Experiment 5
Enzyme-Linked ImmunoSorbent Assay (ELISA)

I. Aim

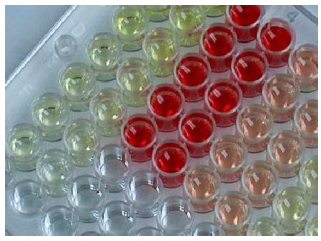
- Master the principle of ELISA;
- Understand the procedure of ELISA.

II. Principle

Enzyme-Linked ImmunoSorbent Assay (ELISA), also called Enzyme ImmunoAssay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

This method combines the specificity of Ab-Ag interaction and the high efficiency of enzyme, by using Abs or Ags coupled to an easily assayed enzyme.

Characteristics of ELISA

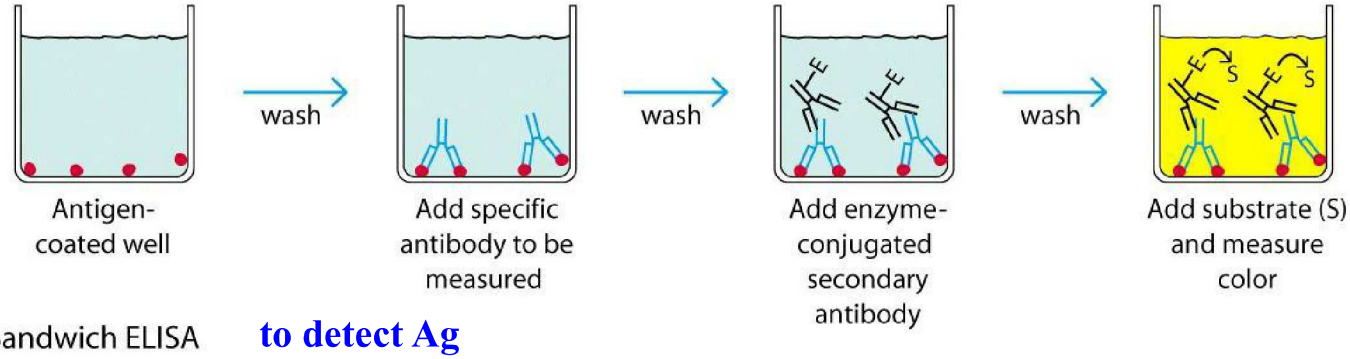


- Quantitative.
- Very sensitive.
- Commonly used in medicine and scientific research.

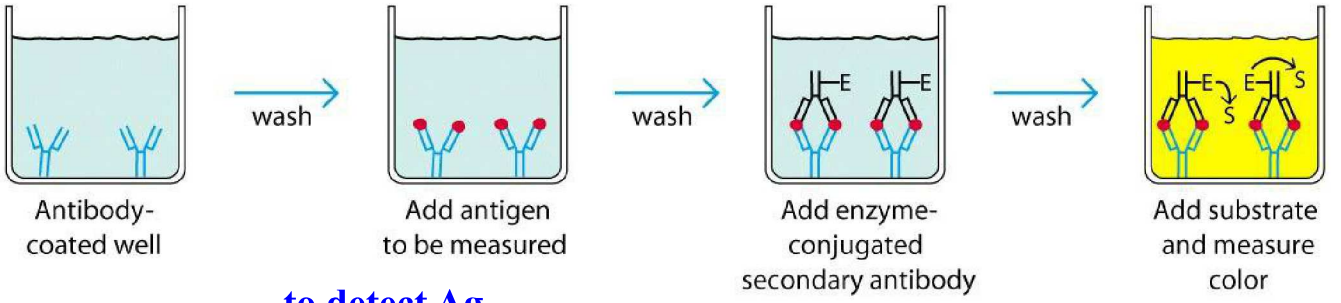
Classifications

- **Indirect ELISA:** Ab concentration
- **Sandwich ELISA:** Ag concentration
- **Competitive ELISA:** Ag concentration
- **……**

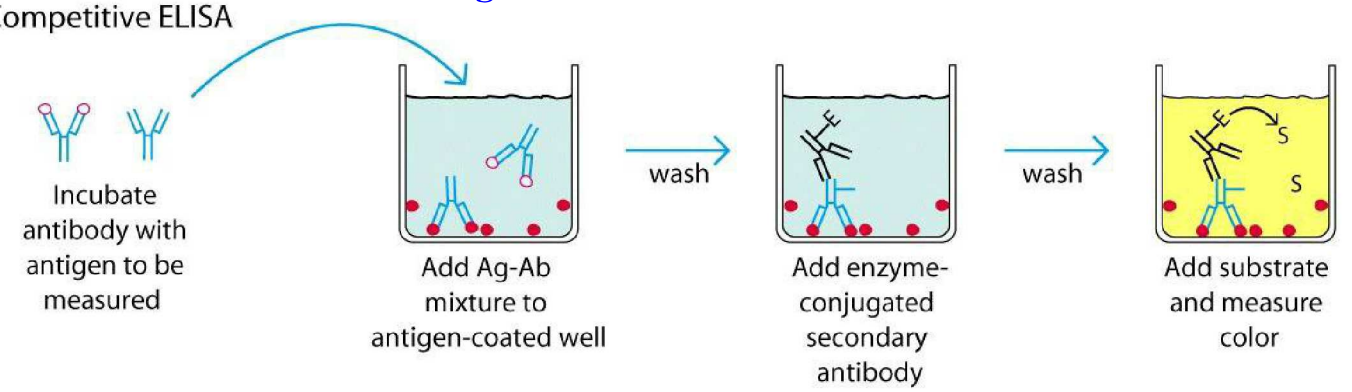
(a) Indirect ELISA **to detect Ab (TG)**



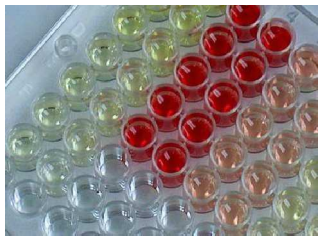
(b) Sandwich ELISA **to detect Ag**



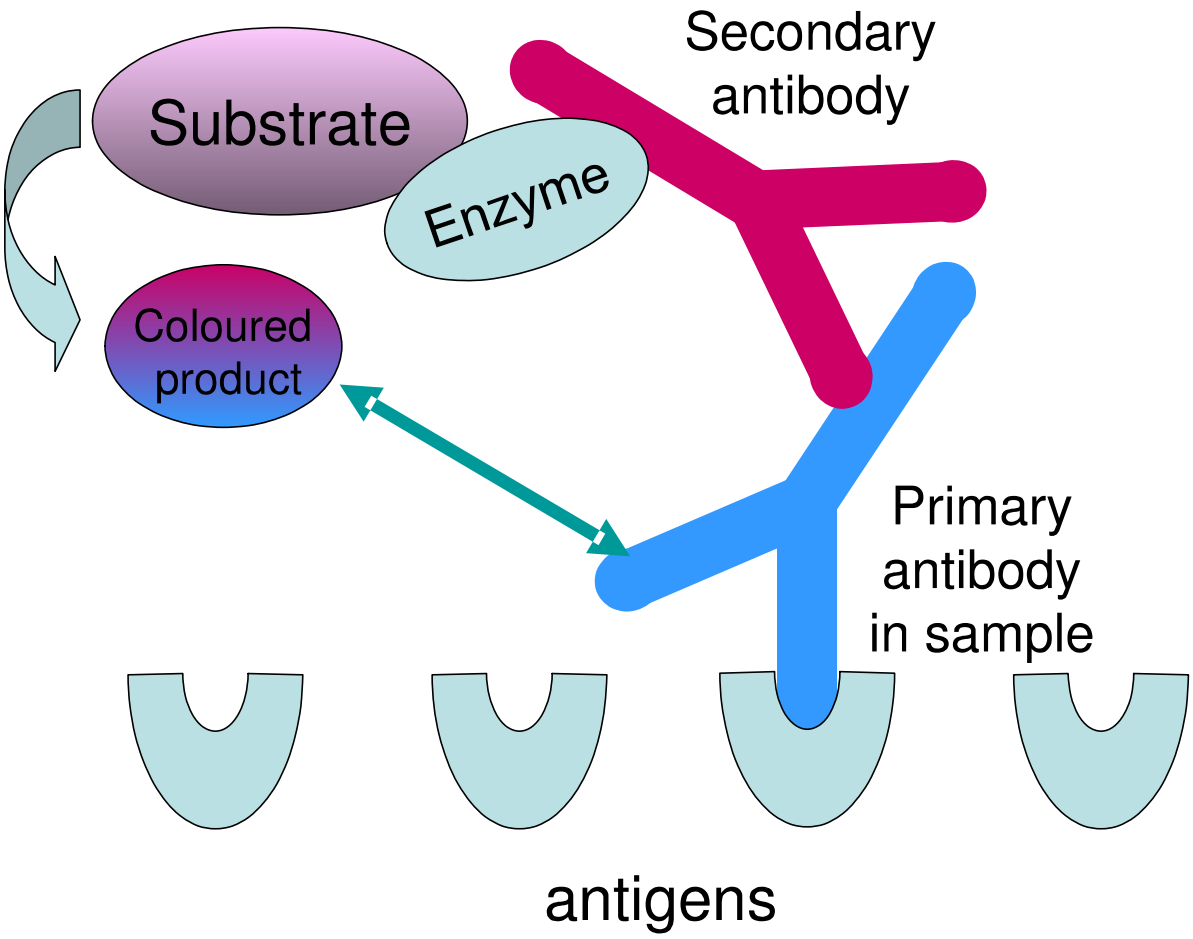
(c) Competitive ELISA **to detect Ag**



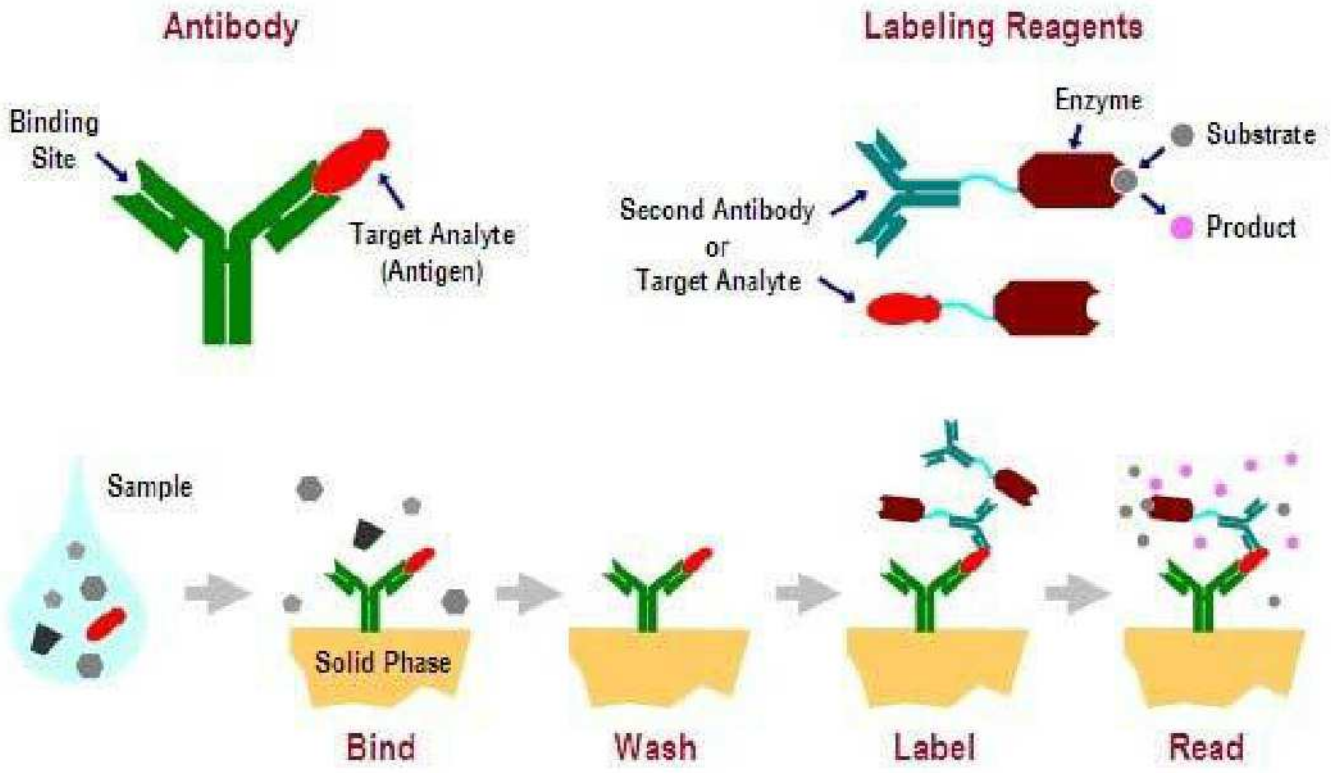
Basic steps of ELISA



1. Antigen of interest is immobilized onto the plastic surface (‘*sorbent*’).
2. Antigen is recognised by specific antibody (‘*immuno*’).
3. This antibody is recognised by second antibody (‘*immuno*’) which has enzyme attached (‘*enzyme-linked*’).
4. Substrate reacts with enzyme to produce product, usually coloured.

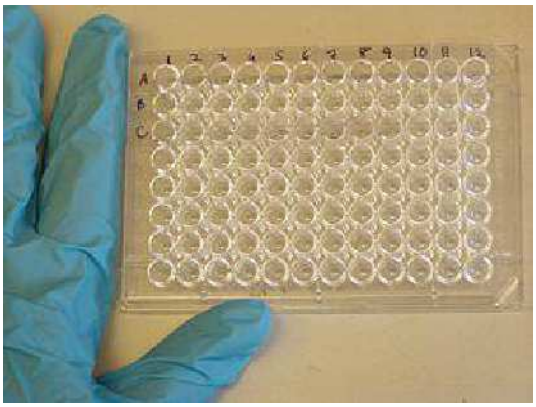

Secondary
antibody
Substrate
Enzyme
Coloured
product
Primary
antibody
in sample
antigens

ELISA


Antibody
Binding Site
Target Analyte (Antigen)
Labeling Reagents
Enzyme
Substrate
Product
Second Antibody or Target Analyte
Sample
Solid Phase
Bind
Wash
Label
Read

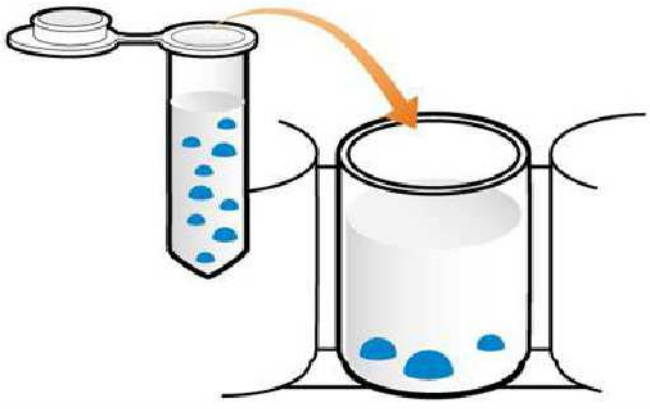
III. Materials and methods

- Thyroglobulin (Tg);
- Positive control serum, negative control serum and serum sample;
- Rabbit anti-human IgG labeled with peroxidase;
- pH9.6 0.05M carbonate buffer;
- pH7.4 0.02M phosphate buffer saline-Tween 20 (PBST);
- 5% BSA
- o-phenylene-diamine(OPD)
- 10% H_2SO_4
- 96-well ELISA plate; dropping pipettes; incubator; ELISA reader,…



1. Coating the wells

   Immobilize the antigen (Tg) on a solid surface (96-well ELISA plate, usually a polystyrene microtiter plate);



2. Block any non specific binding sites on the surface: BSA;

3. Rinse the plate with PBST for 3 times, 1min/time;

- Using pipette, fill wells with PBS-Tween, and then empty out.

- Tap the plate up and down on a pile of paper.

- Repeat twice more, making sure no liquid remains after the last wash.

- Label the pipette with ‘PBST’ and use it for later PBST pipetting.

4. Apply the sera to the plate (the first Ab):

well1: 100μl PBST (blank control)

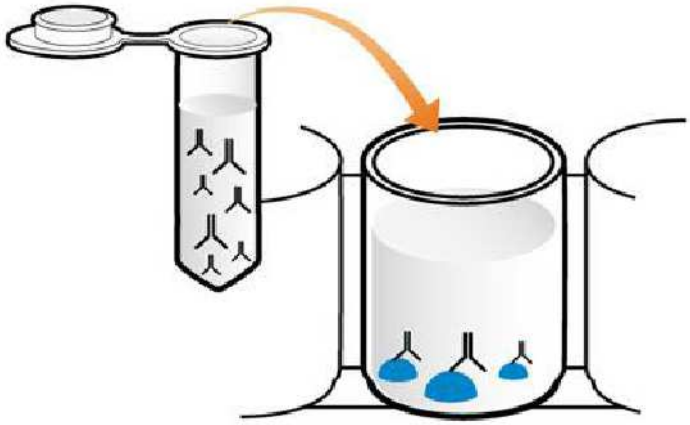
well2: 100μl negative control serum

well3: 100μl positive control serum

well4: 100μl No.1 serum samples

well5: 100μl No.2 serum samples

RT 30min;

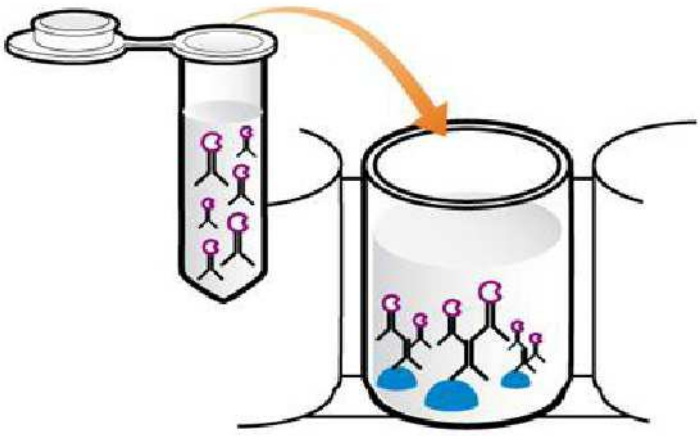


5. Rinse the plate with PBST for 3 times, 1min/time;

6. Apply the secondary Ab to the plate:

100μl peroxidase labeled rabbit anti-human IgG to each well

37℃ 30min;



7. Rinse the plate with PBST for 3 times, 1min/time;

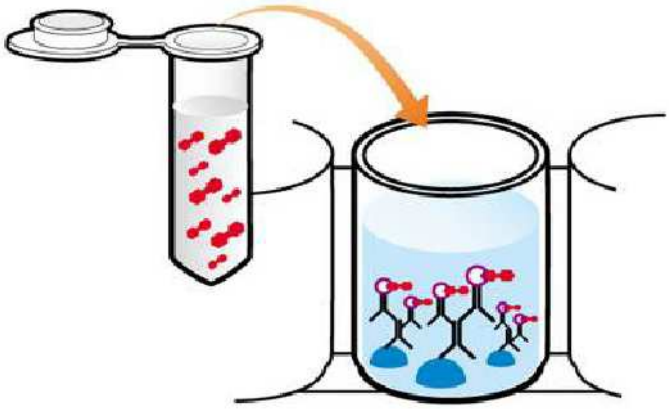
8. Apply the substrate to the plate:

100μl OPD solution to each well;

RT 5-15min(avoid light),

depending on the rate

of color development;

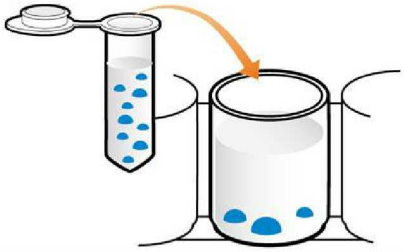


9. Apply the stop solution to the plate:

50 μl 10% H_2SO_4

10. Observe colour development (yellow) product formed.

Usually, results should be the OD492 nm value of each well read with ELISA reader.

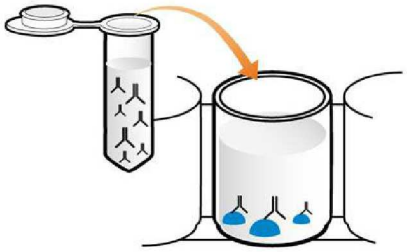


1. Add antigen

2.Wash,block the wells

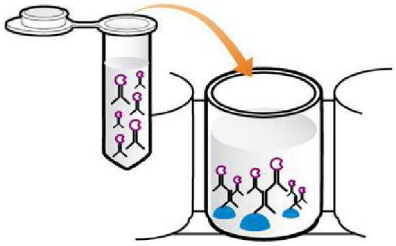
Wash with PBST

We do the test from this step.



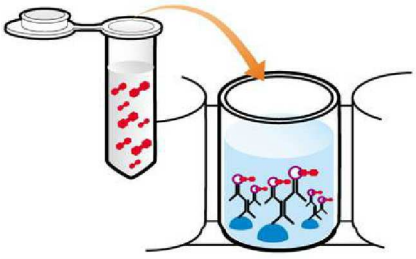
3. Add five samples

. Wash with PBST



4. Add labeled antibody

Wash with PBST



5. Add substrate for enzyme

Observe colour development

stop