Radioimmunoassay
&
Enzyme Linked Immunosorbent Assay

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Principle of Radioimmunoassay

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

Ag + Ag* + Ab → AgAb + Ag*Ab + Ag + Ab*

- Unbound Ag* and Ag washed out
- Radioactivity of bound residue measured
- Ligand conc is inversely related to radioactivity

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

Advantages & Disadvantages of RIA

- Advantages
  - Highly specific: Immune reactions are specific
  - High sensitivity : Immune reactions are sensitive
- Disadvantages
  - Radiation hazards: Uses radiolabelled reagents
  - Requires specially trained persons
  - Labs require special license to handle radioactive material
  - Requires special arrangements for
    - Requisition, storage of radioactive material
    - radioactive waste disposal.

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
- Radiolabelling [Tagging procedure]
  - ^{3}H ^{14}C ^{125}I are used as radioactive tags
  - Antigens are tagged to ^{3}H ^{14}C ^{125}
  - Tagging should NOT affect **Antigenic specificity & Antigenic activity** !

Preparation of the Specific Antibody

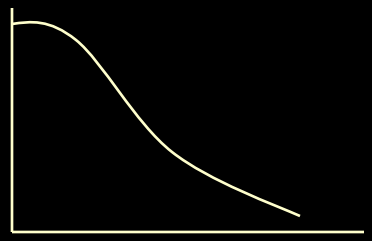
- Antigen injected intradermally into rabbits or guinea pigs → antibody production
- Antibodies recovered from the serum
- Some ligands are not Antigenic
  - Hormones, Steroids, Drugs → *HAPTENS*
  - *Eg:* Gastrin, Morphine,
  - Haptens conjugated to albumin → antigenic

Development of the Assay System

- A crucial step is separation of unbound antigens
- This achieved by binding the antibodies to the microtitre well surface [Solid phase RIA]
- Antigens bound to the fixed antibodies remain stuck to the inner surface
- Decanting & washing the well removes unbound antigens
- Other techniques of separation: Centrifugation

Assay Procedure

- Add known amounts of the test sample + labelled antigen into the microtitre wells
- Incubate → allow the reaction to reach completion
- Decant & wash contents of the well → removes all unbound antigens
- Radioactivity remaining in the Microtitre wells measured by a Counter [GM counter , Scintillation counter etc]
- Intensity of radioactivity is inversely correlated with the conc of antigens in the test sample
- Sensitive to very low conc of antigens



Enzyme Linked Immunosorbent Assay

- Principle:
  - Uses an immune reaction like RIA
  - Differs from RIA in detection method
  - Detection based on
    - Enzyme catalysed reaction OR
    - Fluorescent probe
    - NOT radioactivity [great advantage!]

Advantages of ELISA

- Sensitive: nanogram levels or lower
- Reproducible
- Minimal reagents
- Qualitative & Quantitative
  - Qualitative → Eg HIV testing
  - quantitative assays → Eg Ther. Drug Monitoring
- Greater scope : Wells can be coated with Antigens OR Antibodies
- Suitable for automation →high speed
- NO radiation hazards

Types of ELISA

1. Noncompetitive binding assay or Sandwich method
    1. Antigen measuring system [Titrewells coated with antibodies ; Enzyme labelled antibodies]
    2. Antibody measuring system [Titrewells coated with antigens ; Enzyme labelled antiantibodies]
2. Competitive binding assay [Titrewells coated with antibodies ; Enzyme labelled antigens]

Noncompetitive or Sandwich Assay

- Antigen measuring system
  - Titre wells coated with suitable antibody
  - Add patient sample containing the antigen
  - Incubate: till antigen antibody reaction is complete
  - Wash→ remove unbound antigen
  - Add Antibody labelled with Enzyme
  - Incubate till antigen binds labelled antibody
  - Wash → remove unbound labelled antibody
  - Add substrate ; incubate
  - Enzyme + Substrate → Product → measure colour
  - Colour proportional to antigen in patient sample

Noncompetitive or Sandwich Assay

- **Antibody measuring system**
  - Titre wells coated with suitable antigen
  - Add patient sample containing the antibody
  - Incubate: till antigen antibody reaction is complete
  - Wash→ remove unbound antibody
  - Add **Antiantibody** labelled with Enzyme
  - Incubate till **labelled antiantibodies** binds antigen-antibody complex
  - Wash → remove unbound **labelled antiantibody**
  - Add substrate ; incubate
  - Enzyme + Substrate → Product → measure colour
  - Colour proportional to antibody in patient sample

Competitive binding assay

- Titrewells coated with antibodies
- Known quantities of patient sample containing antigen + antigen labelled with enzyme
- Incubate: till antigen antibody reaction is complete
- Wash→ remove unbound antigens
- Add substrate ; incubate
- Enzyme + Substrate → Product → measure colour
- Colour inversely related to antigen in patient sample

Enzyme labels

- Enzyme labels should have high specific reactivity
- Should be easily coupled to ligands & the labelled complex must be stable
- The reactivity should be retained after linking of the enzyme to the antigen/antibody
- The chosen enzymes should not be normally present in the patient samples
- Examples of enzyme labels
  - Horse radish peroxidase, Alkaline phosphatase, Glucose oxidase

Applications of Immunoassays [RIA & ELISA]

- Analysis of hormones, vitamins, metabolites, diagnostic markers
  - Eg. ACTH, FSH, T3, T4, Glucagon, Insulin, Testosterone, vitamin B12, prostaglandins, glucocorticoids,
- Therapeutic drug monitoring:
  - Barbiturates, morphine, digoxin,
- Diagnostic procedures for detecting infection
  - HIV, Hepatitis A, B etc