



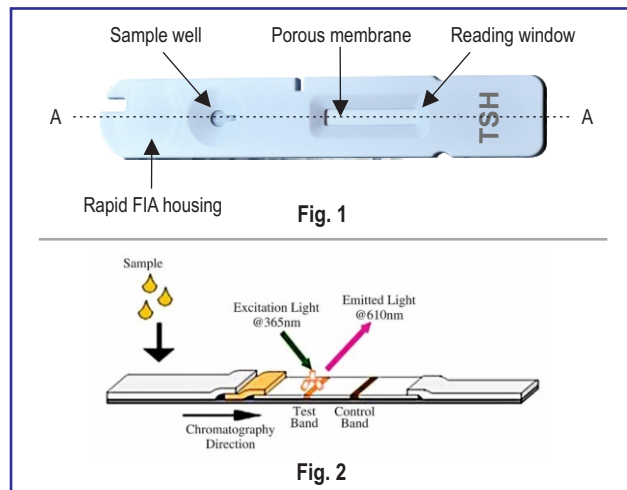
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Editorial

Rapid ICT has gained popularity in recent years amongst all class of Diagnostics laboratories, mainly due to their rapid time to read results and ease of use. Also, no investment in instrument for performing the test. With these advantage Rapid ICT is a perfect choice for many analytes. These Rapid ICT are always optimal for qualitative assay and quantification is a challenge and requires instrumentation for reading and interpretation.

As it's known to all of us, "Necessity is mother of all Invention", a new technology Fluorescence Immunoassay (FIA) has come up as solution to all the requirements. Rapid FIA test system not only gives all the advantages of Rapid ICT but also solve the problem of quantification and documentation. Rapid FIA uses the structure of a general ICT as shown in Figure below. The assay is usually placed inside a plastic housing, which consists of several different functional pads and a porous membrane. There is a backing support at the bottom of the assay. First, the liquid sample is introduced through the sample well of the housing and the sample pad of the assay. It then flows into the conjugate pad, where primary binding of the target analyte and the labeled immunoreagent occurs. Afterwards, the sample is developed through a porous nitrocellulose membrane, where secondary binding of the target analyte and immunofluorescent reagent occurs to form a test line, and another binding independent of the target analyte is induced to form a control line. The control line is used to ensure that a sufficient sample has been added for the test, otherwise the test will be invalidated. Finally, the sample flows into the absorbent pad and the result as fluorescence signal can be measured by the instrument to assign a qualitative or quantitative in terms of numerical value of the analyte concentration in the sample in question.



As is evident this complete issue is dedicated and related to our new product launch based on the technology described above. Hereafter even all class of laboratories shall be able to boast of quantitative assays of parameters/ analytes that they were hitherto outsourcing. What's more it can be used as a POCT testing procedure too.

ENJOY!



TIME RESOLVED FLUORESCENCE IMMUNOASSAY

Introduction

An **Immunoassay** is a biochemical test that measures the presence or concentration of a macromolecule or a small molecule in a solution using an antibody (usually) or an antigen (sometimes). The molecule detected by the immunoassay is often referred to as an "analyte". Immunoassay techniques were used traditionally for the assay of polypeptide, thyroid, and steroid hormones in human serum, contributing most to studies in endocrinology. Increasingly, immunoassays have come to contribute to other areas of clinical biochemistry.

There are mainly 2 types of Immunoassays, Qualitative and Quantitative Immunoassays:

Difference between Qualitative and Quantitative Immunoassays

Assays which detect the presence or absence of analyte in the sample are known as **Qualitative Immunoassays**. E.g., Qualisa Ds-DNA, Qualisa CMV IgG, Qualisa HIV 4.0, Qualisa HBsAg.

Assays that measure/quantify the concentration of an analyte in a sample are known as **Quantitative Immunoassays**. E.g., Qualisa 25 OH Vitamin D.

Most common forms of Immunoassays are:

- ELISA- Enzyme Linked Immunosorbent Assay
- CLIA- Chemiluminescence Immunoassay
- RIA- Radio Immunoassay
- FIA- Fluorescence Immunoassay
- ICT- Immuno-Chromatography Test

Fluorescence Immunoassay (FIA)

Fluorescent Immunoassays are simply a different type of immunoassay. The key variable is the biochemical technique used for detecting the binding of the "detection" antibody and the analyte molecule. The advantages of a fluorescent detection system have been known for many years. These include higher sensitive detection of the analyte, simplified reagents, and simpler assay designs. Several breakthroughs have occurred over the past few years that have enabled the implementation of rapid fluorescence-based immunoassay systems.

A modern fluorescent based immunoassay use as the detection reagent a fluorescent compound which absorbs light or energy (excitation energy) at a specific wavelength and then emits light or energy at a different wavelength. The difference between the wavelength of the excitation light and the emission light is called the **Stokes shift**. The greater the shift or difference in the wavelength the less interference there will be by having the excitation light detected as part of the emission light. Recently several technical improvements have occurred that has enabled the implementation of a high sensitivity Rapid immunoassay systems. These include the availability of narrow wavelength light sources, newer more stable fluorophores that have very wide Stokes shifts, stable solid state light detectors and microprocessors to process and analyse the data from each test.

Fluorescence: The phenomenon in which molecules absorb light at one wavelength and emit light at longer wavelength when it is illuminated by a different wavelength.

Fluorophores: The materials having property of emitting light are known as fluorophores.

Recently Fluorescence technology has been vastly used in development of Immunoassay field.

- Uses antibody/antigen labeled with a fluorophores

Types of Fluorescence Immunoassays

Presently for diagnostic use two types of fluorescence immunoassays are used, Steady state fluorescence and Time Resolved fluorescence.

Steady State Fluorescence:

A type of spectroscopy where the intensity of the fluorescence emitted by molecules excited by constant illumination of ultraviolet light is detected as a function of wavelength.

The applications of steady-state fluorescence include excitation and emission scans, synchronous scans and maps, steady-state fluorescence anisotropy, excitation-emission maps, kinetic measurements, and temperature maps.

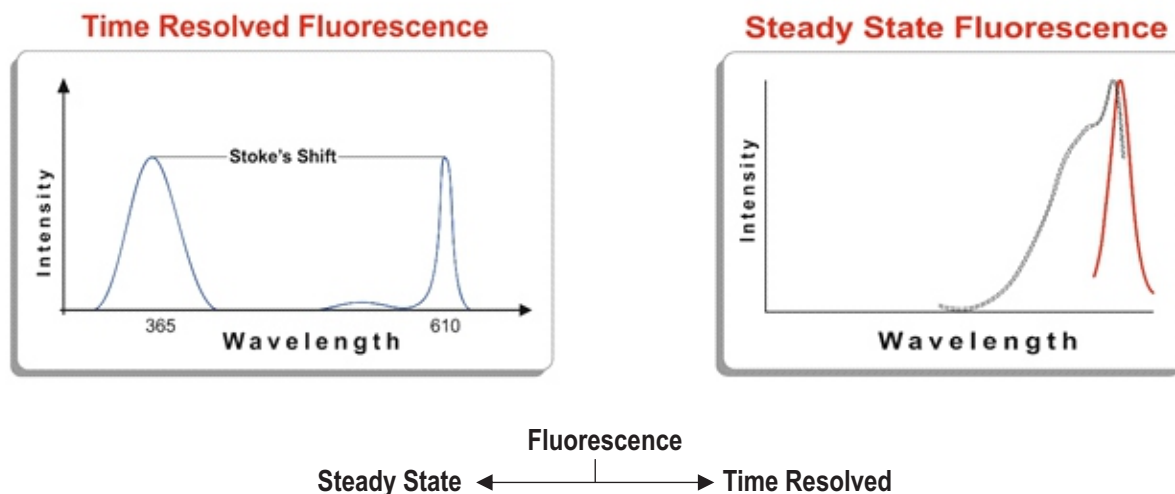
Time Resolved Fluorescence (TRFIA):

Time-resolved fluorescence spectroscopy is a spectroscopy technique used to monitor interactions between molecules and motions that occur in short periods. The ability to measure changes in the picoseconds or nanosecond time range makes it a useful technique in biomolecular structure analysis and dynamics.

Time-resolved fluorescence spectroscopy can detect events within a fluorophore's environment. The events that can be measured are fluorescence decay, indicated by a decrease in fluorescence after excitation, and polarization anisotropic decay, where the reorientation of the emission dipole during excitation is measured.

Measured decay time-resolved fluorescence spectroscopy works based on the duration of the excited state, which can be shortened by certain dynamic events including macromolecular conformation changes, solvent relaxation, side-chain rotation, interactions between neighbouring residues, and other changes in the local environment. The variation is of only a few picoseconds or tens of nanoseconds, meaning measurable changes must occur within this time range. Developments in this type of measurement are making it possible to understand structural information, for example by discerning distances between probes.

Stokes Shift: The difference between the excitation and the emission wavelength is known as Stokes Shift.



Steady State	Time Resolved
The light emitted is detected while excitation is still going on High background signal	The emitted light is detected after the excitation has taken place Low background signal
Stokes shift is relatively very small which can cause self quenching of reagents	Stokes shift is high
Decay time is very less usually in nanoseconds	Decay time is more usually in micro-mili seconds
Biological samples such as serum/tissue often contain autofluorescence particles that can contribute to background signals leading to background noise	Since decay time is more, autofluorescence of samples settles before reading is taken

TRFIA (Time Resolved Fluorescence Immunoassay):

To develop time resolved fluorescence immunoassays special types of fluorophores are used, called **Lanthanides** or Earth materials.

Lanthanides:

- They are a group of uniquely fluorescent chemical elements
- They have very low absorption coefficient and slow emission rates
- This results in prolonged fluorescence decay time (0.1-3) milli seconds
- These elements form trivalent cations (Eu^{3+}) and displays emission in aqueous solution
- Emission peaks are very narrow and very sharp with a large stoke's shift
- Examples: Europium (Eu), Terbium (Tb), Samarium (Sm)

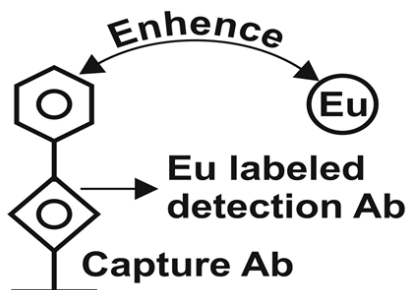
Europium (Eu) is commonly used as labels of TRFIA as along with long decay time it displays large stokes shift; no overlap between excitation and emission wavelength with only single band with a very sharp emission spectrum at 615nm.

Chelates and Cryptates:

- Since the emission of lanthanides is usually too weak for TRF applications, they are generally not directly excited, but are usually embedded in a sort of light collecting cage known as chelates or cryptates.
- It allows both energy collection and energy transfer to the lanthanide ions resulting in higher emission intensities.
- In addition to higher emission signal, chelation makes lanthanide ions conjugation to biological components possible.

Methods:

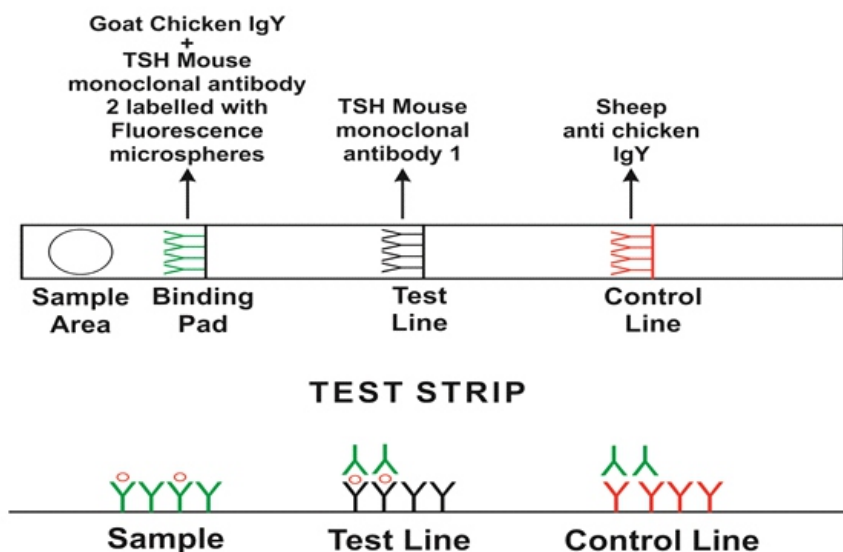
DELFLIA: Dissociation Enhanced Lanthanide Fluorescence Immunoassay



Same as ELISA, only a dissociation step of lanthanide ion is additional.

TRFICA: Time Resolved fluorescence Immuno-Chromatography Assay

(Example of TSH)



Double Antigen Sandwich:

- Test line coated with immobilised TSH monoclonal antibody 1.
- Sheep anti-chicken IgY in the control area.
- TSH mouse monoclonal antibody 2 on building pad along with chicken IgY labeled with fluorescent microspheres.
- TSH in the samples binds to the fluorescent microspheres labeled with TSH mouse monoclonal antibody 2 in the binding pad.
- The Ag-Ab-F moves forward due to capillary action.
- The complex binds to the immobilized antibody on the test area forming complex of fluorescence labeled TSH monoclonal antibody 2-TSH-TSH monoclonal antibody 1
- Fluorescent labeled chicken IgY binds with goat-anti chicken IgG on control line
- When test strip is inserted the analyser scans both T and C and quantify based on the regression graph uploaded by the calibration.

Competitive:

(Example of T3)

- The test line is coated with T3-BSA full antigen complex.
- Sheep anti-chicken IgY coated in the control pad.
- Binding pad coated with T3 sheep monoclonal antibody labelled with fluorescent microspheres and Chicken IgY labelled with fluorescent microspheres.
- T3 in the samples binds to the monoclonal T3 antibody labelled with fluorescent microspheres in the binding pad.
- Unconjugated fluorescent microspheres with T3 antibody binds to the T3-BSA antigen on the test area forming a band.
- The chicken IgY labelled with fluorescent microspheres binds to sheep anti-chicken IgY in the control line forming a band.
- The fluorescence intensity is inversely proportional to the concentration of T3 in the sample.

Advantages of Time Resolved Fluorescence Immunoassay:

- Reduction of background noise
- Large stokes shift
- High intensity signals
- Long assay stability

BOUQUET

In Lighter Vein

From his death bed

The husband called his wife and said, "One month after I die I want you to marry Samy."

"Samy! But he is your enemy !"

"Yes, I know that ! I've suffered all these years so let him suffer now."

Wife: Our new neighbor always kisses his wife when he goes to work, why don't you do that?
Husband: How can I? I don't even know her.



Wife: What is 10 years with me?
Husband: A second.

Wife: What is \$1000 for me?
Husband: A coin.

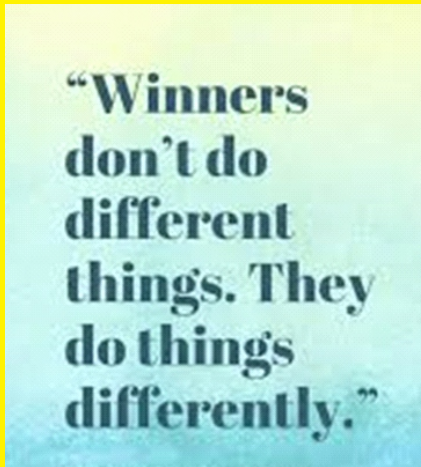
Wife: Ok give me a coin.
Husband: Wait a second

Wisdom Whispers

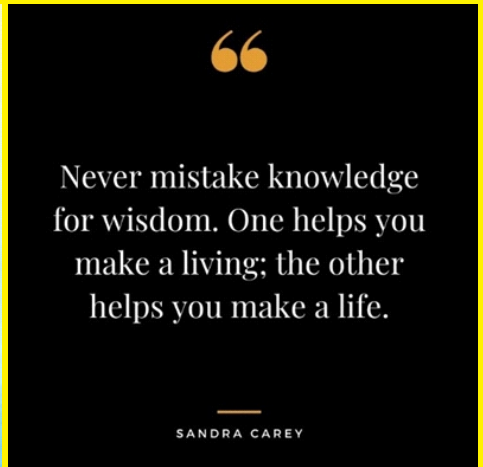


If you want something you've never had, you'll have to do something you've never done.

DAVE RAMSEY



“Winners don't do different things. They do things differently.”



“

Never mistake knowledge for wisdom. One helps you make a living; the other helps you make a life.

SANDRA CAREY

Brain Teasers

- What kind of signal is generated while using FIA technology
 - Fluorescent
 - Colloidal gold based
 - Chemical signal
 - Chemi luminescent
- Which of the following can be used a bedside diagnostic tool
 - ELISA
 - CLIA
 - Molecular tests
 - LFIA
- Which of the following shall be most suitable for primary and secondary peripheral labs ?
 - FIA
 - FISH
 - Molecular pathology
 - Lateral flow immune fluorescent assay
- Which of the following platforms would be fastest and cost effective too?
 - CLIA
 - ELISA
 - Flow cytometry
 - LFIA



Towards The future of Fluorescence

POCT Fluorescence Immunoassay System based on Time Resolved Fluorescence.



FIAcheck™ **1.0**

Time Resolved Fluorescence Analyzer

FIAcheck™ Parameter Menu

Thyroid Markers	TSH	25T
	TT3	25T
	TT4	25T
Fertility Marker	FSH	25T
	LH	25T
	PRL	25T
	β-hCG	25T
	AMH	10T
Cancer Marker	PSA	10T
Rheumatology	Anti-CCP	10T

Diabetic Marker	HbA1C	25T
Inflammation	CRP	25T
	PCT	10T
	IL-6	10T
Anemic Marker	Ferritin	10T
Coagulation	D-Dimer	10T
Cardiac Markers	cTnI	10T
	NT-proBNP	10T
Vitamins	Vitamin D	10T
	Vitamin B12	10T
Allergy	IgE	10T

Not all Fluorescence Analyzers are time resolved

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