بسم الله الرحمن الرحيم

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GENERAL VIROLOGY

4

DIAGNOSIS AND MANAGEMENT OF VIRAL INFECTION

By:

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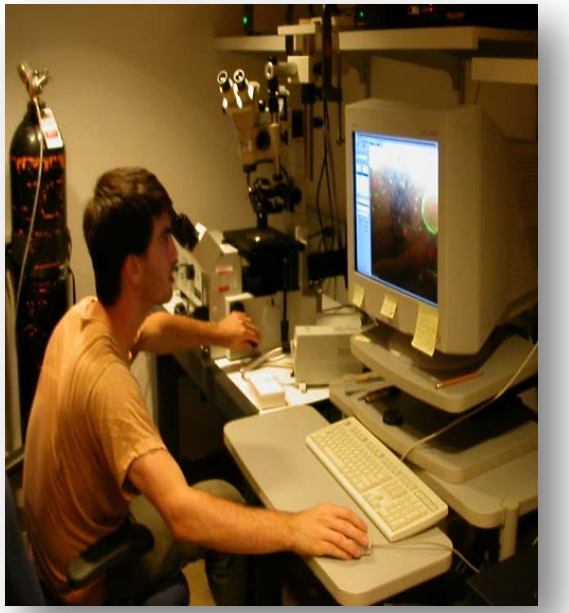
Laboratory viral diagnosis

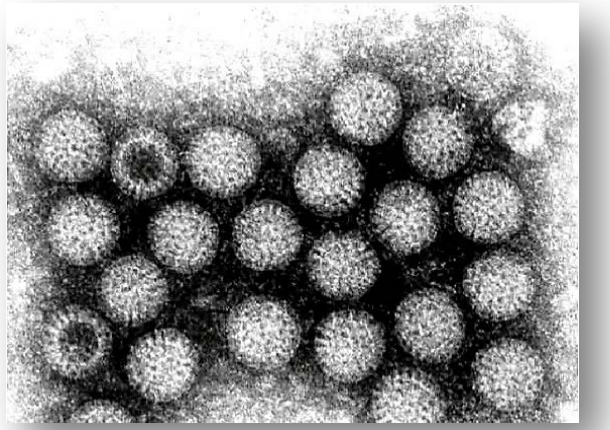
- ***Detection of virus.***
- ***Virus Isolation.***
- ***Detection of viral antigen.***
- ***Detection of anti-viral antibody.***
- ***Detection of virus nucleic acid.***
- ***Gene sequencing.***

Detection of virus

1-**WE** can see virus from inclusion body which is aggregation of virus it can be basophilic or acidophilic (LM)
2-Or pathology such as multinucleated giant cells
3-Ebola isolation is dangers so we use this method

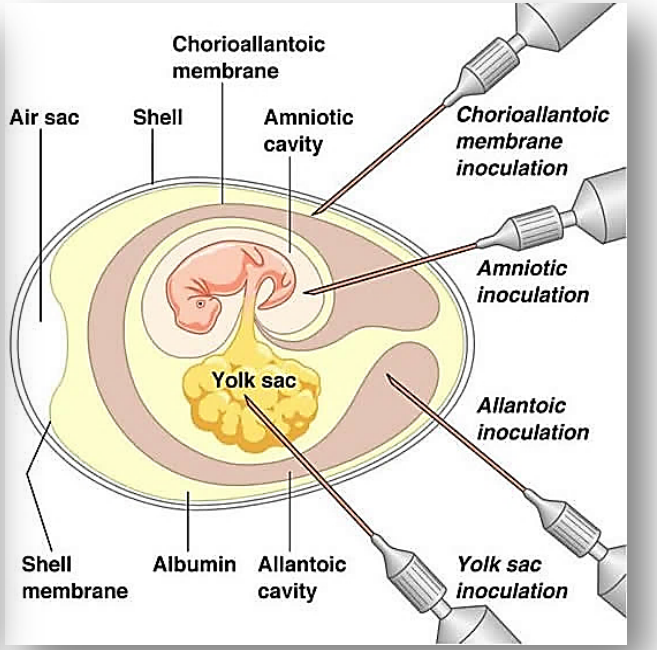


Electron Microscopy detects virus particles, which can be characterized by their size and morphology (e.g. Rota virus, Ebola virus)



Virus Isolation:

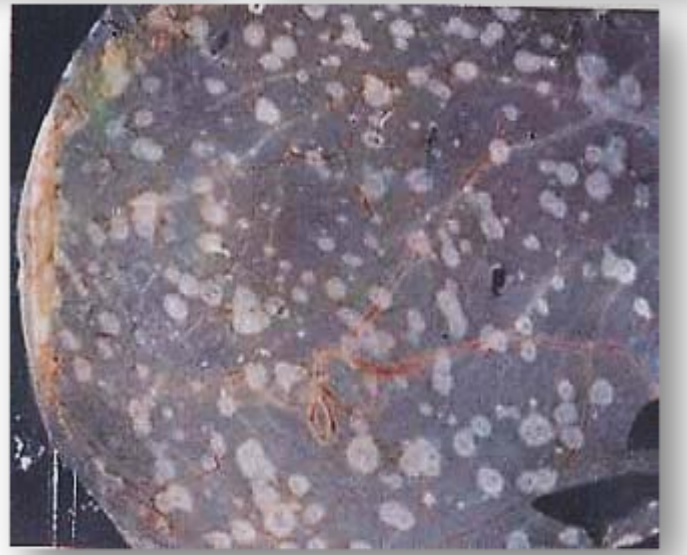
Each pocks is virus particle
Pox virus(by this method)→
injection chorioallontic membrane



❑ Embryonated egg:

- In yolk sac, amniotic cavity, allantoic cavity, chorioallantoic membrane.

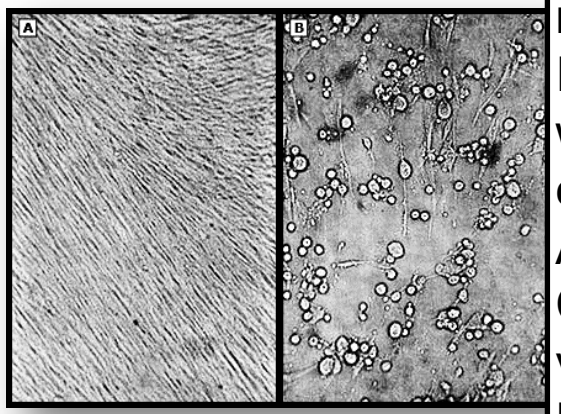
- Test the **presence of Pocks** on chorioallantoic membrane used as **quantitative assay.**



❑ Tissue culture:

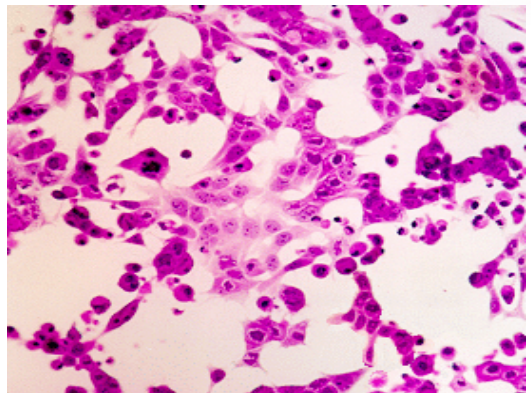
- When growing virus in a cell culture, the cells affected with virus will evolve morphologic changes, called **Cytopathic effect (CPE),** often specific for the type of virus involved.

- CPEs of infected cells can be observed with inverted light microscopes, such as the ballooning of cells or syncytia formation,……

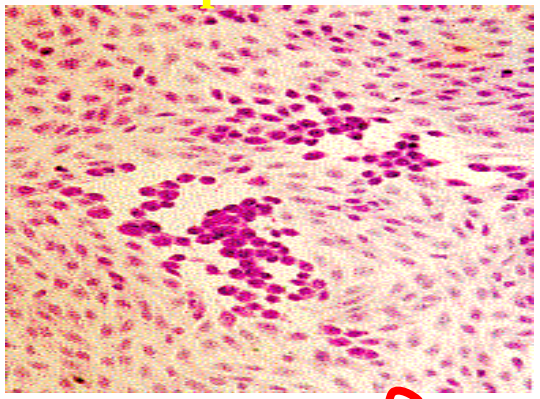


Cells in form monolayer
nutrient by phenol red
I use enzyme to transfer it then we do passage (propagation of culture)
ANOTHER CPE
Complete disriction → entero virus
Focal distruction

Adenovirus



Herpes virus



Cytopathic Effect (CPE)

- **if not producing CPE** , the presence of the virus could be detected by :
  1. **Haemadsorption** (mumps, Influenza, and parainfluenza)as cells acquire the ability to stick to mammalian red blood cells.
  2. **Interference.**
  3. **Characteristic inclusion bodies, immuno-his of viral antigens.**

- Confirmation of the identity of the virus may be carried neutralization, haemagglutination -inhibition or immunc

- Tissue culture in plates showing **CPE in the form of plaque formation** used in **quantitative assay** of virus

Tissue culture is monolayer → above it nutrient →after incubate the sample with virus → then we add semi solid agar → block spread of virus to all culture (stay in its limit)

Used for detection of virus with no CPE
We have two viruses
Virus A → No CPE e.g ***Rubella***
Virus B → have CPE
We think that virus A infect a cells of patient so
We take sample from patient → add virus B to it
There is tow possible result
1- Virus B show its CPE →sample does not have virus A (If it is present it occupied sample prevent virus b action)→ NEGATIVE
2- Virus b does not show its CPE →cell are occupied by virus A→POSITIVE

- 3 types of cell cultures:
  1. Primary cells - Primary Monkey Kidney.
  2. Semi-continuous cells - Human embryonic kidney and skin fibroblasts- human diploid fibroblasts.
  3. Continuous cells - HeLa, Vero, and HEp2 ….

❑ Animal inoculation:

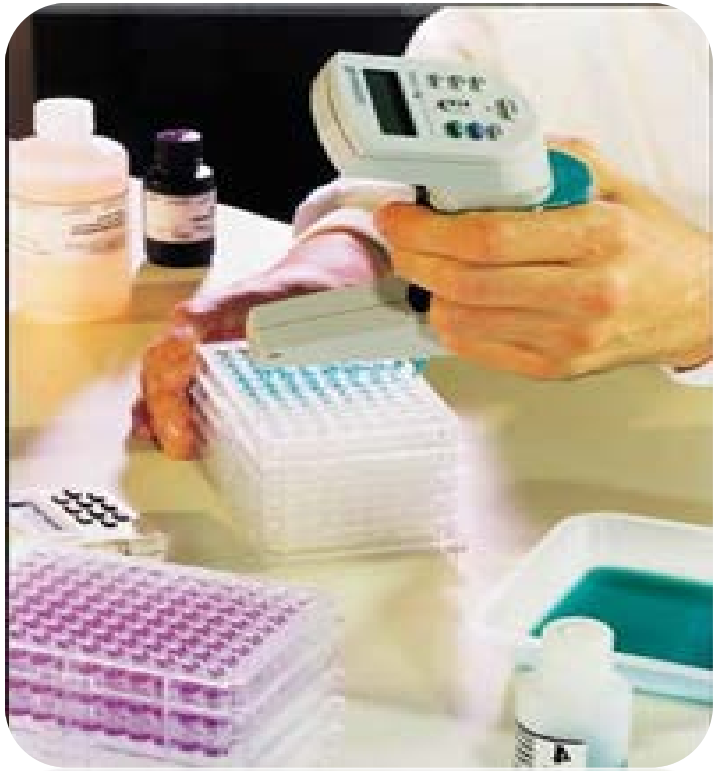
Mice are infected and observe the development of clinical symptoms or death.

Serologic tests:

- To determine **viral antigens** or **antibodies**.

VIRUS ANTIGEN DETECTION:

- Immuno-suppresed patients do not produce antibodies.
- Antibodies take time to be produced (window period).



VIRUS ANTIBODY DETECTION:

expect HCV

- IgM detection to diagnose recent infection.
- IgG antibodies:
  - Indicate past infection or persistent infection
  - Paired blood samples: at the onset and during the recovery, at least a fourfold increase in titer (IgG) to indicate a current infection.
  - Absence of IgG antibodies can determine susceptibility to infection e.g. Rubella in pregnant women.

Types of diagnostic serological tests:

1. Agglutination:

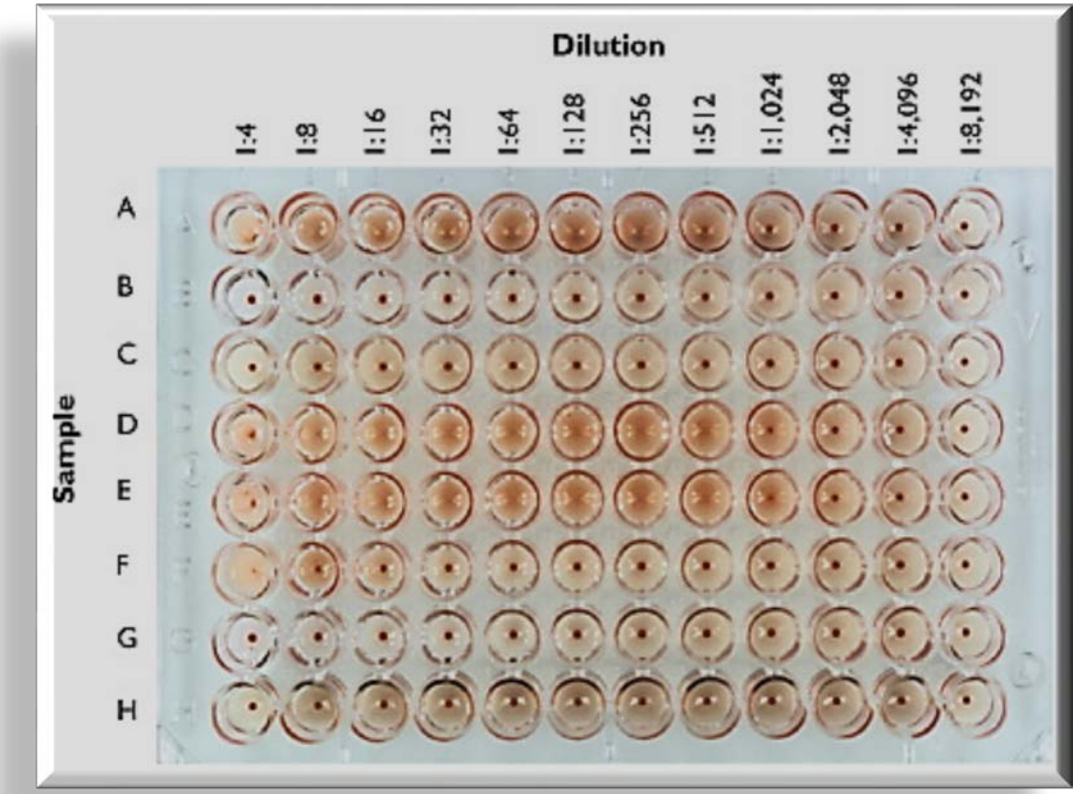
Antigen-Antibody reaction causes visible aggregation

e.g. Rota virus detected in stool by mixing sample with specific antibody coated particles.

2. Haemagglutination Inhibition:

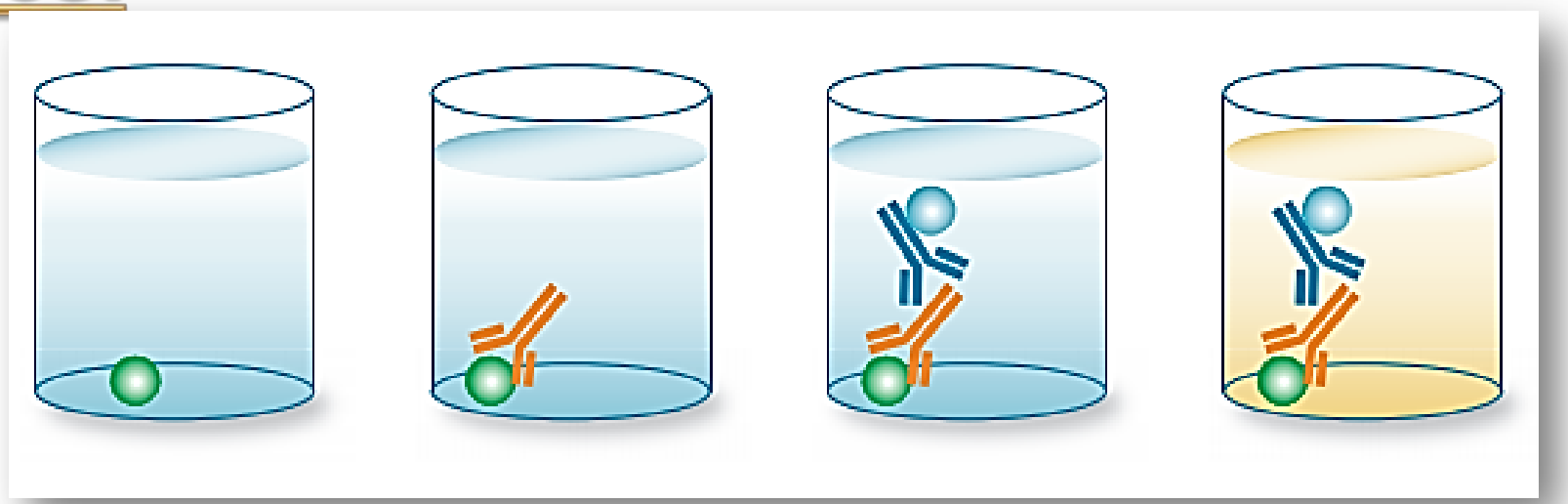
- Some viruses cause RBC agglutination → preventing them from settling.

- Addition of serum sample that contain the type specific antibody will inhibit this haemagglutinantion (e.g. Influenza & Parainfluenza).

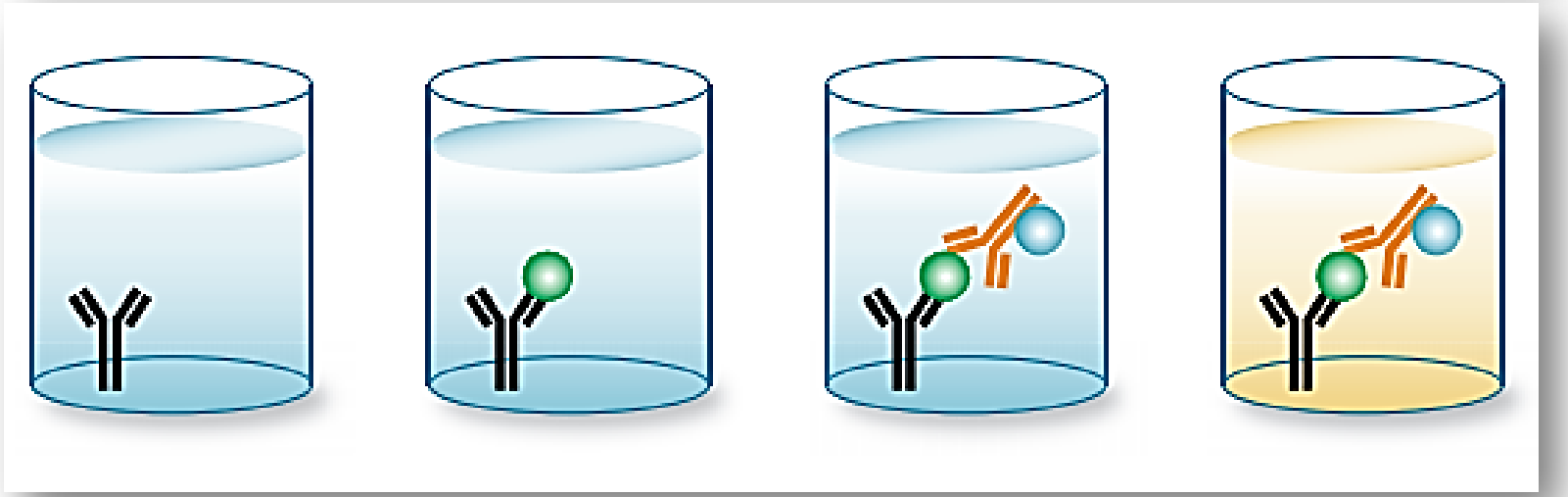


3. ELISA Procedures:

Indirect ELISA

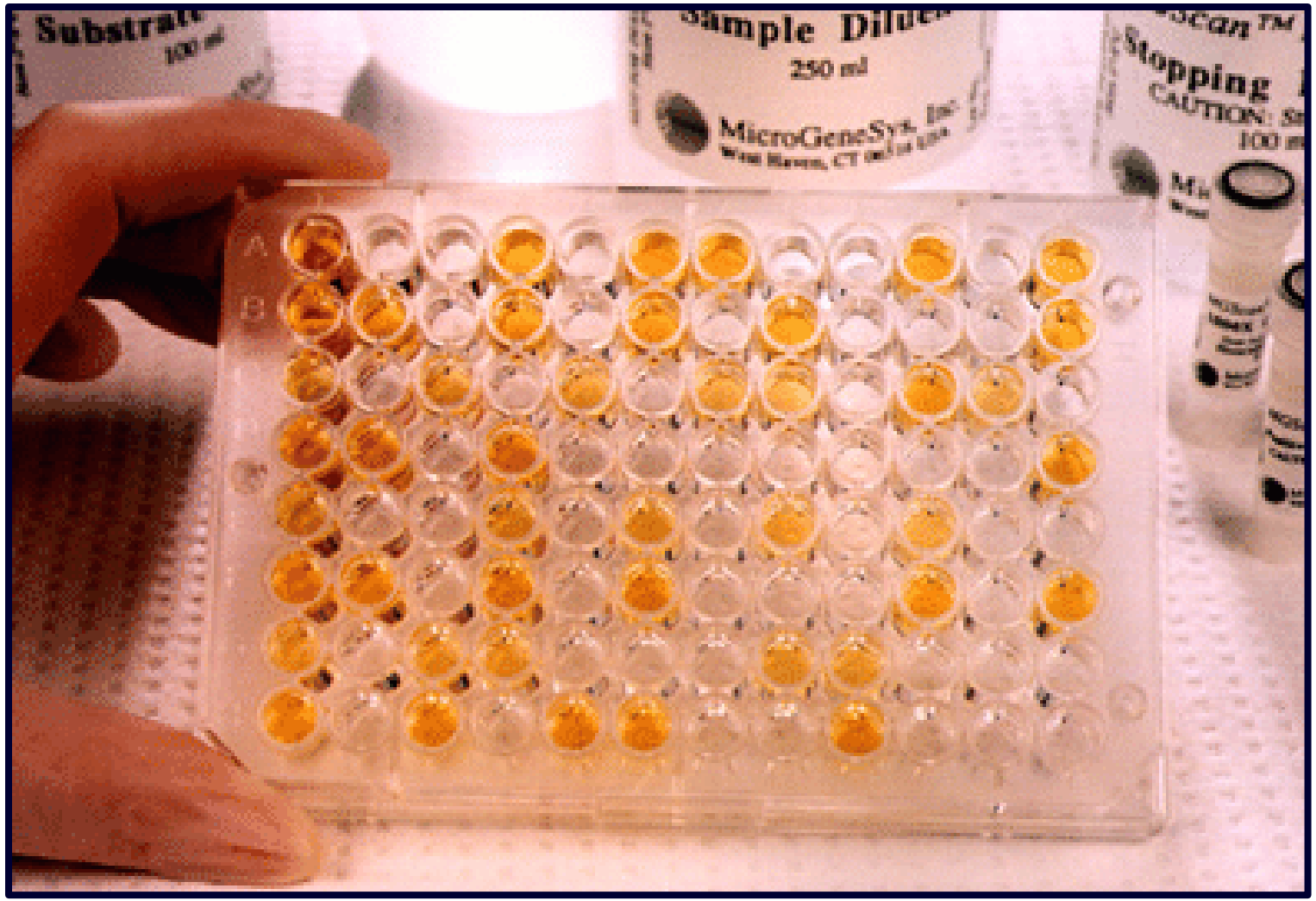


Sandwich ELISA



Indirect ELISA →detect antibody→wells coated with antigen
We add serum to a micro titer plate → then we wash →to get red form antibody that does not Catch antigen (if specific antibody is present it will Catch antigen and stay in micro titer plate and doses not wash out) →add anti-antibody conjugated with enzyme →wash →add substrate → color change→+
No color change → -

Sandwich ELISA →detect antigen →wells coated with antibody to specific antigen → add serum (supposed it contain antigen) → antigen bound to specific antibody →wash →add conjugated antibody (the same antibody in wells)→ wash → substrate
Color change → positive
No color → negative


Substrate
100 ml
Sample Dilue
250 ml
MicroGeneSys, Inc.
Stopping
CAUTION:
100 m
A
B

4. Western Blot:

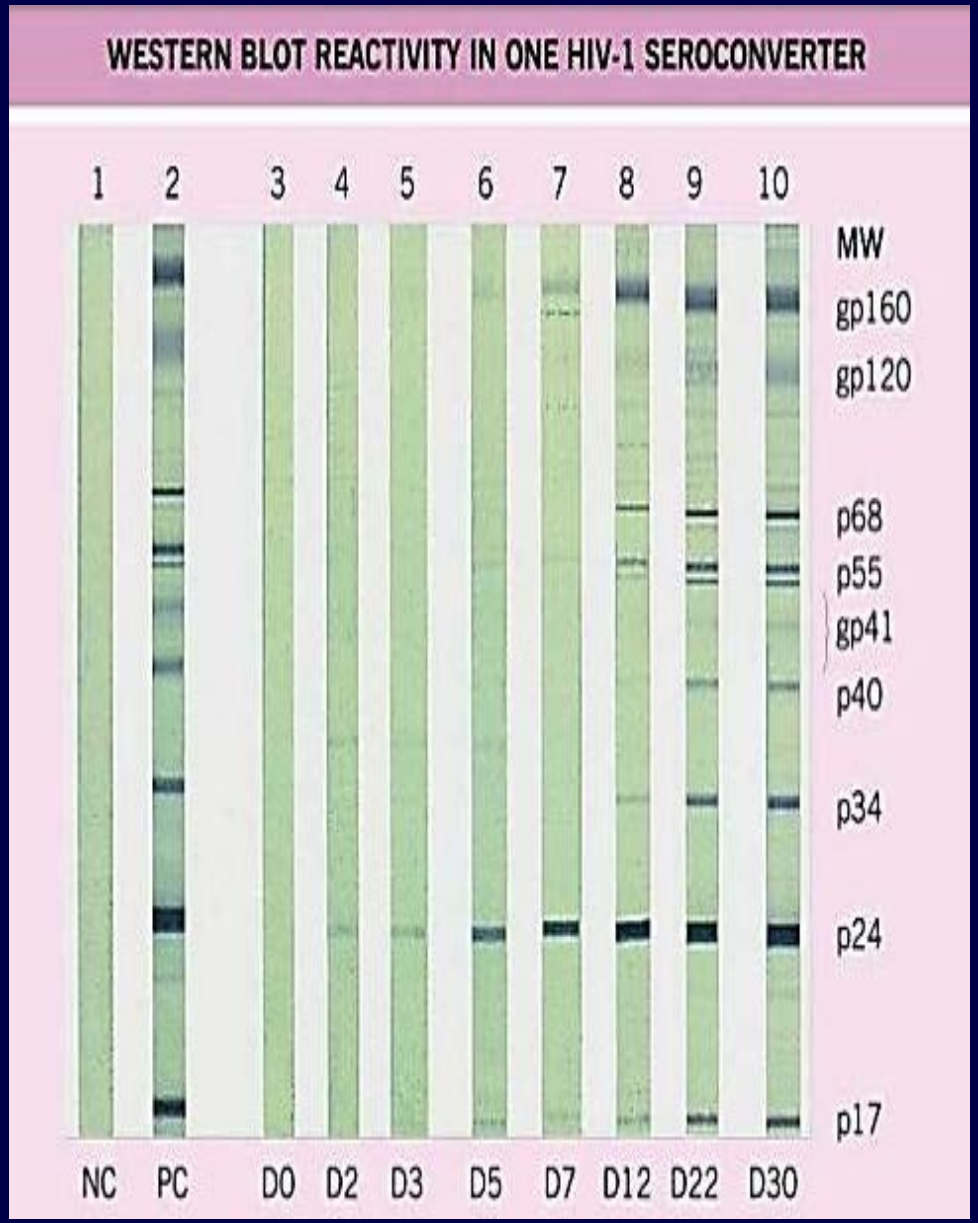
HIV

- **Viral proteins are blotted** on membrane → serum samples are added.
- If serum samples have the **specific antibodies** → interaction → detected.

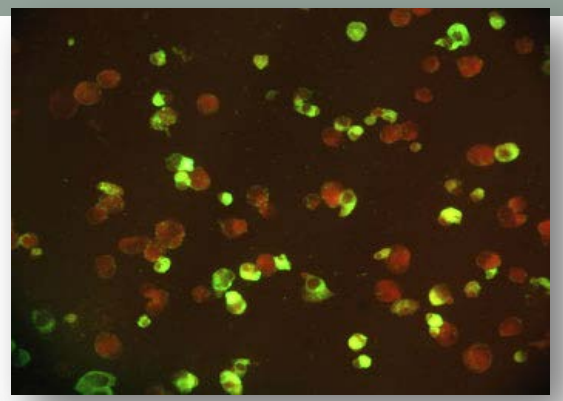
ELISA →more than one antigen for the same virus on micro titer plate and the same antigen may be shared between tow virus (cross reactivity)

اضافه

IN western blot we separate viral antigen protein by MW (ELECTROPHORESIS)

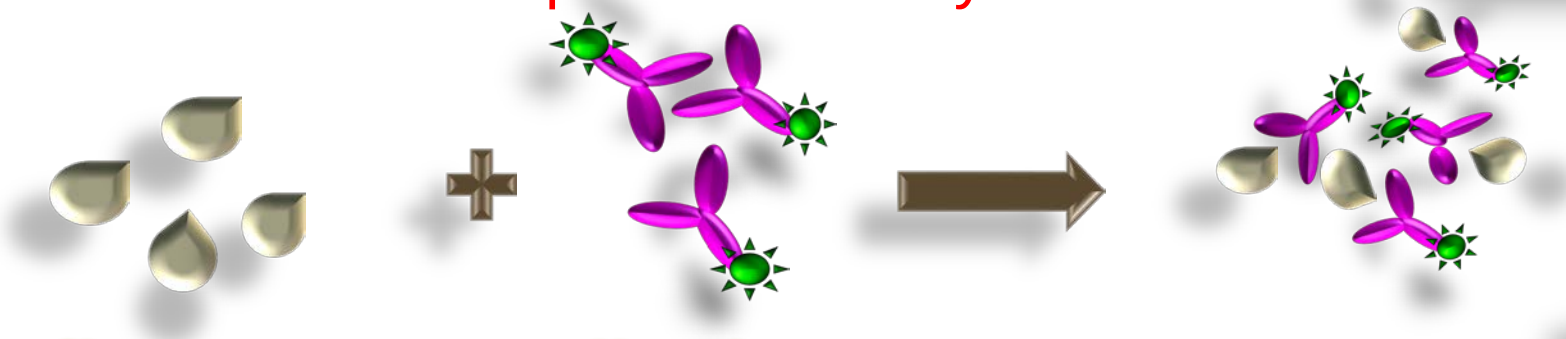


5. Fluorescent antibody tests: (UV)



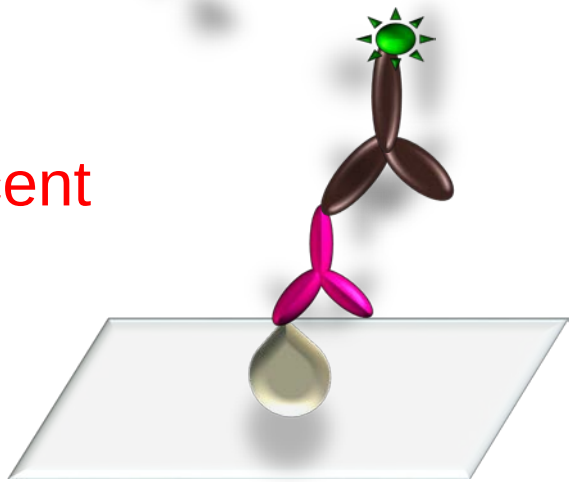
Direct fluorescent antibody test :

Antigen + fluorescent labeled specific antibody

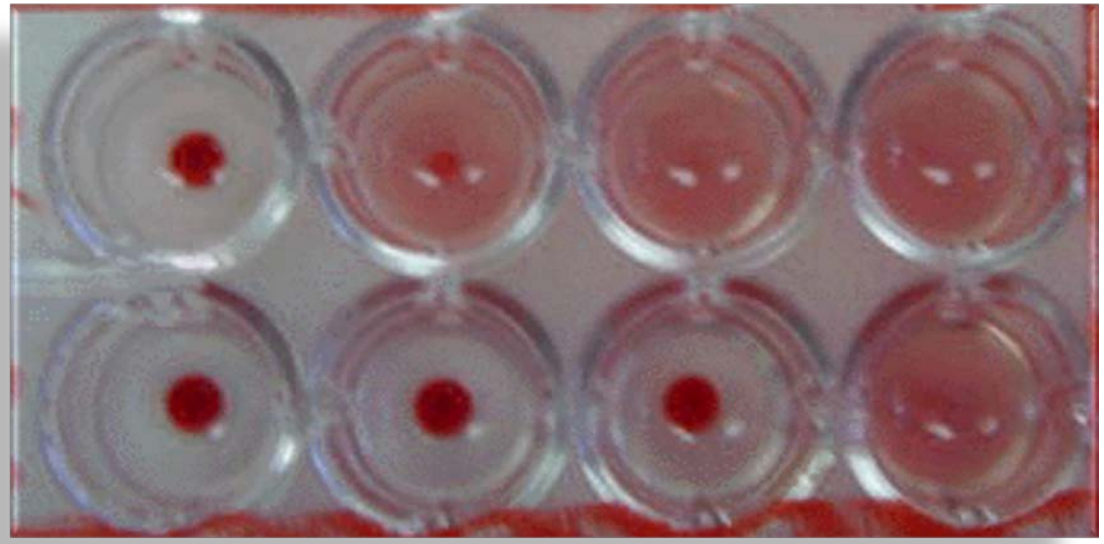


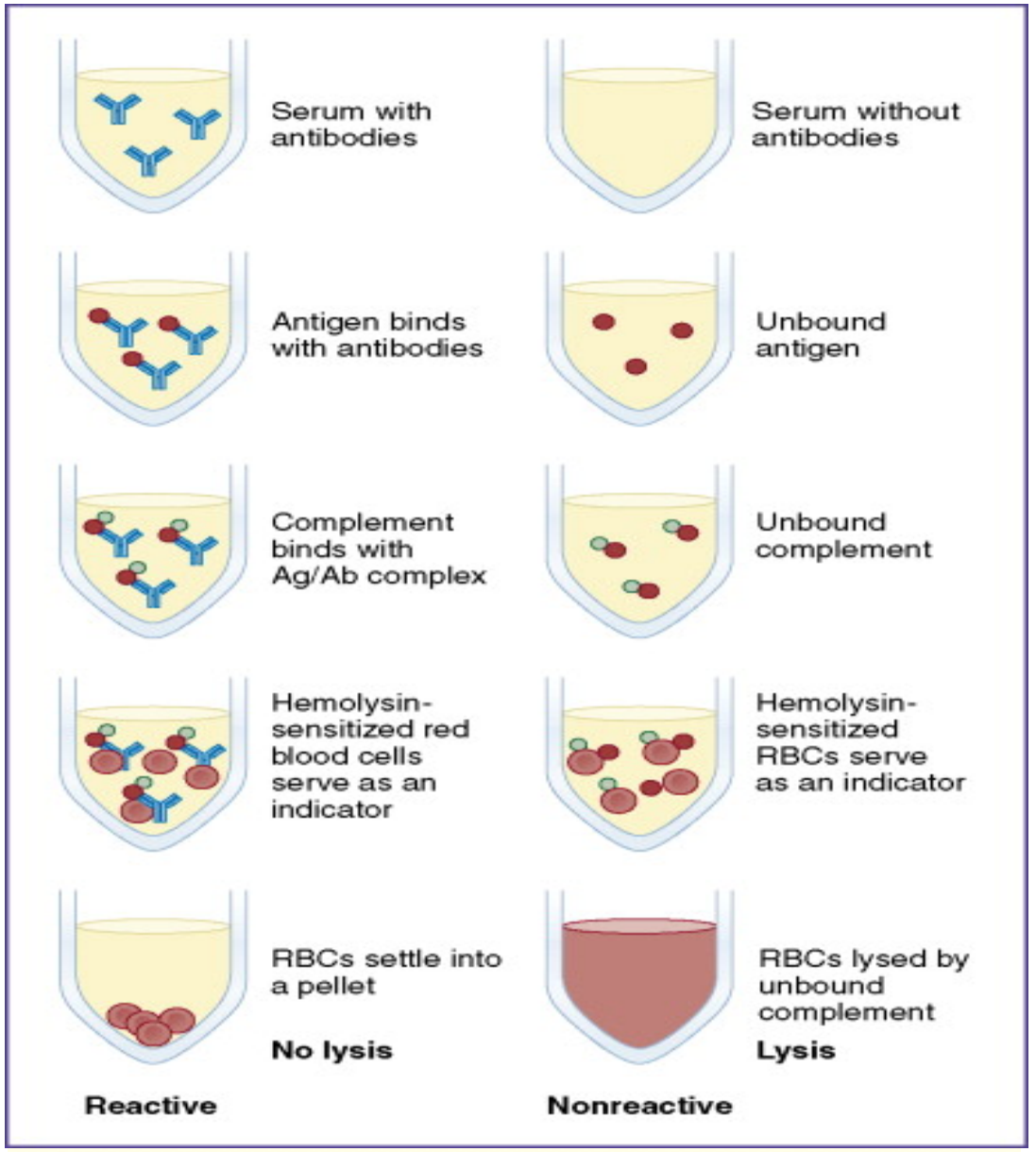
Indirect fluorescent antibody test :

Antigen+ unlabelled antibody → wash → add fluorescent labeled anti-species globulin.



6. Complement Fixation Test:



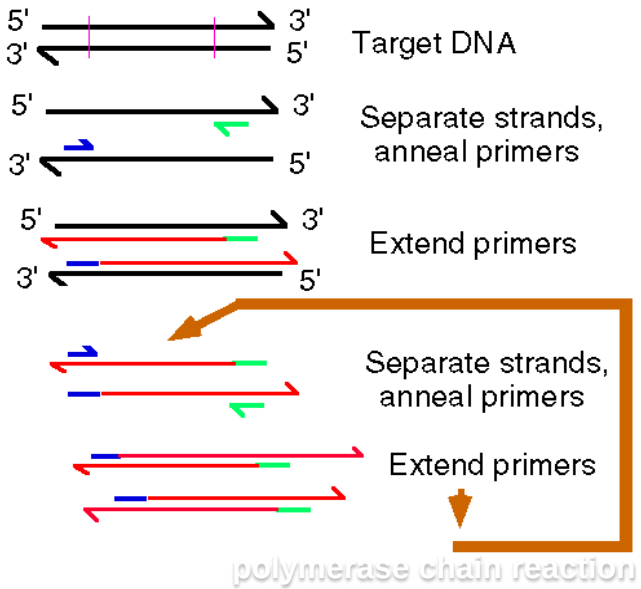


Nucleic acid detection:

Target amplification methods:

- Polymerase chain reaction (PCR).
- Real time PCR LOAD OF VIRUS FOR TRETMENT

Signal Amplification Techniques:

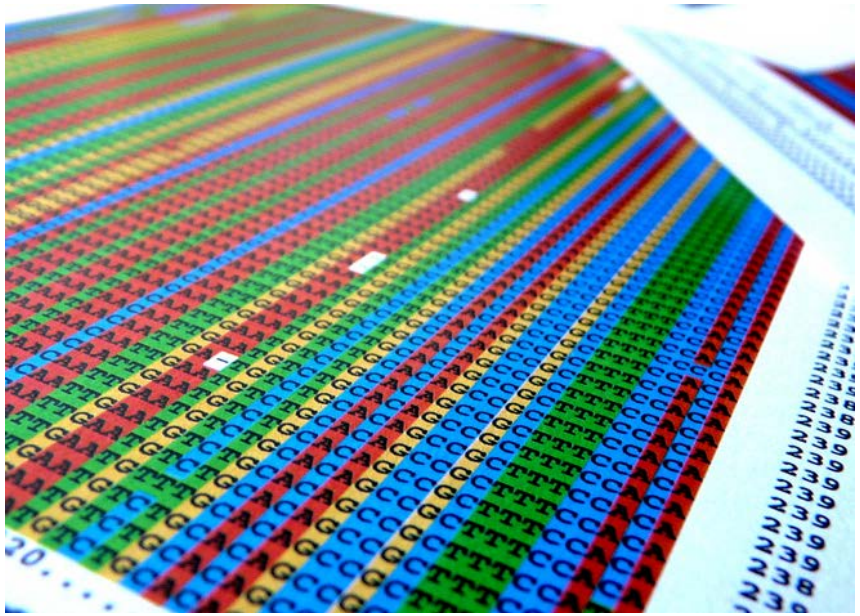


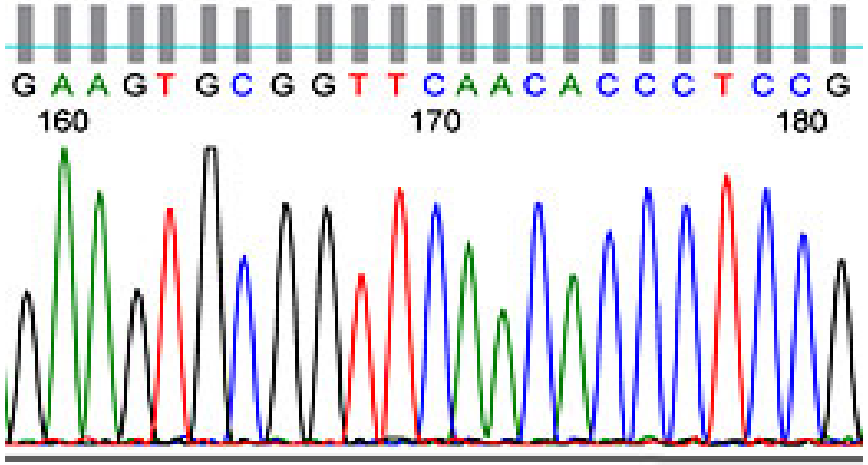
Molecular techniques are usually used to confirm positive serological results due to their **higher sensitivity and specificity**

Gene sequencing:

genotypes

specific mutations





Treatment and Prevention of Virus Infections:

Antiviral agents:

- Selectively inhibit viral replication (selective toxicity)
- Targeting one of the steps in the viral life cycle.
- Only a few viral infections have antiviral agents
- There are no broad-spectrum antivirals.
- Generation of resistant variants.
- At present, no antiviral can eradicate latency.

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Antiviral Targets:

- **Attachment/fusion/un-coating**:
  - **Raltegravir** → **CCR5 co-receptor (HIV)**
  - **Amantadine** bind to the **M2** protein (un-coating) of **influenza A virus**
  - **Enfuvirtide** → **HIV** viral **fusion** protein **gp41**.

FUSION OF ENVOLPE WITH MEMBRANE

- **mRNA inhibitors: Fomivirsen** is an **antisense compound** → CMV retinitis.

HERPES | HERPES+CMV | HIV | HIV

- **Inhibitors of nucleic acid synthesis:** acyclovir, gancyclovir , AZT, lamivudine, ribavirin (nucleoside analogues). **Raltegravir inhibits HIV integrase.**

HCV

RT-INHIBTOR

- **Inhibition of cleavage of precursor polypeptide :**
  - **HIV protease inhibitor : indinavir, ritonavir,…**used for HIV.
  - **HCV protease inhibitor: Simeprevir, grazoprevir**.
- **Inhibition of viral protein synthesis:** Interferon induces expression of translation inhibitory protein (TIP) that binds to ribosome, inhibits host expression of viral proteins.
- **Inhibition of viral release:** **Oseltamivir (Tamiflu)** and **Zanamivir (Relenza)**

→ for **prophylaxis** & **treatment**.

Vaccines and immunisation

- **Active immunity:** Live attenuated [e.g. **measles, mumps, rubella (MMR), poliovirus (Sabin vaccine)]** and killed vaccines [e.g. **poliovirus (Salk vaccine), rabies, influenza]**.

- **Passive immunity:** Preformed antibodies in preparations called immunoglobulins.

- **Passive-active immunity:** Giving both immunoglobulins → immediate protection and a vaccine → long term protection.

RABIES

Inactivated *vs* attenuated vaccines

	inactivated	attenuated
cost	higher (greater mass required)	lower (agent replicates in the body)
administration	parenteral	oral
adjuvant	needed	not needed
stability	good	poor
reversion	absent	possible
immunity	mucosal immunity absent	mucosal immunity present
	antibody-mediated	antibody-mediate and cytotoxic T cells
	short-lasting	long-lasting

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