By: Mahmoud Melhem.

* Diagnosis of viral Infection:

- Clinical signs
- Laboratory
  - Direct Methods
    - Serology
      - ELISA
      - Immuno Fluorescence
      - Complement Fixation test
    - Electron microscope
    - Molecular Methods
      - Ex: PCR
  - Indirect Methods
    - Cell culture
    - Serology
      - EX: Hemaglutination Inhibition test
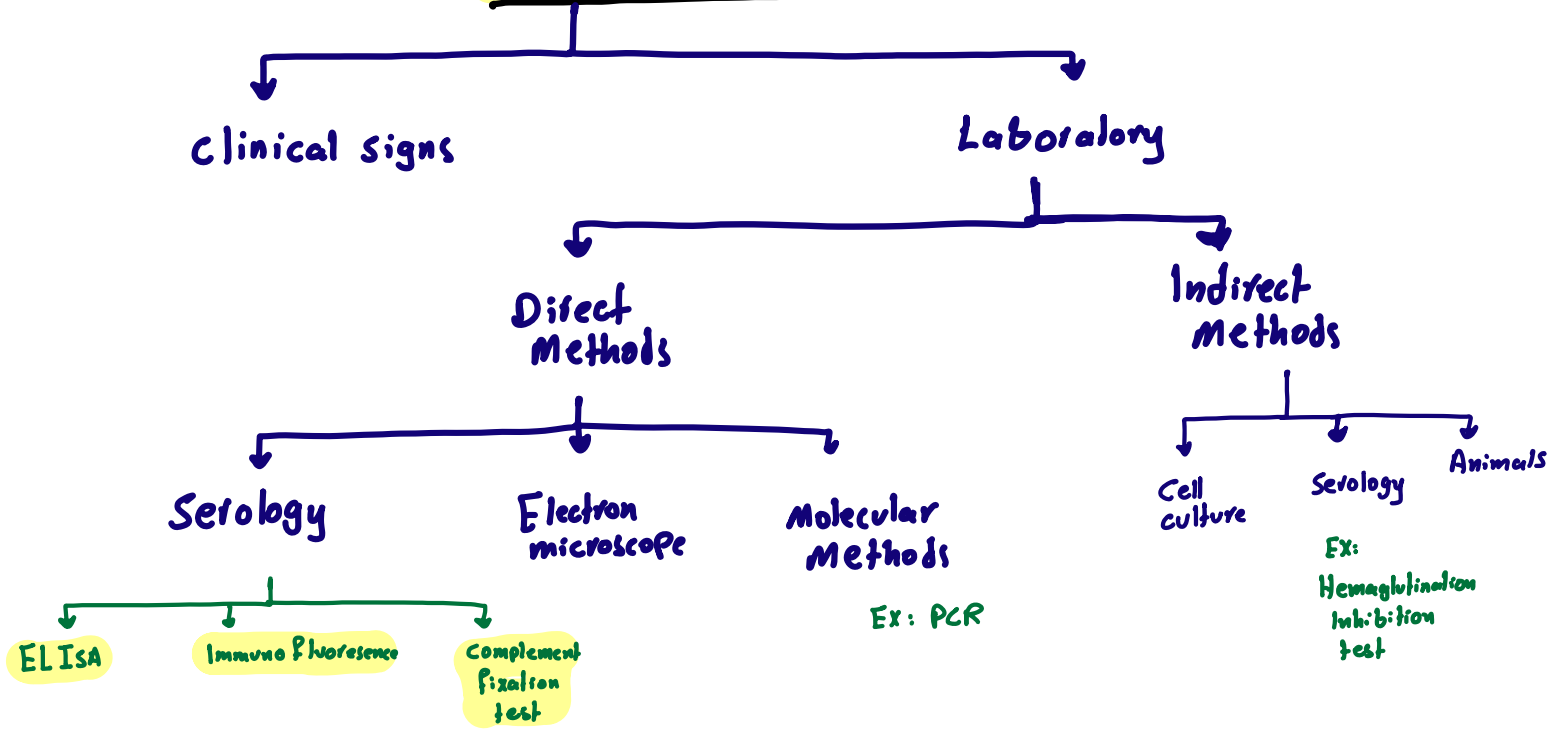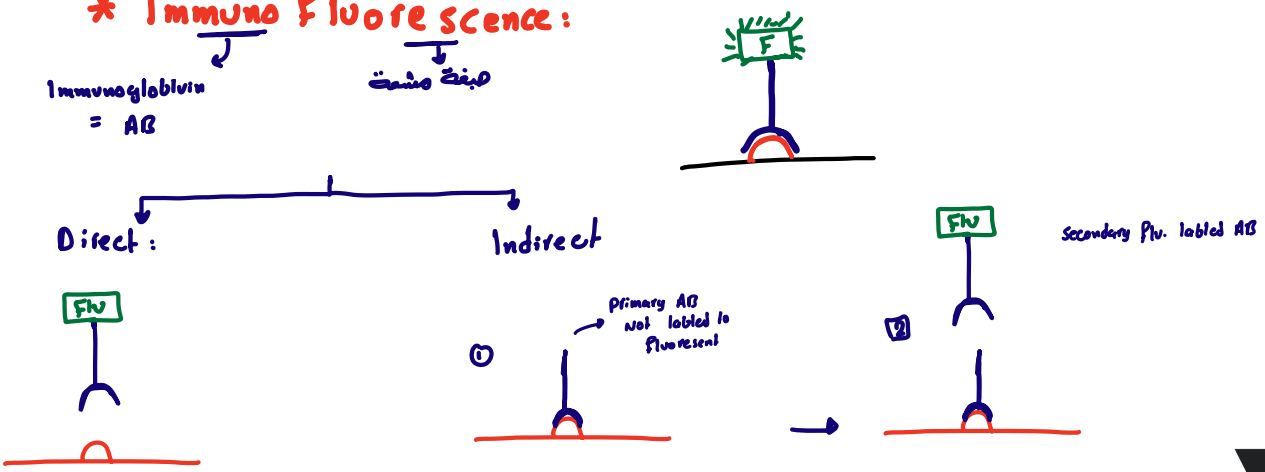   - Animals

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* Direct Methods:

1 Serology:

- Most used

- Detection of Ag in serum (Direct)

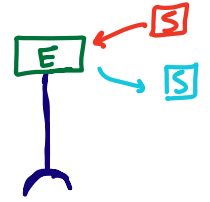- Include: ① Immuno Fluorescence   ② ELISA ③ Complement Fixation test.

* Immuno Fluorescence:

Immunoglobulin = AB   زهرة منبعثة

Direct:
- 1 Antibody.
- Less steps. → Quick.

Indirect
① Primary AB Not labled to Fluorescent
② FIV Secondary Flu. labled AB
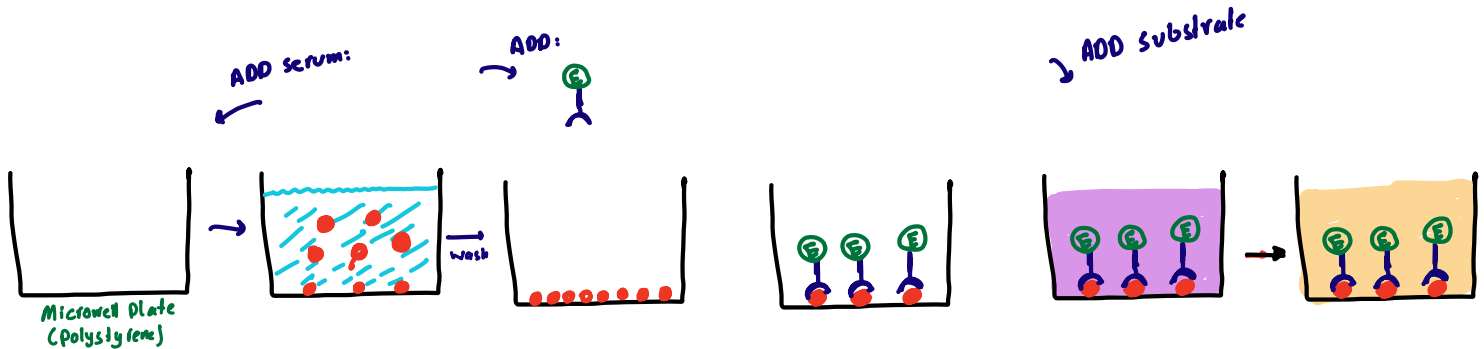- 2 Antibodies { 1° Not labled / 2° labled
- More steps & More Sensitive.

ELISA: Enzyme Labled Immunosorbent Assay.

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

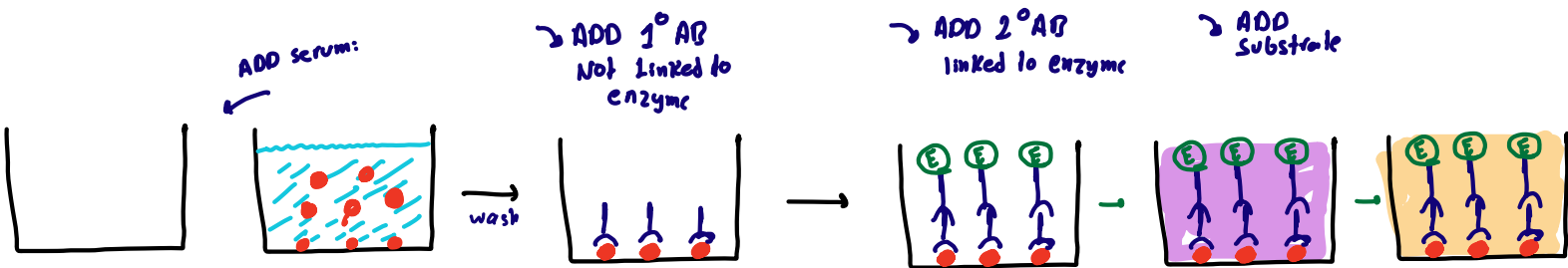- 3 Forms: ① Direct ② Indirect ③ Sandwish
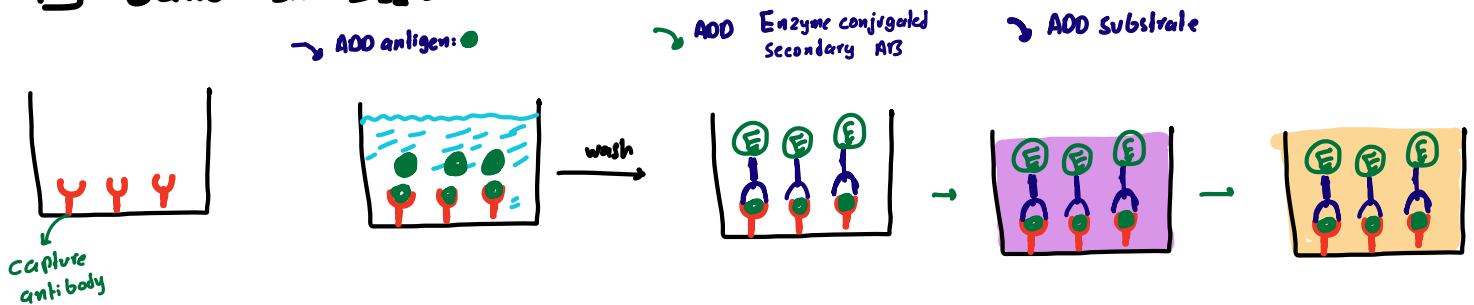
1 Direct ELISA:

ADD serum:

ADD:

ADD Substrate

Microwell Plate
(Polystyrene)

wash

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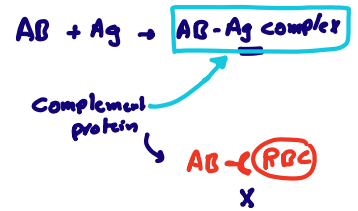 - Intensity of color produced ∝ Amount of Ag.

2 Indirect ELISA:

ADD serum:

ADD 1° AB
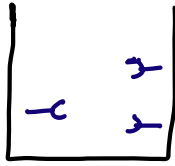Not Linked to
enzyme

ADD 2° AB
linked to enzyme

ADD
Substrate

wash

3 Sandwish ELISA:

ADD antigen: ●

ADD Enzyme conjugated
Secondary AB

ADD Substrate

wash

Capture
antibody

NOVA

* Complement fixation test:

- to test the presence of AB in serum.

$$AB + Ag \rightarrow \boxed{AB\text{-}Ag \text{ complex}}$$

Complement protein → AB - ⟨RBC⟩ ✗

Patient A:

ADD: Antigen ●
Complement ●
Proteins

ADD: ○ Sheep RBC
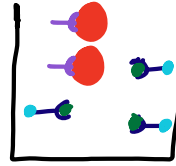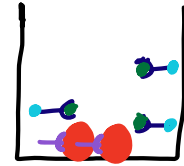AB to RBC

Serum with Antibody
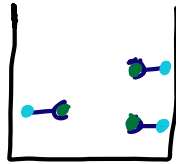
① Ag Bind to AB
② Ag-AB complex
③ Complement protein Fix to the complex
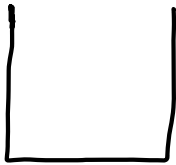
Complement is Fixed.

-ve Hemolysis
∴
√ AB

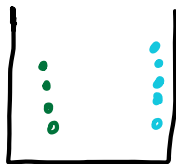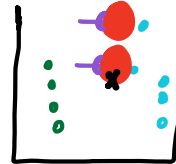**Patient B:**

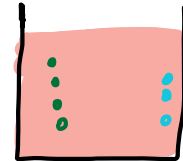ADD: Antigen ●
Complement ●

ADD: ○ Sheep RBC
AB to RBC

Serum without Antibodies

No Ag-AB complex
∴
No complement Fixation

- AB-RBC complex
- complement Bind
↓
Hemolysis.

+ve Hemolysis
∴
✗ AB.

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② Electron microscope:

- 10^6 virus particle / mL   For visualization

- ✗ 50K - 60K.

- Viruses are detected on diff Specimens:

  ① Feaces: Rota virus, Adeno virus

  ② Vesicle Fluid: HSV, VZV (
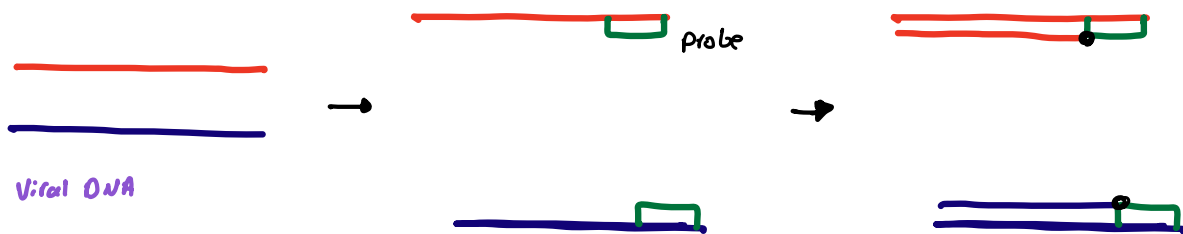
  ③ Skin Scraping: Papilloma virus

- Problems of EM:

  ① Expensive   ② Need Skilled opterator

3 Molecular Methods:

- Detect viral Genome.

↝ Polymerase chain reaction (PCR):

Viral DNA

probe

① Denaturation 95°   ② Annealing: 68°   ③ Elongation: 72°

* PCR	Advantages	VS	dis Advantages
	Highly sensetive		↑ Liable to contamination
	Fast		ϼ Not Qunatitative.
			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 Hemaglutination Inhibition Test
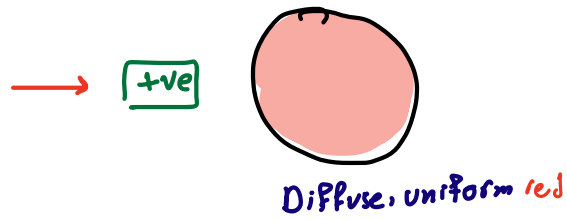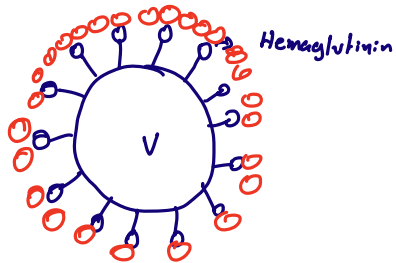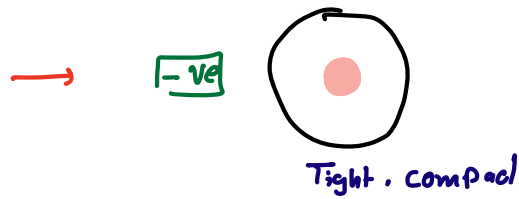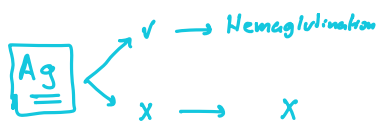
How to diagnose Recent / Primary Infection?

① presence of IgM

② ↑ ≥ 4 of IgG or total AB Between Acute & convalescent.

                                               تعافي   نقاهة

③ Sero conversion.

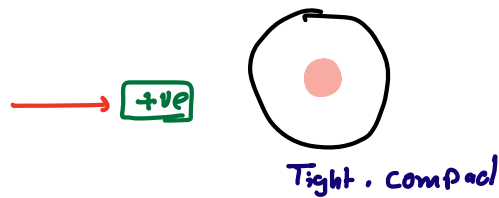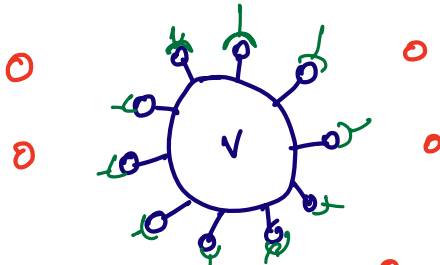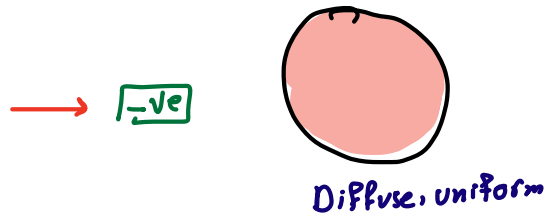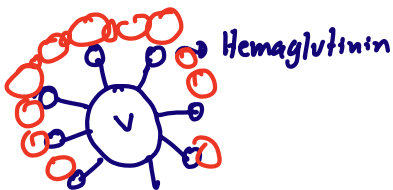   ①   ②

                                  50   ×4 ⟩   10

                                       ×5

Ag
- v → Hemaglutination
- x → x

→ −ve

Tight. compact

Hemaglutinin

V

→ +ve

Diffuse, uniform red

AB
- v → No Hemaglutination
- x → v

Hemaglutinin

V

→ −ve

Diffuse, uniform

V

→ +ve

Tight. compact

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