Introduction to Immunodiagnostics

RIA, ELISA

Dr. Nilansu Das

Dept. of Molecular Biology

Surendranath College

Clarified syllabus. Clarification shown in blue

An overview of immune response : Innate and adaptive immune response, primary and secondary immune response, organs of mammalian immune system

Basic concept on molecular structure of immunoglobulins(antibody), humoral and cellular immune responses, antigen presentation, MHC

Lymphocytes and immune response: cytotoxic T-cell, helper T-cell, suppressor T-cell
Naive B-cell, plasma cell, memory B-cell

Basic concept in Autoimmune diseases, Immunodeficiency-AIDS and vaccination.

Introduction to immunodiagnostics – RIA, ELISA.

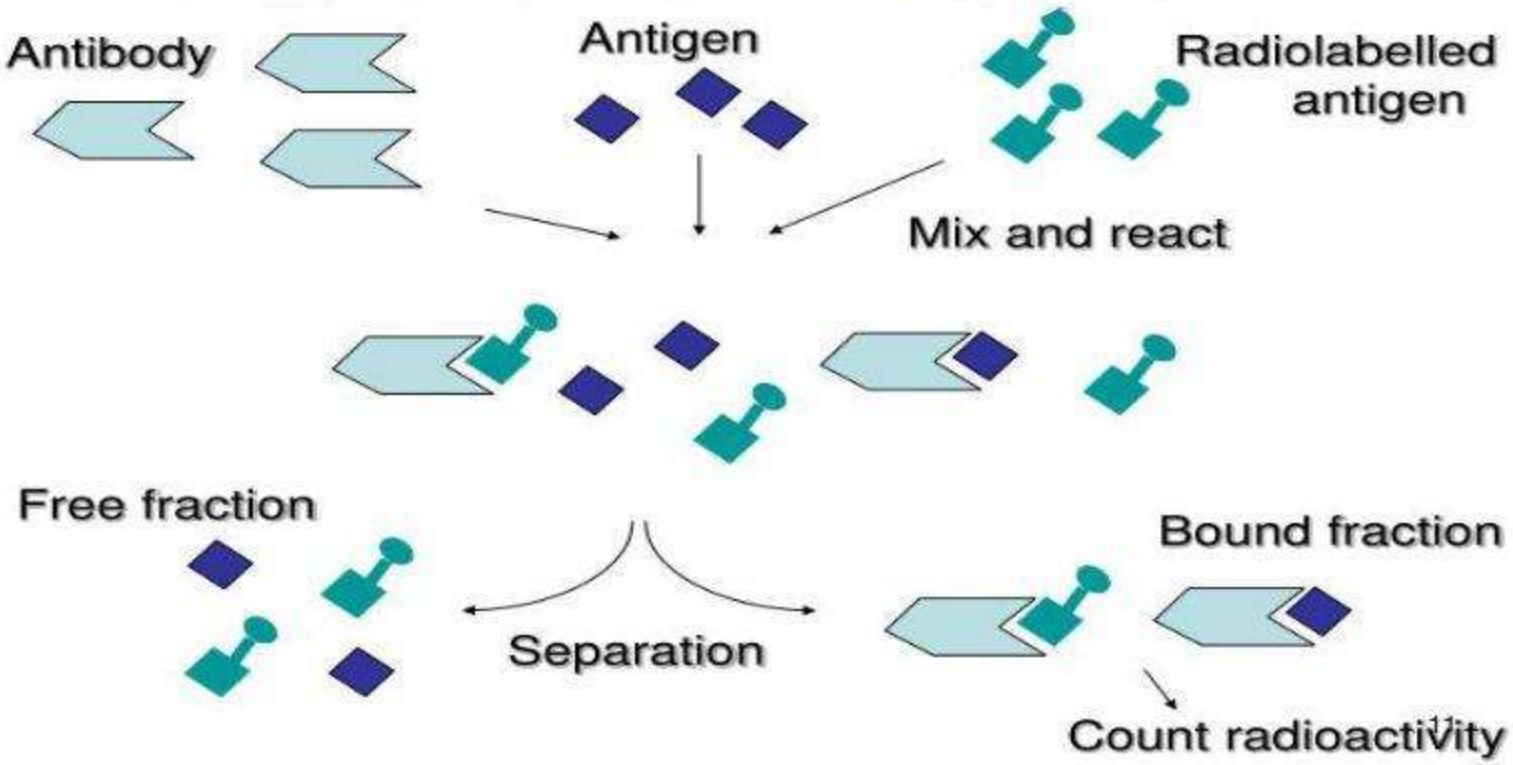
Radioimmunoassay

A radioimmunoassay (RIA) is a very sensitive in vitro assay technique used to measure concentrations of substances (antigen), (for example, hormone levels in blood) by use of antibodies.

Although the RIA technique is extremely sensitive and extremely specific, requiring specialized equipment, it remains among the least expensive methods.

It requires special precautions and licensing, since radioactive substances are used.

Radioimmunoassay (RIA)



Radioimmunoassay Method

To perform a radioimmunoassay, a known quantity of I^{125}- labeled version of the test antigen is mixed with a known amount of cognate antibody and allowed to bind.

Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added.

This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites.

As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen.

The bound antigens are then separated and the radioactivity of the free(unbound) antigen remaining in the supernatant is measured using a gamma counter.

Radioimmunoassay Method ...contd

This method can be used for any biological molecule in principle and is not restricted to serum antigens.

The RIA would begin with the "cold" unlabeled antibody being allowed to interact and bind to the target molecule in solution. Preferably, this unlabeled antibody is immobilized in some way, such as coupled to an agarose bead, coated to a surface, etc.

Next, the "hot" radiolabeled antibody is allowed to interact with the first antibody-target molecule complex.

After extensive washing, the direct amount of radioactive antibody bound is measured and the amount of target molecule quantified by comparing it to a reference amount assayed at the same time.

This method is similar in principle to the non-radioactive sandwich ELISA method.

ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) is a common immunoassay, in which antibodies, peptides, proteins, and small molecules can be detected and quantified.

This assay is the preferred method to determine the titer of antisera and purified antibodies.

ELISA can also be successfully employed for the quantitative assessment of an antigen in a sample, often available in convenient, easy-to-use kit formats.

Although different immunoassay systems operate within common parameters, each configuration differs in the nature and number of stages needed. The common parameters include immobilization to a solid phase, separation of bound and free reagents by washing steps, and readout from colorimetric, fluorescent, or luminescent signals.

ELISA

The following are key characteristics that make ELISA an ideal method of testing:

Versatile: Many formats using different combinations of reagents can be used. ELISA can be run in a variety of settings, with little equipment investment.

Simple: Multiple samples can be tested in a single assay, providing high capacity, rapid, and inexpensive assays.

Sensitive: Readouts from enzyme catalysts or fluorescent tags efficiently amplifies signal and provides broad range of sample detection.

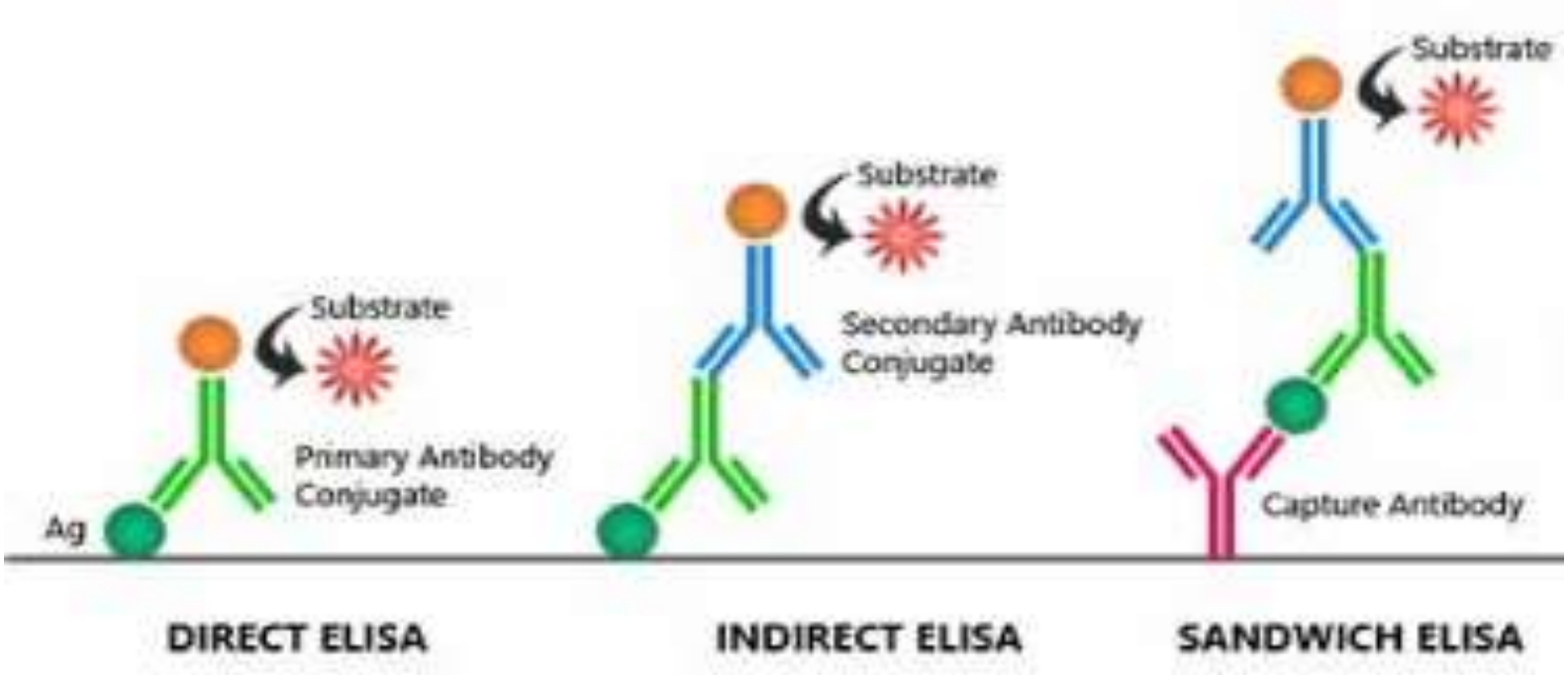
Quantitative:The signal detected by multi-channel spectrophotometers allows data to be stored and analyzed statistically.

ELISA Format

According to the difference of the antigen immobilizing strategy, the antibody labeling strategy, and the type of antibody-antigen reaction (direct recognition or competition), ELISA can be presented in a variety of formats.

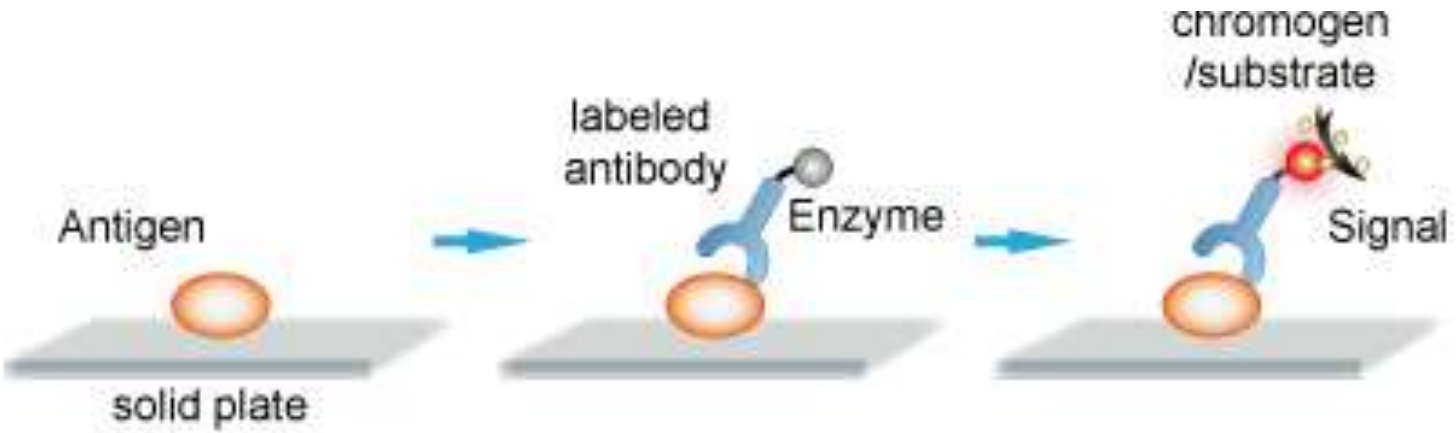
Each has its own advantages and disadvantages.

One can choose an optimal ELISA format flexibly according to the requirements.



Direct ELISA

This is the simplest form of ELISA.
The test antigen is attached to a plastic solid phase by a period of incubation.
Washing step to remove unbound antigen, if any.
Antigen is detected by the addition of an antibody covalently linked to an enzyme.
Incubation to allow binding and washing to remove unbound antigen.
Addition of a chromogen/substrate whereby enzyme activity produces a color change.
Enzyme activity is stopped by chemical means at a defined time.
Color is read in a spectrophotometer



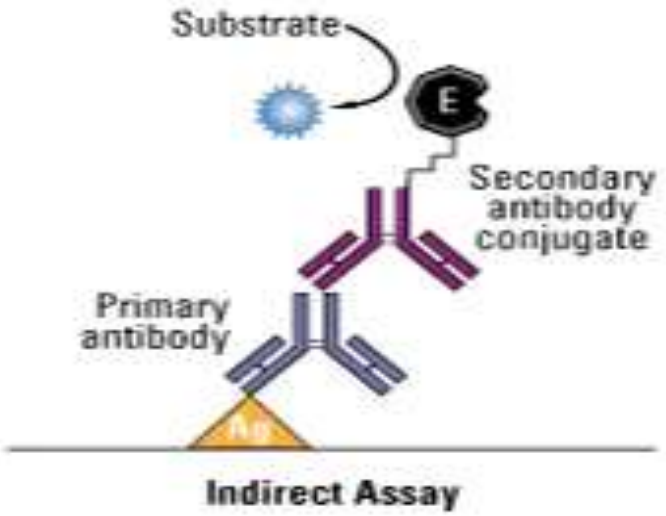
Indirect ELISA

(1) Plate is coated with the antigen to be tested;

(2) The primary antibody is added, which binds specifically to the test antigen coating the well;

(3) The secondary antibody with an attached (conjugated) enzyme is added, which binds specifically to the Fc region of the primary antibody;

(4) substrate is added, and is converted by enzyme to detectable form.



Sandwitch ELISA

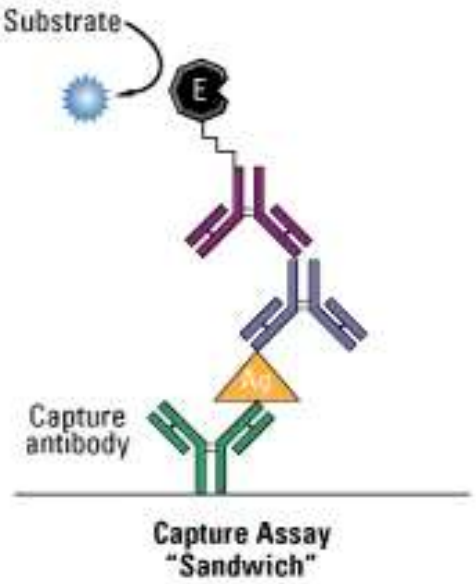
(1)Plate is coated with a capture antibody

(2) sample is added, and any antigen present binds to capture antibody;

(3) detecting antibody is added, and binds to antigen;

(4) enzyme-linked secondary antibody is added, and binds to detecting antibody;

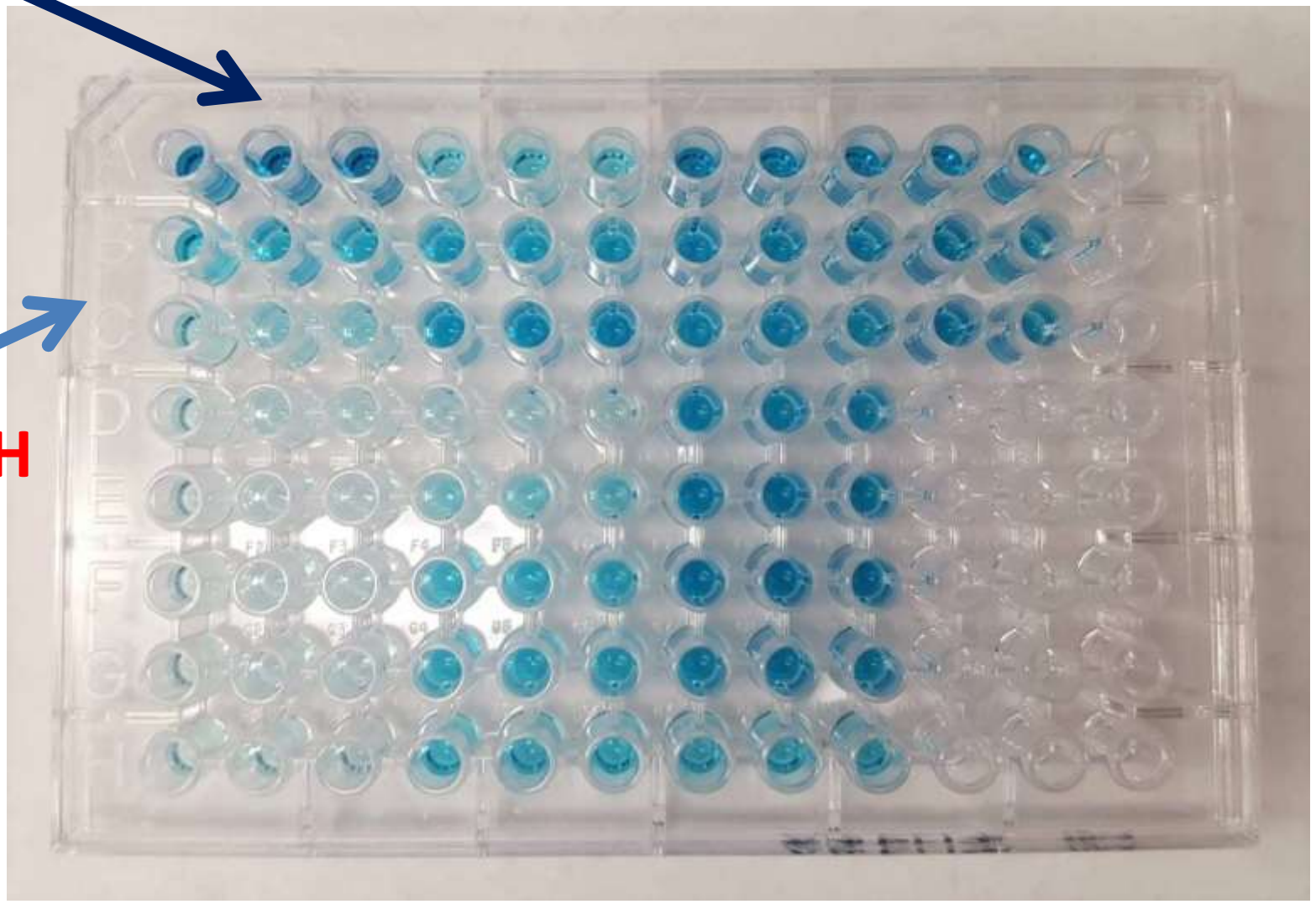
(5) substrate is added, and is converted by enzyme to detectable form.



A typical 96 well ELISA plate

1 - 12

A - H



Comparison of direct, indirect, and sandwich ELISA detection methods

Direct ELISA

Direct ELISA detection	
Advantages	• Quick because only one antibody and fewer steps are used. • Cross-reactivity of secondary antibody is eliminated.
Disadvantages	• Immunoreactivity of the primary antibody might be adversely affected by labeling with reporter enzymes or tags. • Labeling primary antibodies for each specific ELISA system is time-consuming and expensive. • Limited number of conjugated primary antibodies available commercially. • No flexibility in choice of primary antibody label from one experiment to another. • Minimal signal amplification.

Comparison of direct, indirect, and sandwich ELISA detection methods

Indirect ELISA

Indirect ELISA detection	
Advantages	• A wide variety of labeled secondary antibodies are available commercially. • Versatile because many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection. • Maximum immunoreactivity of the primary antibody is retained because it is not labeled. • Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification. • Different detection methods can be used with the same primary antibody (colorimetric, chemiluminescent, etc.).
Disadvantages	• Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal. • An extra incubation step is required in the procedure.

Comparison of direct, indirect, and sandwich ELISA detection methods

Sandwich ELISA

Sandwich ELISA	
Advantages	• Highly sensitive and highly specific for target antigen as two antibodies are used for capture and detection. • Different detection methods can be used with the same capture antibody.
Disadvantages	• Requires more optimization to identify antibody pairs and to ensure there is limited cross-reactivity between the capture and detection antibodies.


Thank You
Dr. Nilansu Das
Dept. of Molecular Biology
Surendranath College