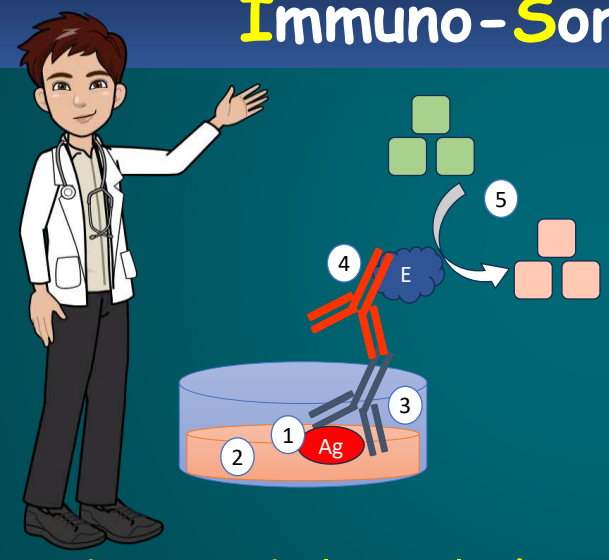
Enzyme Linked Immuno-Sorbent Assay



- ✓Theory/Principle
- ✓Steps
- ✓Types
  - ✓Direct ELISA
  - ✓Indirect ELISA
  - ✓Sandwich ELISA
  - ✓Competitive ELISA
- ✓Application

Instrumental Analysis

/pharmacologyconcepts

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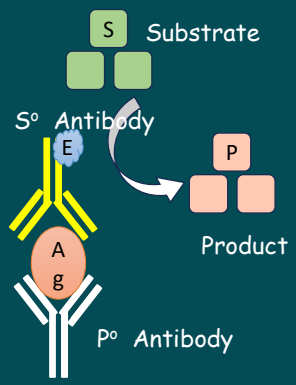
ELISA

Introduction

- The enzyme-linked immunosorbent assay (ELISA) or Enzyme immunoassay (EIA) is an immunological assay commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples.
  - diagnosis of HIV infection,
  - pregnancy tests, and
  - measurement of cytokines or soluble receptors in cell supernatant or serum.
- The term ELISA was first used by Engvall & Perlmann in 1971.
- The ELISA test or the enzyme immunoassay , was the first commonly employed for HIV. It has high sensitivity.



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ELISA

Introduction

- Immuno-assay Method
- Antigen-Antibody Reaction occurs
- Assay components samples are either antigen or antibody
- Enzyme are commonly linked with an antibody
  - Enzymes: Horse Reddish Peroxidase (HRP), Alkaline Phosphatase (ALP)
- Substrate Molecules (chromogen): Tetra methyl benzidine (TMB), para-nitro phenyl phosphate
- Detection: Luminescence (ELISA Reader)
- Highly sensitive up to picko gran (pg)
- Common Antibody- IgG and monoclonal Antibody

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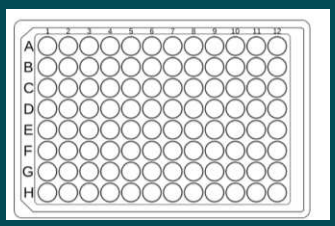
ELISA

Equipment



1. **Microwell Plate and Washer:**
   - 96 well plate (8x12), 350 uL each well
2. **ELISA Reader**

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ELISA

Equipment

3. Reagents Used

Reagent	Composition
Coating Buffer	0.01 M Phosphate Buffer + 0.15 M NaCl
Diluting/Washing Buffer	0.01 M Phosphate Buffer + 0.50 M NaCl + 0.1% Tween 20
Blocking Buffer	Bovine Serum Albumin (BSA)
Enzyme	Horse-redish peroxidase (HRPO)
Chromogenic Substrate	Trimethyl benzidine (TMB)
Stop Solution	0.5 M H2SO4

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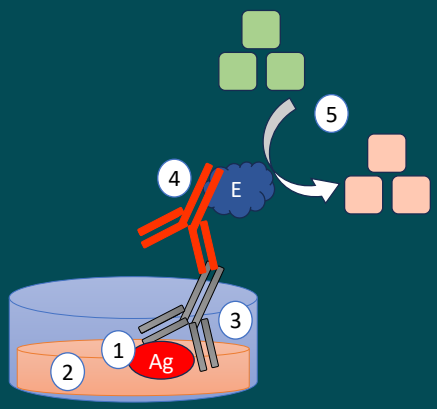
ELISA

Steps (Indirect ELISA)

1. Sample/Antigen added to plate
2. Blocking Buffer is added to block remaining protein binding site
3. Primary Antibody is added
4. Secondary Enz-linked antibody is added which recognise and bind with Primary antibody
5. TMB Substrate is added and its converted by HRPO to detectable form



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ELISA

Types

1. **Direct ELISA:** antigen-coated plate; screening antibody
2. Indirect ELISA: antigen-coated plate; screening antigen/antibody
3. **Sandwich ELISA**: antibody-coated plate; screening antigen
4. **Competitive ELISA:** antigen-coated plate, screening antibody for specific antigen

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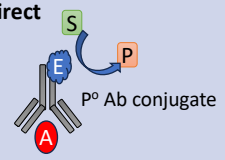
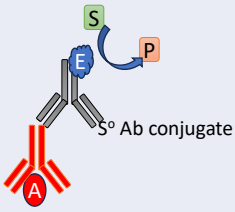
ELISA

Type	Key Points & Steps	Advantages	Disadvantages
Direct 	➢ antigen-coated plate ➢ Screening of Antibody ➢ in a sample directly to the plate. An enzyme-conjugated antibody is then added as a probe for the desired analyte. And washed to remove free Conjugated Pº Ab ➢ A specific substrate is added which gives a colored product and Read	➢ Rapid ➢ Only one Antibody is used, no cross reaction	➢ Low Sensitivity ➢ No specific binding of antigens
Indirect 	➢ antigen-coated plate ➢ Screening of Antibody/ Antigen ➢ A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen and then washed to remove free Pº Ab ➢ The antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody and then washed to remove free Sº Ab. ➢ A specific substrate is added which gives a colored product and Read	➢ High Sensitivity	➢ Risk of cross reactivity ➢ Non-specific binding of sample antigens

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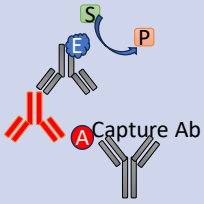
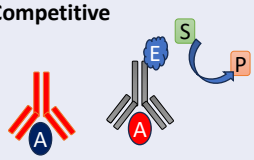
ELISA

Type	Key Points	Advantages	Disadvantages
Sandwich 	➢ antibody-coated plate; screening antigen ➢ Sandwich the Ag with two antibodies ➢ Ag must have at least two binding site ➢ The sample containing the antigen is added to the well and washed to remove free antigens. ➢ Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies. ➢ The enzyme-specific substrate is added to the plate to form a coloured product, which can be measured.	➢ Highly sensitive and specific	➢ Selection of right antibody pair is time consuming
Competitive 	➢ Involves competition for binding to antigen between the serum antibody (produced by Ag) and conjugated antibody. ➢ The presence of a color change means that the test is negative because the enzyme-conjugated antibody bound the antigens (not the antibodies of the test serum). ➢ The absence of color indicates a positive test and the presence of antibodies in the test serum	➢ Rapid	➢ Low specific

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ELISA

Application

1.The presence of antibodies and antigens in a sample can be determined.

2. Used in diagnose various infective disease like:

- Ebola
- Pernicious anaemia
- AIDS
- Rotavirus
- Lyme disease
- Syphilis
- Toxoplasmosis
- Zika virus
- Carcinoma of the epithelial cells

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ELISA

Application

3. It is used in the food industry to detect any food allergens present.

3.To determine the concentration of serum antibody in a virus test.

4. During a disease outbreak, to evaluate the spread of the disease, e.g. during recent COVID-19 outbreak, rapid testing kits are being used to determine presence of antibodies in the blood sample.

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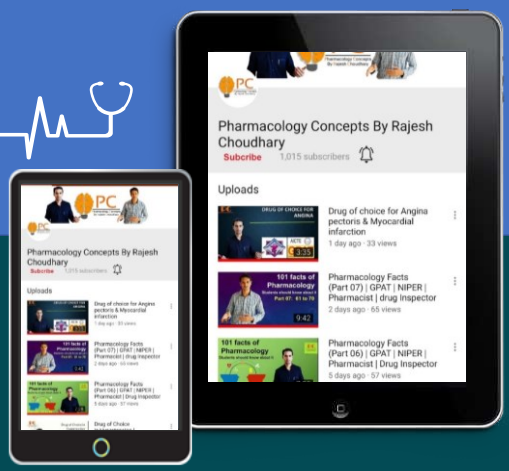

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