in person's bloodstream without the need for a needle and this process takes less than 30 seconds so instead of pricking your finger so it is similar to the biometric device just keep your finger on the device within 30 seconds it will estimate the blood glucose.

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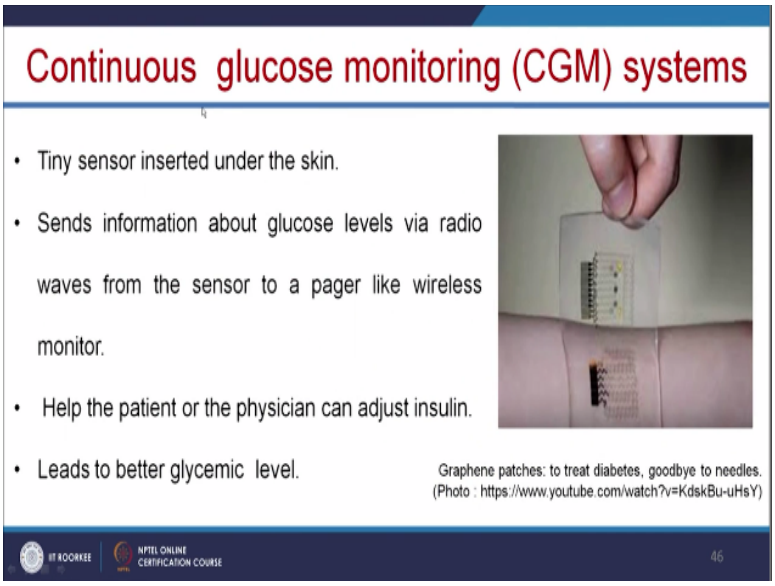

So next one is continuous glucose monitoring systems so this is the tiny sensor so which can be inserted under the skin and it will sends the information about glucose levels via radio waves from the sensor to a pager like wireless monitor and it will helps the patient with doctor and accordingly they can take the insulin.

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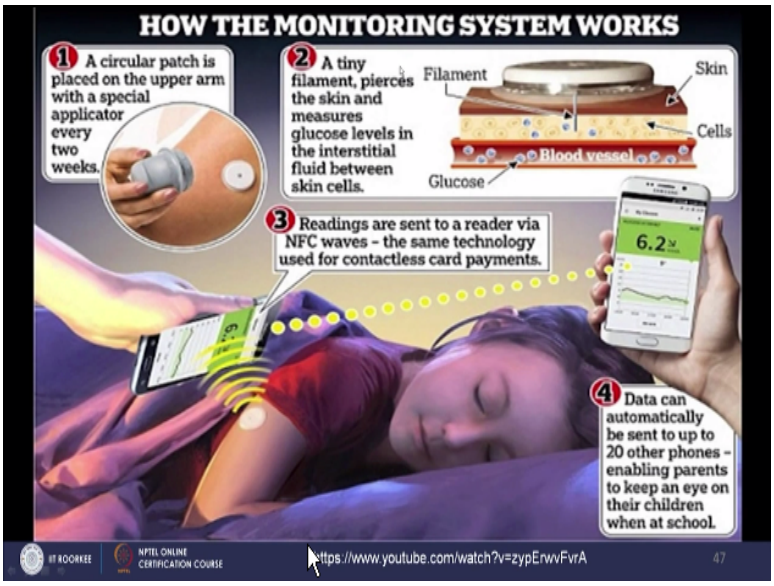

So how the monitoring system works so here circular patch is placed on the upper arm with a special applicator every two weeks okay and this tiny filament pierces the skin and measures the glucose level in the interstitial fluid between the skin cells and the readings are sent to a reader through the NFC waves that is the near field communication waves and this is the same technology which we used for contactless card payments okay and these data payments can automatically be sent to up to 20 other phones okay it can enabling parents to keep an eye on their children when at school.

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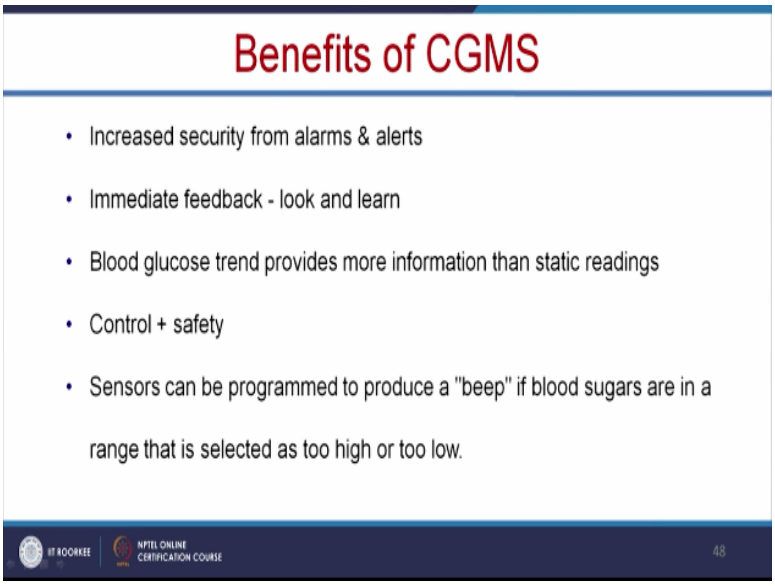

So here the benefits of CGMS is like a it has increased security from alarms and alerts and here we are getting immediate feedback okay and also the blood glucose trend provides more information than static readings and here the sensors can be programmed if it is a low glucose or high glucose it can make some alarm

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So recently this google made a smart contact lenses so this contact lenses have sensors okay and this can detects a glucose in the tears so this technology uses a small nanoparticles which is embedded into the hydrogel lenses okay and these engineered nanoparticles react with glucose molecules found in tears and it causing a chemical reaction that changes the color of the lenses. This tiny lenses will send the information about the blood glucose to the mobile phone and also the color of the contact lenses gets changed based on the color also we will get the idea so what is the sugar level in the blood.

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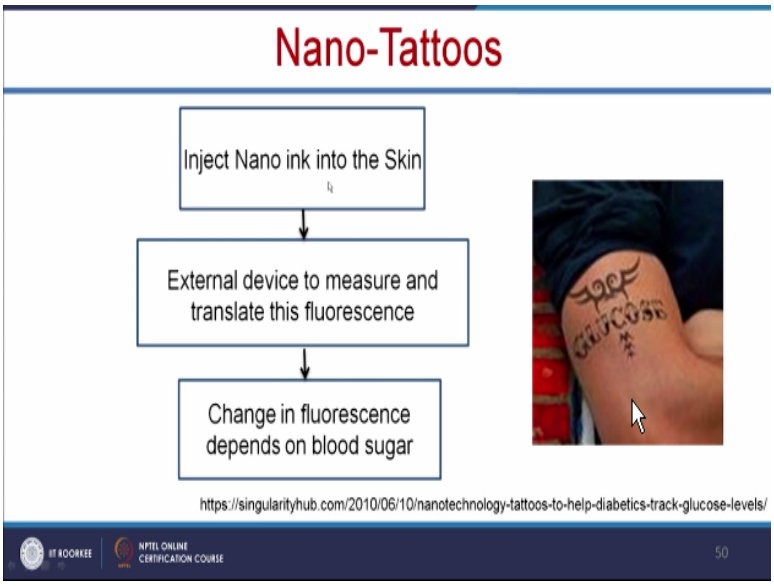

So in another thing is also nano tattoos so we can inject the nano ink into the skin and external device to measure and translate this fluorescence so here this change in fluorescence depends on the blood sugar.

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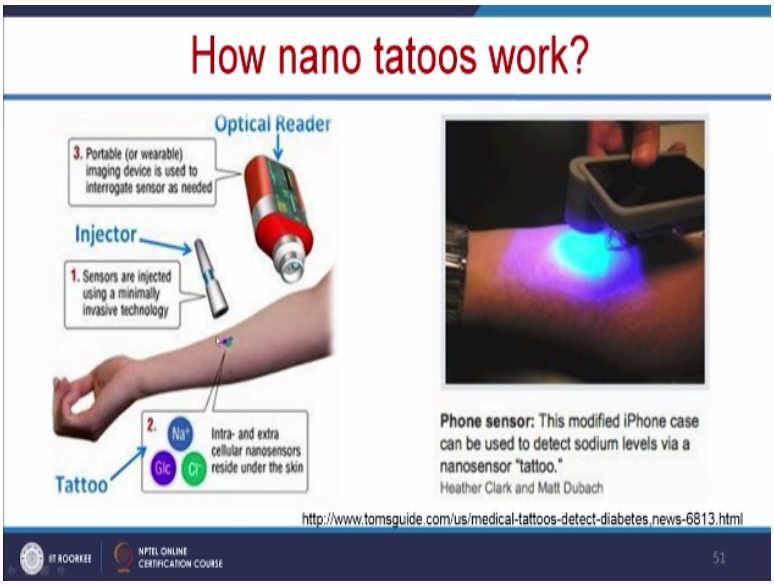

So recently we have so we can inject this sensor using the minimum insentive technology okay so once it is injected that tattoos are made and it can measure the sugar level as well as well as the sodium level and we can have this portable optical reader for example use this modified I phone case which you deduct this sodium levels through this nanosensor tattoos so next one is we can also.

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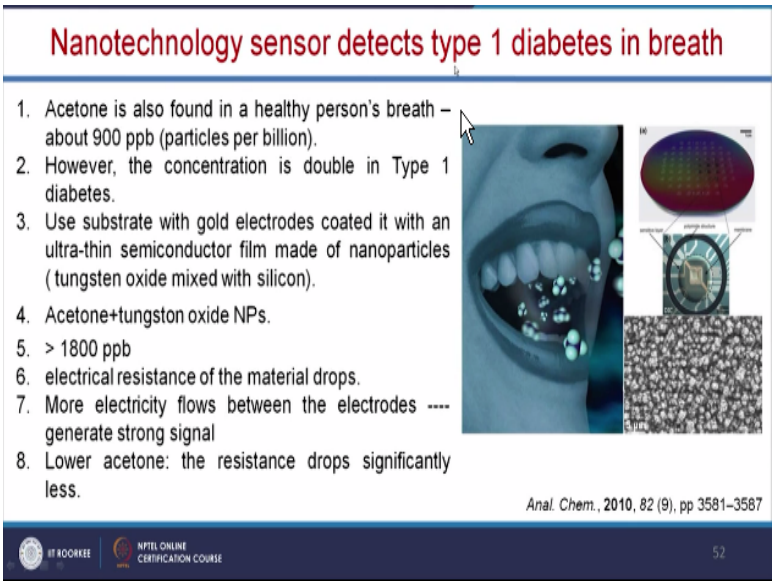

So next one is we can also use this nanotechnology sensor to detect the type a diabetes in the breath so acetone is usually found in a healthy person's breath approximately 900ppb it is a particles per billion okay. but in the concentration is double in the case of type 1 diabetes patient so here will be using the substrate with gold electrodes coated it with an ultra-thin semiconductor film made of nanoparticles and here this tungsten oxide mixed with silicon is mainly used.

In presence of acetone the electric resistance of the material drops okay so depends on the electrical resistance it can easily detect the diabetes in the breath of the particular person so based on this electrical resistance based inn breath resistance you can easily identify the person with the type 1 diabetes.

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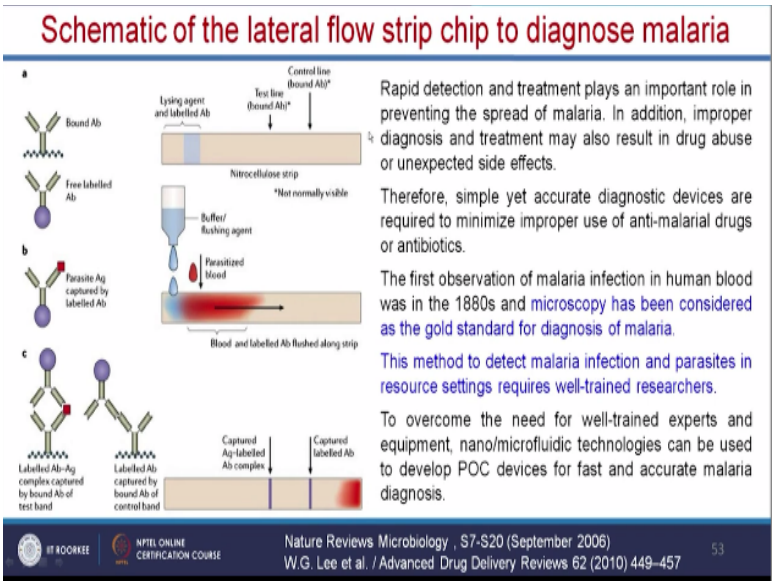

And we can also use this lateral flow strip chip to diagnose malaria so usually microscopy has been considered as the gold standard for diagnose of malaria but for using microscope we need a lab setting as well as well trained researchers so to overcome the drawback we need this nano microfludic technologies so here will be using this natibdy which is specific for the malaria parasite and here will be using this blood smaple okay so this is the two lines.

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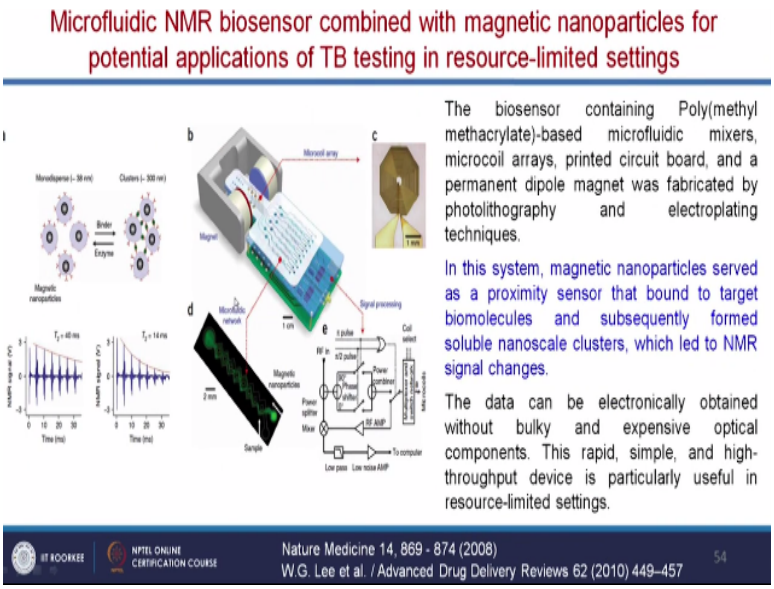

So let us how this fill the magnetic nanoparticles for detecting the TB that is tuberculosis bacteria and this usually antibody dispersed in person of TB in form like a cluster due to the clusters there is a change in the signals so this is the microfludic biosensor which could be useful for detecting the change in the signals.

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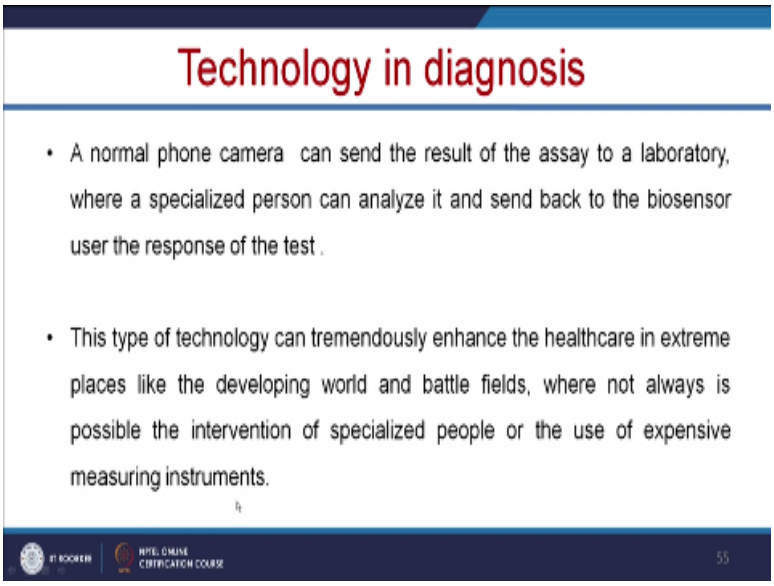

So let us see how we can use this technology diagnosis so we can use a simple camera that can send the results of the assay to a laboratory where a specailised person can analyse and that can send the result of the assay to laboratory so where as specialized person can analyze it and send back the result, and this type of technology can tremendously enhance the health care in extreme places like the developing world and battle fields, so where not always possible intervention of specialized people or the use of expensive measuring instruments.

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So here we can use the normal camera is the imaging device so that will digitize the result and transmit them to an expert and an expert will interprets the result and replace with the treatment.

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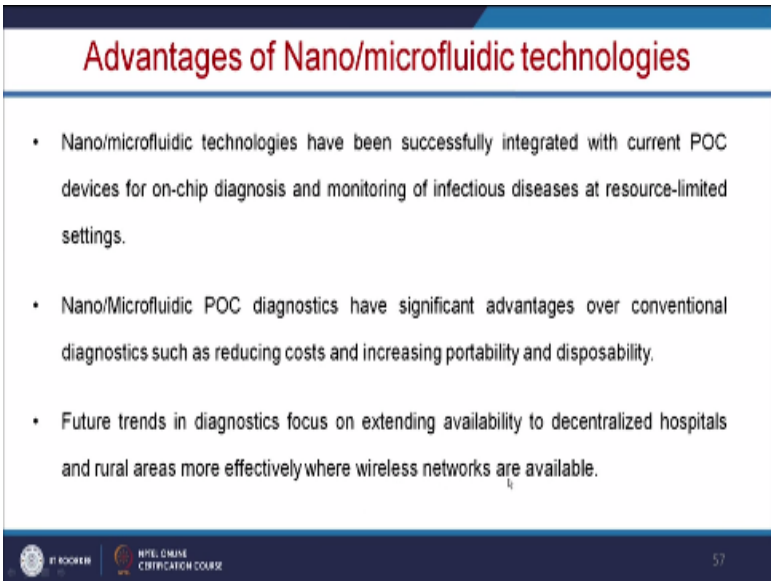

Let us see the advantages of nano or micro fluidic technologies so here this nano or micro fluid technologies have been successfully integrated with the current POC devices for on-chip diagnosis and also for monitoring various infection diseases at resource limited settings and this POC diagnostics have significant advantages over conventional diagnostics such as reducing the costs as well as increasing the portability and disposability, and again the future trends in diagnostics focus on extending the availability to decentralized hospitals and also to the rural areas more effectively where wireless networks are available.

So last summary of this lecture in the lecture we have learned what is point of characteristics and a various paper based diagnosis and also we all see the demonstration of later floe assay using a simple pregnancy test and we have also learnt a various roles of nanotechnology in this point of characteristics, okay so at the lecture here I thank you all for listening the lecture I will see all in another interesting lecture.

<u>Acknowledgement</u>

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