# Radioimmunoassay (RIA)

RIA is an extremely sensitive method that can be used for the quantitation of any substance that is immunogenic and can be labeled with a radioactive isotope (i.e., idone-125). The method is capable of measuring picogram quantities or less, depending on the substance being assayed.

1. Liquid-phase RIAdepends on the competition between labeled (known) and unlabeled (unknown) antigen for the same antibody.

* + A known amount of labeled antigen, a known amount of specific antibody, and an unknown amount of unlabeled antigen are allowed to react together. The antigen-antibody complexes that form are then separated out by either physicochemical or immunologic means (e.g., ammonium sulfate or second antibody precipitation, respectively), and their radioactivity is determined.
  + By measuring the radioactivity still remaining in the supernatant (i.e., unbound, labeled antigen), the percentage of labeled antigen bound to the antibody can be calculated.
  + The concentration of an unknown (unlabeled) antigen can be determined by referring to a standard curve constructed from data obtained by allowing varying amounts of unlabelled antigen to compete.

2. Solid-phase RIA. modifications of the liquid-phase RIA involve absorption or covalent linkage of antibody to a solid matrix (i.e., solid-phase RIA). Unlabeled antigen is then added, followed by labeled antigen. Determination of bound versus free labeled antigen is then made, and the amount of antigen in the unknown sample is calculated by referring to a standard curve.

**RIA has wide application** in the quantitation of a range of biologic substances, including:

* + **a number of hormone** (e.g., insulin, growth hormone, adrenocorticotropic hormone, triiodothyronine, thyroxine, estrogen)
  + **Serum proteins** (e.g., carcinoembryonic antigen, IgE)
  + **Metabolites** (e.g., adenosine 3':5'-cyclic phosphate, folic acid)
  + **Drugs** (e.g., digoxin, digitoxin, morphine)
  + **Other microbial agents and antibodies** [e.g., hepatitis B surface antigen (HBsAg)]

# Immunofluorescence tests

In this test antibodies could be labeled with molecules that have the property of fluorescence. Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength (emission). If antibody molecules are tagged with a fluorescent dye, or **fluorochrome,** immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength.

Disadvantages of these tests are:

* Special training is needed to perform and read Immunofluorescence tests.
* Fluorescence microscope and high quality reagents are required.

A. Direct fluorescent antibody tests

Direct FAT is used to detect and identify an unknown antigen in specimens, for example Viral, bacterial, and parasitic antigens. It is called direct test because the fluorescent dye is attached, or labeled, directly to the antibody. The fluorochrome used is usually fluorescein isothyocynate (FITC), which gives a yellow-green fluorescence. A fluorescent substance is one that, when absorbing light of one wavelength, emits light of another (longer) wavelength.

Interpretation of the results:

Presence of fluorescence: positive test for particular antigen

No fluorescence: negative or absence of particular antigen

Direct FAT can be used;

 To identify bacteria when the numbers are very low

 To detect viruses growing in tissue culture or tissues from infected animals such as rabies virus in the brains of infected animals or antigens of HIV on the surface of infected cells.

## B. Indirect Fluorescent antibody test

In the indirect FAT, unlabelled antibody combines with antigen and the antigen antibody complex is detected by attaching a fluorescent-labeled antispecies globulin to the antibody. The antibody, therefore, is labeled indirectly. Fluorescent-labeled antihuman globulin is used if the antibody is of human origin.

The indirect FAT is used in two main ways:

 To detect and identify unknown antigen in specimens

 To detect antibodies in a patient serum using a known antigen (microorganism).

**I. Indirect FAT to detect antigen**

In this test, a slide preparation of the specimen is made and unlabelled specific antibody is added. After allowing time for the antigen and antibody to combine, the preparation is washed leaving only antibody that has combined with the antigen on the slide. A fluorescent labeled anti- species globulin is added and allowed to combine with the antibody. The excess is washed from the slide. The preparation is examined by fluorescence microscopy using the correct filters. The antigen-antibody complex will be seen fluorescing brightly.

**II. Indirect FAT to detect Antibody**

In this test, a known antigen is placed on the slide and the patient's serum is added. The preparation is then washed and fluorescent-labeled antihuman globulin is added to demonstrate the antigen-antibody reaction. The preparation is examined by fluorescence microscope using the correct filters.

# ELISPOT (Enzyme Linked Immunospot technique)

The enzyme-linked immunospot (ELISpot) assay is a highly sensitive immunoassay that measures the frequency of cytokine-secreting cells at the single-cell level. In this assay, cells are cultured on a surface coated with a specific capture antibody in the presence or absence of stimuli. Proteins, such as cytokines, that are secreted by the cells will be captured by the specific antibodies on the surface. After an appropriate incubation time, cells are removed and the secreted molecule is detected using a detection antibody in a similar procedure to that employed by the ELISA. The detection antibody is either biotinylated and followed by a streptavidin-enzyme conjugate or the antibody is directly conjugated to an enzyme. By using a substrate with a precipitating rather than a soluble product, the end result is visible spots on the surface. Each spot corresponds to an individual cytokine-secreting cell. The ELISpot assay is carried out in a 96-well plate, and an automated ELISpot reader is used for analysis.

