ELISA TEST - enzyme linked Immuno sorbent assay.

⇒ No Radiation hazards like RIA. usually used enzyme → ALP, horse radish peroxidase

⇒ longer shelf life.

⇒ Easy Automation.

⇒ This test is commonly employed to detect Antigen or Antibodies present in small quantity in tissue or blood.

⇒ Eg: detection HIV Antibody.

PRINCIPLE: Ag + enzyme linked Ab (Antibody) ⟹ Ag-Ab (enzyme) complex ⇓ + substrate Chromogen system

Substrate Bind to enzyme that induce chromogen to Produce color.

done on microtiterplate (96 wells).

procedure: Add Antigen (Attaches to surface) ↓ wash Add enzyme linked Antibody ↓ wash Add substrate complex ⇒ produce colour.

colour developed ∝ to the Antibody (one Directly Added (substrate).

Types → Direct (same) → Indirect → sAndwich.

Indirect

⇒ 1° Antibody added dosnot contain enzyme (not linked).

⇒ 2nd Antibody is linked with enzyme.

⇒ then Add substrate chromogen system.

↓ bind with 2nd Antibody enzyme

↓ colour produced.

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enzyme

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Add enzyme linked Antibody
↓ wash
Add substrate complex ⇒ produce colour.

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Types
- Direct (same) - Directly Added (substrate).
- Indirect
- Sandwich.

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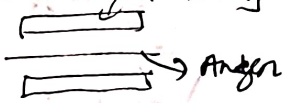
⇒ then Add substrate chromogen system.
↓ bind with 2nd Antibody enzyme
↓ colour produced.

Sandwich, elisa – detect Antigen in serum

Eg: 4



⇒ Microtitre plate is coated with Antibody

⇓

capture Ab.

⇒ the Antigen is Added.

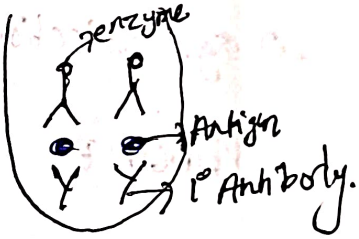
⇒ 2nd Antibody is Added with linked enzyme

⇓

substrate chromogen system Added

⇓

produce colour.



Colour developed is ∝ to the Antigen in serum.

Colorimeter:

⇒ The colorimeter is an electronic instruments that is used for measuring the colour intensity of any solution or Analyse con in a colored solution.

⇒ It measures absorbance of wavelength b/w a visible spectrum of light 400 to 700 nm.

⇒ It is used to estimate Urine, plasma, serum etc.

PRINCIPLE: based on Beer & Lambert law.

Beer's law - "the intensity of the color is directly ∝ to the conc of coloured particles in the solutions"

Lambert's law - The Amount of light Absorbed by a coloured solution depends on the length of the column or depth of the liquid through which light passes.

Absorbance & O.D

Absorbance is defined ~~as~~ which give direct Relationship with con of the substance in solution at constant path length.

T - Transmittance

$A = \log \frac{1}{T}$ or $-\log T$

$A = -2 \log T$.

Absorbance is Also called as O.D - commonly used.

Parts

⇒ Light Bulb
⇒ Aperture / slit
⇒ condenser Lens.
⇒ filter - Absorb unwanted light & only monochromatic light passes theough.

⇒ Cuvette – Glass tube

⇒ Photocell – Detector

⇒ Galvanometer – digital meter.

p- Kit, Im

Blank

Water Blanks

. It is used to set zero in colorimetric Reading.

Reagent Blank

. It is used to minimize Reagent absorbance in colourimetric Readings.