BIOMARKERS AS NOVEL TOOLS FOR EARLY AND RAPID DIAGNOSIS

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WALKATHON

Future diagnosis

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Biomarkers: The Future of Medical Science to Detect Cancer

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Abstract

A biomarker, or biological marker, is in general a substance used as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers play an essential role in much disease detection. Much kind of biomarkers are available in the field of medical science with lots of positive as well as negative effect. Biomarkers will become one of the major driving forces of pharmaceutical research and drug development in the coming years. A specific and ideal biomarker for many unbeaten disease like cancer is still a big challenge.

What is it?

BIOMARKERS

"Biomarker," or "biological marker," generally refers to a measurable indicator of some biological state or condition.

Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers are used in many scientific fields and in clinical practice.

Biomarker- Definition

- A biomarker is a characteristic 'substance', analyte, or otherwise a 'thing' that can be objectively measured as an indicator of normal biological processes, pathogenic processes or a pharmacological response to a therapeutic intervention. These are used for many purposes including disease diagnosis and prognosis, prediction and assessment of treatment response.
- Biomarkers can be characteristic biological properties or molecules that can be detected and measured in parts of the body like the blood or tissue. They may indicate either normal or diseased processes in the body.
- Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones.

Biomarkers - classification

- **Imaging biomarkers**(computed tomography, positron emission tomography, magnetic resonance imaging)
- **Molecular biomarkers**. Molecular biomarkers can be used to refer to non-imaging biomarkers that have biophysical properties, which allow them to be measured in biological samples, and include nucleic acid–based biomarkers such as gene mutations or polymorphisms and quantitative gene expression analysis, peptides, proteins, lipids metabolites, and other small molecules.

BIOMARKER CATEGORIES

Biomarkers can be classified based on their application

- Diagnostic biomarkers
- Staging of disease biomarkers
- Disease prognosis biomarkers (cancer biomarkers)
- Biomarkers for monitoring the clinical response to an intervention.
- Biomarkers includes those used in decision making during early drug development. For instance, pharmaco-dynamic biomarkers are markers of a certain pharmacological response and are of special interest in dose optimization studies

Tumour Biomarkers

- Tumour markers are made by normal cells as well as by cancer cells. These are the substances that are produced by cancer or by other cells of the body in response to cancer or certain begin conditions. These substances can be found in the blood, urine, stool, tumour tissue, or other tissues or bodily fluids of some patients with cancer.
- Most tumour markers are proteins. However, more recently, patterns of gene expression and changes to DNA have also begun to be used as tumour markers. Markers of the latter type are assessed in tumour tissue specifically.

Biomarkers in Oncology

- Oncology biomarkers actually make their way into routine clinical use. These can be detected and measured in parts of the body like blood or tissue. Biomarkers can be specific cells, molecules, or genes, gene products, enzymes, or hormones.
- In the recent years, knowledge about cancer biomarkers has increased tremendously providing great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of treatment. Recent technological advancement has enabled the examination of many potential biomarkers and renewed interest in developing new biomarkers.

Common Serum Markers for Cancer Diagnosis/prognosis

	AFP	CEA	CA15-3	CA19-9	CA125	PSA	PSAf	PAP	hTG	HCGb	Ferr	NSE	B2M	A2M
Lung		x	x	x	x					x	x	x		
Pancreas	x	x	x		x					x				
Kidney	x	x								x			x	
Breast		x	x		x									
Ovarian	x		x	x	x					x			x	
Cervical	x				x									
Uterine		x		x	x					x				
Prostate						x	x	x					x	x
Liver		x	x	x	x					x				
Gastro		x		x	x									
Colon		x		x	x		x			x				
Bladder										x				
Brain										x				
Leukemia								x			x		x	
Myeloma													x	
Thyroid									x			x		
Testicular	x							x		x	x			

Salivary Biomarkers

- Biomarkers in the saliva have the potential to be used for screening purposes in epidemiological studies. The aim of this study was to investigate if certain salivary biomarkers could be used for detection of common systemic diseases.
- For clinical applications such as monitoring health status, disease onset and progression, and treatment outcome, there are three necessary prerequisites:
- A simple method for collecting biologic samples, ideally noninvasively
- Specific biomarkers associated with health or disease
- A technology platform to rapidly utilize the biomarkers. Saliva, often regarded as the 'mirror of the body', is a perfect surrogate medium to be applied for clinical diagnostics. Saliva is readily accessible via a totally non-invasive method.

Serum Biomarkers

- Serum biomarker is used to detect the irritable bowel syndrome (IBS). No single serum biomarker can reliably differentiate irritable bowel syndrome (IBS) from other functional gastrointestinal disorders or organic diseases of the gastrointestinal tract.
- Sepsis, an innate immunological response of systemic inflammation to infection, is a growing problem worldwide with a relatively high mortality rate. Immediate treatment is required, necessitating quick, early and accurate diagnosis. Rapid molecular-based tests have been developed but still suffer some disadvantages. The most commonly studied biomarkers of sepsis are reviewed for their current uses and diagnostic accuracies, including **C-reactive protein, procalcitonin, serum amyloid A, mannan and IFN-γ-inducible protein 10**, as well as other potentially useful biomarkers.

Oxidative Stress Biomarkers

- The potential of oxygen free radicals and other reactive oxygen species (ROS) to damage tissues and cellular components, called oxidative stress. The techniques are as diverse as blood tests for oxidized lipids, volatile hydrocarbons in breath and oxidized DNA bases in urine.
- Increased oxidative/nitrosative stress generally describes a condition in which cellular antioxidant defences are inadequate to completely inactivate the reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated because of excessive production of ROS/RNS, loss of antioxidant defences, or both. A major consequence of oxidative/nitrosative stress is damage to nucleic acid bases, lipids, and proteins, which can severely compromise cell health and viability or induce a variety of cellular responses through generation of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis.

Biomarkers in Clinical Trials

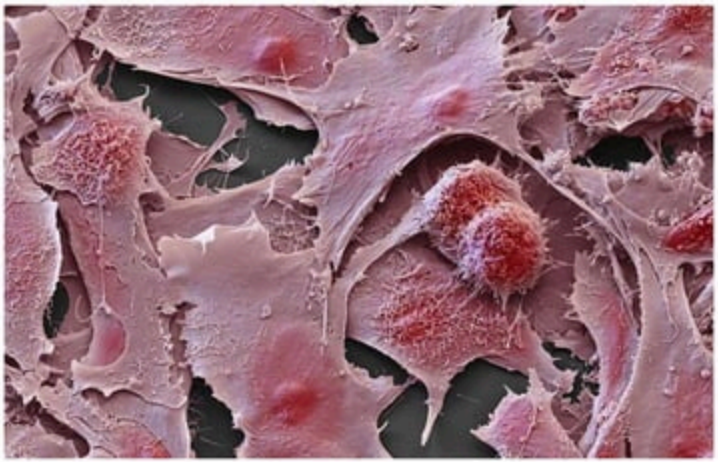
- Biomarkers are likely to be important in the study of Alzheimer disease (AD) for a variety of reasons. A clinical diagnosis of Alzheimer disease is inaccurate even among experienced investigators in about 10% to 15% of cases, and biomarkers might improve the accuracy of diagnosis.
- Biomarkers are the measures used to perform a clinical assessment such as blood pressure or cholesterol level and are used to monitor and predict health states in individuals or across populations so that appropriate therapeutic intervention can be planned.
- Biomarkers are used to predict serious illnesses such as diabetes and cardiovascular disease. Each individual biomarker indicates whether there is a disease or health state and can be combined to provide a detailed picture of how healthy a person is and whether or not a diagnosis needs to be made.

Molecular Diagnostics

- Molecular diagnostics is a technique used to analyse biological markers in the genome and proteome the individual's genetic code and how their cells express their genes as proteins by applying molecular biology to medical testing. The technique is used to diagnose and monitor diseases.

- An ideal biomarker has certain characteristics that make it appropriate for checking a particular disease condition. Ideally, an ideal marker should have the following features:
- Safe and easy to measure
- Cost efficient to follow up
- Modifiable with treatment
- Consistent across gender and ethnic groups

biopsy



Liquid biopsy

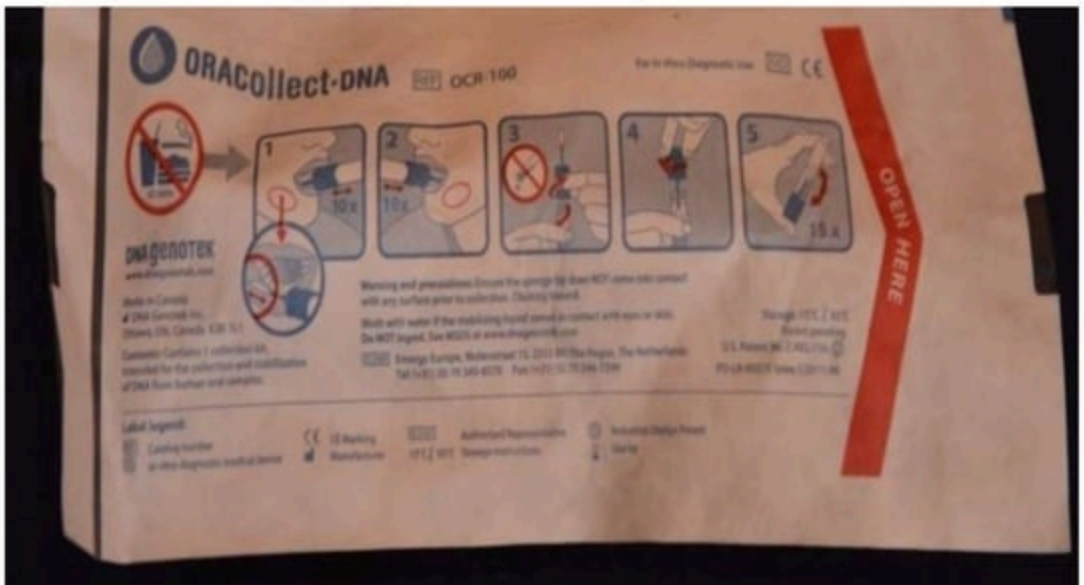
Liquid Biopsy – what is it?

- Until now, a biopsy is considered the best and right method to diagnose cancer. It is a method where a portion or whole of tumour tissue is cut and sent to a laboratory for investigation under a microscope.
- this procedure is invasive, often painful and requires a trained doctor to perform it. But foremost of all, the tumour has to be visible to the doctor, either to the naked eye or through the means of various imaging technologies like a CT Scan, a USG or through a scope..
- For this to happen, quite often the tumour must have progressed through its course and reached a stage which cannot be termed "early".
- This is the primary reason why cancer is often detected late.
- In a developing country like India where annual health check up or cancer screening is yet to catch up with the masses and visit to a doctor is governed by unmanageable symptoms, the battle is already half lost when a cancer is detected first.

Liquid Biopsy targets one of the following

- Free circulating nucleic acids.
- RNA expression and fusion transcripts.
- Circulating tumour cells.
- Multiple DNA abnormalities
- Tumour Emboli
- Insertions and deletions
- Trans-locations and chromosomal abnormalities
- Point mutations

selfy



like Lung cancer and nonmalignant disorders involving Gastrointestinal tissues.

(CA) CYFRA 21-1; LUNG CANCER MARKER

Specimen : 2 mL (0.5 mL min.) serum from 1 SST. Ship refrigerated or frozen. Provide brief clinical history.

Stability : Room 2 hrs | Refrigerated 4 weeks | Frozen 6 months

Method : Electrochemiluminescence

Comment : Also see CEA & NEURONE SPECIFIC ENOLASE (NSE).

Price : ₹ 2650

Report : Sample by Mon 4 pm; Report Same day

Usage : This is a useful marker in the management of Non Small Cell Carcinoma Lung. It is recommended for therapeutic monitoring and recurrences in an already diagnosed case. It may show positivity in certain cases of Squamous cell carcinoma, Large cell carcinoma and Adenocarcinoma.

Stage II / III breast cancer who are clinically free of disease.

CA 15.3 & CEA - See BREAST MONITOR PANEL page 81

CA 19.9: PANCREATIC CANCER MARKER

Specimen : 2 mL (0.5 mL min.) serum from 1 SST. Ship refrigerated or frozen. Provide brief clinical history.

Stability :

Room	Refrigerated	Frozen
8 hrs	1 week	2 weeks

Method : Chemiluminescent Microparticle Immunoassay

Price : ₹ 1240

Report : Daily

Usage : CA 19.9 is useful to monitor the response to treatment and if elevated suggests recurrence in patients with Pancreatic Cancer. Elevated concentrations are not specific. Use in patients with other medical conditions is not advised.

A 27.29: BREAST CANCER MARKER

Price : ₹ 330
Report : Daily
Usage : Prothrombin Time assesses the extrinsic and common coagulation pathway from Factor VII through fibrin formation. Results are interpreted based on INR. A prolonged INR suggests a potential bleeding disorder or if on warfarin therapy, a potential for bleeding complications.

pS2 - See IMMUNOHISTOCHEMISTRY, INDIVIDUAL MARKERS page 206

PSA (PROSTATE - SPECIFIC ANTIGEN), TOTAL

Prostatic Cancer Marker.

Specimen : 2 mL (1 mL min.) serum from 1 SST. Ship refrigerated or frozen. Do not draw sample within 7 days of Digital Rectal Examination (DRE) or Rectal Prostatic Ultrasonography.

Stability :

Room	Refrigerated	Frozen
2 hrs	24 hrs	4 weeks

Method : Chemilumescent Microparticle Immunoassay
Price : ₹ 700
Report : Daily
Usage : This assay is used for monitoring patients with a history of Prostate cancer and as an early indicator of recurrence and response to treatment. The test is commonly used for Prostate cancer screening.

PSA (PROSTATE - SPECIFIC ANTIGEN), FREE

CODE	TEST INFORMATION
	produce mucinous antigens encoded by human muc-1 gene. These antigens include CA 27.29 & CA 15.3. This panel is helpful in predicting early recurrence in women treated for Carcinoma breast.
009	**CA 72.4; GASTRIC CANCER MARKER**
	Specimen : 2 mL (0.5 mL min.) serum from 1 SST. Ship refrigerated or frozen. Provide brief clinical history.
	Stability : Room: 2 hrs; Refrigerated: 4 weeks; Frozen: 3 months
	Method : Electrochemiluminescence
	Price : ₹ 1360
	Report : Sample by Mon / Thur 4 pm; Report Same day
	Usage : CA 72.4 is most useful as a marker for Gastrointestinal cancer, but blood levels may be increased in other malignancies like Lung cancer and nonmalignant disorders involving Gastrointestinal tissues.

concentrations are not specific. Use in patients with other medical conditions is not advised.

RW835 CA 27.29; **BREAST CANCER MARKER**

Specimen : 2 mL (0.5 mL min.) serum from 1 SST. Ship refrigerated or frozen.

Stability : Room 8 hrs | Refrigerated 7 days | Frozen 28 days

Method : Immunoassay

Comment : Also see CA 15.3, BREAST CANCER PANELS.

Price : ₹ 5810 + 1500 (Courier to USA)

Report : Sample by 7th of the month; Report after 2–3 weeks

Usage : CA 27.29 is useful to monitor the response to therapy and if elevated suggests recurrence in women with Stage II or III breast Cancer.

Z306 **CA 27.29 & CA 15.3 BREAST CANCER MARKERS PANEL**

Specimen : 2 mL (1 mL min.) serum from 1 SST. Ship refrigerated or frozen.

Stability : Room 8 hrs | Refrigerated 1 week | Frozen 2 weeks

Method : CMIA, Immunoassay

Price : ₹ 7050 + 1500 (Courier to USA)

Report : LPL: Daily

USA: Sample by 7th of the month; report after 2–3 weeks

Usage : Carcinoma breast is the most prevalent form of cancer in women. These tumors

CD 45 Ro - See IMMUNOHISTO-CHEMISTRY, INDIVIDUAL MARKERS page 206

CD4/CD8 - See IMMUNE DEFICIENCY PANELs page 202

CEA; CARCINO EMBRYONIC ANTIGEN

Specimen : 2 mL (1 mL min.) serum from 1 SST. Ship refrigerated or frozen. Give brief clinical history.

Stability : Room 8 hrs | Refrigerated 1 week | Frozen 2 weeks

Method : Chemiluminescent Microparticle Immunoassay

Price : ₹ 630

Report : Daily

Usage : Incresaed levels of CEA are found in patients with primary Colorectal carcinoma and other malignancies like Medullary thyroid carcinoma and Carcinoma of breast, GI tract, liver, lung, ovarian, pancreatic and prostate. Serial monitoring of CEA should begin prior to therapy to establish a baseline for evaluating possible recurrence. Levels generally return to normal within 1 to 4 months after removal of tumor. Smokers show a higher baseline level of CEA.

CENTROMERE ANTIBODY

Specimen : 2 mL (1 mL min.) serum from 1 SST. Ship refrigerated or frozen. Overnight

VITROS Immunodiagnostic Products CEA Reagent Pack Summary and Explanation of the Test

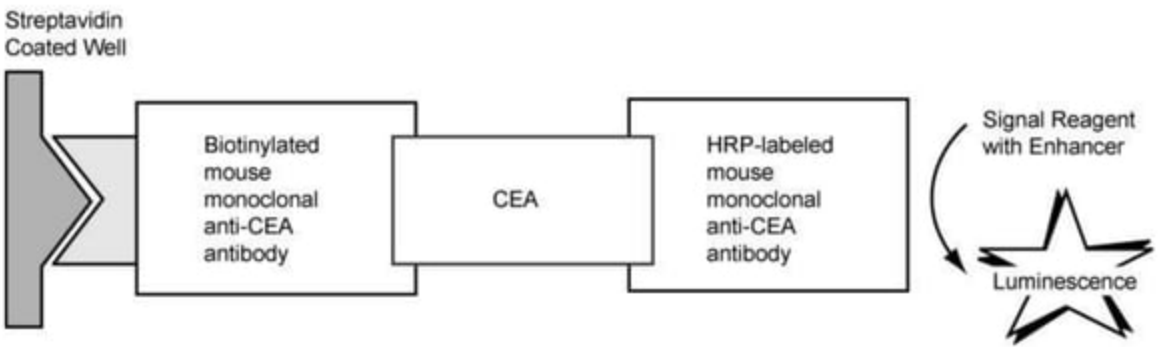
- Carcino-embryonic antigen (CEA) is a glycoprotein with a molecular weight of approximately 180,000 Daltons.
- CEA is present in normal serum at low concentrations. Originally thought to be specific for digestive tract cancers it may also be elevated in other malignancies as well as in some nonmalignant disorders and behaviors CEA testing has become widely accepted in the management of cancer patients.

- A CEA concentration which falls steadily to reach normal concentrations suggests a good prognosis while an increasing concentration is indicative of treatment failure and a poor prognosis.
- Clinical relevance has been shown in the follow-up management of patients with colorectal, breast, lung, prostatic, pancreatic and ovarian carcinoma.
- Prognostic significance has been suggested for preoperative CEA concentrations in patients with colorectal, breast and lung carcinoma.
- The CEA test is **not recommended as a screening procedure** for detection of cancer in the general population or in an otherwise asymptomatic patient but rather as an adjunctive test to aid in predicting prognosis and in management of cancer patients.

Principles of the Procedure

- The VITROS CEA test is performed using the VITROS CEA Reagent Pack and the VITROS CEA Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600Integrated System using Intellicheck® Technology.
- An immunometric immunoassay technique is used.
- CEA present in the sample reacts simultaneously with a biotinylated antibody (mouse monoclonal anti-CEA) and a horseradish peroxidase(HRP)-labeled antibody conjugate (mouse monoclonal anti-CEA).
- The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction.

- A reagent containing luminogenic substrates (aluminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide)increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of CEA present.
- Test Type System * ImmunometricECi/ECiQ, 3600,5600
- Incubation Time 30 minutes
- Time to first result 38 minutes
- Test Temperature 37 °C
- Reaction Sample Volume 20µL


Streptavidin
Coated Well
Biotinylated
mouse
monoclonal
anti-CEA
antibody
CEA
HRP-labeled
mouse
monoclonal
anti-CEA
antibody
Signal Reagent
with Enhancer
Luminescence

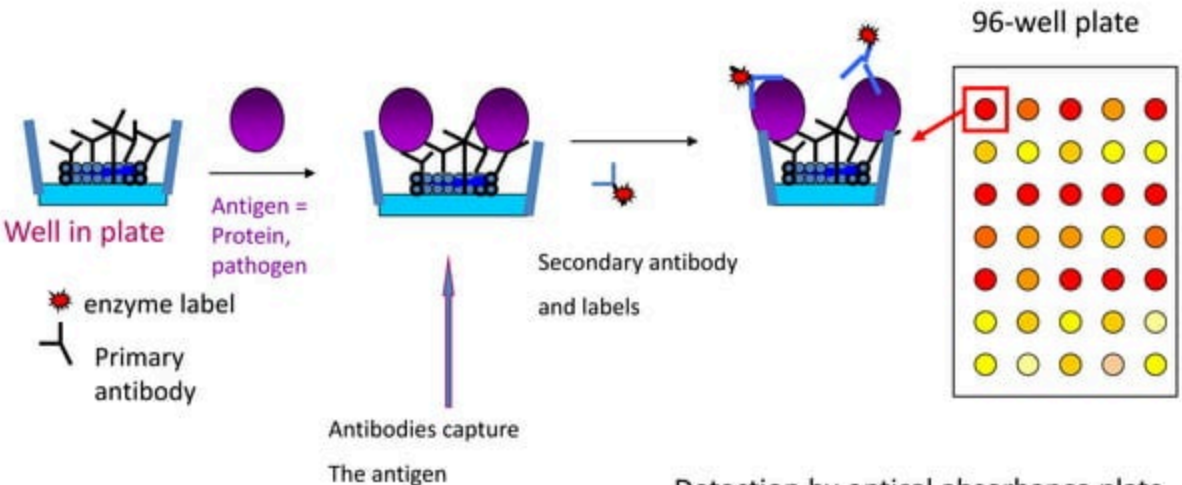
Biomarker Targets: 1. Prostate Specific Antigen

Adapted From Brookhaven Protein Databank

- PSA - Single chain glycoprotein , MW 33 kDa Sensitive, specific biomarker for detection of prostate cancer years before clinical signs of disease
- Detection of PSA in serum: clinical detection of prostate cancer: 4-10 ng/mL
- Led to less invasive treatment protocols, avoid surgery

2. Interleukin 6 (IL-6)
   - prostate and oral cancer biomarker
   - human plasma conc. normal < 6 pg/mL; cancer 20-1000 pg/mL

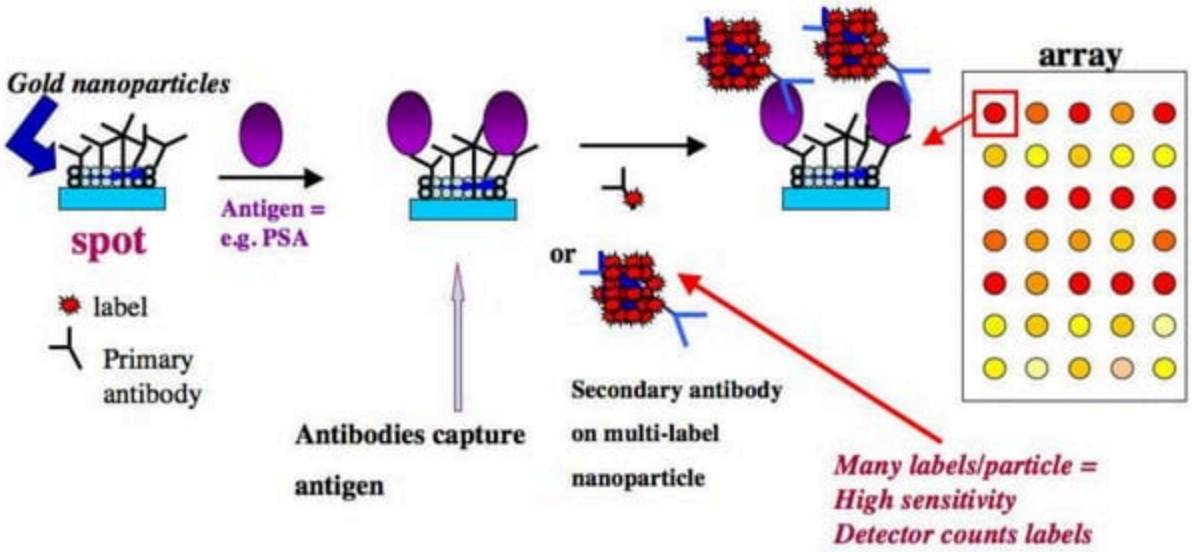
ELISA- enzyme linked immuno-sorbent assay



Detection by optical absorbance plate reader after running enzyme reaction that gives a colored product

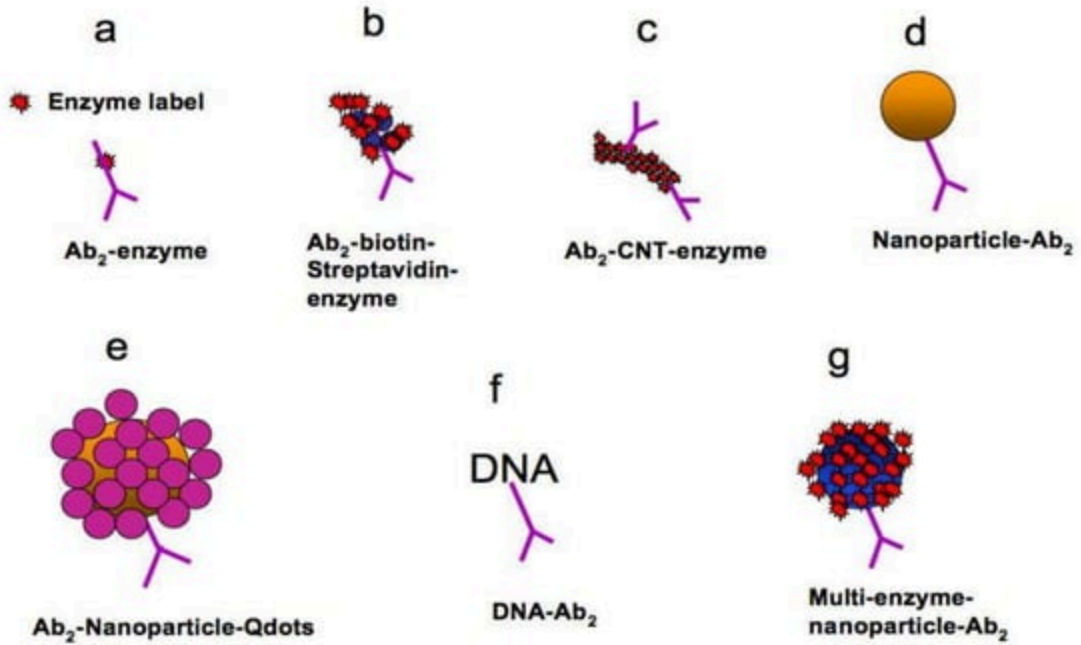
- Reliable method for over 30 years
- Best DL ~ 3 pg/mL in serum
- many commercial assay kits for single proteins
- limitations in sample size, speed, multiplexing

Sandwich immunoassays using nanoparticles

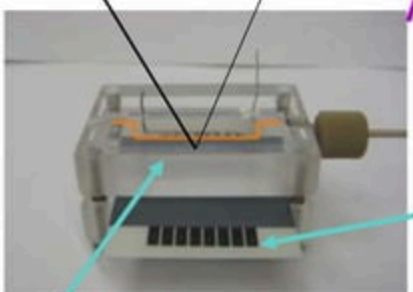


- detection by fluorescence, amperometry, ECL
- non-specific binding must be minimized in any immunoassay

ELISA- enzyme linked immuno-sorbent assay



Off-line capture magnetic particle microfluidic strategy



array electrode

A

Conventional,
single label

B

Multilabel magnetic
Particle, off line capture

Electrical
contacts

8 electrodes
In channel

-0.2V + H_2O_2 + HQ ➔ signal

Ab_2-enzyme

Capture Antibody

AuNP

Protein analyte

1 μm multi-enzyme-
Magnetic particle-Ab_2

7000-400,000 labels

ECLIA & its Advantages in Immunoassay Testing

What Is ECLIA?

E- Electro
C- Chemi
L- Luminescence
IA- Immunoassay

Electrically Controlled Chemiluminescence.

A highly innovative technology that offers distinct advantages over other detection techniques

- Extremely stable non-isotopic label allows liquid reagent convenience.
- High quality assays and fast result turnaround.
- Large measuring range minimizes dilutions and repeats, reducing handling time and reagent costs.
- A solid platform for menu expansion.

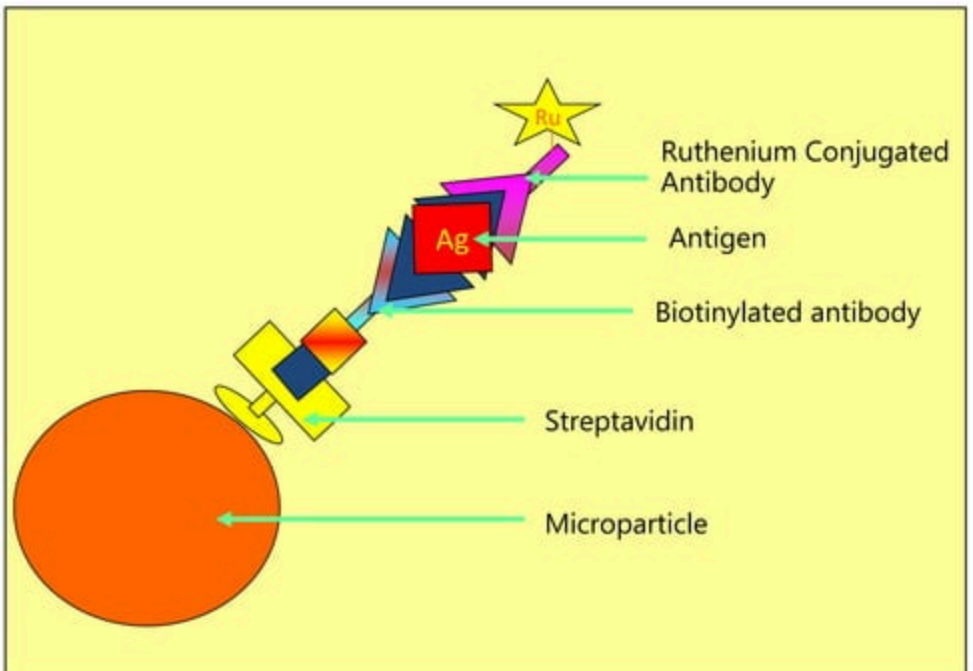
Roche Elecsys 2010 /cobas e411,
Electrochemiluminescence Immunoassay Analyzer

e411 Analyzer:

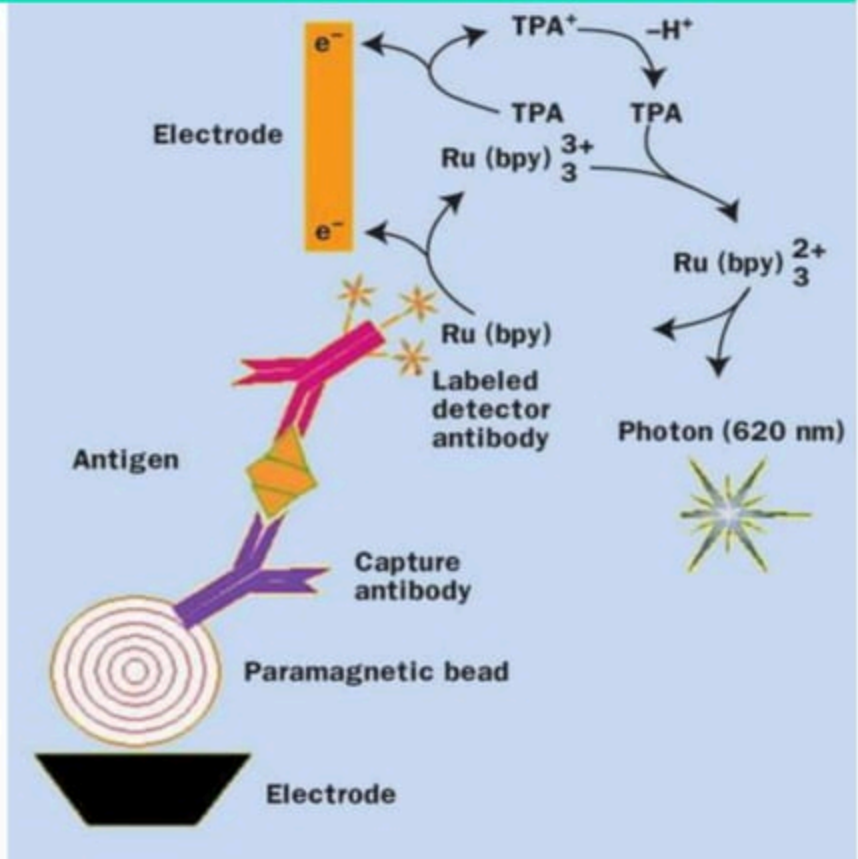
- ECLIA sample selective bench top Analyzer
- Random access system with continuous loading.
- Medium to high volume throughput automated sys.

cobas e411
Electrochemiluminescenc
e Analyzer.

ECL - Electrochemiluminescence Technology



ECL - Electrochemiluminescence Technology



Steps involved in ECL (Electrochemiluminescence) Measurement

Fig 1- Addition of R1+R2+Serum

Fig 2- Addition of M, specific binding

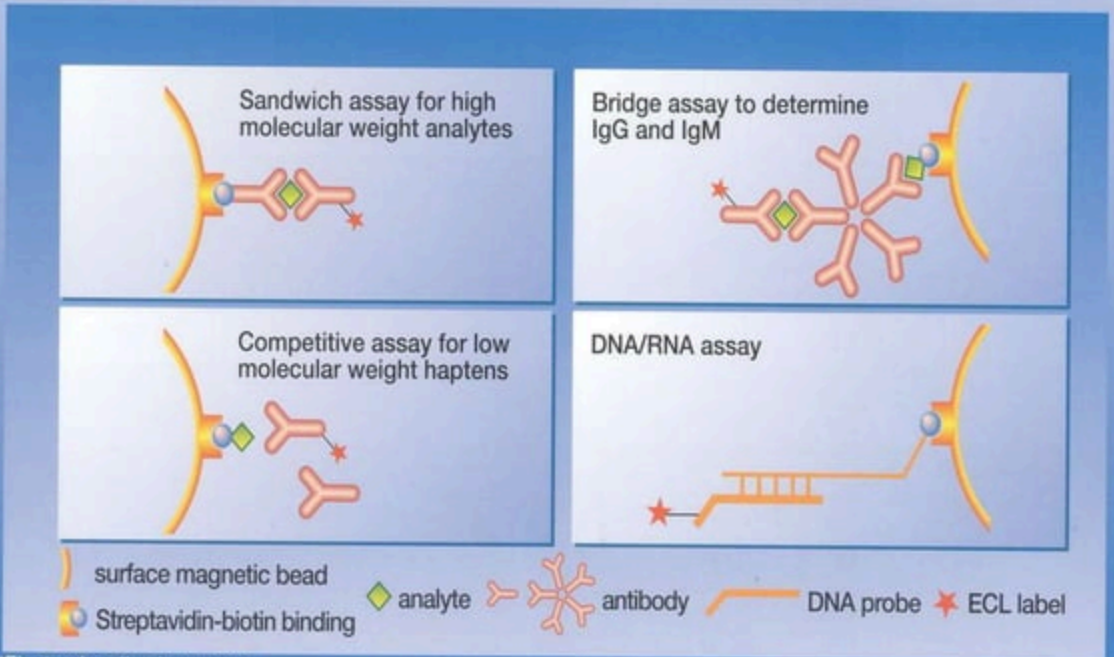
Fig 3- Measuring cell

Step 4- Addition of TPA (Procell),

Step 5- Emission of light

Step 6- Flushing by Clean cell

All immunoassay principles including DNA/RNA assays can be performed with the ECL* bead technology

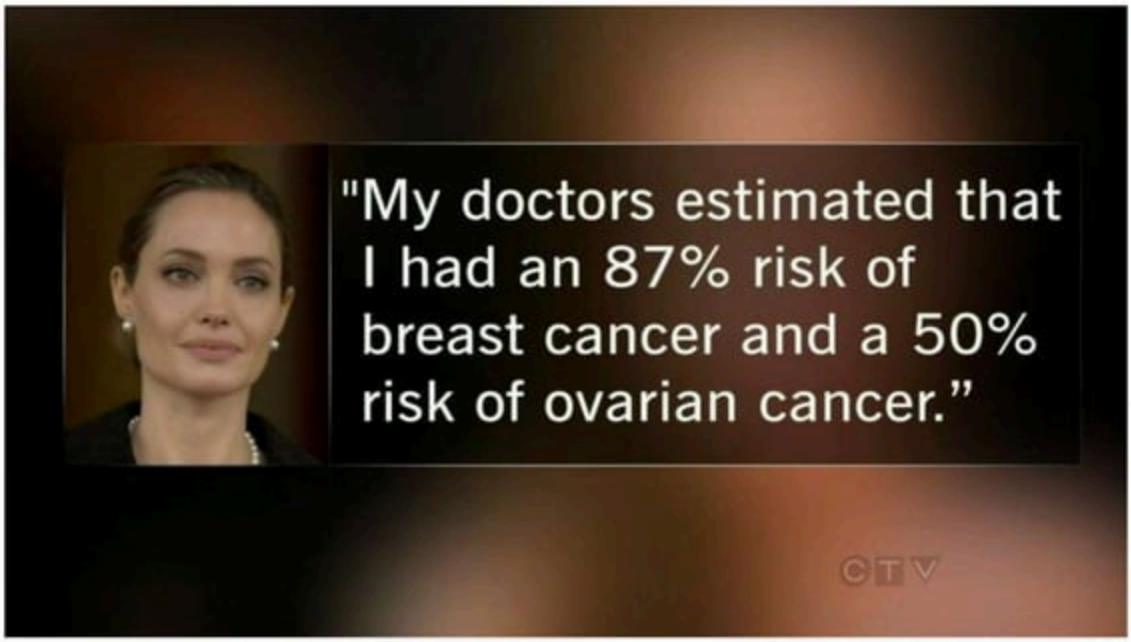


*Electrochemiluminescence

Key aspects and needs:

- Ultrasensitive detection of multiple cancer biomarkers
- Long term objectives
  1. early cancer detection and monitoring
  2. tools for cancer research and surgical decisions
- point-of-care (POC) clinical assays – need to be cheap, simple, fast, accurate, multiplexed

Expensive, and complex methodologies such as LC-MS/MS, some automated optical-based methods are currently not competitive for POC


"My doctors estimated that I had an 87% risk of breast cancer and a 50% risk of ovarian cancer."
CTV


THE
ANGELINA
EFFECT
Angelina Jolie's double mastectomy puts genetic testing in the spotlight. What her choice reveals about calculating risk, cost and peace of mind
BY JEFFREY KLUGER & ALICE PARK

- Cancer screening received a jolt last year when Richard Ablin, who developed the prostate-specific antigen (PSA) test 40 years ago, said he regretted developing it. "PSA testing can't distinguish between the two types of prostate cancer, the one that will kill you and the one that won't," he said, recommending that the test be scrapped.

- A tome written in 1973 by two doctors from Parel's KEM Hospital also don't offer much hope. Dr Manu Kothari and Dr Lopa Mehta, while tracing the history of cancer, summed up in 'The Nature of Cancer' that the disease was incurable. Thirty-eight years later, Kothari still believes so.
- "For 5,000 years, mankind has learnt nothing about how to defeat cancer.'' A website set up recently on the basis of their book ominously states that "early detection is a myth".


THANK YOU