

**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 16<sup>th</sup> lecture of this course this 16<sup>th</sup> lecture is on Nanotechnology in Point of Care Diagnostics.

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## Contents

- Point-of-care tests
- Why point of care diagnostics?
- Paper based diagnosis
- Demonstration of Lateral flow assay
- Nanotechnology in Point of care testing



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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## Point-of-care (POC) tests

- Point-of-care tests are simple medical tests that can be performed at any place by any person.
- This contrasts with the historical pattern in which testing was wholly or mostly confined to the medical laboratory, which entailed sending off specimens away from the point of care and then waiting hours or days to learn the results, during which time care must continue without the desired information.
- Many point-of-care test systems are realized as easy-to-use membrane-based test strips, often enclosed by a plastic test cassette.
- This concept often is realized in test systems for detecting pathogens.

So let us see what is point of care test so it is simple medical test that can be performed at any place by any person without the help of any medical technician and 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 continued without the desired 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 rapid detection of pathogens.

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## Why POC Diagnostics?

### **Time:**

POC measurements provide results rapidly, where needed, and often with major time savings.

### **Patient Responsibility and Compliance:**

In primary care settings, patients are supervised by a medical team responsible for administering medications and monitoring responses. Although often administered by medical professionals, POC tests are also widely self-administered, making patients far more responsible for managing their own condition(s).

### **Cost:**

POC diagnostic cost parameters differ from those of conventional laboratory analysis. Readers (instruments) are smaller and more specialized than laboratory systems, so they cost less but do only one or a few different tests.





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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## FDA definition of a simple test

FDA definition of a simple test
Fully automated instrument or unitized, self-contained test
Uses direct unprocessed specimens/capillary blood (fingertick), nasal swabs, or urine
Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination
Needs only basic, non-technique-dependent reagent manipulation, such as "mix reagent A and reagent B"
Needs no operator intervention during the analysis steps
Needs no technical or specialized training
Needs no electronic or mechanical maintenance
Produces results that require no operator calibration, interpretation, or calculations
Produces results that are clear to read, such as positive or negative, a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations
Has test performance comparable to a traceable reference method, as demonstrated by studies in which intended operators perform the test
Contains a quick reference instruction sheet written at the educational level of the user

P. Yager, G. J. Domingo, J. Gentes. Point-of-care diagnostics for global health, *Annu Rev. Biomed. Eng.* 10 (2008) 107-144

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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## Paper-based diagnosis

- Paper fabrication is one of the most important technologies in human history.
- Paper is widely manufactured from renewable resources.
- Inexpensive.
- Combustible and biodegradable.
- Porous structure of paper enables wicking of liquid which is important for lateral flow assays and chromatography applications.



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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## Paper-based diagnostics-historical perspective

- The first paper-based sensor can be considered the invention of paper chromatography by Martin and Synge, who were awarded with the Nobel Prize in chemistry in 1952.
- Another milestone in the field was the commercialization of the pregnancy test, which can be considered one of the most used Point-of-care (POC) biosensors.

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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## Rapid diagnostics

- An array of rapid tests.
- The majority of these tests are simple and provide a yes/no answer where response time is critical to the user.



Lab Chip, 2013, 13, 2210

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 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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## Example of rapid tests

- First paper-based diagnostic device created was for urinalysis.
- These paper-based urinalysis devices utilize colorimetric assays to measure glucose and protein concentration in urine. Urinalysis using a paper-based diagnostic device is shown in Figure.
- The white colour of the paper provides a strong contrast, which enhances the results of the colorimetric assays.



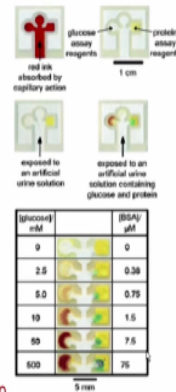
So these are some of the examples of commercial rapid test kits so for example we have the kits for HIV, TB influenza and malaria so these are the various paper based test kits which is a commercial available in the market so let us see of the examples of rapid paper based test kits so first paper based 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 contrast so which enhances the result of the colorimetric assays.

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## Example of rapid tests

- Concentration of protein and glucose in the urine sample is determined by analyzing the colour intensity in the detection zone
- Example of a paper-based urinalysis device. 5 $\mu$ L of artificial urine flowed into two detection areas, where it reacted with assay reagents. The colorimetric assay was scanned and the color intensity was processed by a computer to determine the concentration



Martinez, A.W., et al., Angewandte Chemie-International Edition, 2007, 46(8): p. 1318-1320.



So you can see here this is your simple paper based rapid kit for measuring the glucose protein concentration in a urine sample and when we add the sample here so if you get the colour in this side it will indicate the 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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## Why paper-based diagnostics?

- Sensitive and specific
- User-friendly
- Rapid and robust
- Equipment free, they are mainly read with the naked eye, or, if a quantitative detection is required, the equipment is small and cheap
- Deliverable to end-users
- Can be developed using inkjet, wax printing or screen printing technology, making them amenable to in-situ fabrication.



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 equipment 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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## Types of paper-based diagnostics

Three main categories:

- Dipstick assays
- Lateral flow assays (LFAs)
- Microfluidic paper analytical devices ( $\mu$ PADs).

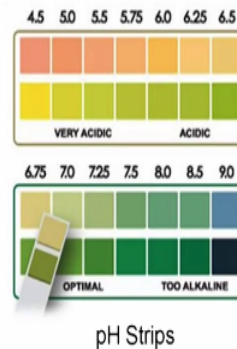
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So there are 3 types of paper based diagnostics dipstick assays lateral flow assays and micro fluidic paper analytical devices so let us see one by one in detail.

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## Dipstick assays

- Dipstick assays are the simplest ones, since they are based on the blotting of the sample onto a paper pre-stored with reagents.
- Dipsticks, such as pH test strips and urine test strips, are simple to design, easy to manufacture and convenient to use.
- pH test strips are manufactured by soaking a piece of filter paper into a mixture of acid-alkali indicators with a certain concentration ratio. After dried, the paper is impregnated with detection reagents.



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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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## Dipstick assays

- When an unknown sample is dispensed on the paper, the detecting reagents react with the analyte ( $H^+$ ) and develop a colour. By referring to a standard indicator card, the pH value of the solution can be indicated and thus the concentration of  $H^+$  is semi-quantified.
- Urine test strips have been designed to detect metabolic products in urine, which have become basic diagnostic tools to indicate pathological changes.
- For instance, urinary metabolic products (e.g., protein, glucose, and salt) from patients with nephritic or diabetic diseases can be detected using a standard urine test strip.



Urine test Strips

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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 indicators 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 3<sup>rd</sup> one is reduction pad and 4<sup>th</sup> one is absorbent pad.

So the sample pad is mainly made up of cellulose and it will filter the sample from impurities 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 reagents 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 nitrocellulose the capture reagents are fixed and the signal is developed in this detection pad and 4<sup>th</sup> one is absorbent 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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## Lateral flow assays (LFAs)

- There are two formats, i.e., sandwich and competitive (or inhibition) formats, for LFAs.
- In general, sandwich format assays are utilized for an analyte with multiple antigen epitopes, while competitive format assays are designed to detect an analyte with a single antigen epitope.

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

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**A typical lateral flow rapid test strip consist of the following components**

**Lateral Flow Assay Architecture**

- Sample pad** - an adsorbent pad onto which the test sample is applied.
- Conjugate or reagent pad** - this contains antibodies specific to the target analyte conjugated to coloured particles (usually colloidal gold nanoparticles, or latex microspheres).
- Reaction membrane** - typically a nitrocellulose or cellulose acetate membrane onto which anti-target analyte antibodies are immobilized in a line that crosses the membrane to act as a capture zone or test line (a control zone will also be present, containing antibodies specific for the conjugate antibodies).
- Wick or waste reservoir** - a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it.

<http://www.cytodiagnosics.com/store/pclateral-flow-immunoassays-d6.htm>

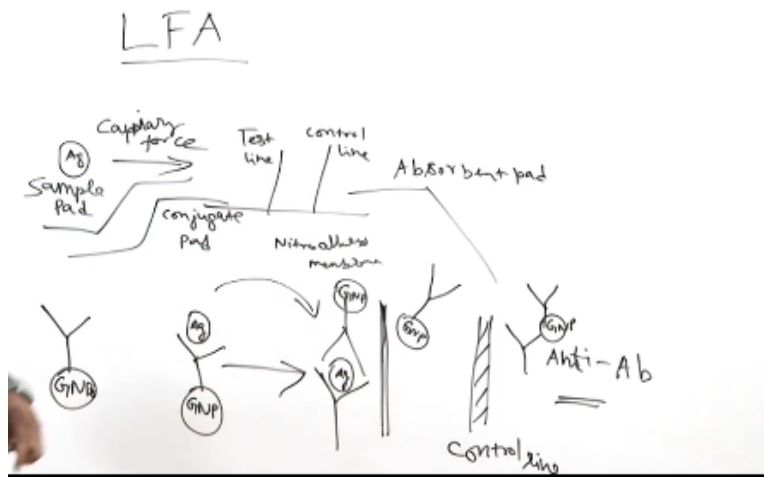
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So let us see the lateral flow as in detail okay so this lateral flow as it contains 4 different pads sample pad conjugate pad and membrane and wicking pad so in this sample pad we will be adding this sample and in the cogitate pad it contains antibodies 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.

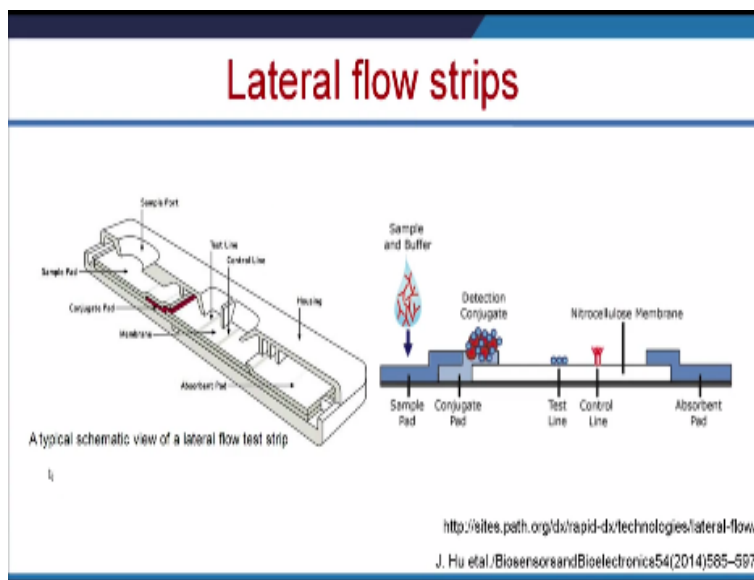
So in the test line you will be having antibody specific for your antigen, so this antigen antibody complex will come and bind here, so antigen antibody complex will come and bind and your antigen will be sandwich between the two antibodies okay. So here you will get a color line and this unbound antibody 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 flow 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.

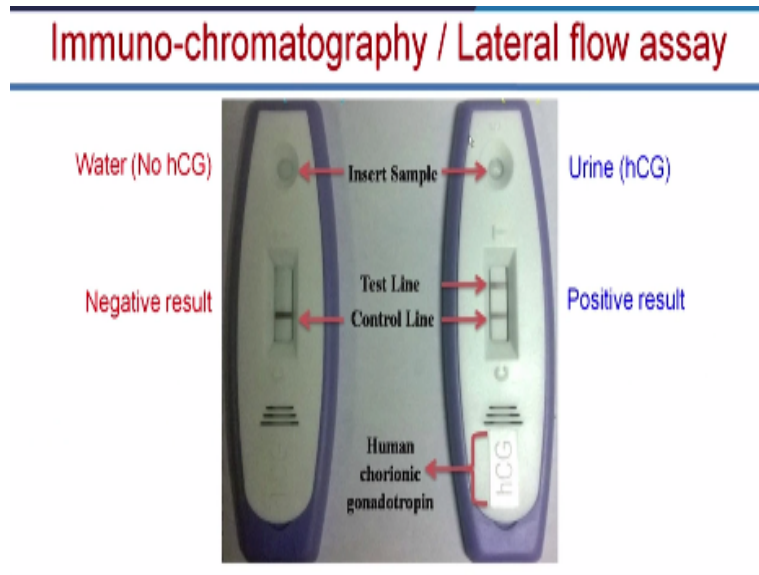
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So this is a typical schematic view of lateral flow tested okay so it is having this kind of arrangement if you open the kit you will have this kind of arrangement I will open and show you how it look like, so the sample pad you can see the arrangement 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.

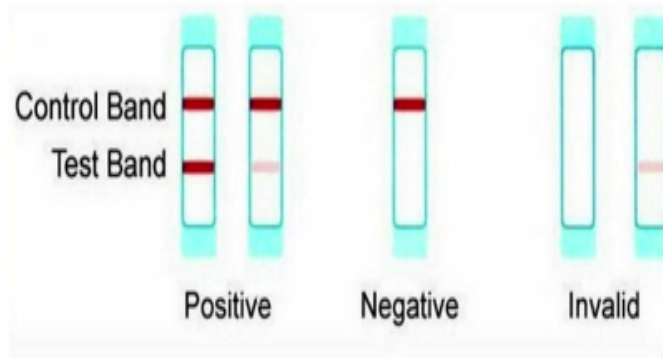
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So let us see the simple experiment to understand the lateral flow assay using the pregnancy test kit, so here we will be using this urine sample of pregnant women so the urine sample of pregnant women contain hCG that is Human chorionic gonadotropin, 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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## Immuno-chromatography / Lateral flow assay



<https://www.medicaisdisposables.us/hCG-pregnancy-midstream-test-pw1-ml.htm>

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 testing line, so you will get the line 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 testing 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 devices, okay.

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## Microfluidic paper analytical devices ( $\mu$ PADs)

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- $\mu$ PADs are made by patterning paper with a variety of assay designs, mainly based on capillary force to drive aqueous fluid movement.
- Two- dimensional (2D) and three-dimensional (3D)  $\mu$ PADs have been developed.
- 2D  $\mu$ PADs are made by patterning physical or chemical hydrophobic boundaries to form microchannels on paper.

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So this micro pads are made by patterning 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 patterning physical or chemical hydrophobic boundaries to form the micro channels on paper.

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## Microfluidic paper analytical devices ( $\mu$ PADs)

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- Various approaches, including cutting, photolithography, plotting, inkjet etching, plasma etching, wax printing, etc., have been used to create channels and barriers in paper.
- The dimensions of the resulting channels together with the characteristics of paper and ambient conditions (temperature and humidity) can affect the wicking rate of fluid.

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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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## Microfluidic paper analytical devices ( $\mu$ PADs)

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- The reagents required for biochemical reactions can be immobilized on paper with different patterns (e.g., four-leaf clover) by hand dispensing or inkjet printing.
  - Functional chemical or biological molecules can be immobilized on paper by physical absorption, chemical coupling, and carrier-mediated deposition.
  - When the reagents are dried, the paper-based devices can be used for biochemical analyses.
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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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## Microfluidic paper analytical devices ( $\mu$ PADs)

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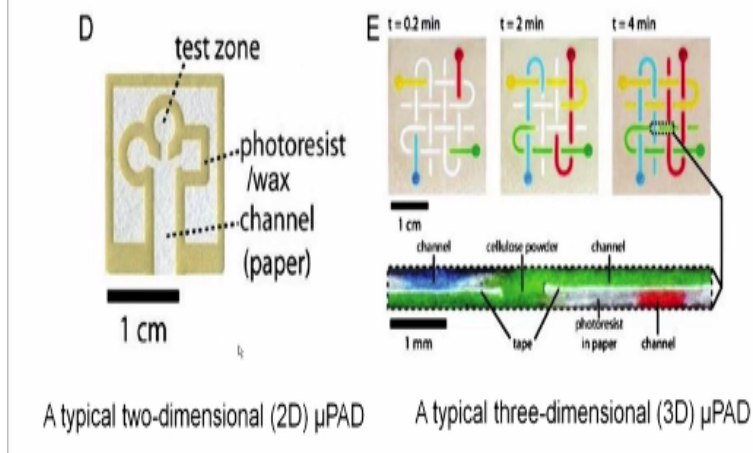
- 3D  $\mu$ PADs are produced by stacking layers of patterned paper in such a way that channels in adjacent layers of paper connect with each other.
- Compared with 2D  $\mu$ PADs, 3D  $\mu$ PADs have several advantages due to their capability to incorporate complex networks of channels, thus providing multiple functionalities.

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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## Microfluidic paper analytical devices ( $\mu$ PADs)



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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## Comparison of different paper-based diagnostic techniques

Type of paper-based biosensor	Possible detection methods	Advantages	Disadvantages
Dipstick	<ul style="list-style-type: none"> <li>• Optical</li> </ul>	<ul style="list-style-type: none"> <li>• Easy design</li> <li>• Fast optimization</li> </ul>	<ul style="list-style-type: none"> <li>• Just one step</li> <li>• Only optical detection</li> <li>• Mostly no quantification</li> </ul>
LFA	<ul style="list-style-type: none"> <li>• Optical</li> <li>• Electrochemical</li> </ul>	<ul style="list-style-type: none"> <li>• Versatile</li> <li>• Flow</li> <li>• Electrochemical detection</li> <li>• Possible quantification</li> </ul>	<ul style="list-style-type: none"> <li>• Long optimization times</li> <li>• Long fabrication</li> <li>• Sample volume (around 100 <math>\mu</math>L)</li> </ul>
$\mu$ PAD	<ul style="list-style-type: none"> <li>• Optical</li> <li>• Electrochemical</li> <li>• Chemiluminescence</li> <li>• MEMS</li> </ul>	<ul style="list-style-type: none"> <li>• Versatile</li> <li>• Flow</li> <li>• Different detection methods</li> <li>• Quantification</li> <li>• Small sample volume (less than 10 <math>\mu</math>L)</li> <li>• Massive production</li> </ul>	<ul style="list-style-type: none"> <li>• Long optimization times</li> </ul>

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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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## Reaction mechanism in paper-based diagnosis

According to the reaction mechanism, the tests can be categorized into

- Chemical
- Biological
- Electrochemical



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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## Chemical reaction: colour change

- Most chemical reactions with colour change can be achieved on paper, such as acid-alkali reaction, precipitation reaction, redox reaction and enzymatic reaction.
- Generally involve a one-step procedure.
- pH test strips can be dispensed with several compounds to exhibit different color changes in response to different pH values.
- Semi-quantitative detection of  $H^+$  concentrations of solutions can then be achieved by grading the pH values of solutions from 1 to 14.

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

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## Biological reaction- Antigen–antibody binding

- Antigen–antibody binding based immunoassays detect either antigen or antibody present in a clinical sample.
- Home pregnancy test strips have been one of the most successful diagnostic paper –based immunoassays so far.
- It measures a hormone, human chorionic gonadotropin (hCG), in urine from pregnant women.
- hCG is a heterodimeric glycoprotein with  $\alpha$  and  $\beta$  subunits.



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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## Biological reaction- Antigen–antibody binding

- Home pregnancy test strips just make use of  $\beta$  subunit (unique to hCG) and contain three kinds of antibodies, i.e., anti-hCG antibody, monoclonal antibody (MAb) and immunoglobulin G (IgG).
- Anti-hCG antibody, conjugated with coloured particles, can specifically recognize and bond with hCG in the sample. MAb and IgG can bond to hCG and anti-hCG antibody, respectively, thus forming the test and control lines.
- This idea has been used to measure tumor markers, e.g., primary hepatic carcinoma and to diagnose infectious diseases, e.g., AIDS.



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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## Biological reaction- Nucleic acid hybridization (NAT)

- NAT assay requires two types of oligonucleotide probes, i.e., detector probe and capture probe.
- Detector and capture probes are both complementary with target nucleic acid sequence, while the detector probe is used to combine with a tag to make the reaction visible or measurable.

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.

(Refer Slide Time: 23:47)

## Biological reaction- Nucleic acid hybridization (NAT)

- The tag can be colored particles (e.g. AuNPs) for colorimetric assays or electroactive molecules (e.g. thionine) for electrochemical measurements.
- Since genetic and infectious diseases can be determined by a fragment of gene-specific nucleic acids, NAT based strips promise a great potential for rapid and reliable diagnosis.



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.

(Refer Slide Time: 24:08)



## Electrochemical reaction

- Electrochemical detection can be achieved on the basis of both redox reactions and non-redox reactions.
- Redox reactions are involved in electrons transfer between molecules or particles (e.g., enzyme and nanoparticles), while non-redox reactions are related with the changes of electrical properties, such as impedance, resistance, conductance, and potential.
- The most successful example of electrochemical detection is the blood glucose meter and test strip for diabetic patients.



So here this electro chemical reactions can be achieved on the basis of both redox reactions and non-redox reactions so the redox reactions are involved in electrons transfer between the molecules or particles and this non-redox reactions are related 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.

(Refer Slide Time: 24:34)

## Electrochemical reaction

- The glucose meter is an amperometer, and it measures the quantity of electroactive species as a result of the oxidation of glucose by reagents stored in the test strips.
- Test strip is impregnated with glucose oxidase and other components (e.g., ferrocyanide). When a drop of blood is added, 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 blood.



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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## Nanotechnology for bio sensing

- In the paper biosensors the nanomaterial's are mainly used as labels or carriers.
- Gold nanoparticles (AuNPs) have been mostly used.
- AuNPs have many properties, which make them excellent labels: easy functionalization, easy manipulation, biocompatibility, a strong red color, a characteristic surface plasmon resonance and electrochemical activity.
- Magnetic nanoparticles, quantum dots, liposomes, carbon and ceria nanoparticles have also been explored.



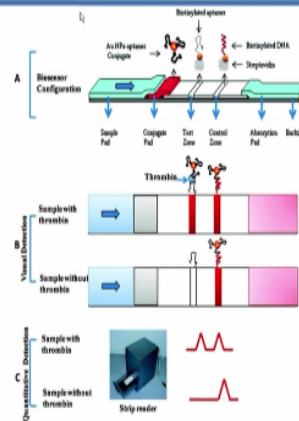
So let us see the role of nanotechnology in point of care testing so in the paper based biosensors so the nanometer are mainly used as a labels or carriers so the gold nanoparticles have been mostly used and because this gold nanoparticles have been excellent labeling properties and easy functionalization 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.

(Refer Slide Time: 25:35)

## Nanobiosensors for protein detection

Schematic illustration of the configuration and measurement principle of the aptamer-based strip biosensor: (A) configuration of the biosensor; (B) the principle of visual detection in the presence and absence of thrombin; (C) quantitative detection with a portable strip reader.

Xu et al., *Anal. Chem.*, 2009, 81, 669–675.



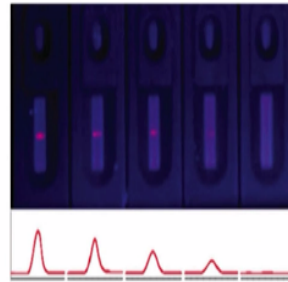
So let us say example of nanobiosensors for protein detection and here we can detect the thrombin okay so this is also similar to the your pregnancy test only and let us flow sink you can see a sample pad and conjucted pad okay and if you are interrupt antibody you will using this aptamer so aptamer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

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

(Refer Slide Time: 26:31)

## Nanobiosensors for protein detection

Fluorescence imaging of QD-based LF for (from the left to the right) 10  $\mu\text{g mL}^{-1}$ , 1  $\text{mg mL}^{-1}$ , 100  $\text{ng mL}^{-1}$ , 10  $\text{ng mL}^{-1}$  nitrated ceruloplasmin and 10  $\text{mg mL}^{-1}$  ceruloplasmin without nitration. The bottom curves are the corresponding readout using a strip reader. *Li et al., Anal. Chem., 2010, 82, 7008–7014.*



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

(Refer Slide Time: 26:42)

## Nanobiosensors for cell detection

- Paper-based devices that integrate cells either as receptors for indirect detection of proteins or other species or for their direct detection also have been developed.
- Most of the cells cannot run intact through the pores of the membranes used, but they can be attached to the surface of the paper.
- Detection of whole-cell antigens of *Pseudomonas aeruginosa* and *Staphylococcus aureus* based on the use of AuNPs functionalized with specific antibodies as labels within a detection range of the 500–5000 CFU mL<sup>-1</sup>.



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.

(Refer Slide Time: 27:06)

## Common diagnosis for diabetic patient

1. Urine test – Qualitative
2. Blood test -- Quantitative test

### Finger pricking blood test

- sample is (Whole Blood) placed on a chemically coated stripe of paper which is visually tested



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.

(Refer Slide Time: 27:38)

## Non-invasive device could end daily finger pricking for people with diabetes



At the heart of the new technology is a piece of **nano-engineered silica glass** with ions that fluoresce in infrared light when a low power laser light hits them. 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. **The device measures the length of time the fluorescence lasts for and uses that to calculate the glucose level in a person's bloodstream without the need for a needle.** This process takes less than 30 seconds.



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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## Continuous glucose monitoring (CGM) systems

- Tiny sensor inserted under the skin.
- Sends information about glucose levels via radio waves from the sensor to a pager like wireless monitor.
- Help the patient or the physician can adjust insulin.
- Leads to better glycemic level.

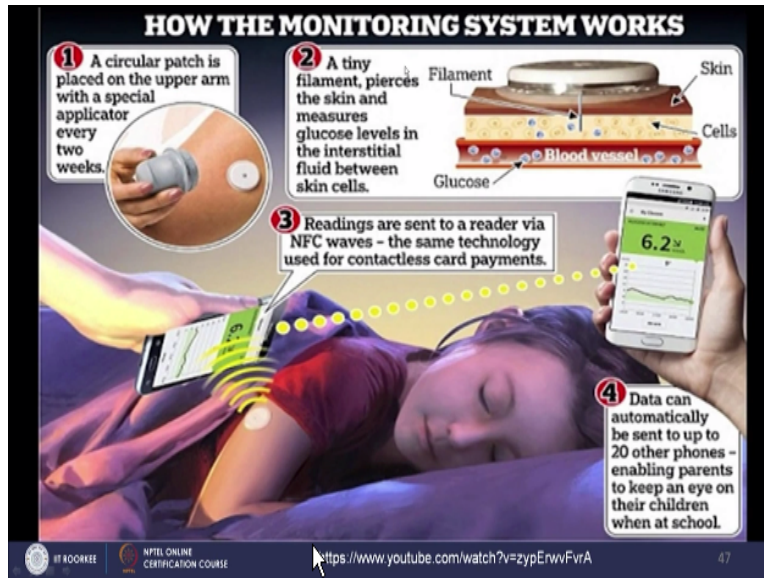


Graphene patches: to treat diabetes, goodbye to needles.  
(Photo : <https://www.youtube.com/watch?v=KdskBu-uHsY>)



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 send the information about glucose levels via radio waves from the sensor to a pager like wireless monitor and it will help 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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## Benefits of CGMS

- Increased security from alarms & alerts
- Immediate feedback - look and learn
- Blood glucose trend provides more information than static readings
- Control + safety
- Sensors can be programmed to produce a "beep" if blood sugars are in a range that is selected as too high or too low.



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

(Refer Slide Time: 29:50)

## Tears of joy for diabetics

- Uses extremely small nanoparticles embedded into the hydrogel lenses

Google  
Smart Contact Lenses



- These engineered nanoparticles react with glucose molecules found in tears, causing a chemical reaction that changes the color of the lenses

Soft contact lens

encapsulates electronics

Sensor

detects glucose in tears

Chip & antenna

receives power and sends info



[http://bigcommunity.net/big\\_news/google-contact-lens/](http://bigcommunity.net/big_news/google-contact-lens/)



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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## Nano-Tattoos

Inject Nano ink into the Skin

External device to measure and translate this fluorescence

Change in fluorescence depends on blood sugar




<https://singularityhub.com/2010/06/10/nanotechnology-tattoos-to-help-diabetics-track-glucose-levels/>



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.

(Refer Slide Time: 30:43)

## How nano tatoos work?



**Optical Reader**

3. Portable (or wearable) imaging device is used to interrogate sensor as needed

**Injector**

1. Sensors are injected using a minimally invasive technology

**Tattoo**

2. Intra- and extra-cellular nanosensors reside under the skin

**Phone sensor:** This modified iPhone case can be used to detect sodium levels via a nanosensor "tattoo."  
Heather Clark and Matt Dubach

<http://www.tomsguide.com/us/medical-tattoos-detect-diabetes.news-6813.html>

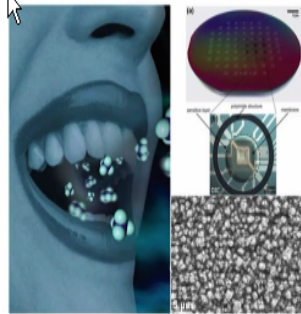
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So recently we have so we can inject this sensor using the minimum insentive technology okay so once it is injected that tattoos are made and it can measure the sugar level as well as well as the sodium level and we can have this portable optical reader 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.

(Refer Slide Time: 31:10)

## Nanotechnology sensor detects type 1 diabetes in breath

1. Acetone is also found in a healthy person's breath – about 900 ppb (particles per billion).
2. However, the concentration is double in Type 1 diabetes.
3. Use substrate with gold electrodes coated it with an ultra-thin semiconductor film made of nanoparticles (tungsten oxide mixed with silicon).
4. Acetone+tungsten oxide NPs.
5. > 1800 ppb
6. electrical resistance of the material drops.
7. More electricity flows between the electrodes ---- generate strong signal
8. Lower acetone: the resistance drops significantly less.



*Anal. Chem.*, 2010, 82 (9), pp 3581–3587



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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## Schematic of the lateral flow strip chip to diagnose malaria



Rapid detection and treatment plays an important role in preventing the spread of malaria. In addition, improper diagnosis and treatment may also result in drug abuse or unexpected side effects.

Therefore, simple yet accurate diagnostic devices are required to minimize improper use of anti-malarial drugs or antibiotics.

The first observation of malaria infection in human blood was in the 1880s and microscopy has been considered as the gold standard for diagnosis of malaria.

This method to detect malaria infection and parasites in resource settings requires well-trained researchers.

To overcome the need for well-trained experts and equipment, nano/microfluidic technologies can be used to develop POC devices for fast and accurate malaria diagnosis.

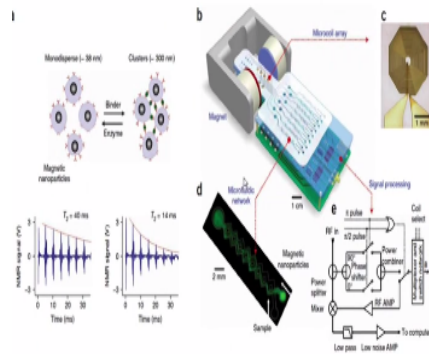


 Nature Reviews Microbiology, S7-S20 (September 2006)  
 W.G. Lee et al. / Advanced Drug Delivery Reviews 62 (2010) 449–457

And we can also use this lateral flow strip chip to diagnose malaria so usually microscopy has been considered as the gold standard for diagnosis 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 microfluidic technologies so here will be using this technology which is specific for the malaria parasite and here will be using this blood sample okay so this is the two lines.

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## Microfluidic NMR biosensor combined with magnetic nanoparticles for potential applications of TB testing in resource-limited settings



The biosensor containing Poly(methyl methacrylate)-based microfluidic mixers, microcoil arrays, printed circuit board, and a permanent dipole magnet was fabricated by photolithography and electroplating techniques.

In this system, magnetic nanoparticles served as a proximity sensor that bound to target biomolecules and subsequently formed soluble nanoscale clusters, which led to NMR signal changes.

The data can be electronically obtained without bulky and expensive optical components. This rapid, simple, and high-throughput device is particularly useful in resource-limited settings.

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 microfluidic biosensor which could be useful for detecting the change in the signals.

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## Technology in diagnosis

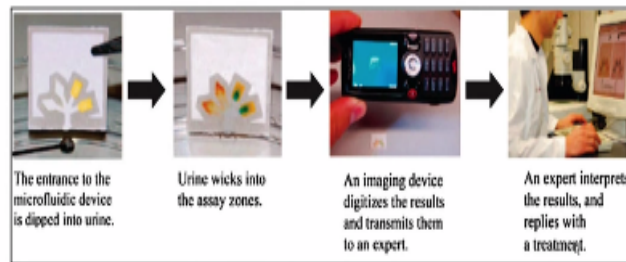
- A normal phone camera can send the result of the assay to a laboratory, where a specialized person can analyze it and send back to the biosensor user the response of the test .
- This type of technology can tremendously enhance the healthcare in extreme places like the developing world and battle fields, where not always is possible the intervention of specialized people or the use of expensive measuring instruments.



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 specialised 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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## Technology in diagnosis



General strategy for performing inexpensive bioassays in remote locations and for exchanging the results of the tests with offsite technicians.

*Martinez et al., Anal. Chem., 2008, 80, 3699–3707.*



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

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## Advantages of Nano/microfluidic technologies

- Nano/microfluidic technologies have been successfully integrated with current POC devices for on-chip diagnosis and monitoring of infectious diseases at resource-limited settings.
- Nano/Microfluidic POC diagnostics have significant advantages over conventional diagnostics such as reducing costs and increasing portability and disposability.
- Future trends in diagnostics focus on extending availability to decentralized hospitals and rural areas more effectively where wireless networks are available.



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.

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