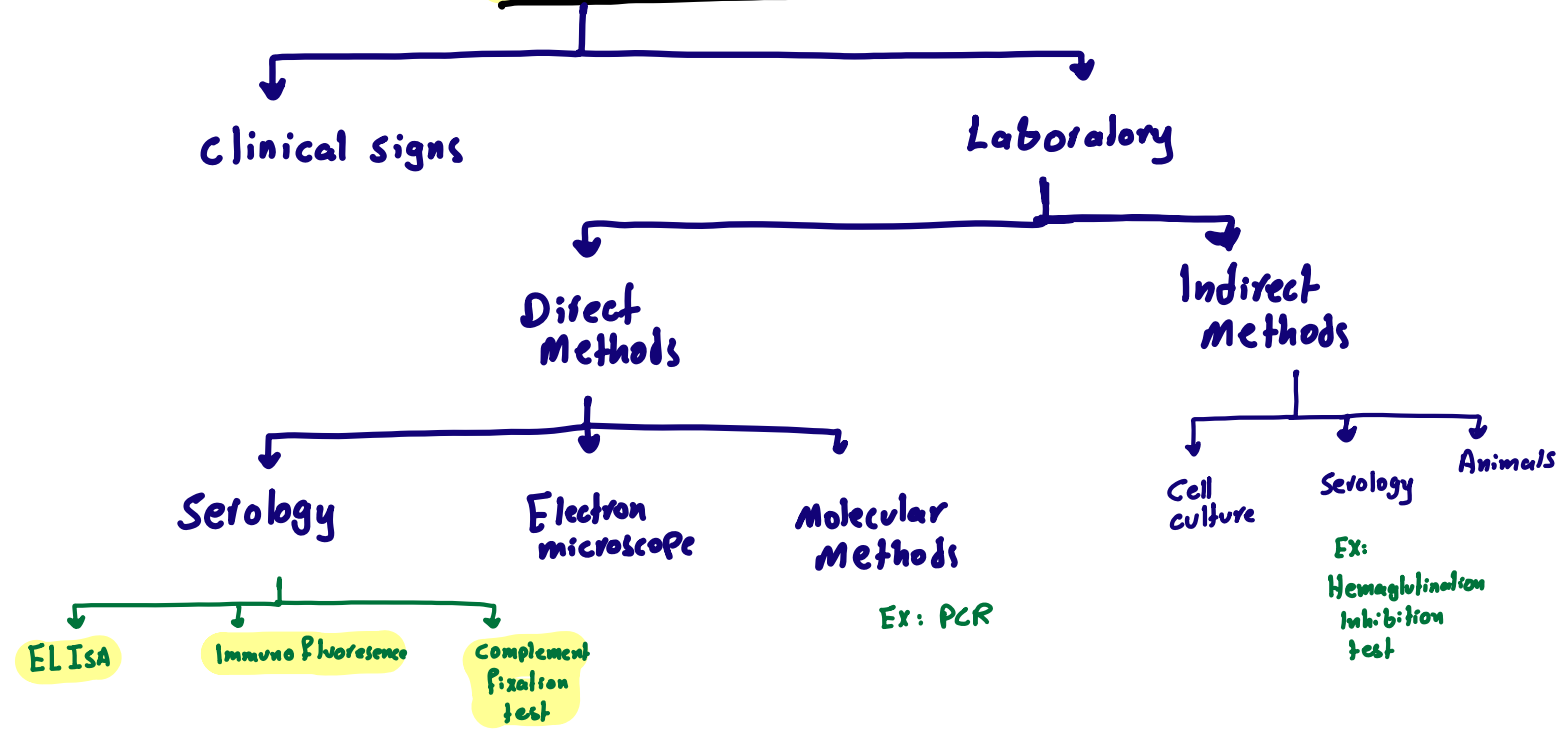


# \* Diagnosis of viral Infection:

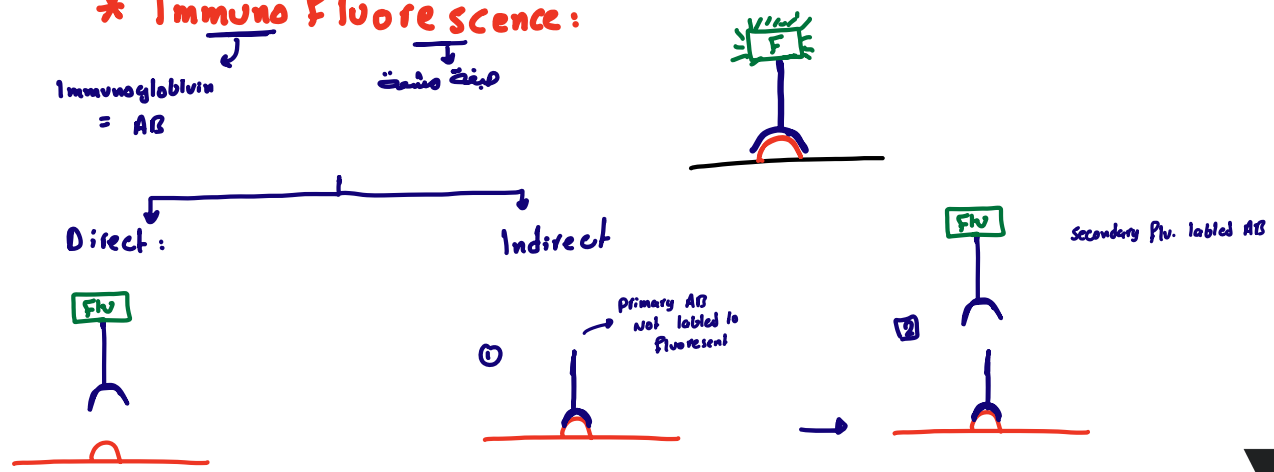


## \* Direct Methods:

### 1 Serology:

- Most used
- Detection of Ag in serum (Direct)
- Include: ① Immuno Fluorescence ② ELISA ③ Complement fixation test.

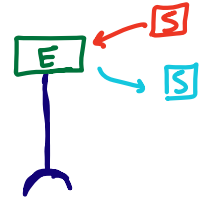
### \* Immuno Fluorescence:



- 1 Antibody.
- Less steps. → Quick.
- 2 Antibodies
  - 1° not labeled
  - 2° labeled
- More steps & More sensitive.



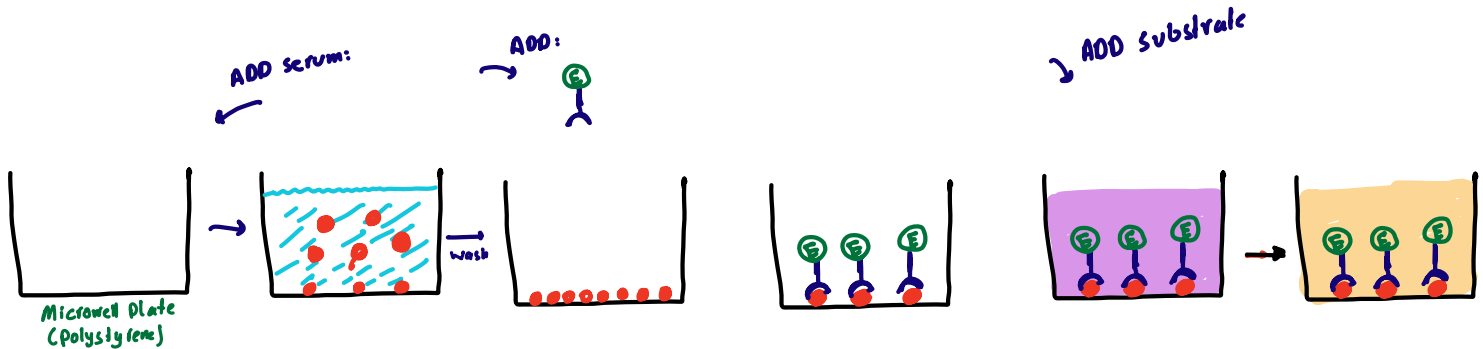
# ELISA: Enzyme Labeled Immunosorbent Assay.



- Detect Antigen or Antibody in serum by Enzymatic color change.

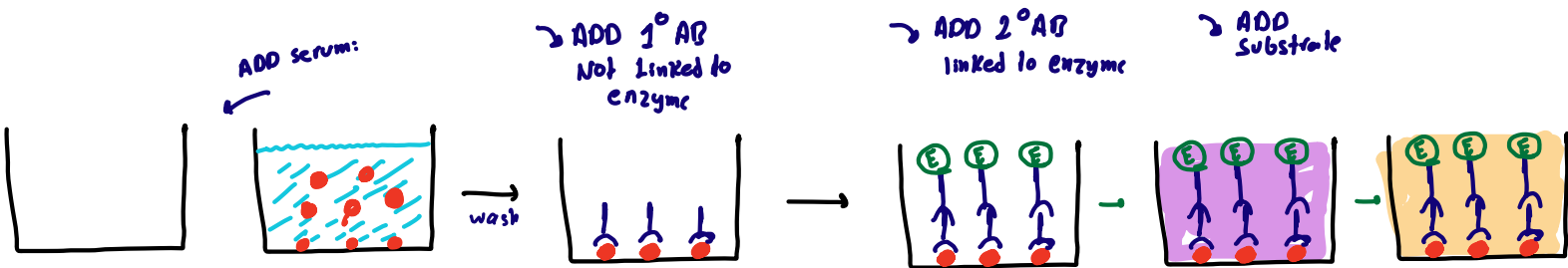
- 3 Forms: ① Direct ② Indirect ③ Sandwich

## ① Direct ELISA:

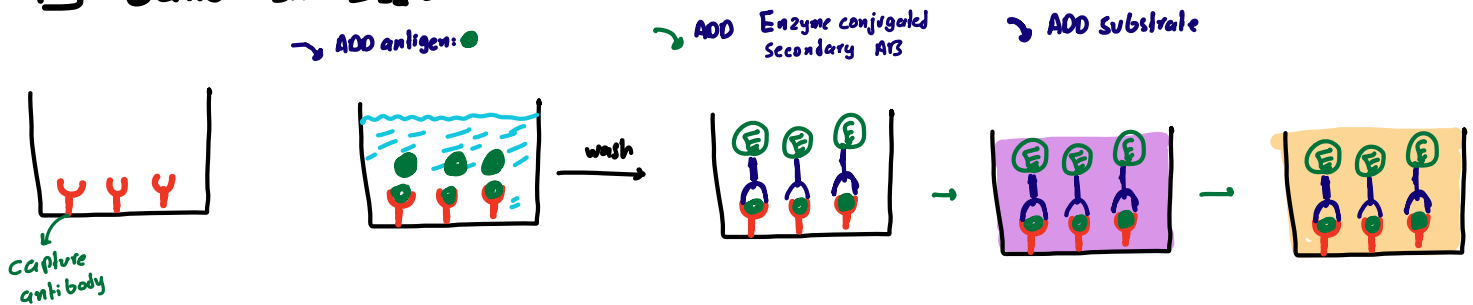


- why Direct? Enzyme linked Antibody Directly Bind to the Antigen.
- Intensity of color produced  $\propto$  Amount of Ag.

## ② Indirect ELISA:

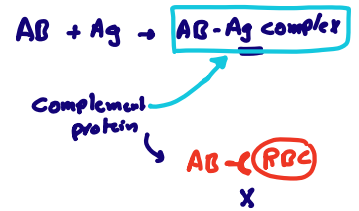


## ③ Sandwich ELISA:



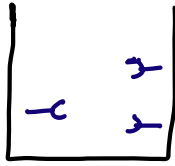
## \* Complement Fixation test:

- to test the presence of AB in Serum.

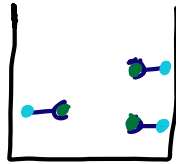


Patient A:

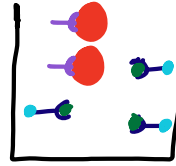
ADD: Antigen ●  
Complement proteins



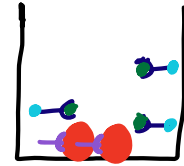
Serum with Antibody



- ① Ag Bind to AB
- ② Ag-AB complex
- ③ Complement protein Fix to the complex



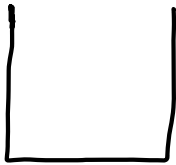
Complement is fixed.



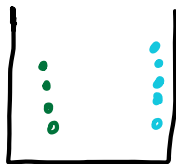
-ve Hemolysis  
∴  
✓ AB

Patient B:

ADD: Antigen ●  
Complement

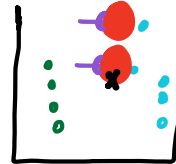


Serum without Antibodies

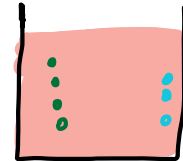


No Ag-AB complex  
∴  
No complement fixation

ADD: Sheep RBC  
AB to RBC



- AB-RBC complex  
- complement bind & Hemolysis.



+ve Hemolysis  
∴  
X AB.

## ② Electron microscope:

- $10^6$  virus particle / mL For visualization
- X 50K - 60K.
- Viruses are detected on diff specimens:

① Faeces: Rotavirus, Adenovirus

② vesicle fluid: HSV, VZV

③ skin scraping: Papilloma virus

- Problems of EM:

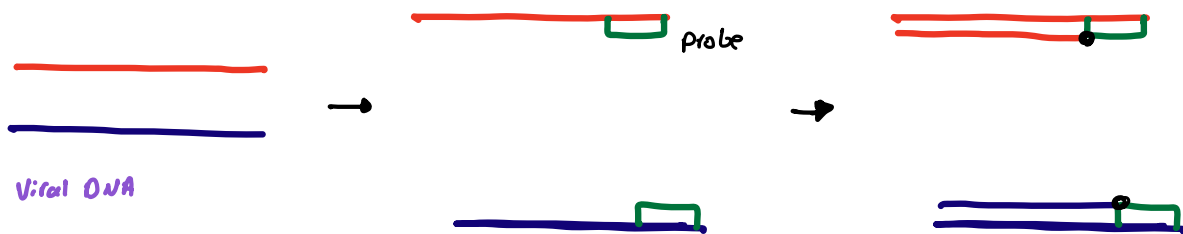
- ① Expensive
- ② Need skilled operator



### 3] Molecular Methods:

- Detect viral Genome.

→ Polymerase chain reaction (PCR):



① Denaturation  $95^{\circ}$

② Annealing:  $68^{\circ}$

③ Elongation:  $72^{\circ}$

* PCR	Advantages	vs	Dis Advantages
	Highly sensitive		↑ Liable to contamination
	Fast		Not Quantitative.
			need skilled operator

### \* Indirect Methods :

#### 1] Cell culture:

- ↓ ↓ used ?
- ① Long Period ( $> 4$  week)
  - ② very expensive
  - ③ poor sensitivity

#### 2] Serology:

- Detect AB in Serum (indirect) by Hemagglutination Inhibition Test

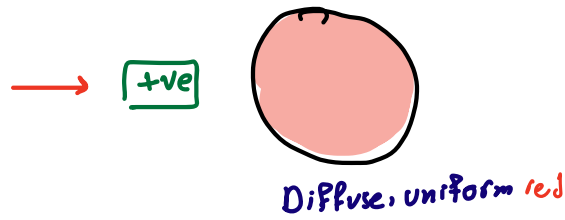
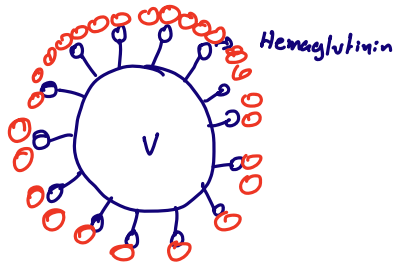
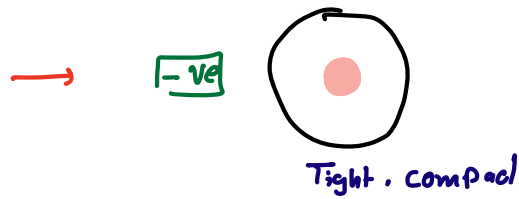
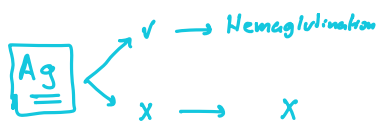
How to diagnose Recent / Primary Infection?

- ① presence of IgM
- ②  $\uparrow > 4$  of IgG or total AB Between Acute & convalescent.
- ③ Seroconversion.

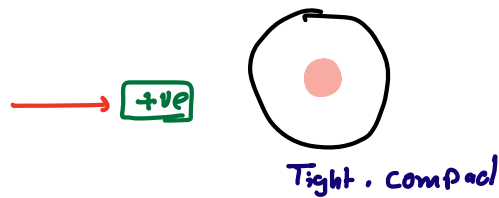
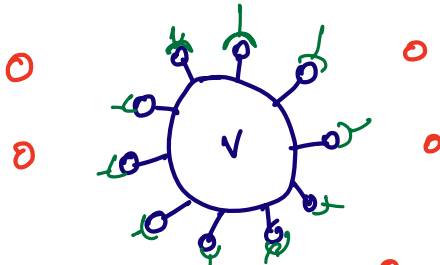
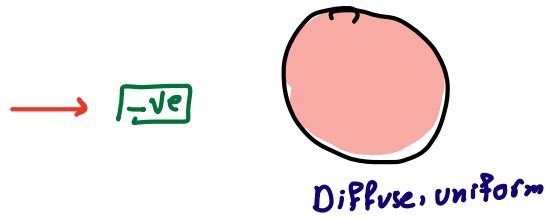
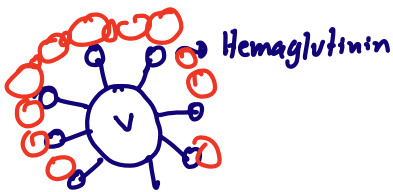
①	x4	②
50	x47	10
	x5	



## \* Hemagglutination Test:



## \* Hemagglutination Inhibition Test:



Feature	Hemagglutination Test	Hemagglutination Inhibition Test
Purpose	Detect viral antigens/proteins.	Detect antibodies against the virus.
Mechanism	Exploits viral ability to agglutinate RBCs.	Uses antibodies to inhibit this process.
Result Interpretation	Agglutination indicates a positive result.	Inhibition of agglutination indicates a positive result.
Applications	Diagnosing presence of a virus.	Assessing immunity or confirming infection.

Test Type	Positive Test	Negative Test
HA Test	Diffuse, uniform reddish "mat."	Tight, compact "button."
HI Test	Tight, compact "button."	Diffuse, uniform reddish "mat."

## \* Problems of Serology:

- in Immunocompromised Patient. X False Positive
- Mild Infection. X
- Patient given Blood. X ~~+ve~~

