RADIO IMMUNOASSAY (RIA)

Introduction:

- An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result.
- An antibody antigen complex is also known as an immune-complex.
- Immunoassays are different from other types of laboratory tests, such as colorimetric tests, because they use antibody: antigen complexes to generate a signal that can be measured.
- RIA is an exquisitely sensitive assay method that is capable of measuring with great accuracy concentrations in nanograms and picograms utilizing very small volume of the sample.

PRINCIPLE OF RADIOIMMUNOASSAY

Principle:

- Uses an immune reaction [Antigen – Antibody reaction] to estimate a ligand

$$\mathbf{Ab + Ag + Ag^* \rightarrow Ag\,Ab + Ag^*Ab + Ag + Ag^*}$$

- The labeled Ag* and free Ag are saparated and washed out.
- Radioactivity of bound residue is measured
- Ligand concentration is inversely related to radioactivity

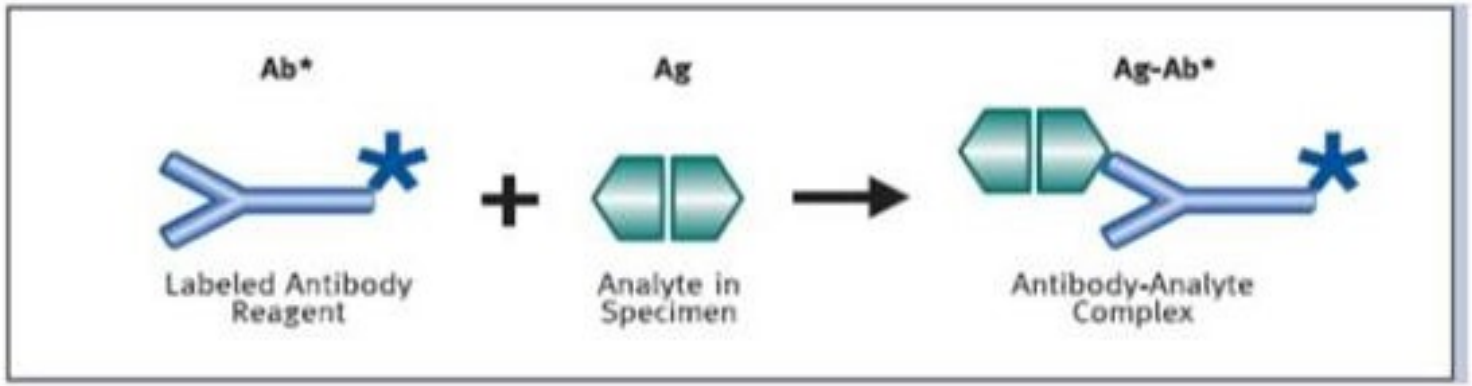
[Ag : ligand to be measured ; Ag* radiolabelled ligand]

What is a label?

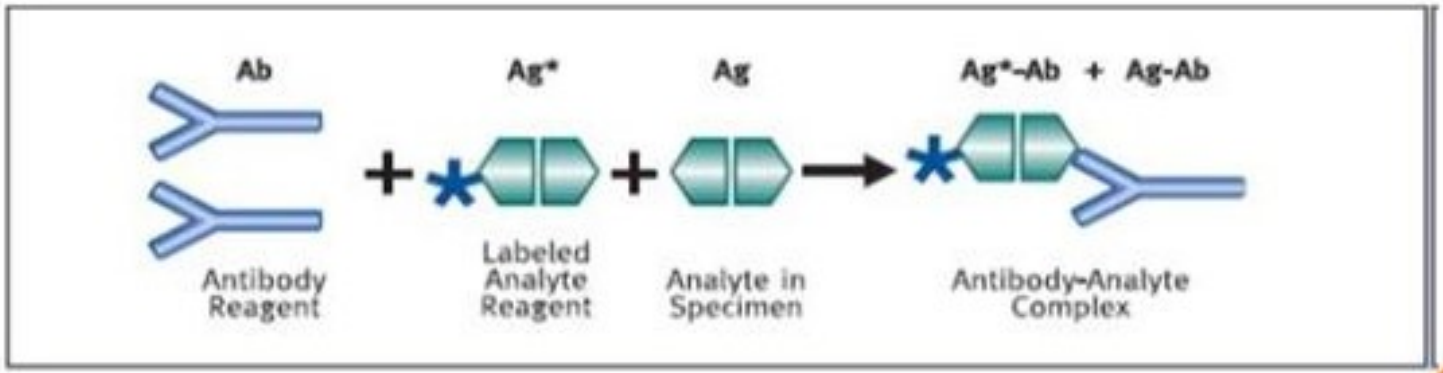
- All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present.

- A label is a molecule that will react as part of the assay, so a change in signal can be measured in the blood : reagent solution.

- Examples of a label includes-
  - ✓ A radioactive compound,
  - ✓ An enzyme that causes a change of colour in a solution,



Labeled antibodies allow detection of antigen/antibody complexes in immunoassays



Labeled antigen also allows detection of antigen/antibody complexes in immunoassays

General procedure for RIA:

A known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine attached to tyrosine.

This radio labeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two chemically bind to one another.

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 radio labeled antigen for antibody binding sites.
- 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 radio labeled antigen to free radio labeled antigen.
- The radioactivity falls because unlabelled antigen dilute it.
- The count obtained from the radioactivity are used to determine the hapten concentration in the sample, the interpretetion being done on the standard curve.

REQUIREMENTS FOR RIA

1. Preparation & characterisation of the Antigen [Ligand to be analysed]
2. Radiolabelling of the Antigen
3. Preparation of the Specific Antibody
4. Development of Assay System

PREPARATION & RADIOLABELLING OF THE ANTIGEN

- Antigens prepared by..
  - Synthesis of the molecule
  - Isolation from natural sources
- Radio labelling [Tagging procedure]
  - ^{3}H ^{14}C ^{125}I are used as radioactive tags
  - Antigens are tagged to ^{3}H ^{14}C ^{125}I
  - Tagging should not affect Antigenic specificity & Antigenic activity

PREPARATION OF THE SPECIFIC ANTIBODY

- Antigen injected intradermally into rabbits or guinea pigs → antibody production
- Antibodies recovered from the serum
- Some ligands are not Antigenic
  - Hormones, Steroids, Drugs → *HAPTENS*
  - *Eg:* Gastrin, Morphine,
  - Haptens conjugated to albumin → antigenic

ASSAY PROCEDURE

Add known amounts of the test sample + labelled antigen into the microtitre wells

↓

Incubate → allow the reaction to reach completion

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Decant & wash contents of the well → removes all unbound antigens

↓

Radioactivity remaining in the Microtitre wells measured by a Counter [Gamma counter , Scintillation counter etc]

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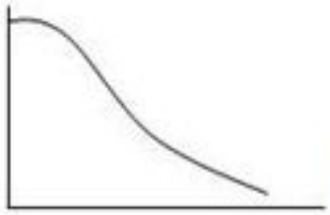
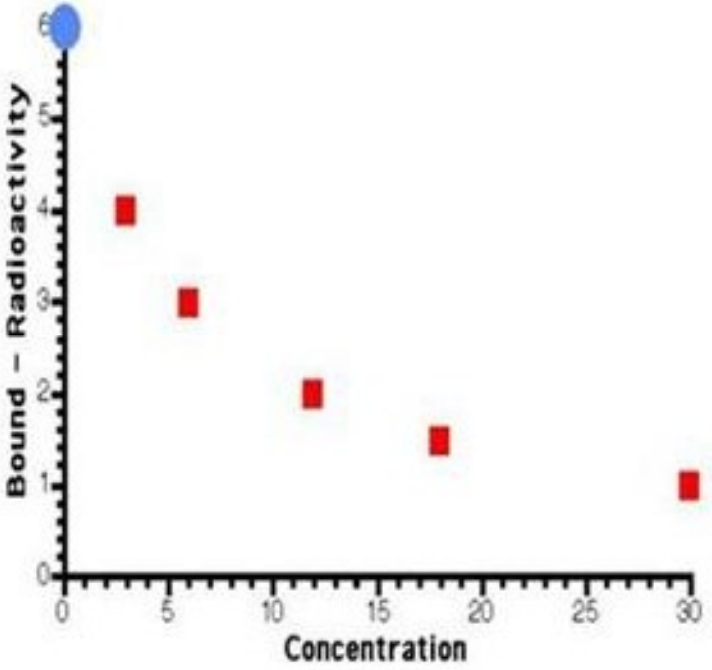
Intensity of radioactivity is inversely correlated with the concentration of the antigens in the test sample

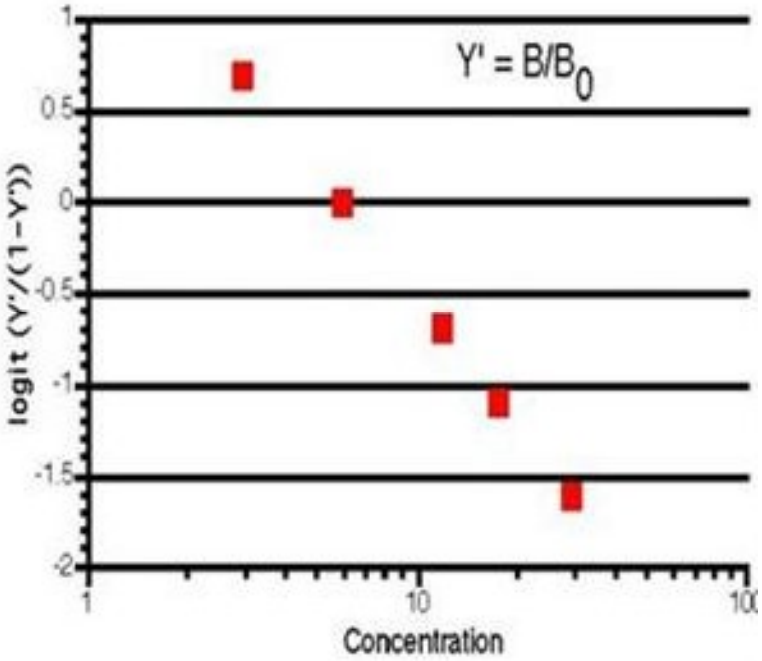
↓

Sensitive to very low concentration of antigens





Plot of Bound versus Total Drug Concentration



Logit versus Log Total C Plot

INSTRUMENTATION:

1)Centrifuge :

swing bucket rotor : 1200-2500 rpm.

Fixed angle head rotor : 3500-4000 rpm.

2) Radioactive counter:

A) gamma counter : which is used for agmma energy emiting isotopes. E.g. 125I .

B) scintilation counter : It is used for beta energy emitting isotopes . Eg. Tritium 3H and 14 C isotopes.

ADVANTAGES & DISADVANTAGES OF RIA

- **Advantages:**

  Highly specific: Immune reactions are specific

  - High sensitivity : Immune reactions are sensitive

- **Disadvantages** :
  - Radiation hazards: Uses radiolabelled reagents
  - trained persons
  - special license
  - Requires special arrangements for Requisition, storage of radioactive material
    - radioactive waste disposal.

APPLICATION:

- RIA has been used to assay plasma levels of :most of hormones :Insulin in human plasma, -β-HCG in females, vasopressin.
- Digitoxin or Digoxin in patients receiving these drugs.
- Certain abused drugs,
- eg:Morphine,
- Anti-DNA antibodies in systematic lupus erythematosus(SLE)

Thank You