Al-Mustaqbal University College
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Class: 2nd
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No. : 14

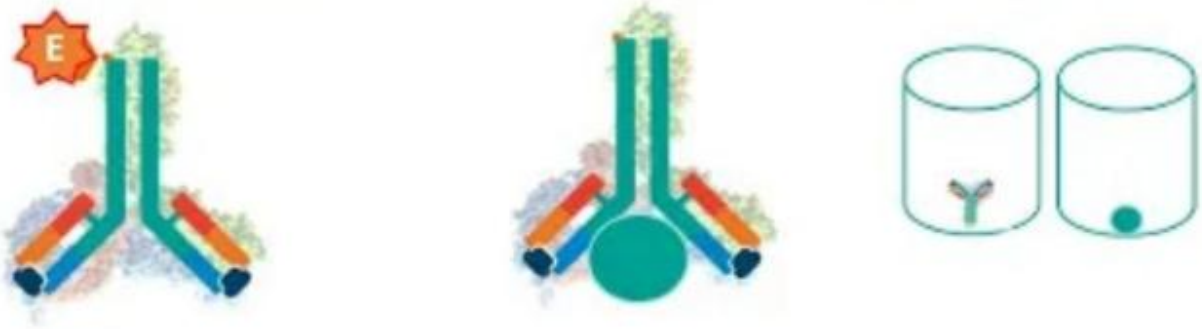
Elisa Instrument and its uses

- The enzyme-linked immunosorbent assay (**ELISA**) is a common laboratory technique which is used to measure the concentration of an analyte (usually antibodies or antigens) in solution.

مقايسة الامتصاص المناعي المرتبط بالإنزيم

ELISA

Enzyme Linked Immuno Sorbent Assay



- **Antigen:**

  يثير

  Any molecule that elicits the production of antibodies when introduced into body.

- **Antibodies:**

  Proteins produced in response to antigenic stimuli.

  البروتين المنتج استجابة للمحفزات المستضدة

- **Enzyme conjugate:**

  An enzyme that is attached irreversibly to an antibody.

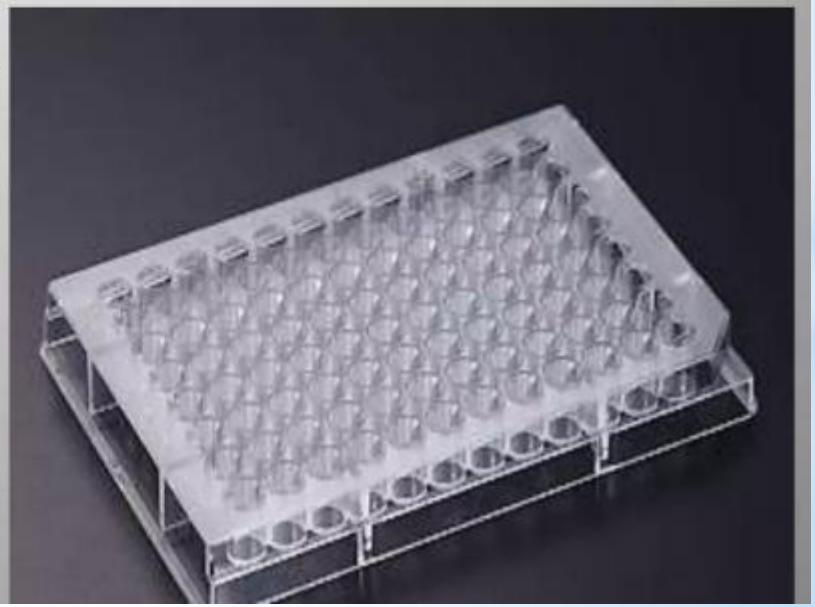
- **Solid Phase:**

  Usually a micro-well-plate , having 8 ✖ 12 well format.

•The equipment of ELISA consists of a micro-plate which consists 96 wells. The wells are arranged in the form of 8 rows and 12 columns. By this arrangement we can detect 96 samples at a time.

•Marked on one side alphabetically

•Numerically on the other side



Basic Terms:

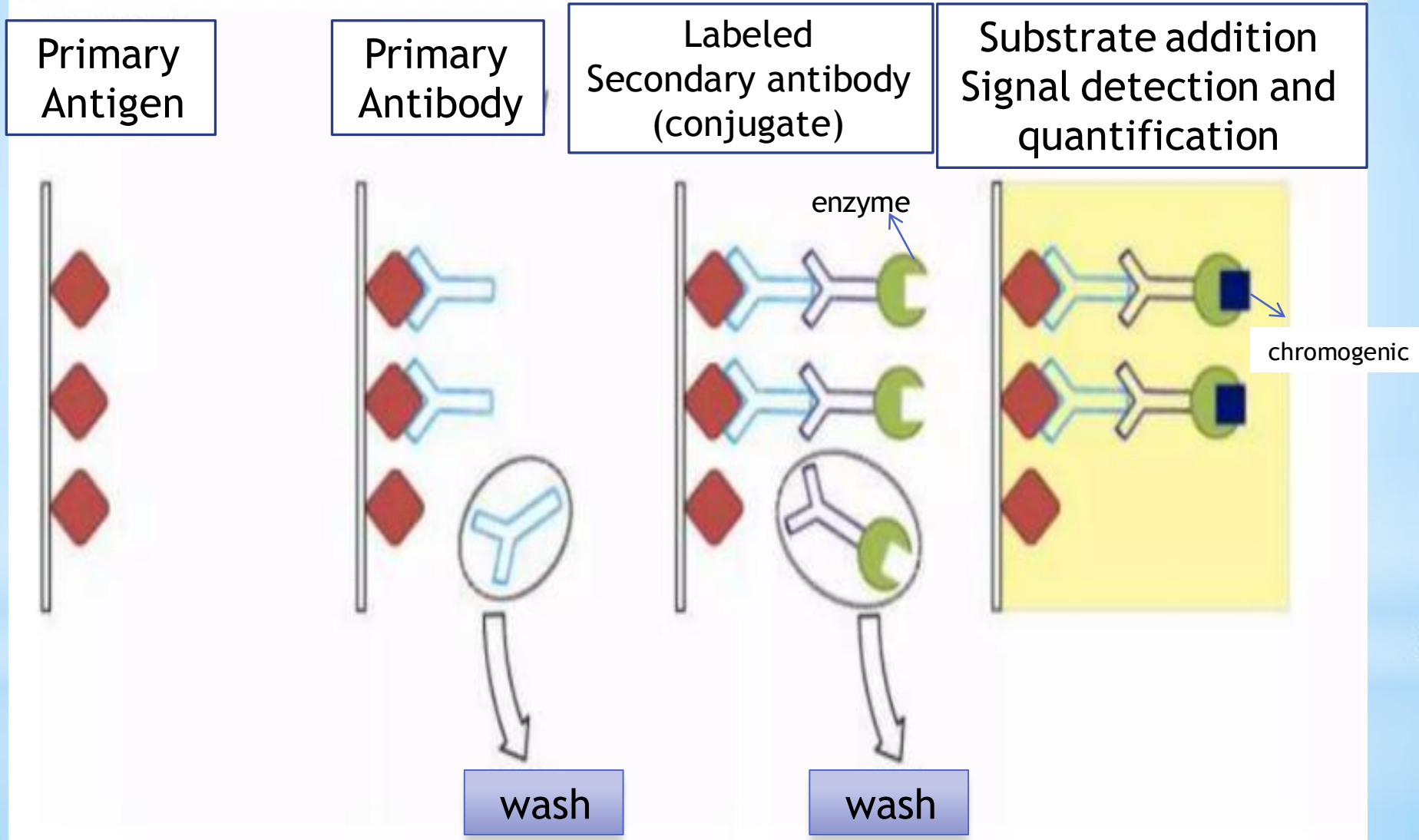
- **Adsorption:**

  The process of adding an antigen/antibody, diluted in buffer, so it attaches to the solid phase on incubation.

- **Washing:**

  The simple flooding & emptying of wells with a buffered solution to separate bound from un-bound reagents in ELISA.

Assay to Determine the Antibody Concentration.



- **Chromogen:**

  A chemical alters color as a result of an enzyme interaction with substrate (color reaction used as signal) e.g Trimethyl benzidine (TMB).

- **Stopping:**

  The process of stopping the action of an enzyme on a substrate. Sulphuric acid

- **Reading:**

  Spectrophotometric measurement of color developed in ELISA.

- It is a device which used to read the result of ELISA test utilizing theory of spectrophotometer.
- ELISA-(Enzyme-Linked Immunosorbent Assay)

Biochemical technique used mainly in Immunology to detect the presence of an antibody or an antigen in a sample.

- Also called Photometric Microplate reader, ELISA reader

قارئ صفيحة مايكروية ضوئية

Principle of Elisa

- **Based on Basic Immunology Response**
- Lock and Key Concept:

1) Antigen (key) 2) Antibody (lock):

–Key fits into the lock

- Enzyme conjugate substrates
- Bound to a secondary antibody that binds with the antibody-antigen complex.

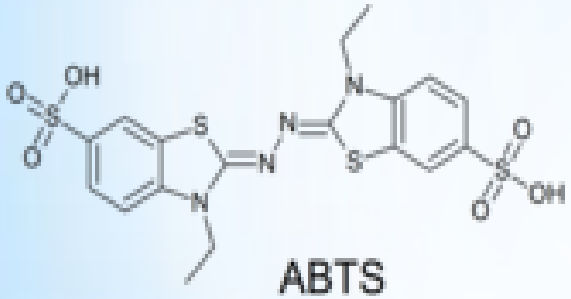
3) Washing Device:

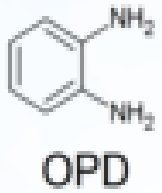
- manually operated washing devices.
- may be of use particularly when there is a risk that the samples tested in ELISA contain infectious material, so must be collected for subsequent disinfection.

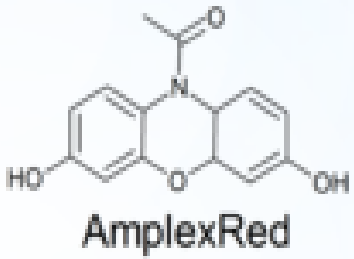
Reagents Used:

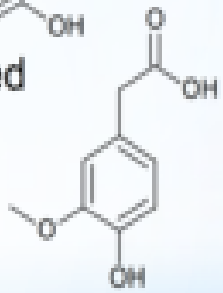
Reagent	Composition
Coating Buffer	0.01 M Phosphate Buffer + 0.15 M NaCl (PBS)
Diluting/Washing Buffer	0.01 M Phosphate Buffer + 0.50 M NaCl + 0.1% Tween 20
Blocking Buffer	Bovine Serum Albumin (BSA)
Enzyme	Horse-redish peroxidase (HRPO)
Chromogenic Substrate	Trimethyl benzidine (TMB)
Stop Solution	0.5 M H_2SO_4

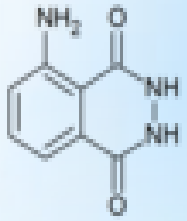
HRP(Horse-reddish peroxidase) is used in immunohistochemistry and ELISA because it generates colored compounds. For detection of an antigen or protein molecule, HRP substrates have been designed so that they will generate a chemiluminescent, chromogenic, or fluorescent signal upon oxidation

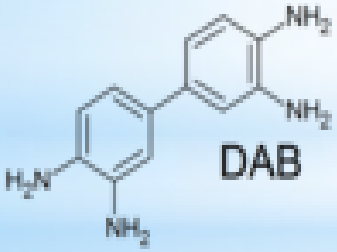


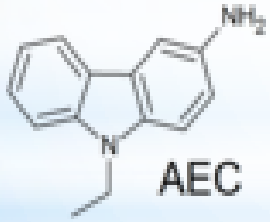


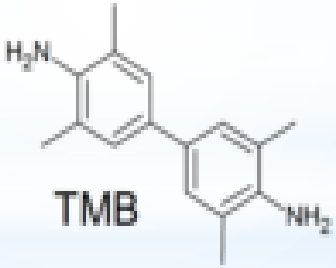


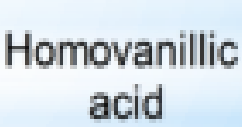










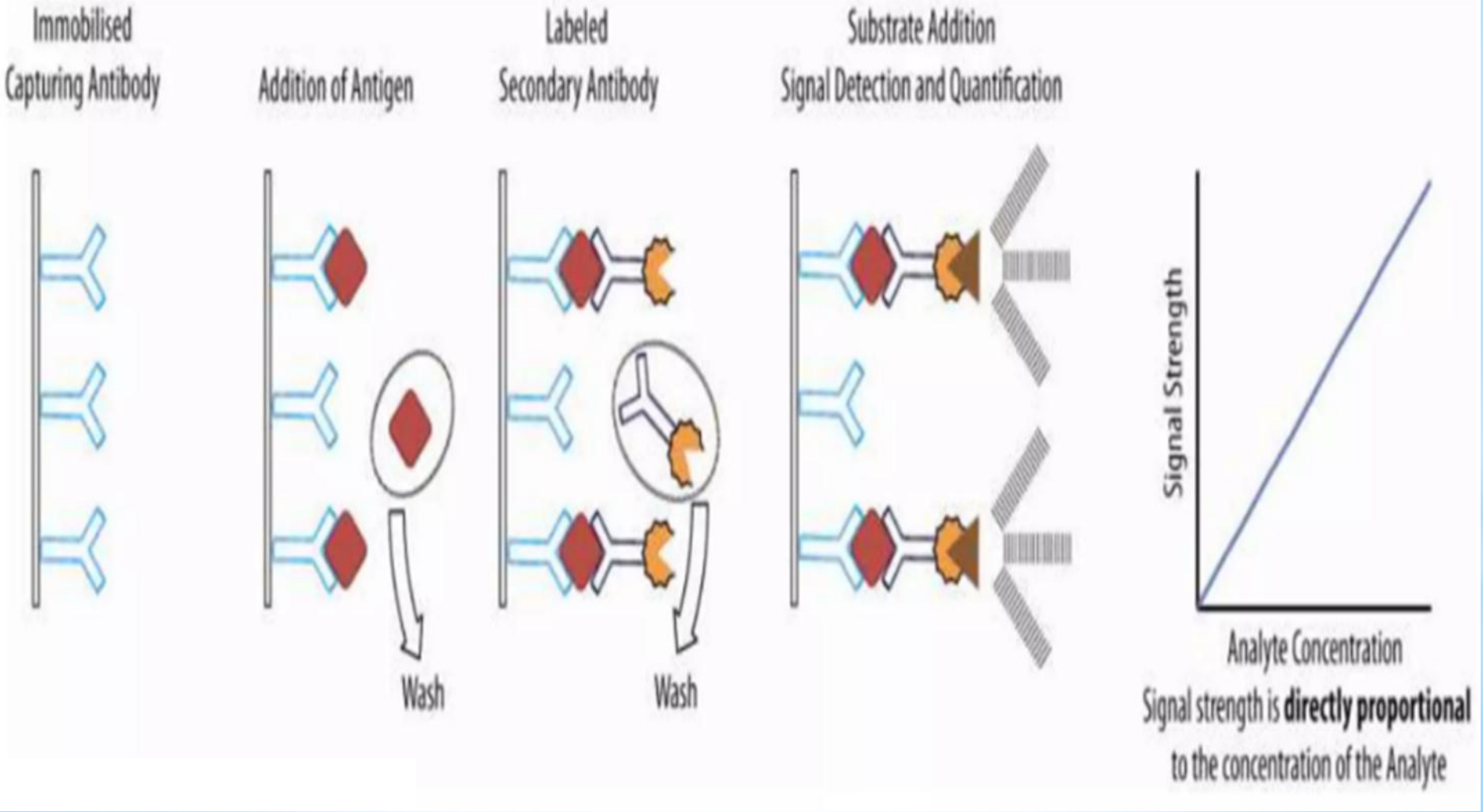


Some of the most common HRP chromogenic substrates.

General procedure:

1-Antigen/sample is added to plate
2-Blocking buffer is added to block remaining protein –binding site
3-Next a suitable primary antibody is added
4-A suitable secondary antibody –HRPO conjugate is then added which recognizes and binds to the primary antibody
5-TMB substrate is added and is converted by HRPO to detection form

Assay for the Quantitation of an Antigen in a Biological Sample.



Types of ELISA

1) Direct ELISA:

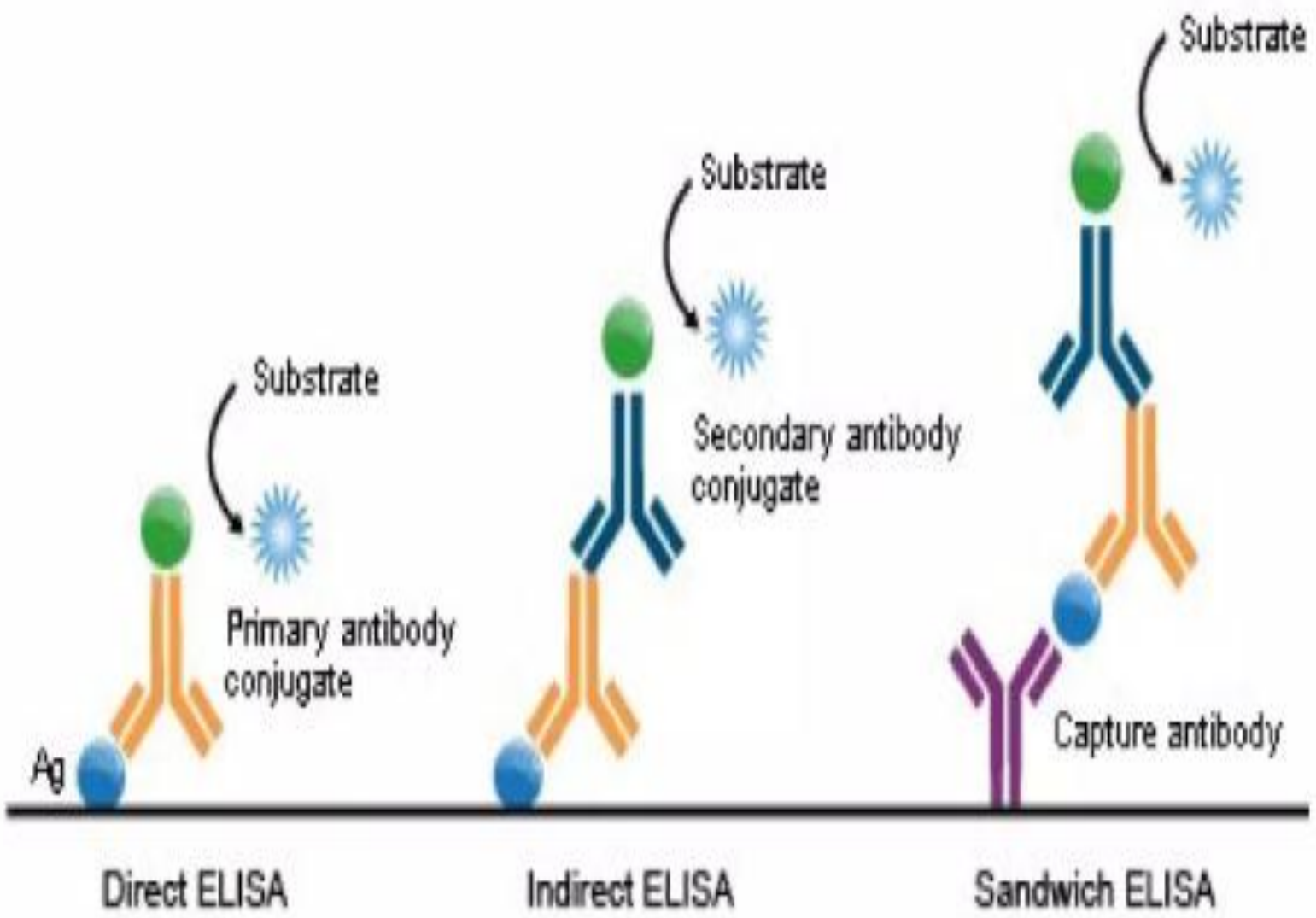
- It uses a primary labeled anti-body that react directly with the antigen.
- It can be performed with the antigen that is directly immobilized on assay plate.
- Not widely used but common for immuno-histochemical staining of cells & tissues.

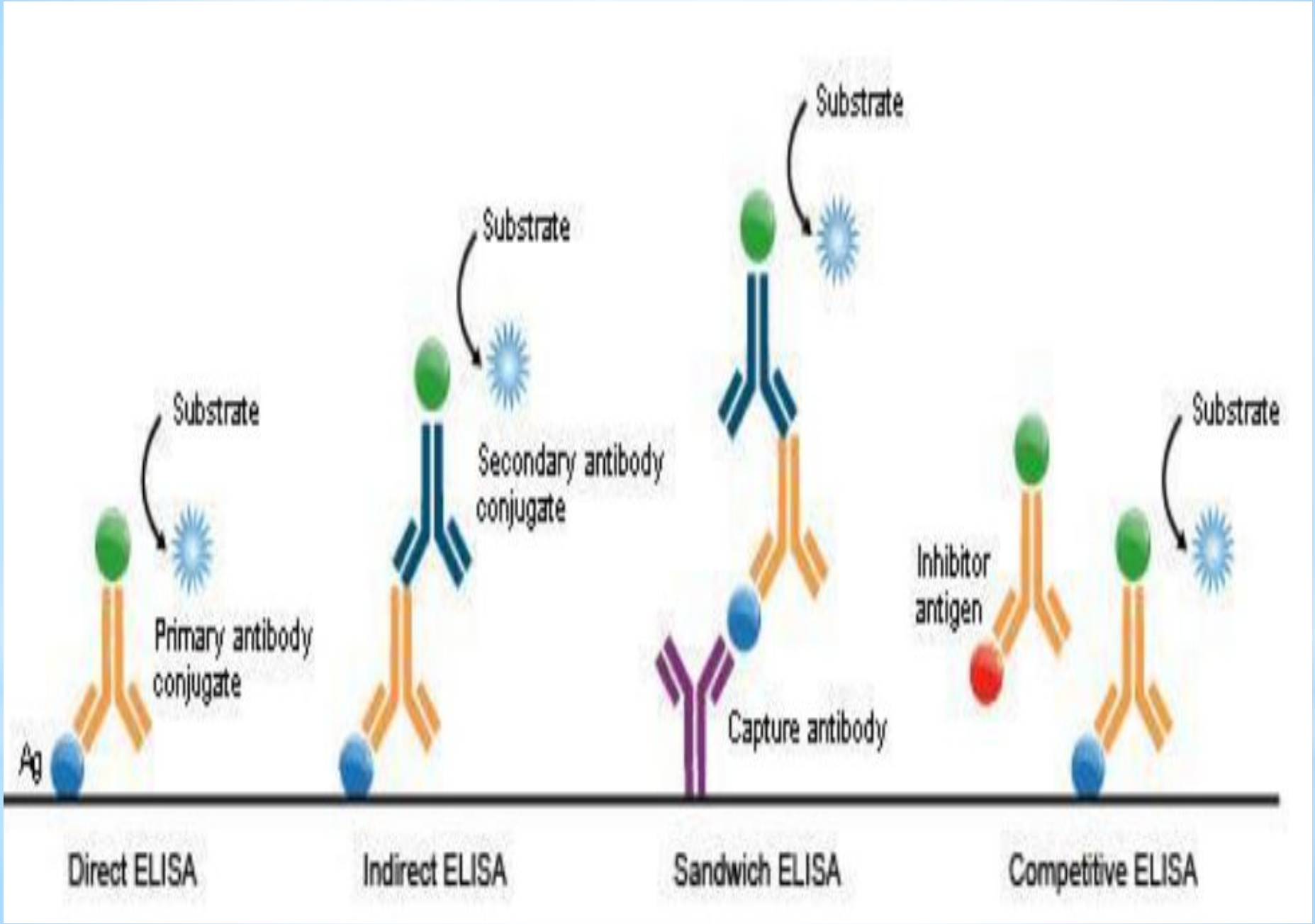
2) Indirect ELISA:

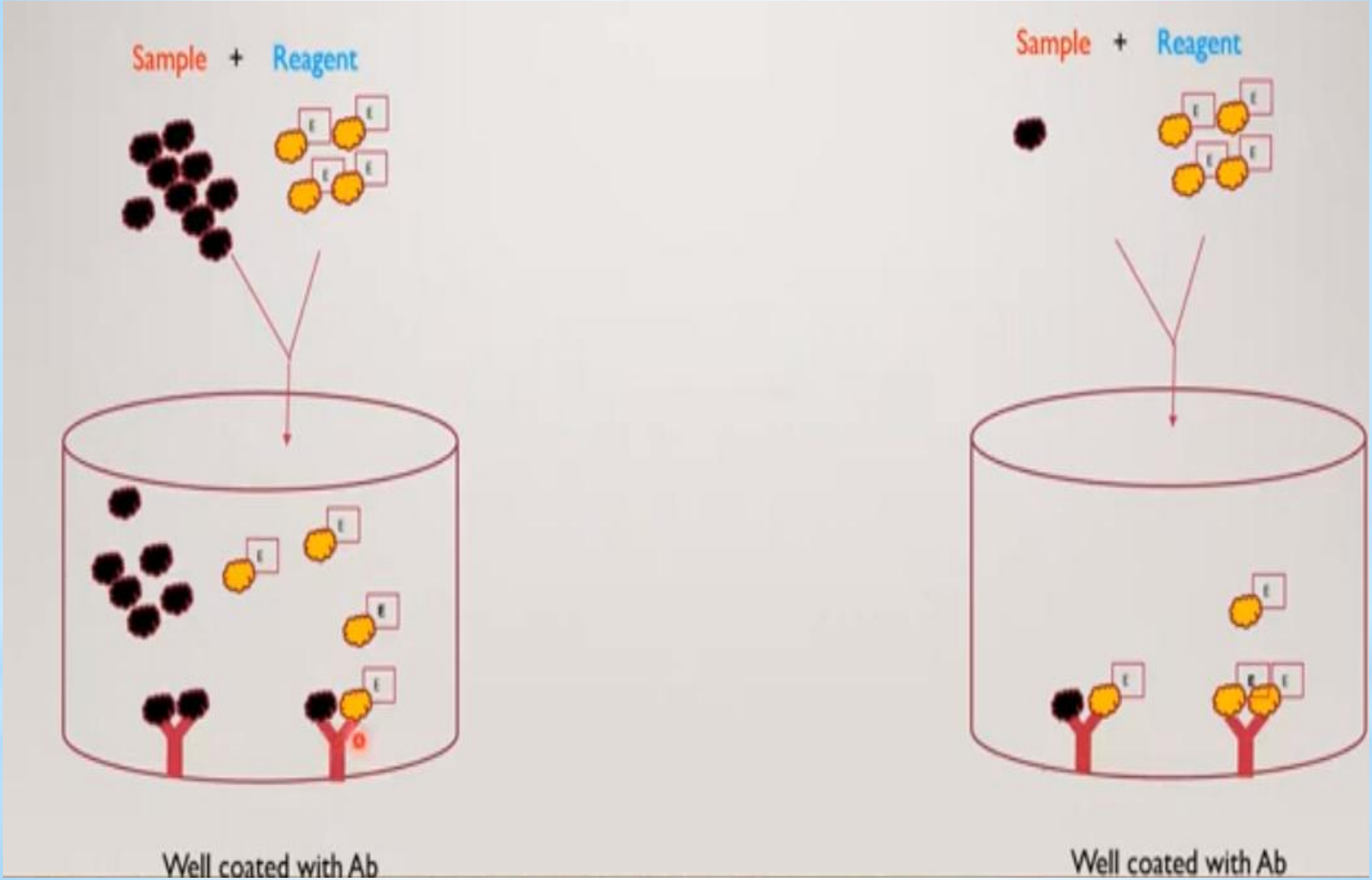
- It utilizes a primary un-labeled antibody in conjunction with a labeled secondary antibody.
- Secondary antibody has specificity for primary antibody.

3) Sandwich ELISA:

- Antigens like Tumor markers, hormones, serum proteins may be determined.
- Antigens in the sample bind with the capture antibody & become immobilized.
- The antibody of the enzyme conjugate bind with the immobilized antigen to form a sandwich of Ab-Ag-Ab/ enzyme bound to microwell.


Substrate
Primary antibody conjugate
Ag
Substrate
Secondary antibody conjugate
Substrate
Capture antibody
Direct ELISA
Indirect ELISA
Sandwich ELISA


Substrate
Primary antibody conjugate
Ag
Substrate
Secondary antibody conjugate
Substrate
Capture antibody
Inhibitor antigen
Substrate
Direct ELISA
Indirect ELISA
Sandwich ELISA
Competitive ELISA


Sample + Reagent
Well coated with Ab
Sample + Reagent
Well coated with Ab

After washing

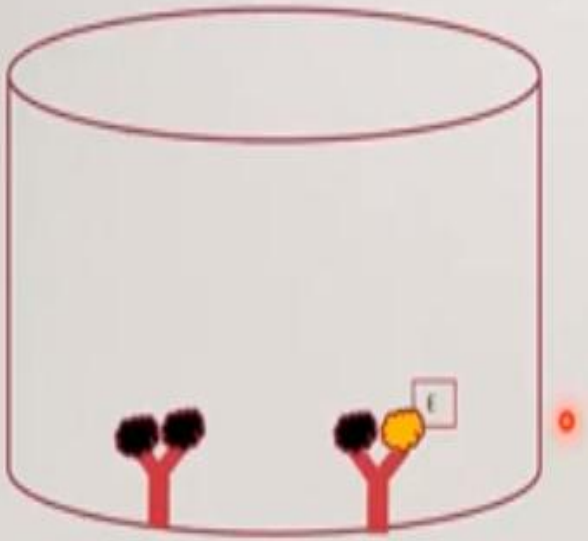


Plate coated with Ab

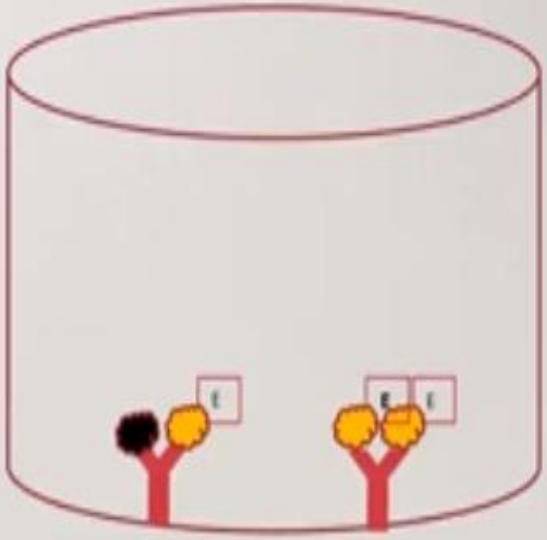


Plate coated with Ab

اضافة Substrate

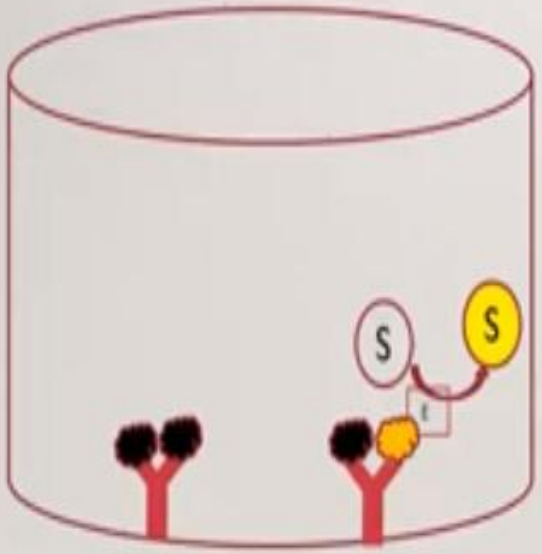


Plate coated with Ab

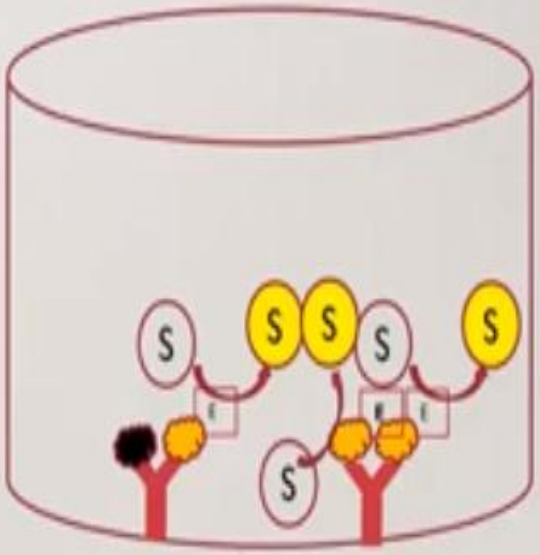


Plate coated with Ab

كمية اللون الناتجة من التفاعل تتناسب عكسيا مع كمية البروتين الموجود في العينة

antigen

Plate coated with Ab

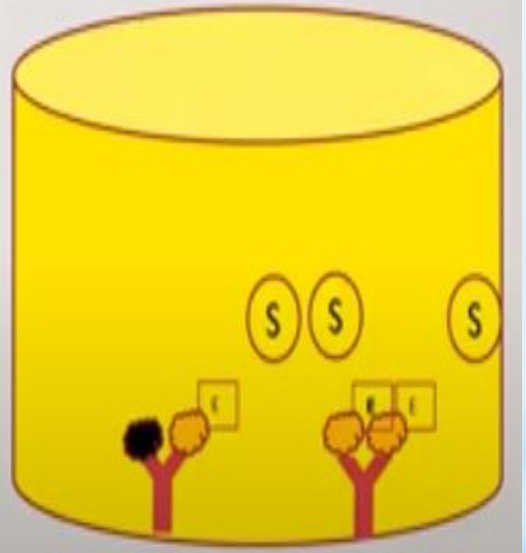
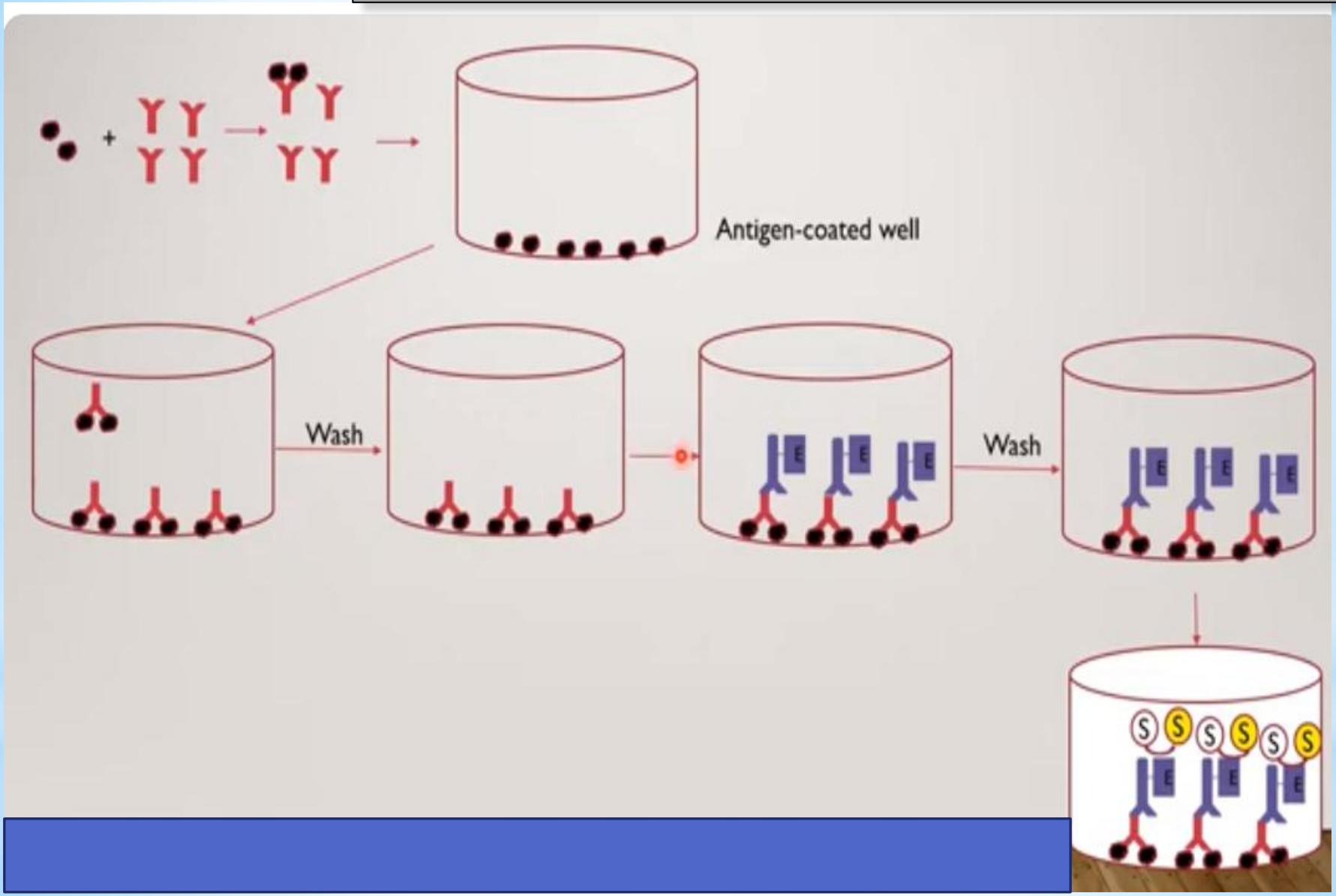
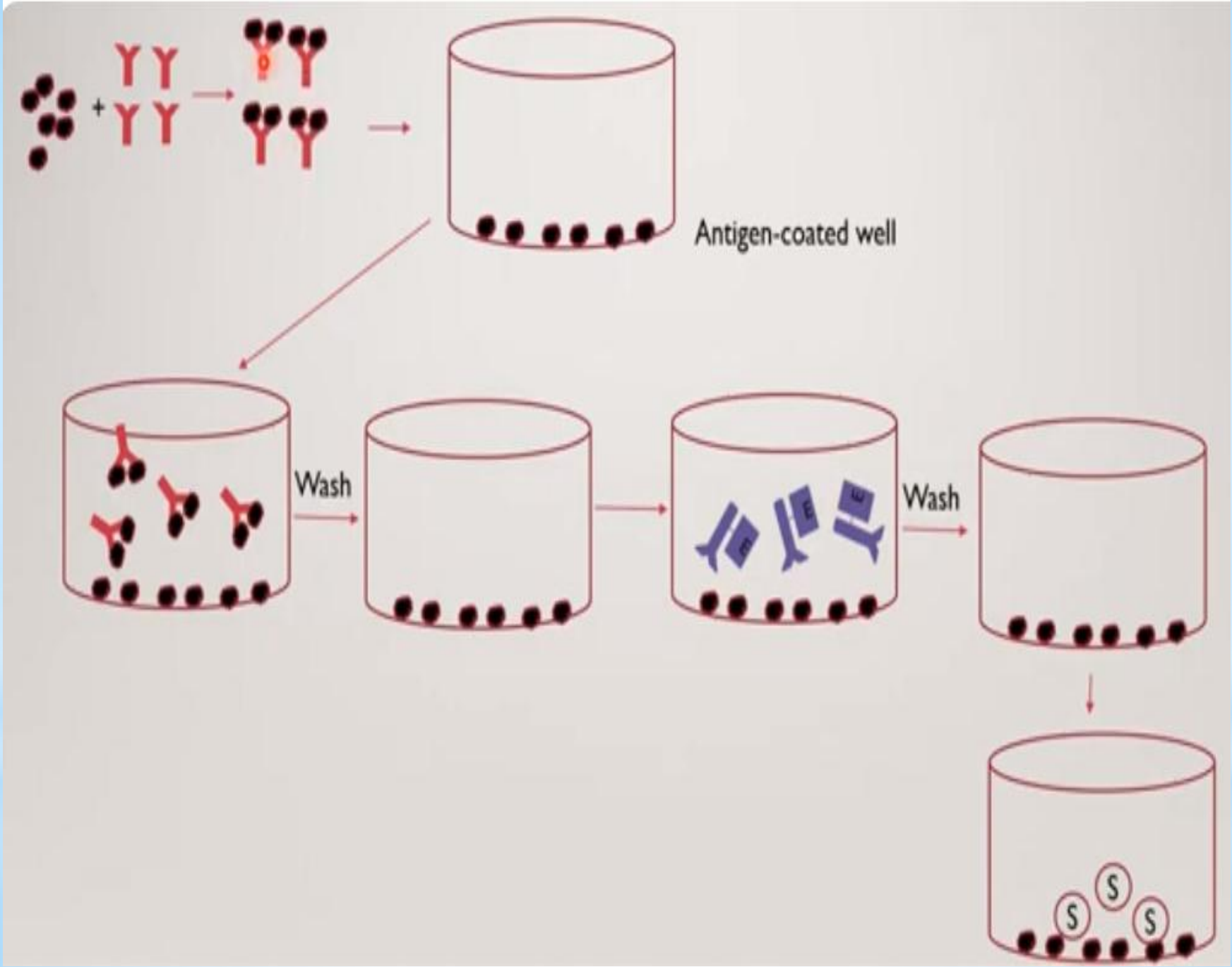


Plate coated with Ab

طريقة ثانية للاليزا التنافسية يمزج ال antigen مع ال antibody




Antigen-coated well
Wash
Wash
S
S
S

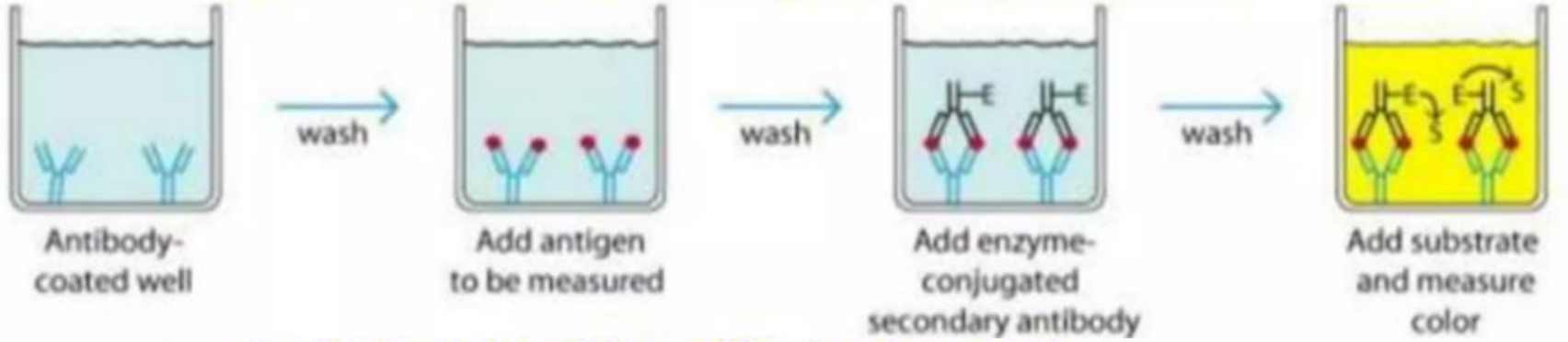
Competitive:

- Antibody coated microwell.
- Serum antigen & labeled antigen added together .... Competition
- Ab-Ag enzyme complex bound is inversely related to the conc. of antigen present in sample.
- Increased serum antigen results in reduced binding of Ag-enzyme conjugate with the antibody producing less enzyme activity & (yellow) color formation.

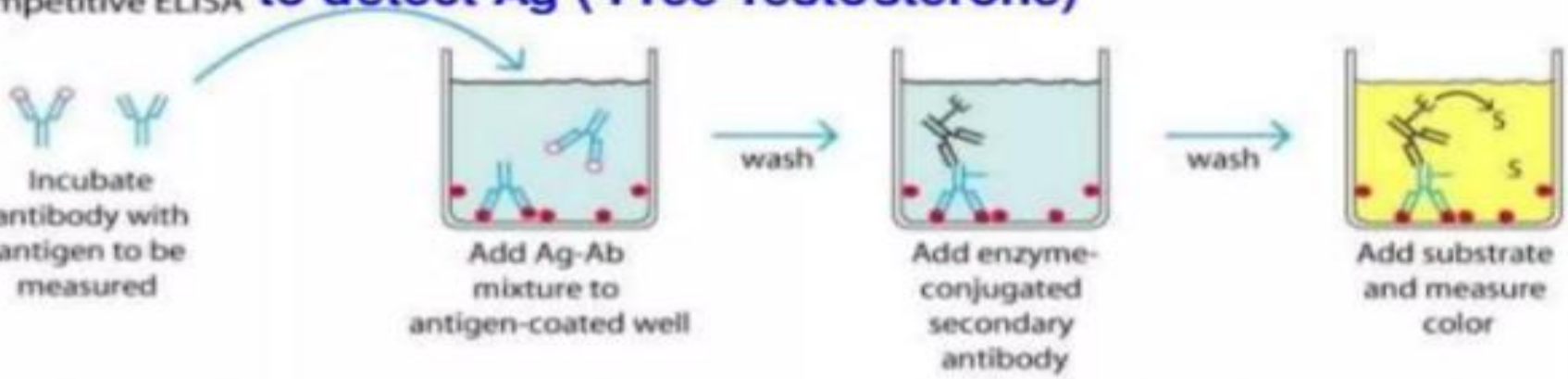
Used to determine small molecules like T_3 , T_4 & Progesterone.

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

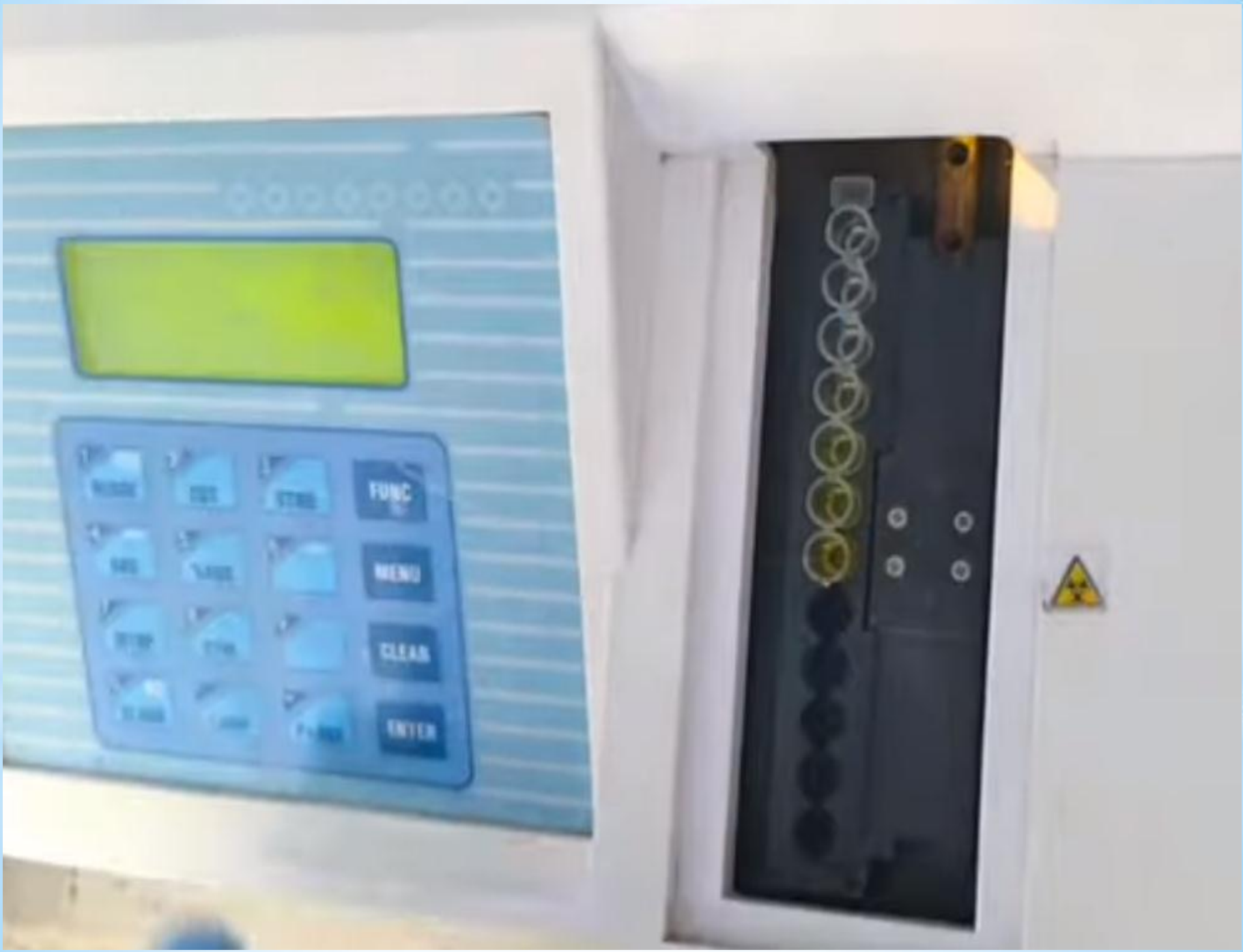
(b) Sandwich ELISA **to detect Ag (Tumor Markers, Hormones)**

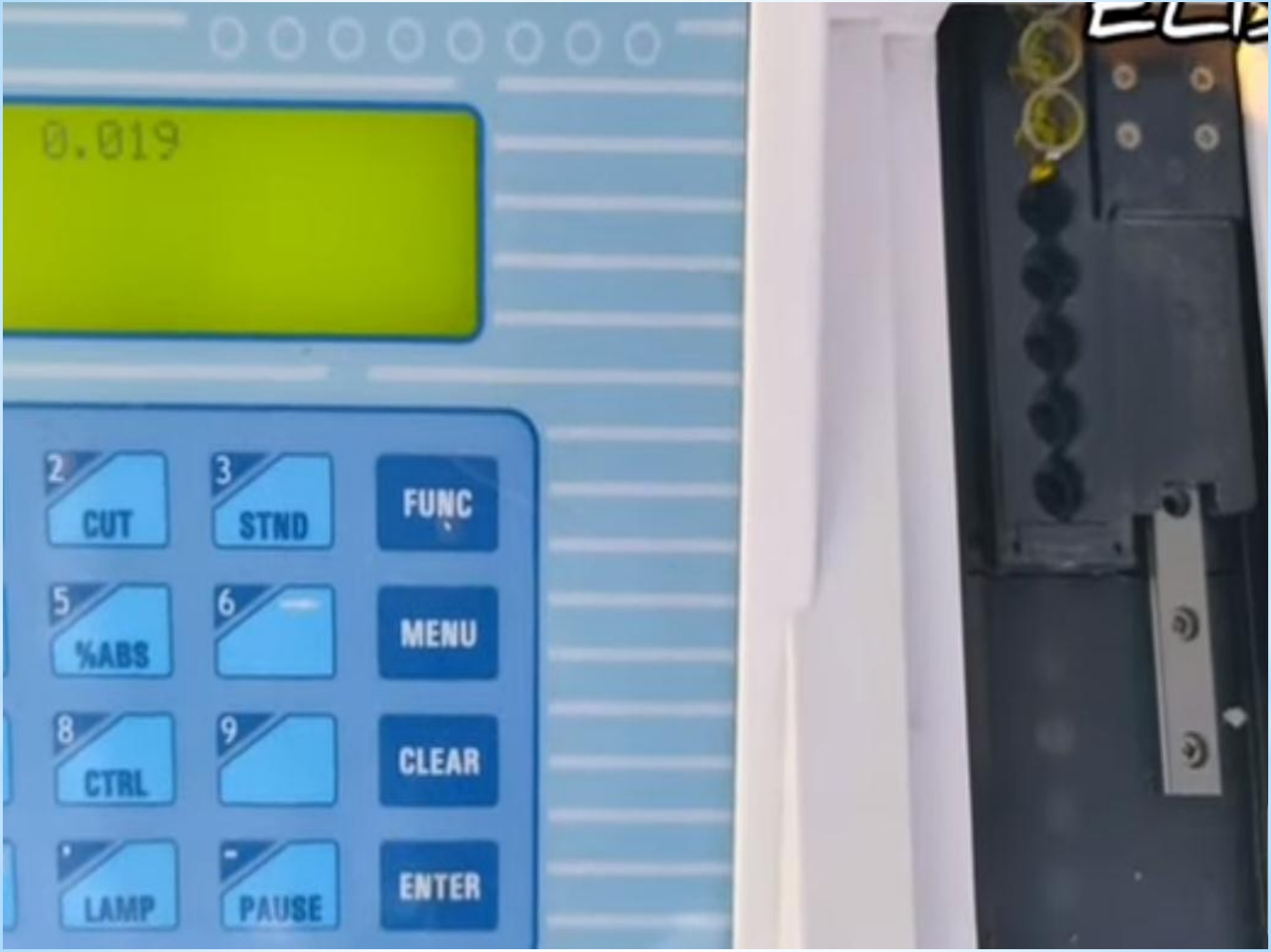


(c) Competitive ELISA **to detect Ag (Free Testosterone)**




TSH


FUNC
MENU
CLEAR
ENTER


0.019
2
CUT
3
STND
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CLEAR
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PAUSE
ENTER


1 CAL1 0.017 0.00
1 CAL2 0.019 0.50
1 CAL3 0.068 2.50
1 CAL4 0.186 7.50
1 CAL5 0.383 20.00
1 CAL6 0.926 40.00
1 1S_ 3.295 127.26