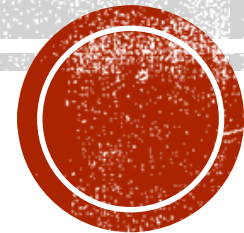


# **LAB-5- RADIOIMMUNOASSAY**

**Submitted by**  
**assist.Lec.Hiba Hadi Rashid**



- **History**
- **Definition**
- **Principle**
- **Requirements**
- **Methods**
- **Applications**
- **Advantage**
- **Disadvantage**



# HISTORY

- In 1959 This type of technique firstly was developed by two endocrinologist Soloman Berson & Rosalyn Yalow in New York when perform their experiment for detection the levels of Insulin Complexes in diabetes.
- In 1977 Dr.Yalow award Nobel Prize for the significance of this technique.
- In Recent years this technique used for detect types of Materials present at concentration measured by( picograms,nanograms and micrograms/ml)



# DEFINITION OF RIA

- **RADIOIMMUNOASSAY(RIA):** It is Serological Method based on immunological antigen-antibody reactions. This Immunoassay uses Antigens Labelled with Radioisotopes.
- This technique is very specific and very sensitive in Vitro for detecting Micro quantities of proteins, Viral antigens, Structural Proteins, Vitamins , Drug and their Metabolites and Antigen Present at Concentration of <math><0.001</math> micrograms/ml.

▪ Radio



Use of radio active material

Immuno



Antigen-Antibodies binding  
Complex

Assay



Detection of Compound



# PRINCIPLE OF RIA

❖ The principle of RIA involves in three Notes Which Make(RIA) More Specific and Sensitive than other Immune- assays

A. An Immune Reaction i.e Antigen-Antibody Binding.

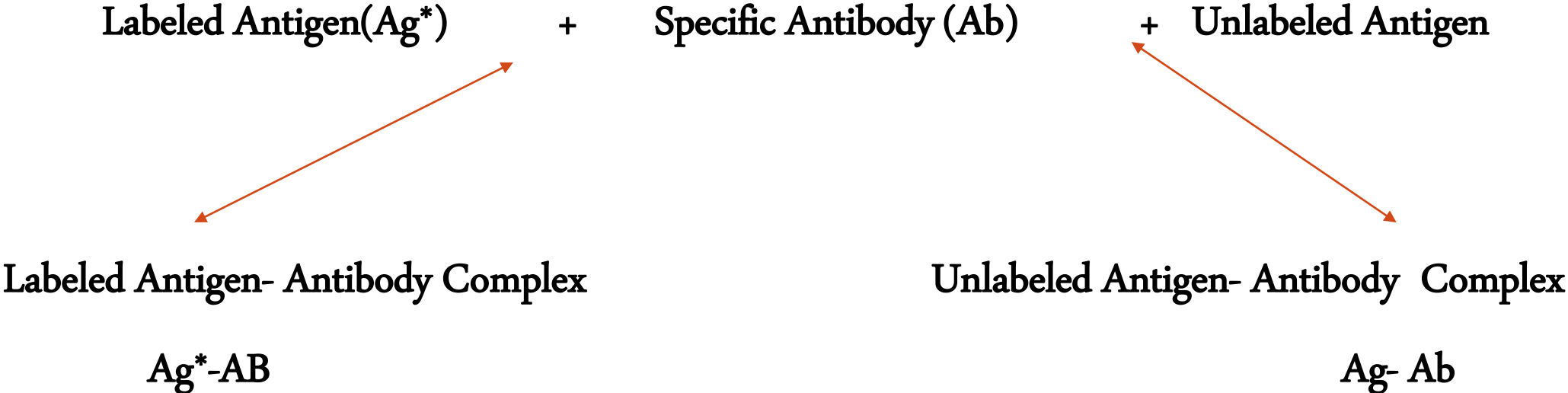
B. A competitive binding or Competitive displacement Reaction(It gives Specificity).

Competitive Binding of Radiolabeled antigen and unlabeled antigen to high affinity antibody. The Antibodies does not Distinguish between Labeled and unlabeled Antigens so the Two Kinds of Antigens Compete for available Binding Sites on the Antibodies. The Concentration of unlabeled Ag increases, more Labeled Ag will be displaced from the binding site.

C.Measurment of Radioemission . .(It gives Sensitive)



# PRINCIPLE OF RIA

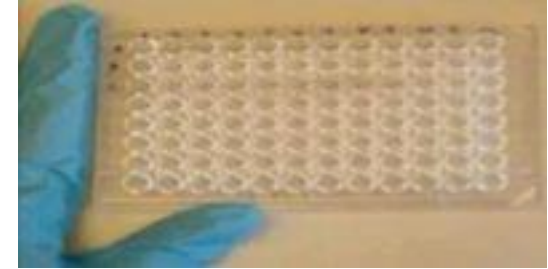


\* Labeled antigen



# REQUIREMENTS

- Microtiter plate: It could have 6, 24, 96, 384 or even some times 1536 wells arranged in rows, each wells can hold very small amounts of Liquid.



- Pure Antigen: may be obtained from biological sample or by synthetic form.

Radiolabeled Antigen: **The Antigen generally Labeled with:-**

**Gamma- Emitting Isotope such as 125I.**

**Beta- Emitting Isotope such as tritium 3H and C14.**

- **Specific antibodies are obtained by injecting the Ag to animals**



# REQUIREMENTS

Radio active counters

- Two types of counters used :

a) Gamma Counter: These used for counting gamma energy .



b) Scintillation Counter : used for counting beta energy.





# METHODS OF RIA

■ There are two Types of RIA :

1. Direct RIA

2. Indirect RIA

you can explain the procedure of these Methods by using the HepatitisB –virus kit in blood sample

a) Microtitter wells are coated with constant amount of antibody specific for HBsAg.

■ A serum Sample and (125I) HBsAg are then added.

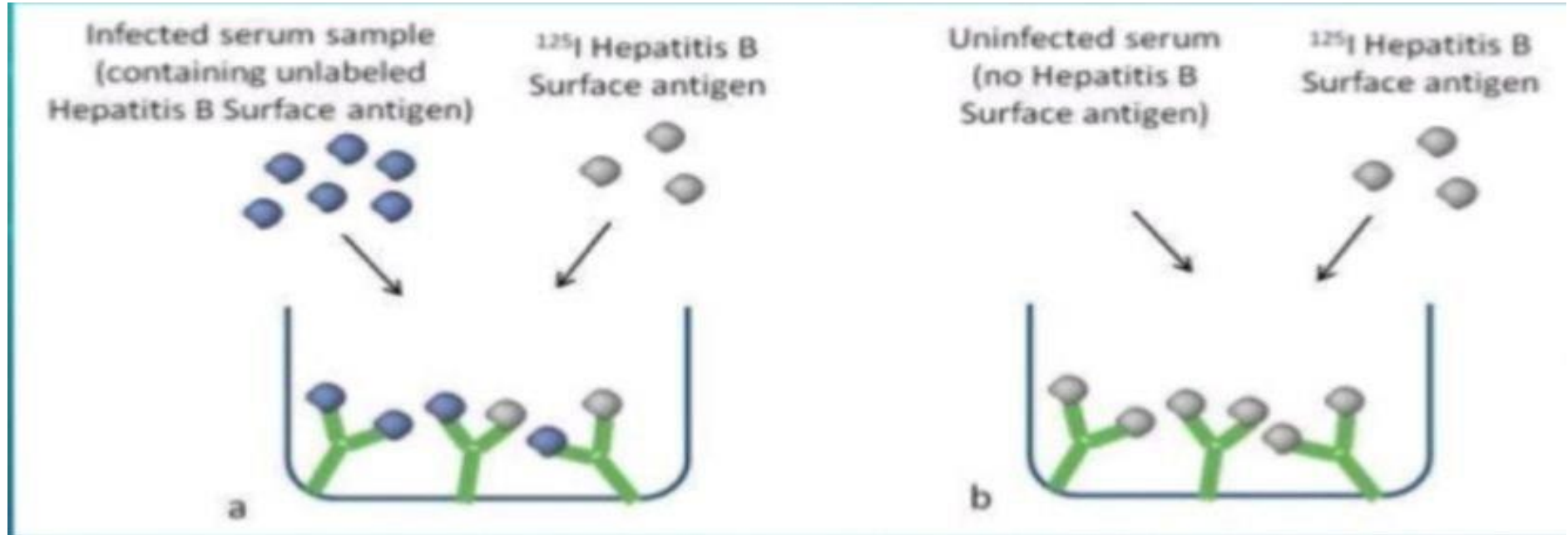
■ After incubation, the supernatant is removed and the radioactivity of the Antigen-antibody complexes is measured.

b) Standard curve is obtained by adding increasing Concentrations of un labeled HBsAg to affixed quantity of (125I) HBsAg and Specific antibody.



# METHODS OF RIA

Direct RIA:



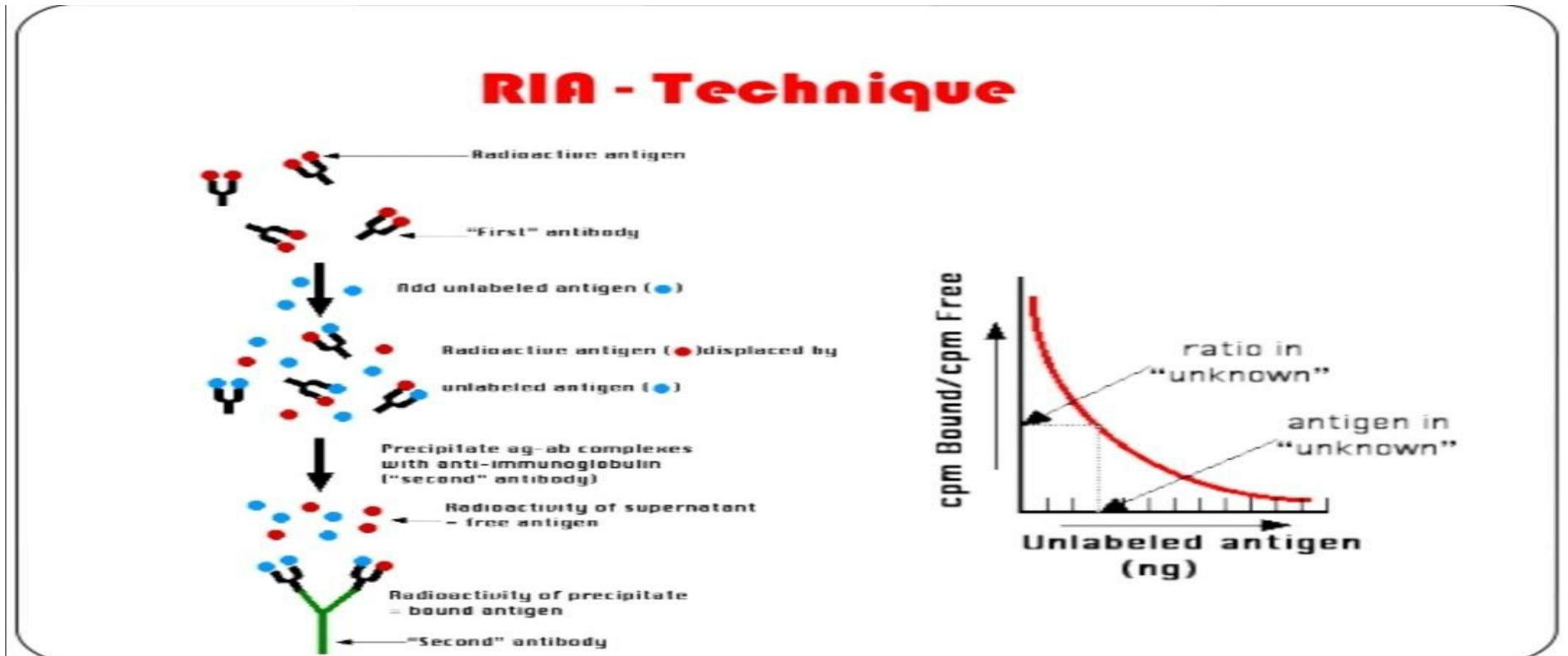
Fig(1)-a) Sample Containing high amount of Antigen, the unlabeled antigen Compete for binding to Ab in the tube or well.

b) Sample Containing no or Low amount of Ag , Ab is bound by the Radiolabeled Ag(tracer).



# METHODS OF RIA

- Indirect RIA



- Fig(2) Indirect RIA, Radioactive Antigen(tracer) is added to the antibody, followed by addition of unlabeled antigen from sample or from standard. The Ag-Ab complexes formed are precipitated using a precipitating reagent (in the figure shown, a secondary antibody) to separate bound and free tracer.



# APPLICATIONS OF RADIOIMMUNOASSAYS

## Epidemiology

Hepatitis B virus & HIV Antigens

## Clinical Immunology

Antibodies for inhalant Allergens

Allergy Diagnosis

## Oncology

Early Cancer Detection and Diagnosis



# ADVANTAGES OF RIA

- Highly specific.
- Radioimmunoassay its widely used because of its highly Sensitive.
- Using Antibodies of high affinity, it used to detect a few picograms of materials to the tube



# DISADVANTAGES

- Radioimmunoassay hazards: Uses radio labeled reagents
- Required specially trained persons.
- Labs require special License to handle radioactive material.
- Requires of special arrangements for requisition.

Storage of radioactive material.

Radioactive waste disposal.



**THANK  
YOU**