

Measurement System and Diagnostic Devices

Biomedical Instrumentation

- Diagnosis and therapy depend heavily on the use of medical instrumentation.
- Medical procedures:
Medicine can be defined as a multistep procedure on an individual by a physician, group of physician, or an institute, repeated until the symptoms disappear

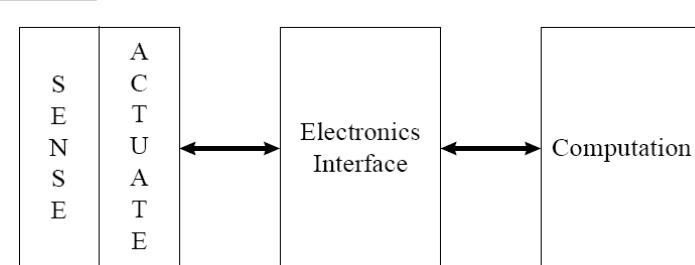
The Importance of Biomedical Instrumentation

• Medical procedure

- 1 Collection of data - qualitative and/or quantitative
- 2 Analysis of data
- 3 Decision making
- 4 Treatment planning based on the decision

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Biomedical Instrumentation System



- All biomedical instruments must interface with biological materials. That interface can be direct contact or by indirect contact

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Sensor

- A sensor converts one form of energy to another, and in so doing detects and conveys information about some physical, chemical or biological phenomena.
- More specifically, a sensor is a transducer that converts the **measurand** (a quantity or a parameter) into a signal that carries information.

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What is a Biosensor

- Biosensors are ‘analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment’ [Vo-Dinh and Cullum, 2000].
 - Antibodies
 - Enzymes
 - DNA, RNA
 - Whole cells

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Components of BM Instrumentation System...

- A sensor
 - Detects biochemical, bioelectrical, or biophysical parameters
 - Provides a safe interface with biological materials
- An actuator
 - Delivers external agents via direct or indirect contact
 - Controls biochemical, bioelectrical, or biophysical parameters
 - Provides a safe interface with biologic materials

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...Components of BM Instrumentation System...

- The electronics interface
 - Matches electrical characteristics of the sensor/actuator with computation unit
 - Preserves signal to noise ratio of sensor
 - Preserves efficiency of actuator
 - Preserves bandwidth (i.e., time response) of sensor/actuator
 - Provides a safe interface with the sensor/actuator
 - Provides a safe interface with the computation unit
 - Provides secondary signal processing functions for the system

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...Components of BM Instrumentation System

- The computation unit
 - provides primary user interface
 - provides primary control for the overall system
 - provides data storage for the system
 - provides primary signal processing functions for the system
 - maintains safe operation of the overall system

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Classifications of Biomedical Instruments

- The sensed quantity
- The principle of transduction
- The organ system for measurement
- The clinical medicine specialties
- Based on the activities involved in the medical care, medical instrumentation may be divided into three categories:
 - Diagnostic devices
 - Therapeutic devices
 - Monitoring devices

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Accuracy and precision...

- Resolution

- the smallest incremental quantity that can be reliably measured.
 - a voltmeter with a larger number of digits has a higher resolution than one with fewer digits.
- However, high resolution does not imply high accuracy.

- Precision

- the quality of obtaining the same output from repeated measurements from the same input under the same conditions.
- High resolution implies high precision.

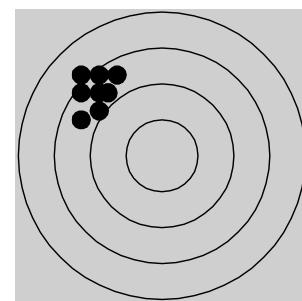
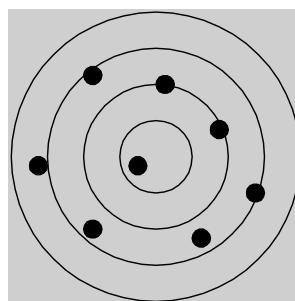
- Repeatability

- the quality of obtaining the same output from repeated measurements from the same input over a period of time.

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...Accuracy and precision...

- Data points with
 - (a) low precision and (b) high precision.



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...Accuracy and precision...

- Accuracy

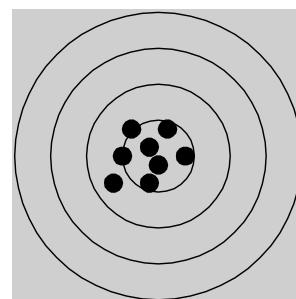
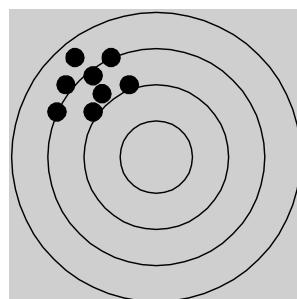
- the difference between the true value and the measured value divided by the true value.

- **Obtaining the highest possible precision, repeatability, and accuracy is a major goal in bioinstrumentation design.**

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...Accuracy and precision...

- Data points with
 - (a) low accuracy and
 - (b) high accuracy



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The Ideal POC Test

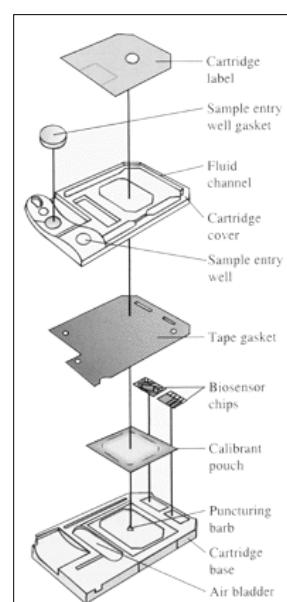
Affordable
Sensitive
Specific
User-friendly
Rapid and robust
Equipment-free
Deliverable to end-users



POCT “Device/Cartridge”

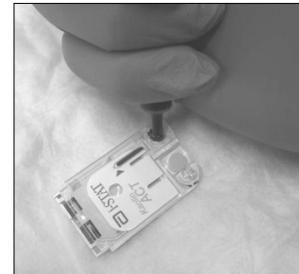


i-STAT “system”



POCT device & cartridges

- **Electrolytes**
- **Creatinine**
- **Glucose**
- **Free calcium**
- **pH, pO₂, pCO₂**
- **Lactate**
- **Activated Clotting Time**
- **Prothrombin Time**
- **Troponin, Creatine Kinase-MB**



MiRO



MiRO (Endoscopic Microcapsule) :
To take images in body and transmit to receiver

PC/PDA :
Real time display images taken by MiRO



Jacket :
To receive the image signals and to
the capsule's location

Receiver :
To store images and transmit images to PC/PDA

MiRo

- The prototype capsule, MiRo, was 10.8 × 24 mm, weighed 3.3 g, had a field of view of 150°, resolution power of 320 × 320 pixels, and a battery life of 9 to 11 hours in preclinical tests (Fig. 1). A white-light-emitting diode (LED) was used as the illumination source, and the optical system focused reflected optical rays onto a complementary metal oxide silicon (CMOS) sensor. The sensitive, low-power CMOS image sensor converted the optical rays to electrical voltages. The capsule uses 2 serial silver oxide batteries as a power source. Only one chip is used in the capsule, which includes the CMOS image sensor and a parallel-to-serial data converting logic block. The serial digital signal is limited in power to a safe level in the human body and propagates as a low-power electric field outside of the body via 2 gold plating bands on the housing. The capsule transmitted continuous video images at 2 frames/s during passage through the GI tract. Images were recorded by using a solid-state recorder and an aerial system applied to the skin of the body.

MiRo

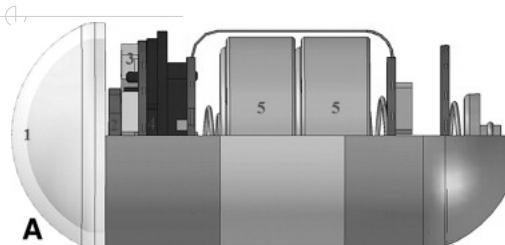
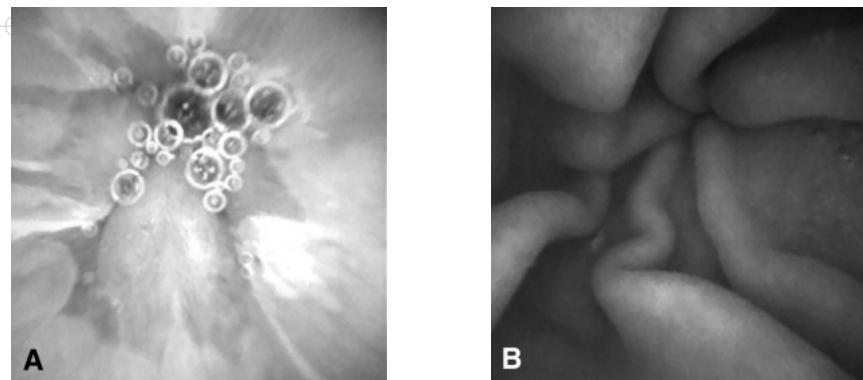


Figure 1.

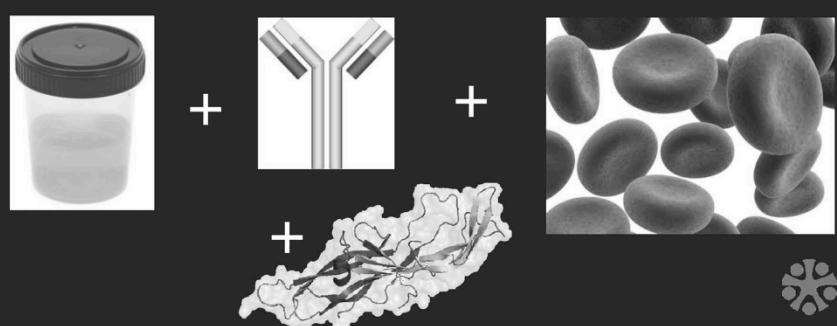
A, Schematic diagram of MiRo: optical dome (1) lens (2), LEDs (3), image sensor (4), battery (5). B, Photograph of MiRo.

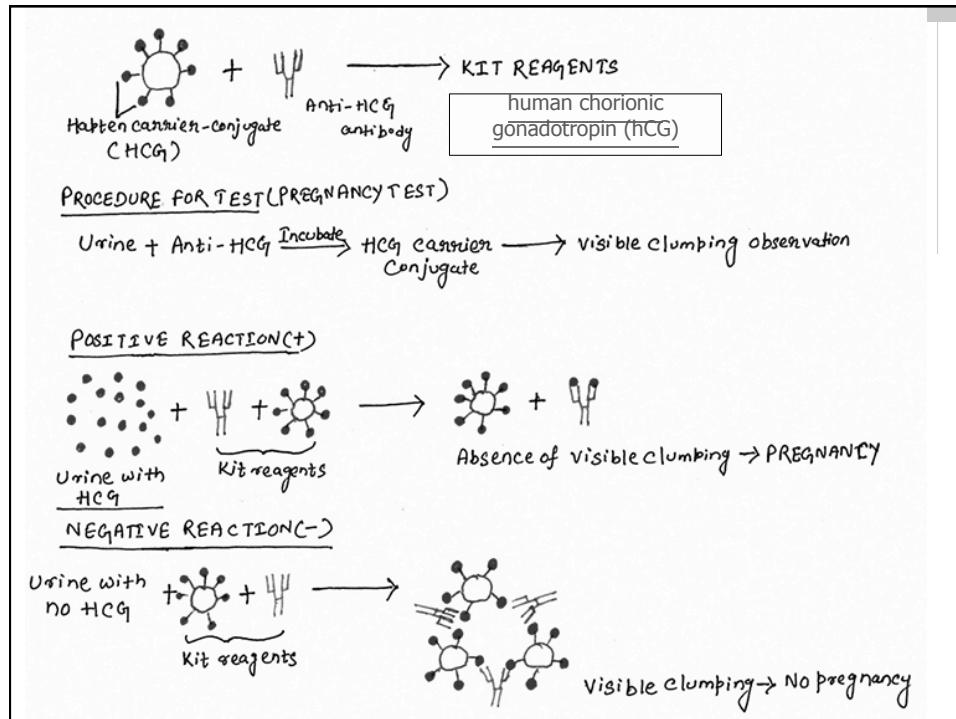


Samples of images taken by MiRo that were scored as outstanding. A, Esophagogastric junction. B, Antrum of stomach.

Pregnancy Test Evolution

- 1960 First Immunoassay for Pregnancy
- Hemagglutination inhibition assay
- Significantly faster and cheaper





Pregnancy Test Evolution

- 1970's

"For your \$10 you get pre-measured ingredients consisting of a vial of purified water, a test tube containing, among other things, sheep red blood cells...as well as a medicine dropper and clear plastic support for the test tube, with an angled mirror at the bottom."

The test took two hours

97% Sensitive, 80% Specific

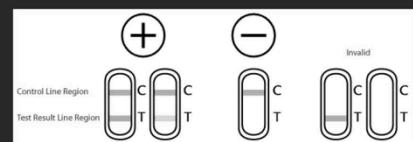
1976 FDA approval

1977 First marketed



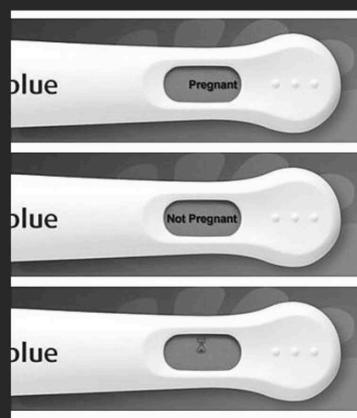
Pregnancy Test Evolution

- 1980's-1990's
 - Expansion of pregnancy tests to the home market with single step testing procedures



Pregnancy Test Evolution

- 2003 Digital Pregnancy Test Introduced



Lateral Flow Immunoassay

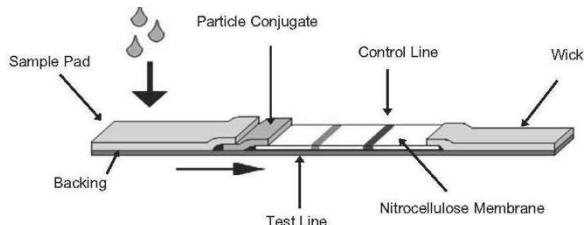


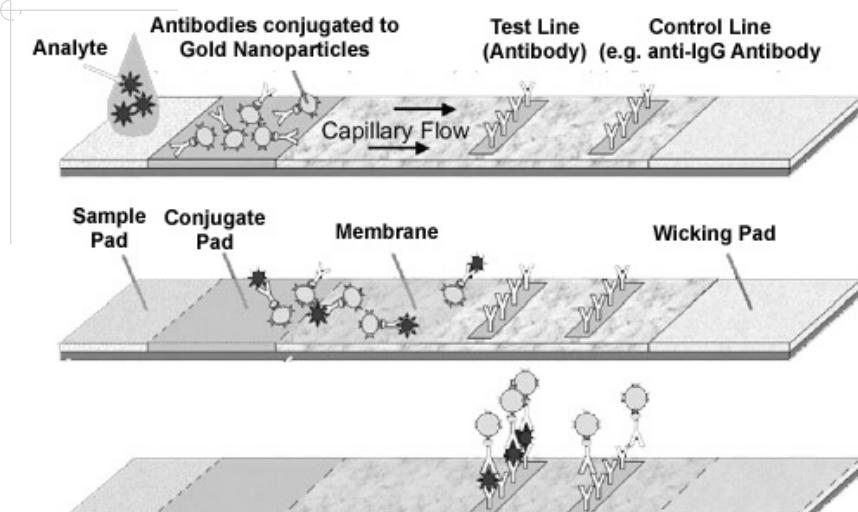
FIGURE 1 Typical lateral flow test strip configuration. (The color version of this figure may be viewed at www.immunoassayhandbook.com).

Lateral flow tests also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence (or absence) of a target analyte in sample (matrix) without the need for specialized and costly equipment, though many lab based applications exist that are supported by reading equipment. Typically, these tests are used for medical diagnostics either for home testing, point of care testing, or laboratory use. A widely spread and well known application is the home pregnancy test.

Lateral Flow Immunoassays

- A typical lateral flow rapid test strip consist of the following components:
- 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 fluorescent particles).
- 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.
- The components of the strip are usually fixed to an inert backing material and may be presented in a simple dipstick format or within a plastic casing with a sample port and reaction window showing the capture and control zones.

Lateral Flow Immunoassays



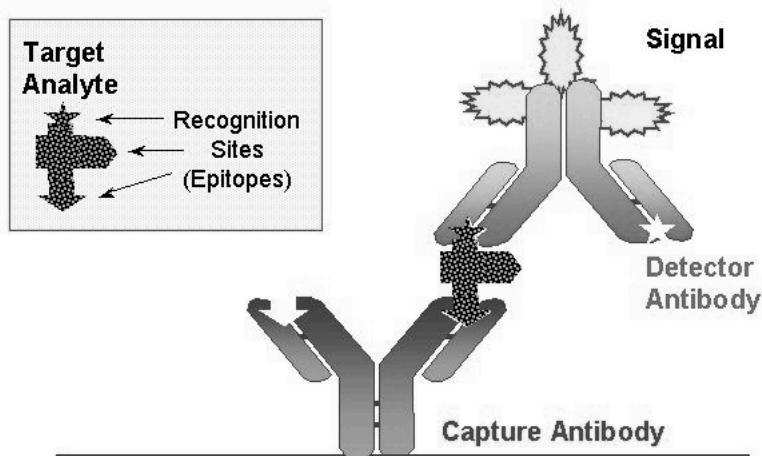
Double antibody sandwich assays

- In this format the sample migrates from the sample pad through the conjugate pad where any target analyte present will bind to the conjugate. The sample then continues to migrate across the membrane until it reaches the capture zone where the target/conjugate complex will bind to the immobilized antibodies producing a visible line on the membrane. The sample then migrates further along the strip until it reaches the control zone, where excess conjugate will bind and produce a second visible line on the membrane.

Double antibody sandwich assays

- This control line indicates that the sample has migrated across the membrane as intended. Two clear lines on the membrane is a positive result. A single line in the control zone is a negative result. Double antibody sandwich assays are most suitable for larger analytes, such as bacterial pathogens and viruses, with multiple antigenic sites.

Double Antibody Sandwich Immunoassay



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Competitive assays

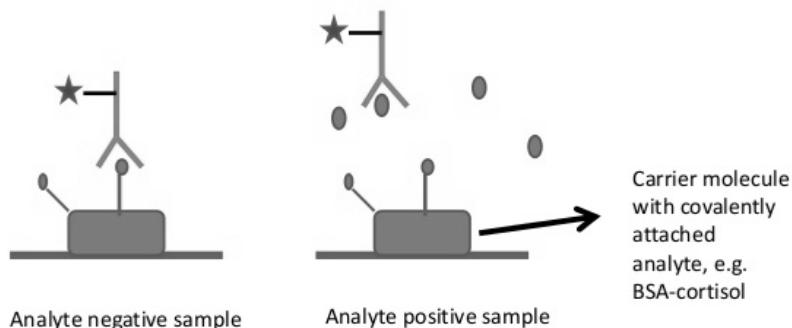
- Competitive assays are primarily used for testing small molecules and differ from the double antibody sandwich format in that the conjugate pad contains antibodies that are already bound to the target analyte, or to an analogue of it. If the target analyte is present in the sample it will therefore not bind with the conjugate and will remain unlabeled. As the sample migrates along the membrane and reaches the capture zone an excess of unlabeled analyte will bind to the immobilized antibodies and block the capture of the conjugate, so that no visible line is produced. The unbound conjugate will then bind to the antibodies in the control zone producing a visible control line.

Competitive assays

- A single control line on the membrane is a positive result. Two visible lines in the capture and control zones is a negative result. However, if an excess of unlabeled target analyte is not present, a weak line may be produced in the capture zone, indicating an inconclusive result. Competitive assays are most suitable for testing for small molecules, such as mycotoxins, unable to bind to more than one antibody simultaneously. There are a number of variations on lateral flow technology. The capture zone on the membrane may contain immobilized antigens or enzymes - depending on the target analyte - rather than antibodies. It is also possible to apply multiple capture zones to create a multiplex test.

Competitive assays

Competitive Inhibition LF Assay



Intuitive Results: Alpha- Numeric Symbols

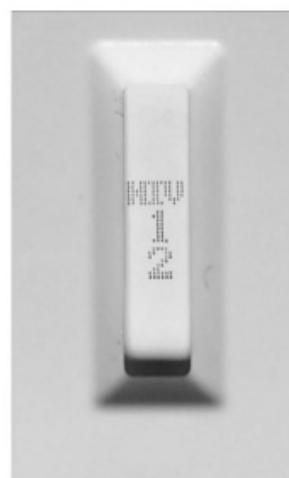




FIGURE 13 ESEQuant™ lateral flow strip reader from Qiagen Lake Constance. (The color version of this figure may be viewed at www.immunoassayhandbook.com).



Most tests are intended to operate on a purely qualitative basis. However it is possible to measure the intensity of the test line to determine the quantity of analyte in the sample.

Handheld diagnostic devices

known as lateral flow readers are used by several companies to provide a fully quantitative assay result. By utilizing unique wavelengths of light for illumination in conjunction with either CMOS or CCD detection technology, a signal rich image can be produced of the actual test lines. Using image processing algorithms specifically designed for a particular test type and medium, line intensities can

Multiplexing
If there is an intention to multiplex, early consideration should be given to whether to attempt to multiplex on the strip (i.e., multiple analytes on one strip) or whether to generate multiple strips within a single cartridge. Whether a single strip can be used depends completely on the assays and reagents, and whether the analytes can be presented to each assay using a single set of conditions that allows each set of



FIGURE 12 Benchtop imaging system for lateral flow tests from Axin Inc. (The color version of this figure may be viewed at www.immunoassayhandbook.com).



FIGURE 17 Multiplex cassettes: single strip vs multiple strips in one cassette. Source: Symbiont Product Development. (The color version of this

HIV Infection and its Detection

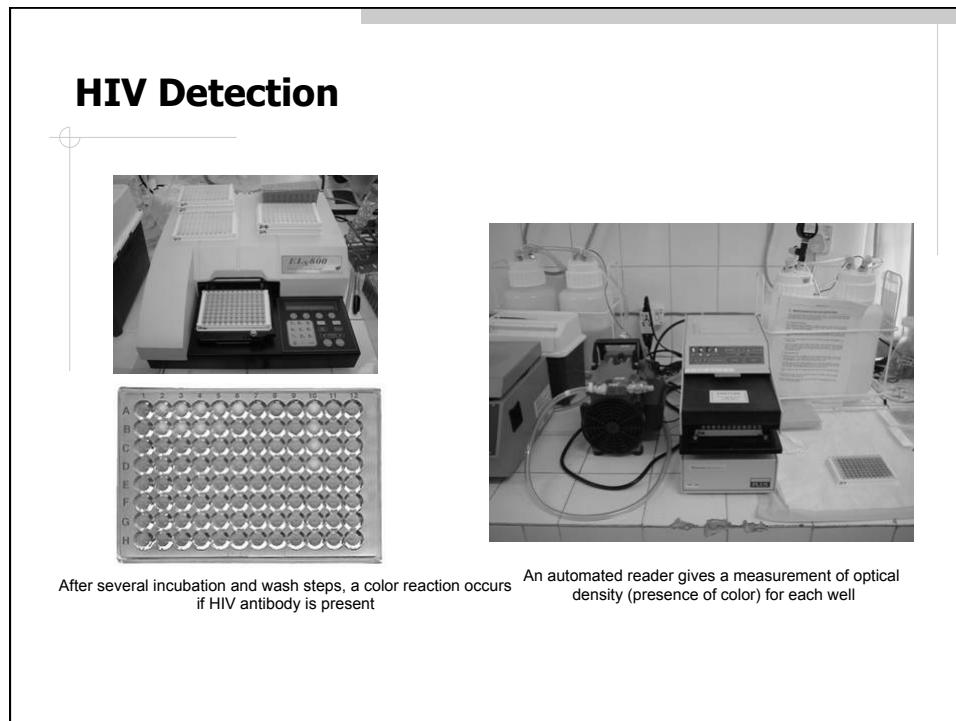
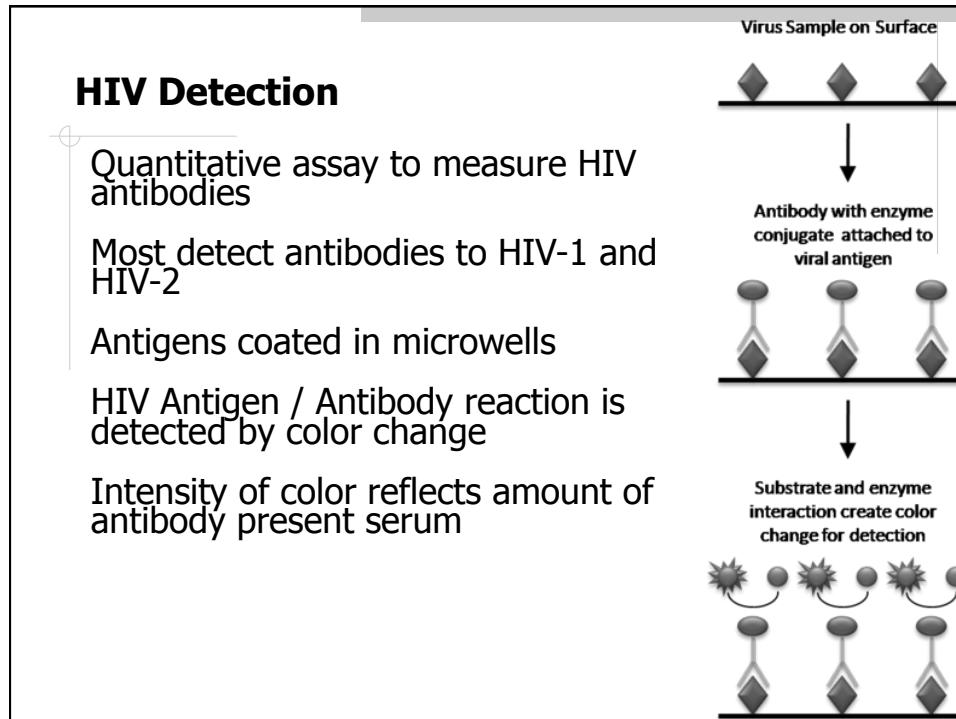
- Human Immunodeficiency Virus (HIV) causes Acquired Immune Deficiency Syndrome (AIDS). Of the two types of HIV (HIV type 1 and HIV type 2), HIV -1 is far more prevalent within North America and in most regions worldwide. HIV is known to be transmitted through contact with the body fluids of an infected individual.
- Infection with HIV-1 and/or HIV-2 elicits an immune response resulting in the production of corresponding anti-HIV antibodies. Antibody detection tests for HIV-1/HIV-2 antibodies provide a means to aid in the diagnosis of HIV-infected individuals 1,2.

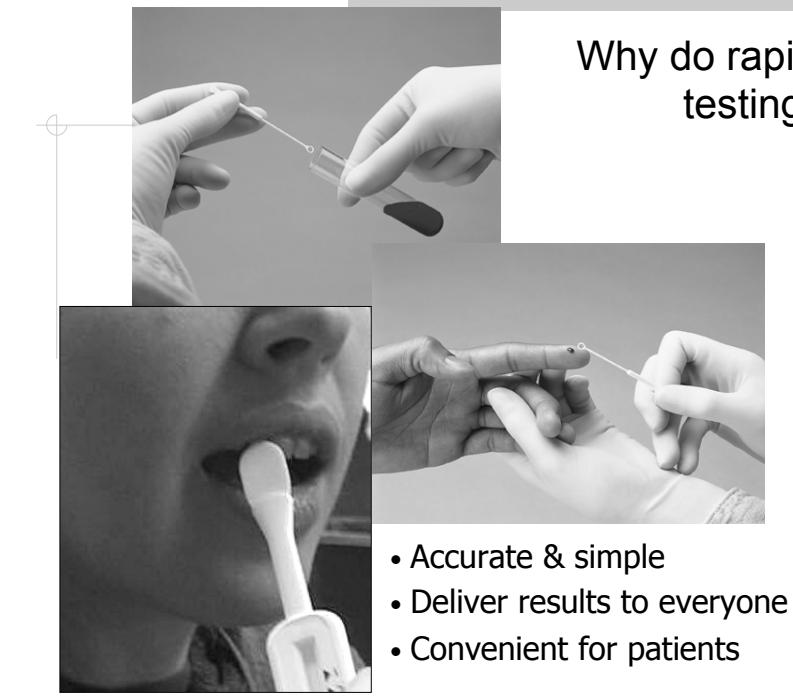
HIV Infection and its Detection

- Conventional laboratory testing for antibodies to HIV utilizes enzyme immunoassays (EIAs) followed by confirmation of repeatedly reactive EIAs using supplemental tests such as the Western blot test, both of which are complex, multi-step procedures. Rapid immunoassay technology has proven to be extremely useful in the diagnosis of infection and is widely utilized as a screening tool. Although use of an EIA screening test is well-suited for batch testing, the turnaround time could be several days to a few weeks. Additionally, the complexity and cost of EIA screen testing and the required equipment may prohibit its universal utilization in medical settings with limited resources and personnel.

Enzyme-linked immunosorbent assay

- The enzyme-linked immunosorbent assay is a test that uses antibodies and color change to identify a substance.
- ELISA is a popular format of "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.
- Antigens from the sample are attached to a surface. Then, a further specific antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate.
- Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.





Why do rapid HIV testing?

- Accurate & simple
- Deliver results to everyone
- Convenient for patients

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HIV Rapid Tests

- Qualitative assay to detect HIV antibodies
- Most detect HIV 1 and HIV 2
- As reliable as EIAs
- Issues:
 - Small volumes
 - Validation of use
 - Appropriate training

HIV Rapid Tests: Advantages

- Increases access to prevention and interventions
- Supports increased number of testing sites
- Same-day diagnosis and counseling
- Robust and easy to use
- Test time under 30 minutes
- Most require no refrigeration
- None or one reagent
- Minimal or no equipment required
- Minimum technical skill

Body Fluids Used for HIV Rapid Testing

- Serum
- Plasma
- Whole blood
- Oral fluids

Three Formats of HIV Rapid Tests

- Immunoconcentration (flow-through device)
- Immunochromatography (lateral flow)
- Particle agglutination

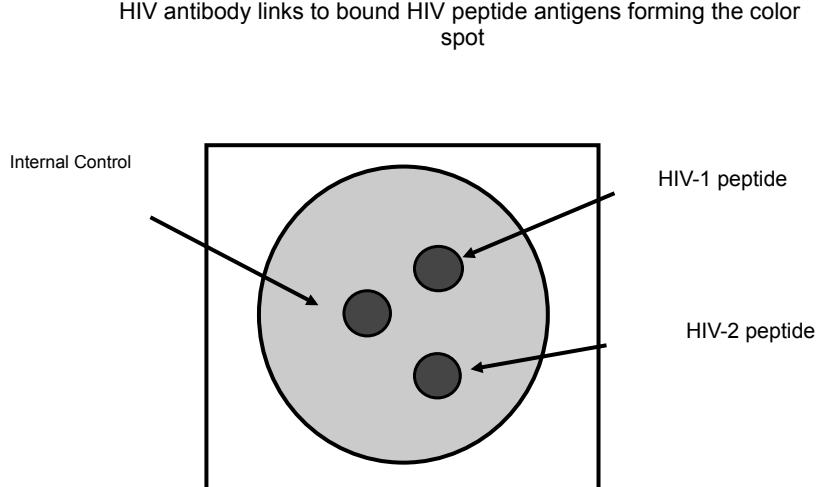
Immunoconcentration

- Microscopic particles are separately coated with the antigens that represent portions of the transmembrane proteins HIV-1 and HIV-2, respectively. The microparticles are immobilized on the reaction membrane of the Cartridge and form the Test Spots. The reaction membrane also contains a Procedural Control Spot that serves as a control spot to ensure that the entire test procedure was properly executed. If antibodies against HIV-1 and/or HIV-2 are present in the specimen, they bind to the antigens on the microparticles in the specific spots on the cartridge membrane. The Conjugate, which contains alkaline phosphatase-labeled goat anti-human IgG, is then added to the Cartridge. The Conjugate binds to the human antibody-antigen complexes that are immobilized in the spots on the cartridge membrane.

Immunoconcentration

- Next, Development Reagent is added to the Cartridge. A purple color develops on the Test Spots in proportion to the amount of antibodies against HIV-1 and/or HIV-2 that have been bound to the antigen-coated microparticles and detected by the Conjugate. A purple color will also develop on the Procedural Control Spot when the test has been performed correctly. Color development is stopped by the addition of Stop Solution. The membrane is examined visually for the presence of purple color on the Procedural Control Spot and on the Test Spots.

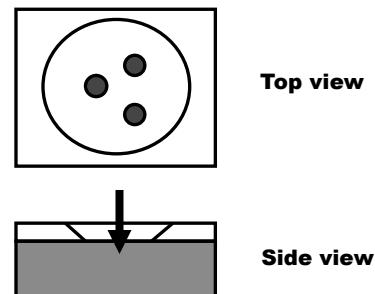
How Immunoconcentration Works



Tests Based on Immunoconcentration

Flow-Through Devices:

- Multi-Spot
- Genie II



Multispot HIV-1/2

- Serum
- Plasma



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Multispot

- Pros

- can distinguish between HIV-1 and 2
- approx. 10 minutes to complete process
- can read results immediately or up to 24 hours after completion

- Cons

- more difficult processing steps
- once test process started, must complete

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Reading Results: Genie II



Non-reactive



Reactive

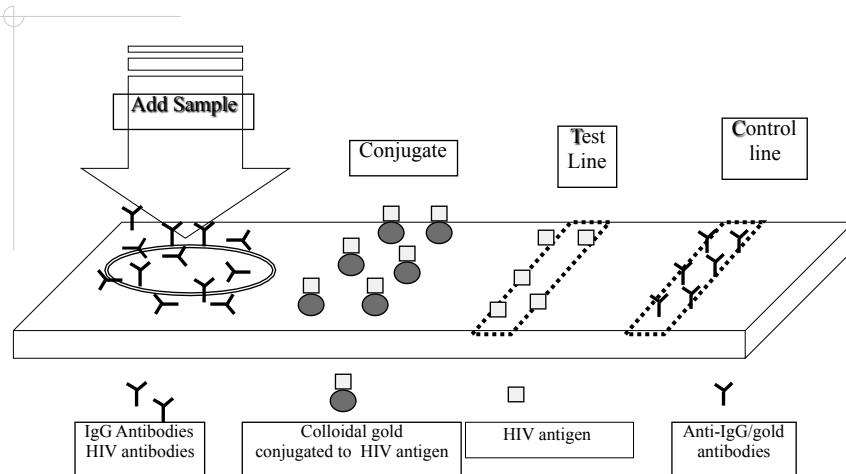
FDA-approved Rapid Tests

- Reveal (MedMira)
- OraQuick (OraSure Technologies)
- Uni-Gold (Trinity Biotech)
- Multispot (Bio-Rad)

All test for HIV antibodies

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How Immunochromatography Works

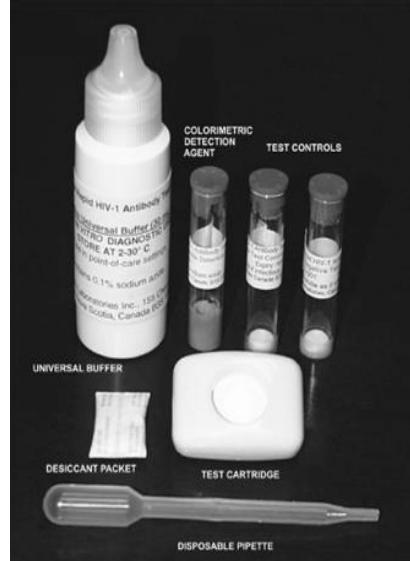


Reveal G-2 HIV-1 Test

- The RevealTM G2 Rapid HIV-1 Antibody Test is a manually performed, visually interpreted, rapid immunoassay. The RevealTM G2 Rapid HIV-1 Antibody Test is comprised of a single-use test cartridge containing an immunoreactive test membrane. The immunoreactive test membrane is comprised of a combination of synthetic peptides corresponding to conserved regions of HIV structural proteins coated onto a membrane matrix, which functions to capture anti-HIV-1 antibodies present in human serum or plasma when a drop of the specimen is applied. In addition, the test membrane has a procedural and reagent Control Line comprised of protein A. Following the application of the sample, the membrane is washed with MedMira Universal Buffer to remove any non-specifically bound antibodies. Captured anti-HIV-1 antibodies are visualized through a reaction with the MedMira Colorimetric Detection Agent (a proprietary protein A-colloidal gold conjugate) followed by a second washing step with MedMira Universal Buffer for clarification of the test result. A Reactive test result occurs only when the protein A portion of the conjugate binds to the captured antibodies, producing a distinctive red dot in the test (T) zone and a vertical red Control Line in the control (C) zone of the test membrane upon completion of the test procedure. In contrast, a Non-Reactive test result, due to the absence of the HIV-1 antibody/antigen complex, is indicated by the presence of only the vertical red Control Line on the test membrane. If the vertical red Control Line is not present, the test result is considered invalid and testing must be repeated with a new cartridge

Reveal G-2 HIV-1 Test

- Serum & Plasma



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Reveal G2



- Pros:
 - Fastest processing time
- Cons:
 - Somewhat complicated
 - Lower specificity
 - Serum or plasma only - requires centrifuge equipment
 - Requires operator attention during entire process

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OraQuick Advance



- Oral Fluid
- Whole blood
- Plasma

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OraQuick Advance

- The OraQuick ADVANCE Rapid HIV-1/2 Antibody Test is a manually performed, visually read, 20 minute immunoassay for the qualitative detection of antibodies to HIV-1 and HIV-2 in human oral fluid, whole blood obtained from a finger puncture or a venipuncture, and plasma.
- The OraQuick ADVANCE rapid test is comprised of a single use test device and a single use vial containing a pre-measured amount of a buffered developer solution.
- Each component is sealed in separate compartments of a single pouch to form the test. The OraQuick ADVANCE rapid test utilizes a proprietary lateral flow immunoassay procedure. The device plastic housing holds an assay test strip comprised of several materials that provide the matrix for the immunochromatography of the specimen and the platform for indication of the test results.



Reading Results: OraQuick

Non-Reactive



Reactive

OraQuick

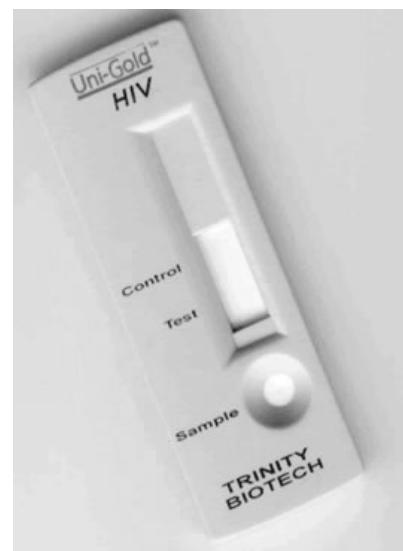
- Pros:
 - Simplest procedure
 - Flexible read time
 - Tests for HIV-2, oral fluid
 - Internal control verifies addition of sample
- Cons:
 - Longest [passive] processing time



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Uni-Gold

- Whole blood
- Plasma & Serum



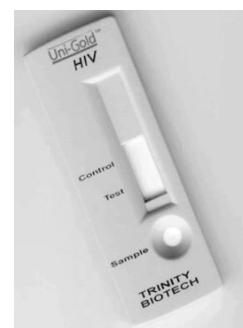
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Uni-Gold

- Uni-Gold Recombigen® HIV-1/2 was designed as a rapid immunoassay and is intended to detect antibodies to HIV-1 and/or HIV-2 in human serum, plasma and whole blood (venipuncture and fingerstick).
- Uni-Gold Recombigen® HIV-1/2 uses proteins representing regions of the HIV virus. If antibodies to HIV-1 and/or HIV-2 are present in the sample, they combine with these proteins and a color reagent and this complex binds to the proteins in the test region of the device forming a visible pink/red band in the test region of the device adjacent to the word 'Test'.
- The control line should always appear as a visible pink/red band in the control region of the device to indicate that the test device is functioning correctly. A reactive result is indicated by a pink/red band in the test region of the device. A non-reactive result occurs in the absence of detectable levels of antibodies to HIV-1 and/or HIV-2 in the specimen; consequently no visually detectable band develops in the test region of the device.

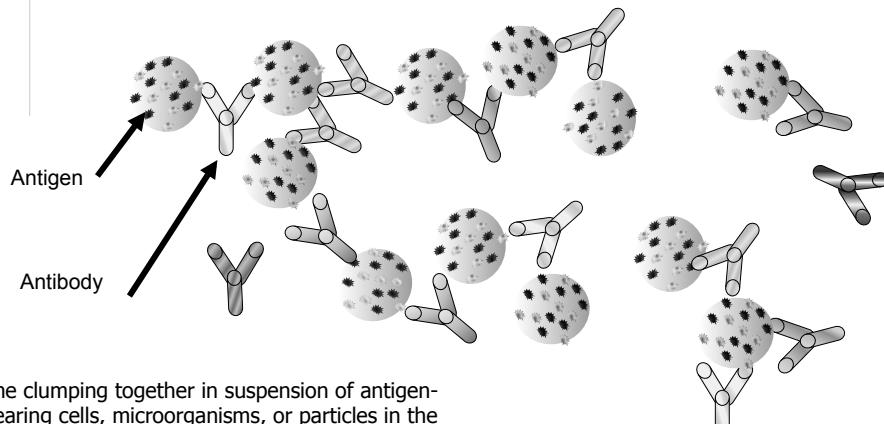
UniGold

- Pros:
 - Relatively simple procedure
 - 10 minute processing time
- Cons:
 - No flexibility in read time
 - Internal control does not verify addition of sample (03.04 PI)



How Particle Agglutination Works

Anti-HIV antibodies bind to the antigen-coated latex particles,



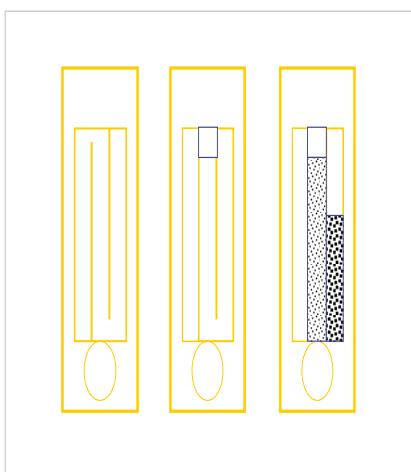
The clumping together in suspension of antigen-bearing cells, microorganisms, or particles in the presence of specific antibodies (agglutinins)

Tests Based On Agglutination

Agglutination devices:

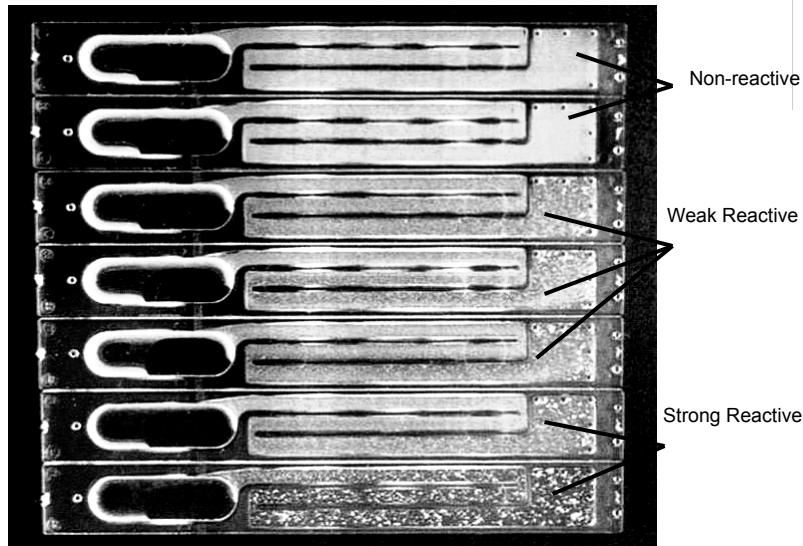
- Capillus
- Serodia

A particle agglutination (PA) test, the Serodia HIV-1/2 test, is a low-cost, simple-to-perform agglutination assay capable of detecting antibodies to HIV-1 and HIV-2. The test incorporates viral lysate antigens coated on gelatin microbeads (particles) that agglutinate in the presence of specific antibodies





Reading Results: Capillus



There Are Only Three Possible Outcomes for Single HIV Antibody Tests

Reactive or "Positive"

- Test band
- Control band

Non-reactive or "Negative"

- Control band only

Invalid

- No control band present
- Test has failed. Repeat with new device.

Sensitivity

A net with very high **sensitivity** would not “miss” (m)any tuna

Specificity

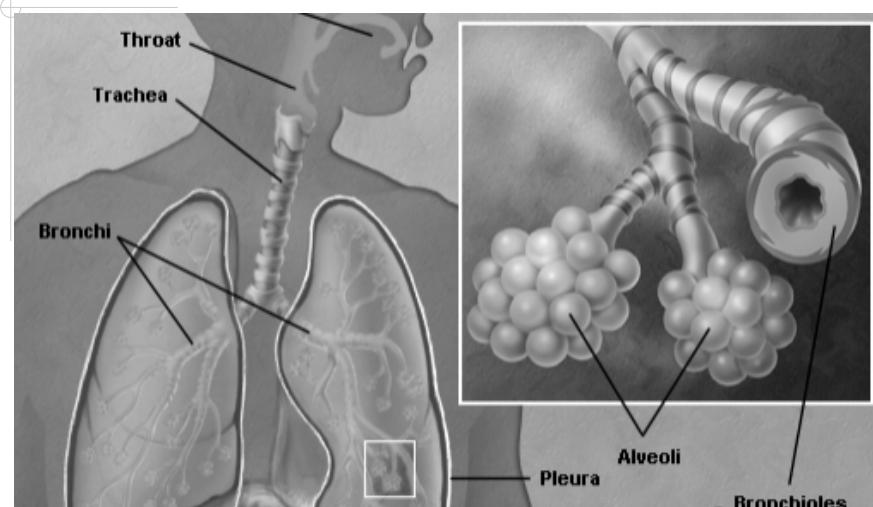
A net with very high **specificity** would not “catch” many dolphins

Sensitivity & Specificity

Test	Sensitivity	Specificity
OraQuick	99.6%	99.9% - 100%
Uni-Gold	100%	99.7% - 99.8%
Reveal	99.8%	98.6% – 99.1%
Multispot	100%	99.9%

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Human Lungs

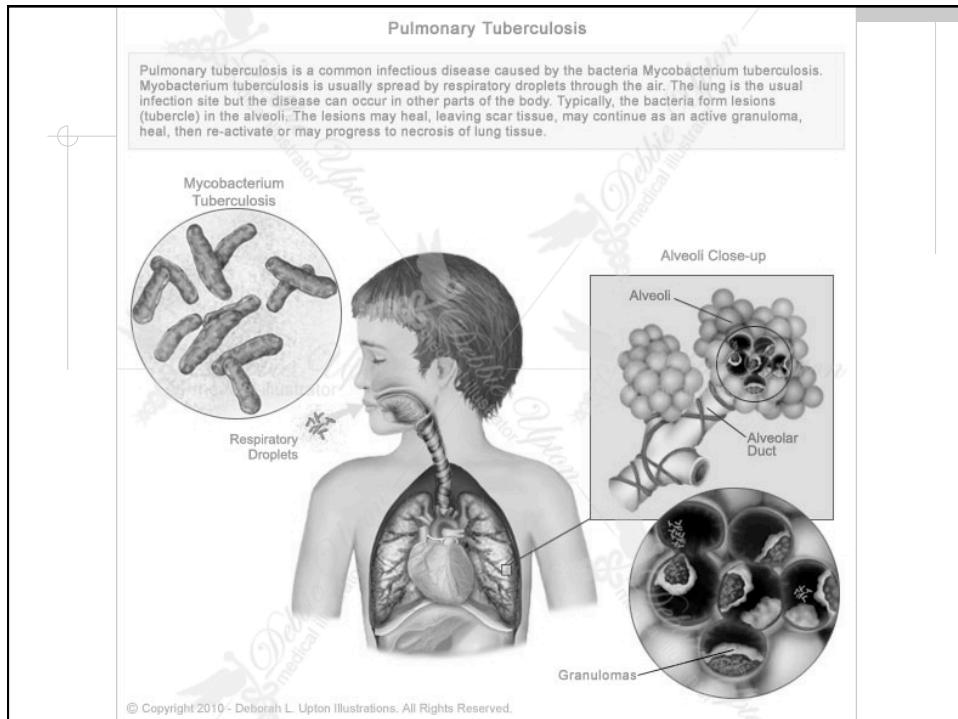


Human Lungs

- The lungs are a pair of spongy, air-filled organs located on either side of the chest (thorax). The trachea (windpipe) conducts inhaled air into the lungs through its tubular branches, called bronchi. The bronchi then divide into smaller and smaller branches (bronchioles), finally becoming microscopic.
- The bronchioles eventually end in clusters of microscopic air sacs called alveoli. In the alveoli, oxygen from the air is absorbed into the blood. Carbon dioxide, a waste product of metabolism, travels from the blood to the alveoli, where it can be exhaled. Between the alveoli is a thin layer of cells called the interstitium, which contains blood vessels and cells that help support the alveoli.
- The lungs are covered by a thin tissue layer called the pleura. The same kind of thin tissue lines the inside of the chest cavity -- also called pleura. A thin layer of fluid acts as a lubricant allowing the lungs to slip smoothly as they expand and contract with each breath.

Lung Diseases - Tuberculosis

- Tuberculosis (TB) is a serious disease caused by breathing in a bacteria called Mycobacterium tuberculosis. TB usually infects the lungs. TB can also infect other parts of the body, including the kidneys, spine and brain.
- People can have TB and not be sick, this is called latent TB. Latent TB is when a person has the TB bacteria in their body but it is not growing. The latent TB can become active TB at any time and make them very sick. If they have inactive TB infection they need to get treatment to cure their TB infection.
- TB is contagious. People who are sick with active TB disease spread TB germs through the air. It's important for people with TB to get treatment right away.
TB treatments can cure TB and prevent it from spreading to others.



Global Burden of TB

- 1/3 world population is latently infected with *M. tuberculosis*
- 10% will develop active disease at some point in their lifetime
- In 2009, there were 9.4 million new cases of TB across the globe, and 1.7 million deaths
- TB –HIV co-infection
- Drug-resistant TB: MDR/XDR-TB

Drug Resistant TB

- Among TB patients notified in 2009, an estimated 250,000 had MDR-TB. Of these, slightly more than 30 000 (12%) were XDR-TB (WHO Global Tuberculosis Control 2010)
- Current phenotypic DST tests for drug resistance can take > 4 weeks, leading to higher mortality and spread of MDR strains
- Need for molecular diagnosis of MDR/XDR-TB
 - Minimizing side effects
 - Highly economic, expensive and ineffective drugs can be avoided
 - Guiding treatment

Treatment for Tuberculosis

Treatment for inactive TB infection

If you have inactive TB infection, there are TB bacteria in your body, but you do not have any symptoms. Inactive TB must be cured to kill the TB bacteria before it becomes active TB and makes you very sick.

Inactive TB is often treated with a medicine called isoniazid (INH). Most people are prescribed this medication daily for 9 months. Inactive TB is also treated with the medicine isoniazid (INH) for 6 months and 3 months with INH and rifampin (PMP) for 3-4 months.

It's very important to take your TB medicine exactly as your doctor or nurse says, for as long as they say. If you stop taking your TB medicine or skip doses, these things could happen:

Your TB infection could come back.

Your TB infection could turn into active TB disease. With active TB, you will have symptoms and feel sick and you can pass TB on to your friends and family.

You could accidentally make the TB germ even stronger, so your TB infection is harder to treat. This is called drug-resistant TB and it is very dangerous and it can be deadly.

Treatments for active TB infection

If you have active TB, your doctor will prescribe medicine to cure you. To get TB medicine, you need a prescription. TB medicine and treatment are free for most people in India.

Some antibiotic medicines (antibiotics) can cure TB. They kill the tuberculosis germs. It usually takes two or more TB medicines to cure active TB disease.

These are the most common medicines to cure TB:

Isoniazid (INH), also called Dom-Isoniazid®, Isotamine®, or PMS-Isoniazid®. It comes as pills or syrup.

Rifampin (RMP), also called Rifadin® or Rofact®. It comes as pills.

Pyrazinamide (PZA), also called PMS-Pyrazinamide® or Tebrazid®. It comes as pills.

Ethambutol (EMB), also called Etibi®. It comes as pills.

Your doctor may put you on all four medications at first. Your doctor will decide which medicines are best for you, and how long you must take them to be cured. TB germs are hard to kill. That's why it's very important that you take all your medicine.

TB Diagnostics

- For decades, researchers and the industry had pinned their hopes on serological antibody-detection methods for POC test development. Indeed, dozens of serological rapid (lateral flow assays) and ELISA tests got commercialized, even though no international guideline recommended their use. Today, these tests are on the market in at least 17 of the 22 highest tuberculosis burden countries, and millions of patients in the private sector undergo serological testing. Unfortunately, TB serological tests are neither accurate nor cost-effective, prompting the WHO to issue a strong negative recommendation against their use. The WHO policy, announced on July 20, 2011, states that, since the "the harms/risks [of commercial serodiagnostic tests] far outweigh any potential benefits (strong recommendation) ...these tests should not be used in individuals suspected of active pulmonary or extra-pulmonary TB, irrespective of their HIV status".

TB Diagnostics

- The failure of antibody-based approaches spurred interest in antigen-detection. While many candidate antigens have been evaluated, urine lipoarabinomannan (LAM) detection assay was the first and, to date, the only antigen detection test to be commercialized, based on promising results from early studies. Two recent studies have evaluated the Determine® TB-LAM (Alere Inc., Waltham, MA, USA), a low-cost, POC version of the urine LAM test, in HIV-infected persons in South Africa.

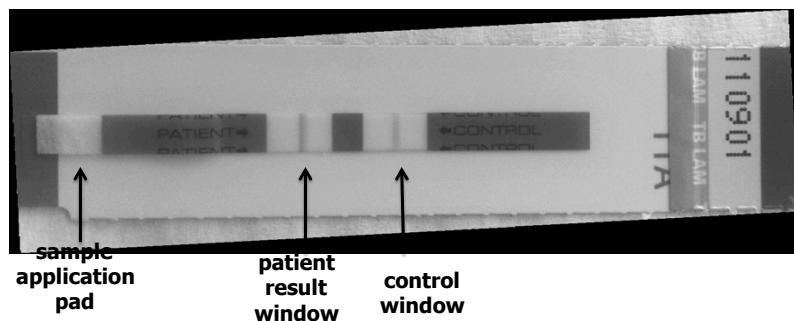


Lipoarabinomannan, also called LAM, is associated with *Mycobacterium tuberculosis*, the bacteria responsible for tuberculosis. Its primary function is to inactivate macrophages and scavenge oxidative radicals.

The inactivation of macrophages allows for the dissemination of mycobacteria to other parts of the body. The destruction of oxidative radicals allows for the survival of the bacteria, as oxidative free radicals are an important mechanism by which our bodies try to rid ourselves of infection.

Determine LAM lateral flow assay (Alere)

- Uses Determine testing platform
- No sample processing; results in 25 minutes
- Analytical sensitivity reported to be 0.25 ng/ml
- Uses urine samples



Determine TB-LAM

Author/ Year	N	Setting	Sensitivity		Specificity
			Overall	CD4<100	
Peter, 2012	335	Inpatients	45%		96%
Lawn, 2012	516	ART clinic	28%	52%	99%
Dorman S, 2012	561	Outpatients Inpatients	45%	80%	90%

TB Diagnostics

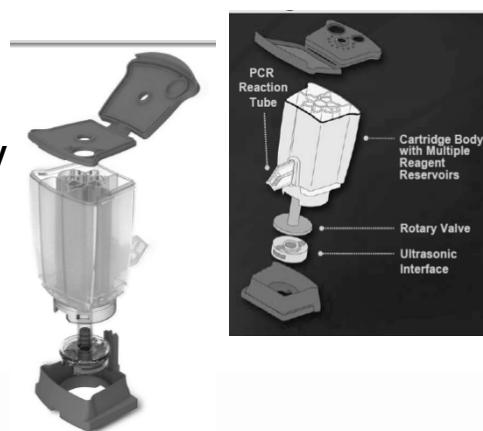
- Consistent with previous studies, the overall sensitivity of Determine® TB-LAM was low in patients with culture-confirmed TB. However, these studies showed that a combination of POC LAM test and sputum smears may offer value in screening for TB among severely immune-compromised HIV-infected patients, a subgroup of high-risk patients for whom diagnostic delays can be fatal. Because the Determine® TB-LAM test may have value only in those with low CD4 counts (CD4 cells or T-cells are a type of white blood cells that play a major role in protecting your body from infection. They send signals to activate your body's immune response when they detect "intruders," like viruses or bacteria), the test must be evaluated as part of an algorithm which includes, ideally, HIV and CD4 testing at the point-of-care.

TB Diagnostics

- The recent WHO endorsement of Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA), an automated, cartridge-based nucleic acid amplification test (NAAT), has greatly stimulated resurgent interest in using molecular tests for rapid diagnosis of active TB and drug. While the Xpert MTB/RIF assay is accurate and can potentially be used outside of a laboratory setting by a minimally trained health worker, it falls short of meeting the ideal POC requirements on two important grounds: at current prices, it is expensive and unaffordable in many settings, and it requires sophisticated equipment that cannot be deployed at the community level. Also, the pricing of Xpert MTB/RIF assay in the private sector in developing countries is substantially higher than the pricing for the public sector, imposing additional barriers for scale-up.

GeneXpert® - a Molecular Lab in a Cartridge Fully-Integrated Sample Preparation, Amplification and Detection

- Universal sample prep**
- Closed test system provides:**
 - Nested PCR**
 - Reflexive test capability**
- Same Basic Cartridge works With all Tests and GeneXpert® Systems**



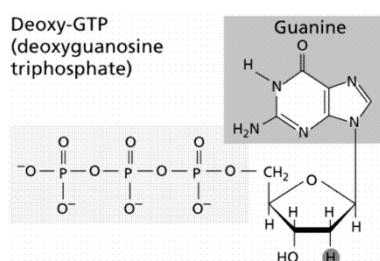
Xpert MTB/RIF

- Detects *M. tuberculosis* and common mutations that confer resistance to rifampin
- Fully automated
- Uses GeneXpert platform (Cepheid, CA)

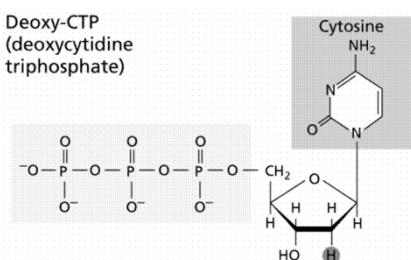
DNA Review

- DNA molecule:
DNA molecules are linear, unbranched polymers composed of nucleic acid molecules – deoxyribonucleic acid molecules.
- Nucleic acid molecule consists of nucleotides: a purine (guanine, G, and adenine, A) or pyrimidine base (thymine, T, cytosine, C) covalently bound to deoxyribose phosphate

Deoxy-GTP
(deoxyguanosine triphosphate)

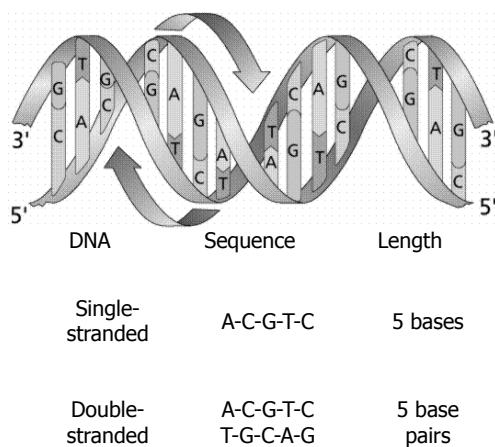


Deoxy-CTP
(deoxycytidine triphosphate)



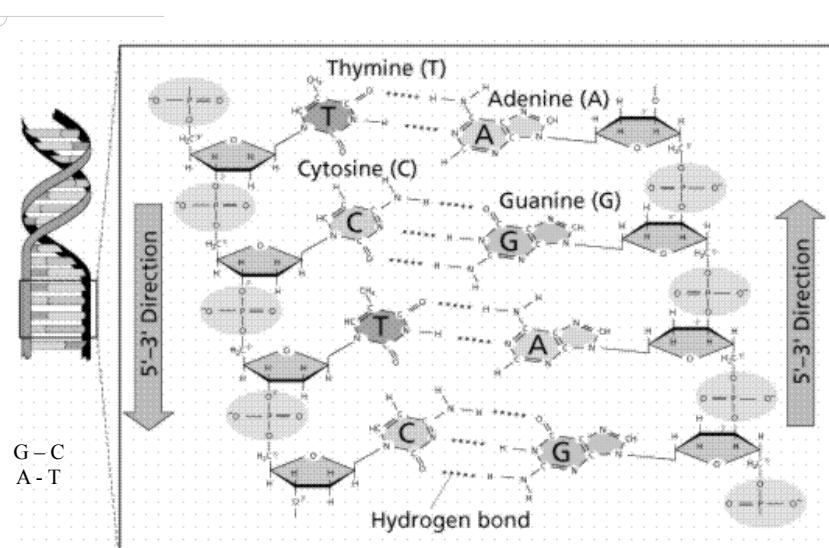
DNA Review

- DNA molecule:
DNA molecule has a double helix structure
- Two DNA strands bound together
- Single stranded DNA – polymer consisting of a series of bases (A, C, G, T)
- Double stranded DNA – base pairs



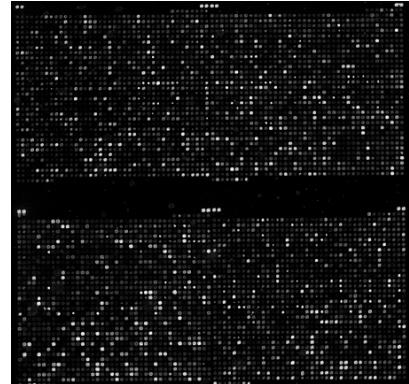
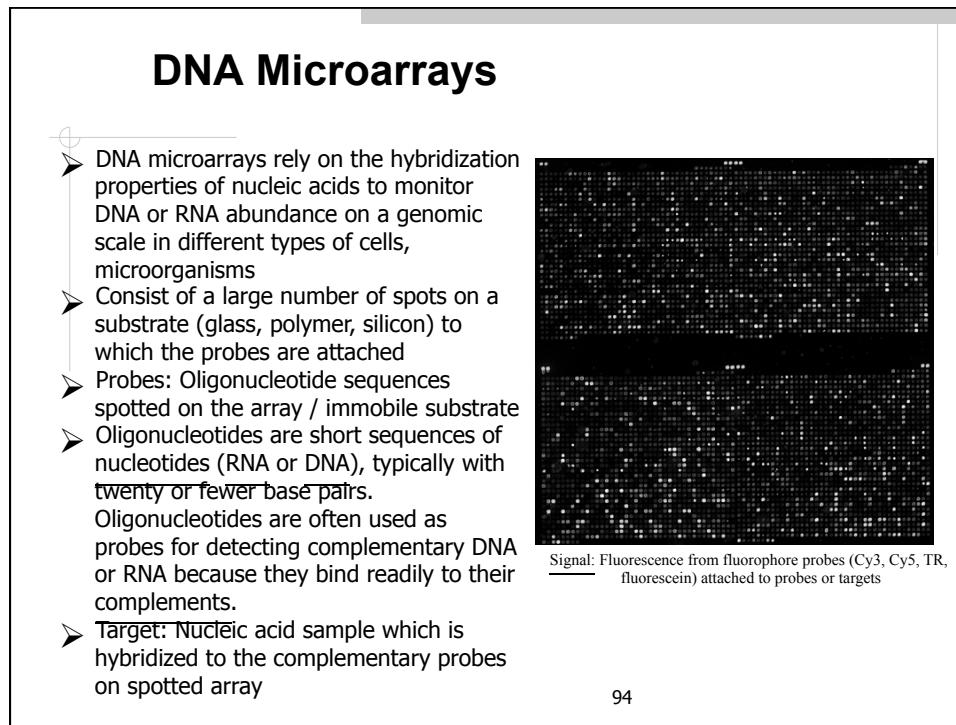
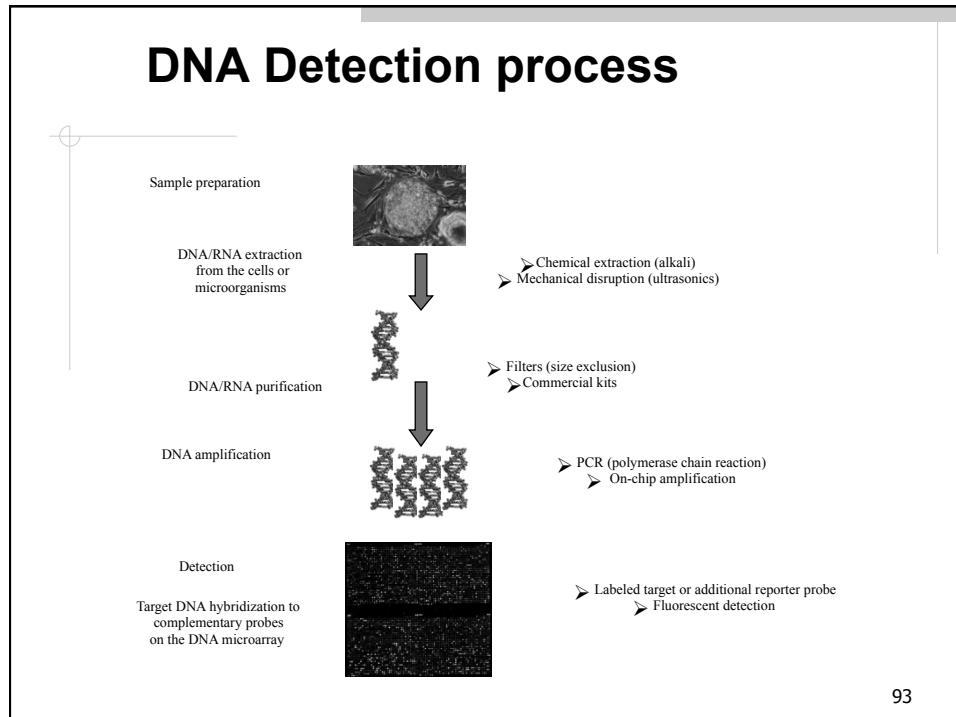
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DNA Review



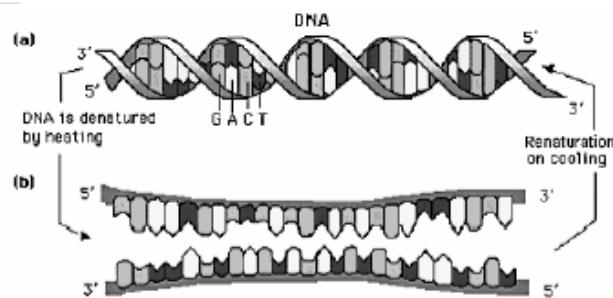
Complementary pair of polymer strands held by hydrogen bonds

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Signal: Fluorescence from fluorophore probes (Cy3, Cy5, TR, fluorescein) attached to probes or targets

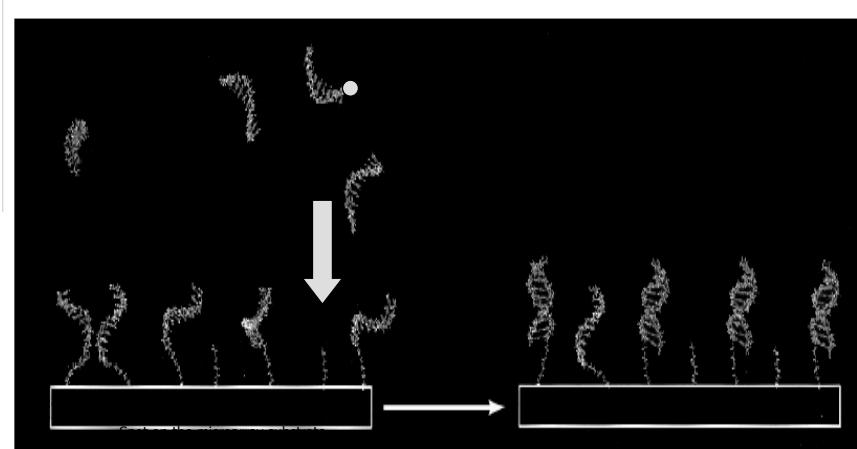
Hybridization



- Hybridization refers to the annealing of two nucleic acid strands following the base-pairing rules.
- Nucleic acid strands in a duplex can be separated, or denatured, by heating to destroy the hydrogen bonds.

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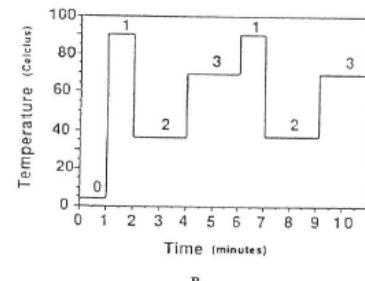
Hybridization



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PCR

- Technique used to produce a large number of copies from a target DNA sequence
- Repetitive 3 step process
 - Denaturation (~95°C)
 - Annealing (~55°C)
 - Chain Extension (~ 72°C)



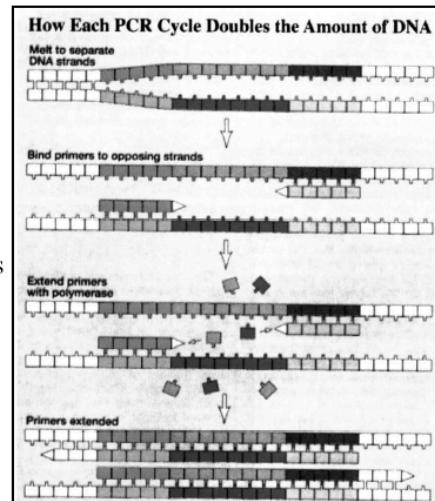
B

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How PCR Works

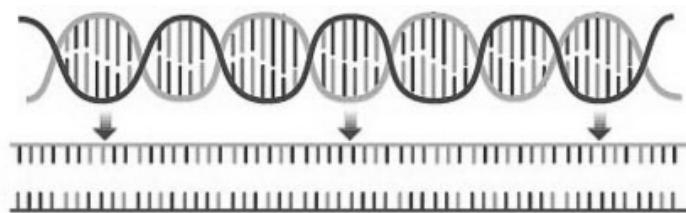
How PCR Works

- High temperature to split strands
- Low temperature to anneal (primers in high concentration)
- Medium temperature to extend
- Repeat



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How PCR Works

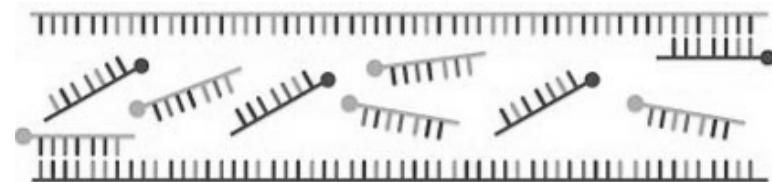


step 1 - Denaturation (optimal temperature is 95°C)

By heating the DNA, the double strand melts and open to single stranded DNA.

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How PCR Works



step 2 - Annealing (optimal temperature is 55°C)

The single-stranded primers bind to their complementary single-stranded bases on the denatured DNA.

100

How PCR Works

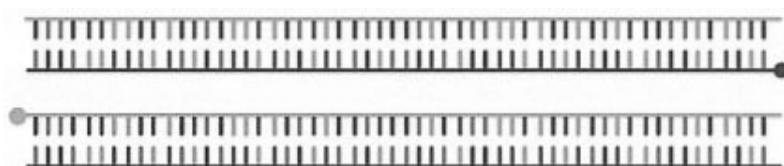


Step 3 - Extension

72°C is the ideal temperature for the Taq polymerase to attach and start copying the template. The result is two new helixes in place of the first.

101

How PCR Works



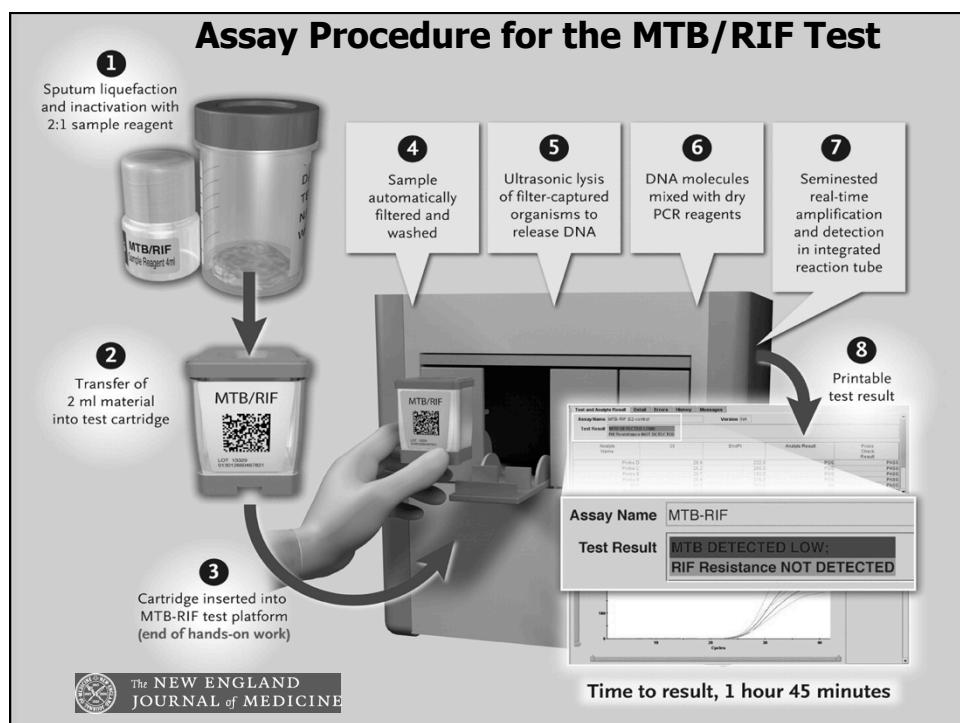
By applying several times this cycle, the quantity of DNA obtained is quickly enough to perform any analysis. Starting with one DNA molecule after just 20 cycles there will be a million copies and after 30 cycles there will be a billion copies.

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Basic PCR Reagents

- Template DNA
- Complementary Primers (~20 nucleotides)
- Thermostable Polymerase Enzyme (TAQ)
- Single nucleotides (A,C,G,T)
- Buffers (pH and ionic concentrations)

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Xpert MTB/RIF

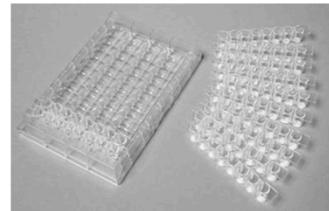
Attributes & Advantages

- Simple to perform, minimal training required
- Not prone to cross-contamination
- Requires minimal biosafety facilities
- “Near-care”

Shortcomings & Disadvantages

- Complex instrument (calibration, power supply)
- Cost for instrument
 - Cost of cartridges reduced to ~\$10
 - Single supplier

T-SPOT.TB Test Kit



- Flexible, 96-well format
- Twelve, 8-well strips
- 4 wells used per patient; 24 patients per kit
- Positive and Negative control for each patient test
- Utilizes standard blood collection tubes
- No special lab equipment required

T Spot TB test

- The T-SPOT.TB test is a revolutionary in vitro diagnostic assay that measures T cells primed to *Mycobacterium tuberculosis* (MTB) antigens. It was developed for diagnosing both latent TB infection and TB disease in humans. The T-SPOT.TB test sets new clinical standards of sensitivity and reliability, even in the immunocompromised. The product was licensed in the European Union in July 2004, received FDA premarket approval in July 2008. It is replacing the tuberculin skin test, bringing effective TB testing to many new patient groups where the skin test gives poor results.

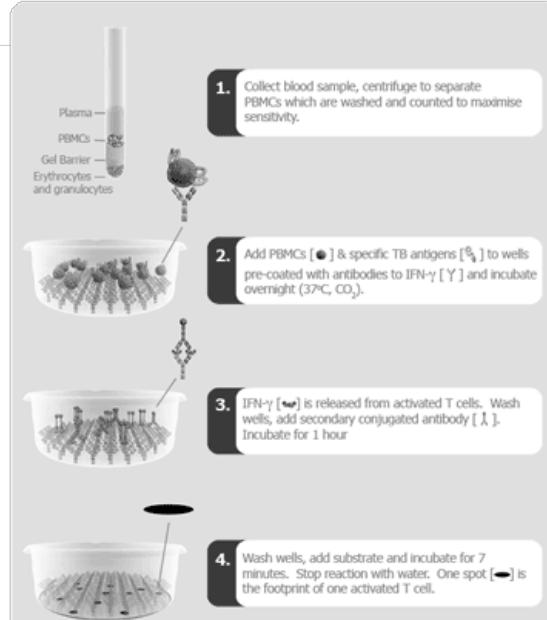
T Spot TB test

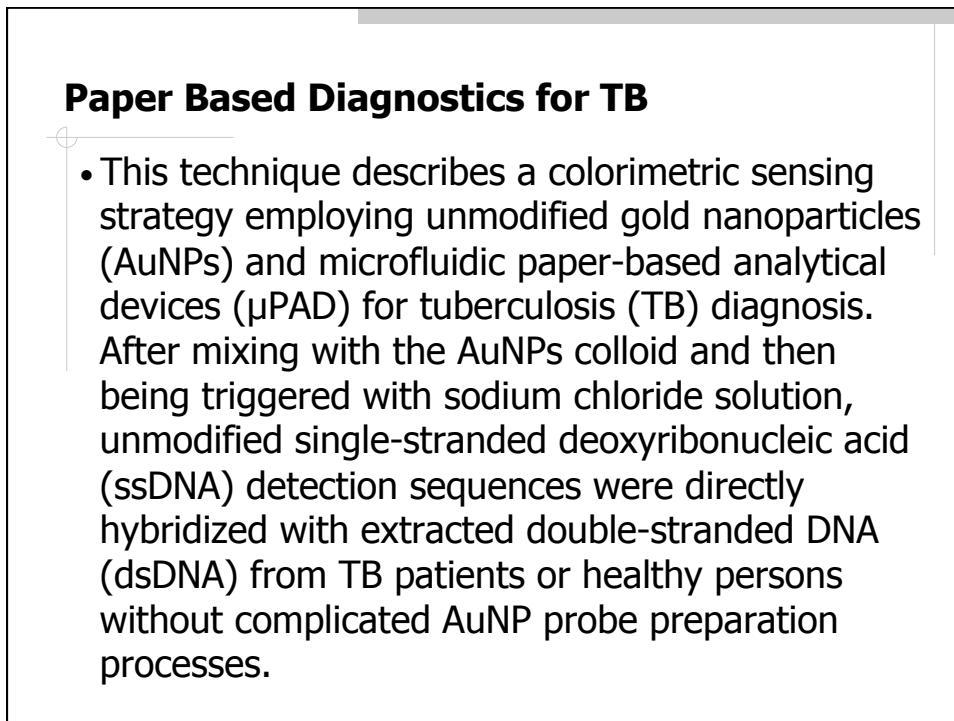
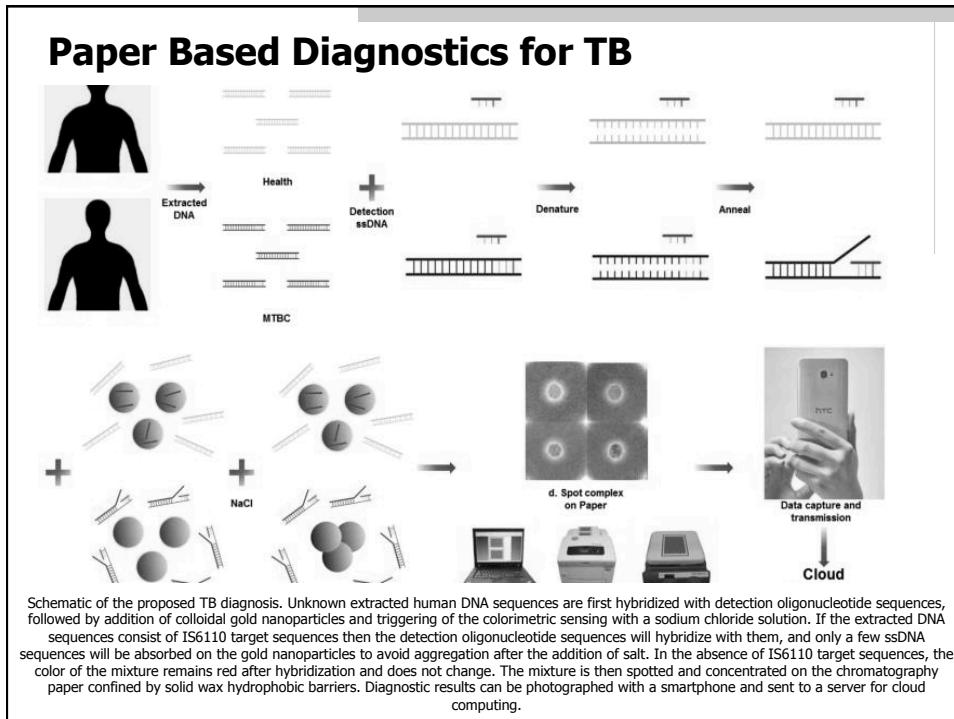
- The principles of T-SPOT assay system, are shown below, using blood as the body fluid in the example. The process starts with a blood sample, from which PBMCs (specifically Peripheral Blood Mononuclear Cells which is any blood cell having a round nucleus (as opposed to a lobed nucleus). For example: a lymphocyte, a monocyte or a macrophage. These blood cells are a critical component in the immune system to fight infection and adapt to intruders) or WBC components containing T cells (T lymphocytes are a type of lymphocyte or a type of white blood cell) that plays a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. They are called T cells because they mature in the thymus (although some also mature in the tonsils) are separated, washed and counted.

T Spot TB test

- A pre-determined number of PBMCs and antigens specific to the disease or condition of interest are then added to the wells of a microtiter plate to which antibodies to interferon-gamma, or IFN- γ , are bound. The test is based on the principle that the T cells of an individual who carries an active infection will respond to the antigens and secrete interferon-gamma. The secretion of interferon-gamma by the T cells of the subject is captured by the anti-interferon-gamma antibodies coated to the floor of each well. The numbers of individual reacting T cells are enumerated through visualizing the footprint of each T cell by this secretion of interferon-gamma.

Principles of the T-SPOT® Assay System





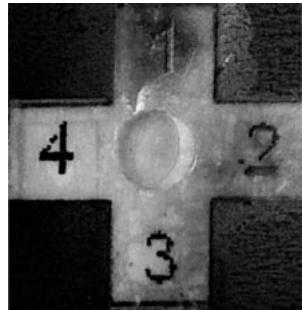
Paper Based Diagnostics for TB

- The specific IS6110 dsDNA sequence of *Mycobacterium tuberculosis* complex (MTBC) was chosen as the diagnostic target for recognition. When the target DNA sequences were absent, the detection ssDNA sequences were absorbed on the AuNP surfaces and protected unmodified AuNPs from aggregation in the high salt solution. The detection ssDNA sequences were hybridized with target DNA when the target DNA sequences were present, and the color of the unprotected AuNPs colloid turned from red to blue.

Compliance

- A new monitoring system that combines cheap, paper-based diagnostics with text-messaging technology could help health organizations, with the cooperation of telecommunications companies, give patients another incentive to adhere to the drug regimen. José Gómez-Márquez, program director for the Innovations in International Health program at MIT, and his collaborators developed a simple paper-based test that detects metabolites of the TB drug in urine. The metabolite reacts with chemicals in the paper, revealing a simple numerical code. A patient would take the test daily and text the code to a central database. Those who take the drugs consistently for 30 days would be rewarded with cell-phone minutes.

Compliance



Take your meds: Paper tests reveal hidden codes (above) when exposed to the urine of patients who have taken tuberculosis medication. The codes can be numerical sequences or bar codes



Why we Fail to Diagnose TB?

- Lack of health infrastructure
- TB control is plagued with lack of accurate, robust and rapid diagnostic methods
- Patients are diagnosed late, many are never diagnosed before death
- In HIV infected patients, on many occasions microscopy may prove negative in spite of presence of bacilli, as only a few bacilli are expectorated

SmartSense – A recent concept won at the Health Care Innovation World Cup 2013 at New York, will now be put to reality with the support of Wellcome Trust, UK. It is going to be an integrated, multianalyte critical care analyzer, indigenously developed to cater the laboratories need of the hour at an affordable cost.

UChek – A mobile based Urine and blood glucose analysis system launched in Apr 2013 registered with US FDA as Class 1 device. Low-cost, efficient, and error proof urine analysis on most of the commercially available urine test strips. The UChek mobile platform saves and generates reports of urine tests done, which can be emailed.

SuChek - Newest addition to glucometers launched on 13th Jan 2014. Indigenous, accurate, low-cost glucometer. As accurate as conventional glucometers, at a fraction of the price. Suchek mobile app helps you save, trend & analyze blood glucose levels at an individual level or track response to treatment at a community level.

SmartSense, Ucheck and SuChek has been developed by Prof Rohit Srivastava and his team at Nanobios Lab, IIT Bombay, in collaboration with Biosense Technologies Pvt. Ltd with the support of Wellcome Trust, DBT-IYBA and ICMR respectively.

NB
NANOBIOS