LAB-7-
ENZYME-LINKED-IMMUNOSORBENT ASSAY

Submitted by

assist.Lec.Hiba Hadi Rashid

- History
- Difinition
- Principle
- Requirements
- Methods
- Advantage
- Disadvantage
- Applications

➢ HISTORY

- In 1971, **Peter Perlmann** and **Eva Engvall** at Stockholm University in Sewden , and Anton Schuurs and Bauke van Weemen in the Netherlands independently published papers that synthesized this knowledge into methods to perform EIA/ ELISA.

DEFINITION OF ELISA

- **ENZYMED LINKED IMMUNO SORBENT ASSAY(ELISA): It is Serological Method based on immunological antigen-antibody reactions. This Immunoassay uses to measure the concentration of an analyte (usually antibodies or antigens) in Solution .**

- Enzyme-Linked

Secondary antibody linked with enzyme

Immuno

Antigen is recognised by specific antibody

Sorbent

Ag- Ab of interest is absorbent on to plastic surface(Sorbent)

PRINCIPLE OF ELISA

The principle of ELISA based on the immunochemical principle of Antigen- Antibody reaction

❖It dependent on Lock and Key concept:

1)Antigen (key) 2) Antibody(Lock)

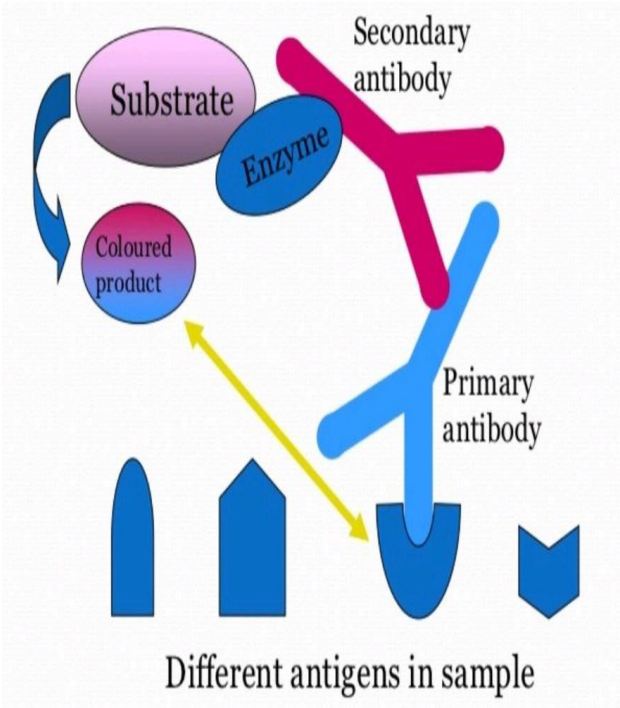
- Key fit into the lock

specificity of Antigen-Antibody complex formation

❖Its detection by a second antibody conjugated with suitable enzyme

❖Enzyme conjugate Substrate(chromogen)

❖ colored product , indicating the prescence of Ag:Ab binding.



REQUIREMENTS

Specimen Sample for ELISA

SERUM

CSF

SPUTUM

URINE

SEMEN

SUPERNATANT OF CULTURE

STOOL

MATERIALS ARE NEEDED

TESTING SAMPLE

ANTIBODY(1^{ST}, 2^{ND})

POLYSTYRENE MICROTITTER PLATE

BLOCKING BUFFER

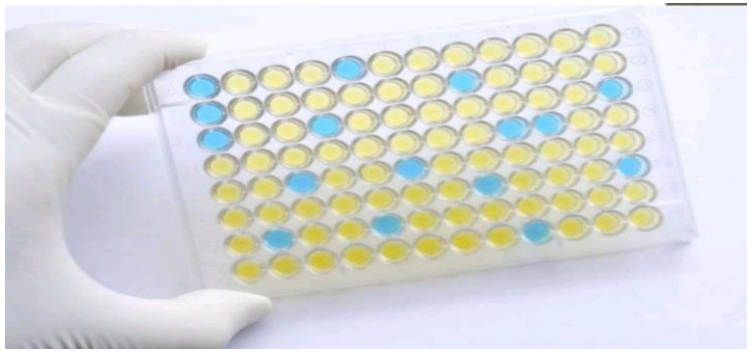
WASHING BUFFER

SUBSTRATE

ENZYME

REQUIREMENTS

- Microtiter plate: Flat bottom polystyrene plate , Contains 8 ×12 wells holding 350µl each.



- Multipipette: An 8-channel 100µl pipette is a good help for even small- scale work

REQUIREMENTS

Washing Device :

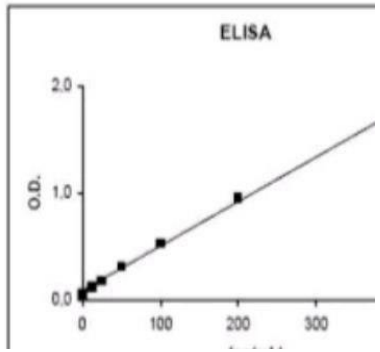
Manually operated washing devises.

May be use particularly when there is a risk that samples tested in ELISA contain infectious material , so must be collected for subsequent disinfection.

- Microplate washer: There are very efficient with unusually low carry – over contamination.

REQUIREMENTS

ELISA READER



- Incubator

METHODS OF ELISA

- There are Four Types of RIA :

1. Direct ELISA
2. Indirect ELISA
3. Sandwish ELISA
4. Competition - Inhibition ELISA.

METHODS OF ELISA

Direct ELISA:

Aim: detect antibody

- An antigen is immobilized in the well of an ELISA plate.
- The antigen is then detected by an antibody directly conjugated to an enzyme such as HRP.
- A substrate for this enzyme is then added , this substrate changes the color upon reaction with enzyme.
- Higher the concentration of antibody , stronger the color changed .
- Often spectrometer is used to give the Quantitative value for color change.

METHODS OF ELISA (DIRECT ELISA)

Advantages

- Much faster
- Less prone to error since fewer reagents and steps are needed.
- Best for analyzing the immune response to an antigen.
- Cross- reactivity of secondary Ab is eliminated

Disadvantages

- Ag immobilization is not specific
- Less flexible.
- No signal amplification – reduces assay sensitivity.
- Labeling of every primary Ab is time – consuming and expensive.

METHODS OF ELISA(INDIRECT ELISA)

- Aim : detect the prescence of a type of antibody.
- Antigen is adsorbed to a well in an ELISA plate.
- Detection is a two – step process . First , an unlabeled primary antibody binds to the specific antigen
- Second an enzyme conjugated secondary antibody that is directed against the host species of the primary antibodies is applied.
- Usage: HIV Infection test.

METHODS OF ELISA (INDIRECT ELISA)

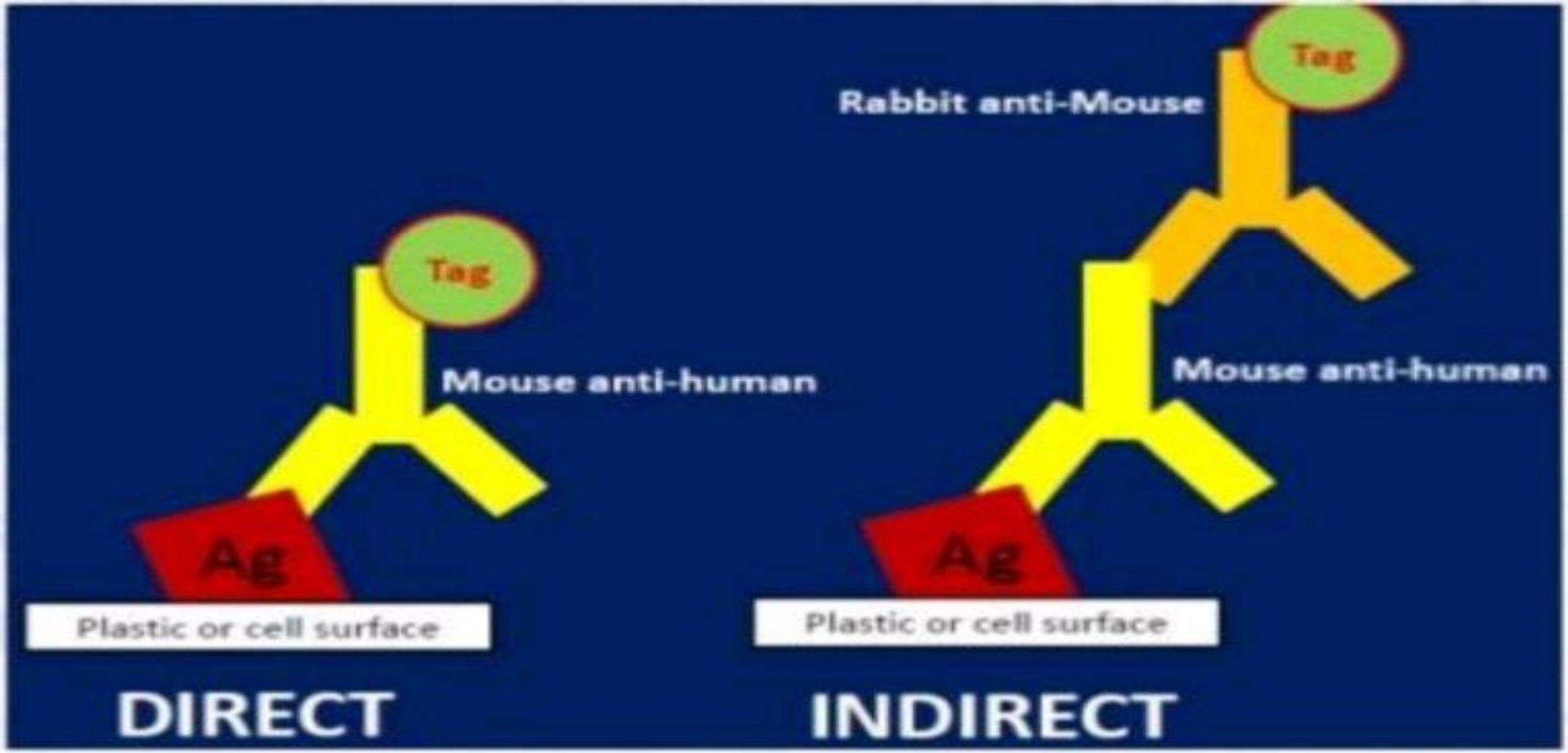
- Advantages

- Economical
- High sensitivity
- Greater flexibility
- Best for determining total antibody concentration in samples.

Disadvantages

- Cross-reactivity may occur with the secondary Ab , resulting non specific signal.
- Longer procedure than direct ELISA technique.
- Additional incubation step for secondary antibody needed.

Direct and Indirect ELISA



SANDWICH ELISA

- **Aim: Detect the prescence the type of antigen.**
- **Sandwich ELISA require the use of matched antibody pairs (capture and detection antibodies).**
- **Each Ab is therefore specific for a different and non-overlapping region or epitope of the Ag.**
- **The capture Ab , binds the antigen that can then be detected in a direct ELISA or in an in direct ELISA Configuration**

METHODS OF ELISA (SANDWICH ELISA)

- Advantages

- Highly sensitivity
- High Specificity
- Flexibility: both direct & in direct detection can be used.
- Best for analysis of complex samples

Disadvantages

- Antibody optimization can be difficult-cross- reactivity may occur between the capture & detection antibodies.

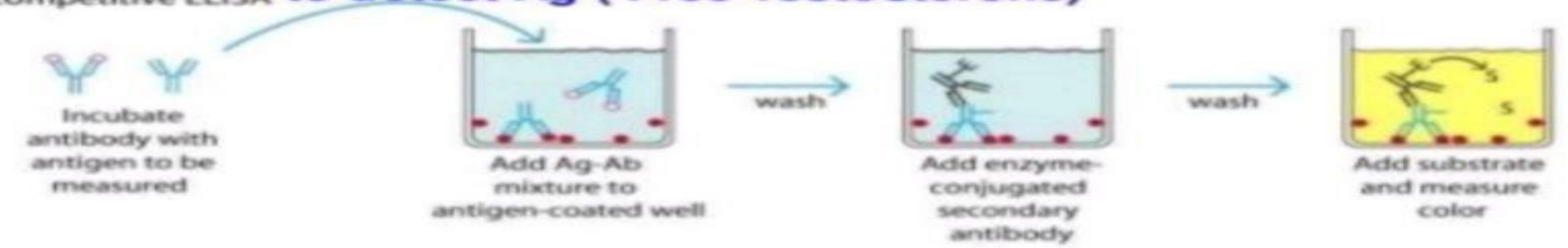
Competitive elisa

- Incubate unlabeled antibody within presence of its antigen.
- Bound antibody/antigens are added to a antigen coated well.
- Wash and remove unbound antibodies.
- Competition results from the fact that the more antigens are present in the sample, the less antibody will be able to bind.
- A secondary antibody that is coupled to a enzyme is added.
- Substrate added for signal
- The weaker colored or fluorescent signal that is released shows that that the original antigen concentration was high.

(a) Indirect ELISA **to detect Ab (HIV, HCV)**

(b) Sandwich ELISA **to detect Ag (Tumor Markers, Hormones)**

(c) Competitive ELISA **to detect Ag (Free Testosterone)**



arison between Indirect Sandwich & Competitive E

APPLICATIONS OF ELISA

Detect the Infectious agent (Viral infections)

Sexually transmitted agents like(HIV)…HIV-1 and HIV-2(presence of anti- HIV antibodies).

Hepatitis C(presence of antibodies)

Hepatitis B (Testing of both antibodies and viral antigen).

Detect (IgG, IgM, IgA)

Tumour test

In Clinical Research


THANK YOU