



النادي  
MC  
الطبي

Done By :  
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لا تنسونا من دعائكم بالتوفيق

شو بنسوي لها نوخذ عينة الفايروسي

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## Specimen storage and transport

- Keep specimens other than blood at 4°C
- If delay >24hrs, freeze at -70°C or below.
- Avoid any storage at -20°C: greater loss in infectivity
- Nonenveloped viruses (adenovirus, enteroviruses) more stable than enveloped (e.g. RSV, VZV, CMV).

enveloped

انتبهوا على هاي النقطة

نصلي عن ال Sample

(- or +) اذا كان -70 or -20 مافرق كثير

بس اذا لبي ال infectivity assastable بعمني هو ويكون بفرق  
slow freezing ← -20 جند بصيرك freezing و جند لا infectivity  
loss the envelope ← بانزى infectivity

See wheel like structure ← Rota *يشوف هال الشكل للفيروس مثل ال Rota*

1. Electron Microscopy morphology of virus particles

modified electron microscopy ← immune electron microscopy  
which involved targeting the Antigen with Fluorescence Antibodies

2. Light Microscopy histological appearance

cytopathic effect inclusion bodies

3. Viral Genome Detection hybridization with specific nucleic acid probes  
polymerase chain reaction (PCR)

# Indirect Examination

↓  
effect *بطلع على ال effect*

1. Cell Culture cytopathic effect (CPE)

haemadsorption *binding*

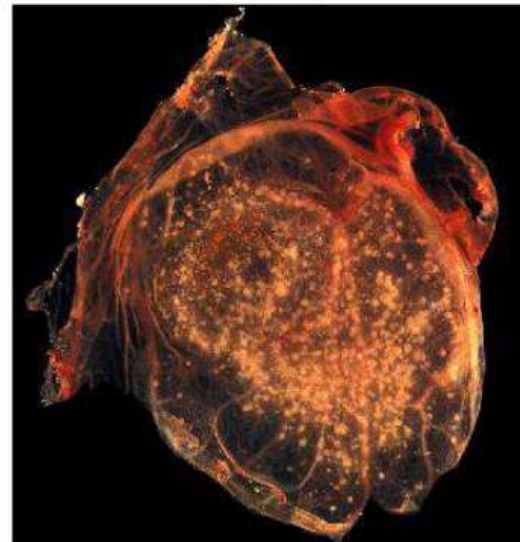
*enveloped virus* *حبيبات وخصيات في culture* ← immunofluorescence → *Fluorescence antibody*  
*going to be left as apart of cell membrane* *بترجع على glycoprotein اذا ظهرت*  
*اذا عنون الخلية بتكون infected*

2. Eggs pocks on CAM

RBC *binding* ← haemagglutination

inclusion bodies

3. Animals disease or death





# Virus Isolation

Primary is considered **the best**, the most expensive, The least used.

Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

Similarity human cell  
هناها الاحسن : لونها اقرب لـ human cell

- 1. Primary cells - 1-2 passages (Monkey Kidney)
- 2. Semi-continuous cells - 20-50 passages (Human embryonic kidney and skin fibroblasts)

بتقتل القرود وتجيبها الكليته تامة - ونقلها  
Trashing مينطها بال Media في Plast  
مستقلتها ← فاي replication

- 3. Continuous cells - Indefinite passages (HeLa, Vero, Hep2, LLC-MK2, MDCK) Tumor cell line

continuous

Providing them in the media capable of replicating and produce new cell

most used in the lab

cell line  
Liquid Nitrogen  
لنوع عدي  
بتحفظ بال

**Primary cell** culture are widely acknowledged as **the best cell culture systems available since they support the widest range of viruses**. However, **they are very expensive** and it is often difficult to obtain a reliable supply. **Continuous cells** are **the most easy to handle but the range of viruses supported is often limited**.

can survive as single layer ← Flask  
بتطلع على الـ flask الـ cell لما احطهم

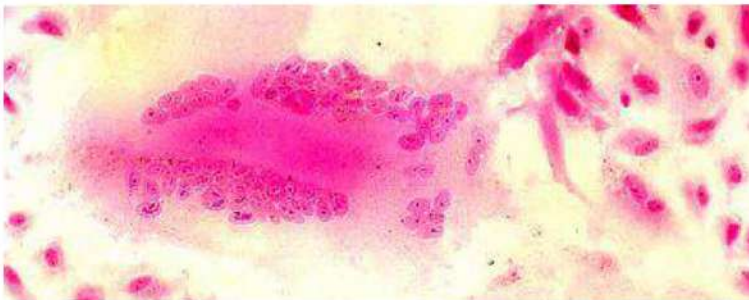
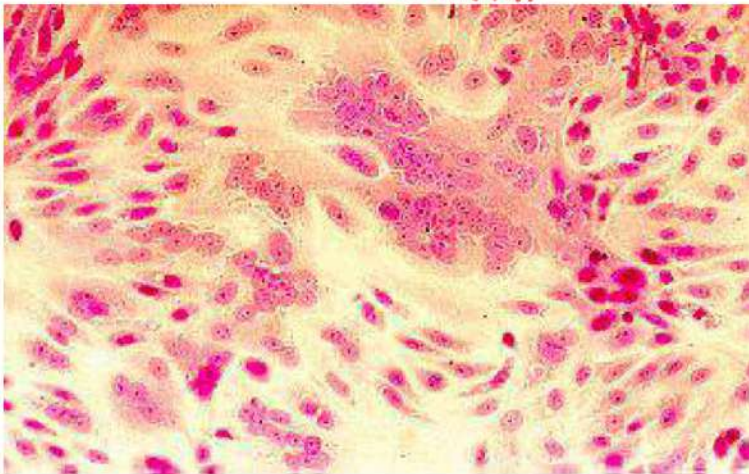
# Cytopathic Effect (2)



Syncytium  
Formation

تلف ال →

- 1 HIV
- 2 herpes virus



Syncytium formation in cell culture caused by<sup>3</sup> RSV (top), and measles virus (bottom).

(courtesy of Linda Stannard, University of Cape Town, S.A.)

Q which of the following is associated with syncytium formation!

هيكه ممكن السؤال يحيي :

الدكتورا يحيي هاي التسئلة المباشرة بعطها بكيس وبشرح الحارة  
ويحيي سوال السؤال

- Many viruses will not grow in cell culture e.g., Hepatitis B, Diarrheal viruses, parvovirus, papillomavirus.

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صاح السلايه ركز عليه الدكتور

# Electron Microscopy



$10^6$  virus particles per ml required for visualization, □ 50,000 - 60,000 magnification normally used. Viruses may be detected in the following specimens.

## Faeces

gastroenteritis

Rotavirus, Adenovirus

Norwalk like viruses

Astrovirus, Calicivirus

## Vesicle Fluid

HSV

→ I above the waist

→ II below the waist

VZV

→ Causes chicken pox


## Skin scrapings

<sup>causes warts</sup>  
papillomavirus, orf

molluscum contagiosum

# Nucleic Acid Detection



- Short length of viral genome makes them ideal candidate for nucleic-acid based diagnosis
- PCR  horizontal — gel — UV light
  - conventional PCR – agarose gel detection of product
  - Real-time PCR- products detected using probes or intercalating dyes within the reaction.



# Serology



## Criteria for diagnosing Primary Infection

- 4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera 50 → 250
- Presence of IgM
- Seroconversion - is the development of detectable specific antibodies to microorganisms in the blood serum as a result of infection or immunization. vaccine ممكن المريض يكون او يكون ماخذ infected by the virus
- A single high titre of IgG (or total antibody) - very unreliable

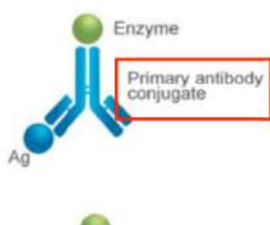
## Criteria for diagnosing Reinfection

- fold or more increase in titre of IgG or total antibody between acute and convalescent sera 100-170
- Absence or slight increase in IgM

## ELISA for HIV antibody

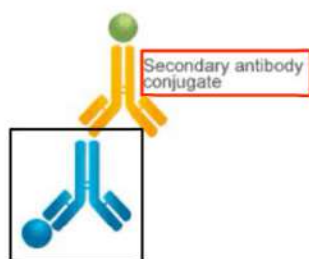
## ELISA types

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In **direct ELISA**, only an enzyme-labeled primary antibody is used, meaning that secondary antibodies are not needed. The enzyme-labeled primary antibody "directly" binds to the target (antigen) that is immobilized to the plate (solid surface). Next, the enzyme linked to the primary antibody reacts with its substrate to produce a visible signal that can be measured. In this way, the antigen of interest is detected.

عنا 4 انواع لل elisa  
اول واحد ال direct اللي بوخذ فيها ال plate بتكون فيه coated by antigen  
و بعدها بنضيف ال antibody اللي بكون معه enzyme  
ال antibody يرتبط مع ال antigen  
ELISA machine اللي راح تعطي لون معين بنقدر نشوفه بال  
بعدها بنضيف



In **indirect ELISA**, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme. The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody. Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured.

antigen  
تبع المريض

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عندك ثاني نوع ال indirect  
ال plate بروض coated by antigen و بروض primary antibody  
و secondary antibody  
و بروض بضيف مادة بتتفاعل مع ال enzyme و بتعطيك اللون

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# Anti-Viral drugs



## Pharmacology of acyclovir and related compounds

- Acyclovir, Valacyclovir, Ganciclovir, Famciclovir, Penciclovir all are guanine nucleoside analogs.

Herpes → DNA virus  
↳ latent virus  
↳ Dorsal nerve ganglia (5th cranial nerve)



# Anti-viral drugs

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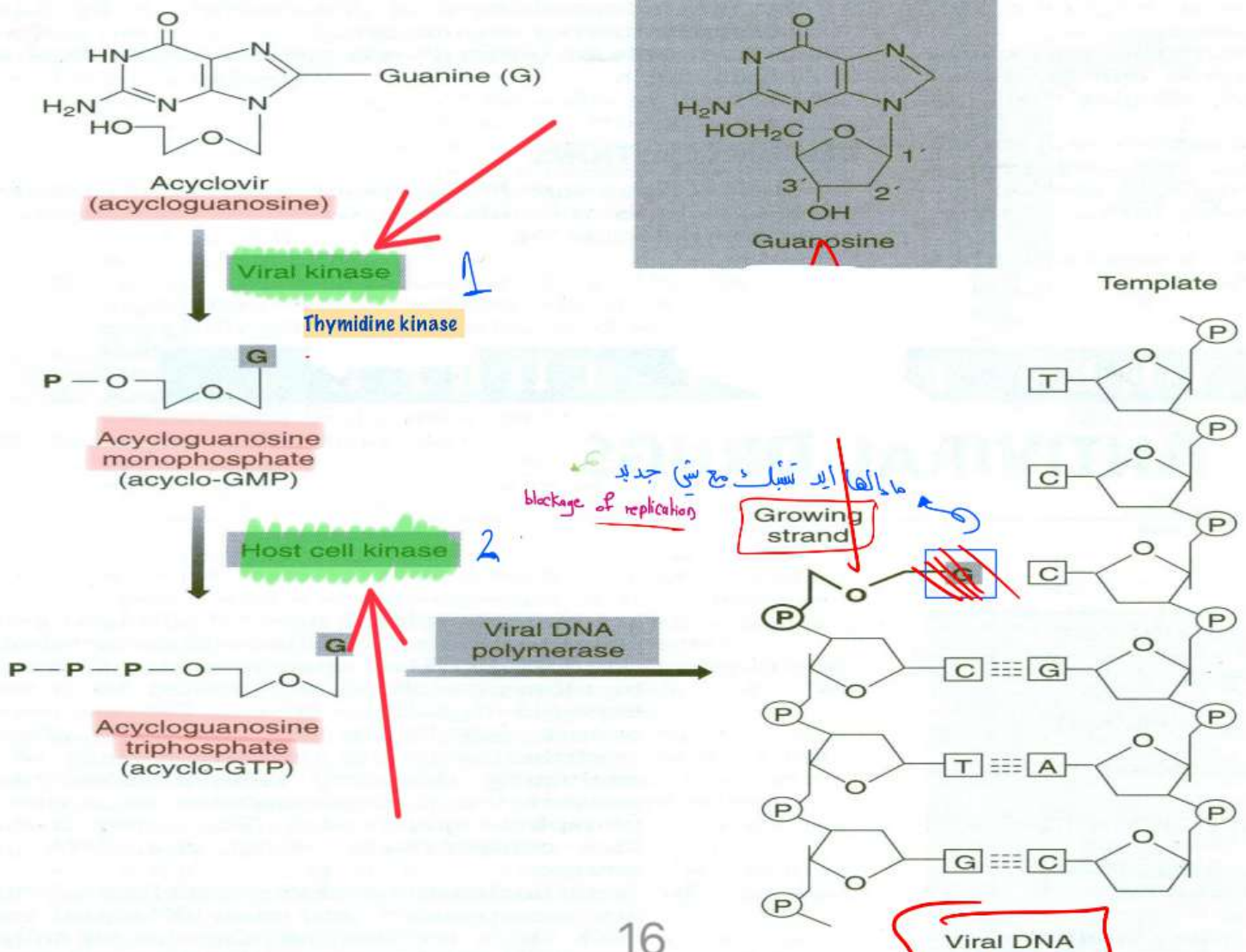
## Mechanism of action of Acyclovir and related compounds :

Firstly activated by viral enzyme then by cellular enzyme

- All drugs are phosphorylated by a viral thymidine-kinase, then metabolized by host cell kinases to nucleotide analogs.
- The analog inhibits viral DNA-polymerase
- Incorporation of acyclovir triphosphate into the growing viral DNA chain
- Only actively replicating viruses are inhibited

replication يوقف شغلها، وما رح بصير

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## Cidofovir ←

• It is approved for the treatment of CMV <sup>Cytomegalovirus</sup> 32  
retinitis in immunocompromised patients and  
Adenovirus infections ?

• It is a nucleotide analog of cytosine – no

# Anti-viral drugs

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+ Cidofovir → Active form.  
won't be phosphorylated

## PHARMACOLOGY OF FOSCARNET

- Foscarnet is an inorganic pyrophosphate analog  
So it's an active form
- It directly inhibits viral DNA and RNA - polymerase and viral reverse transcriptase (it does not require phosphorylation for antiviral activity) → It's in the active form.  
So it will act on HIV

# Anti-viral drugs

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## Therapeutic uses of Foscarnet

- *It is an alternative drug for*
- **HSV** infections (**acyclovir** resistant / immunocompromised patient )
- **CMV** retinitis (**ganciclovir** resistant / immunocompromised patient )

حكيينا هذول الثنين ال drug of choice ل كل من HSV+ CMV ، لو كان المريض عنده resistance الهم، ف بنستخدم ال foscarnet .

## PHARMACOLOGY OF RIBAVIRIN

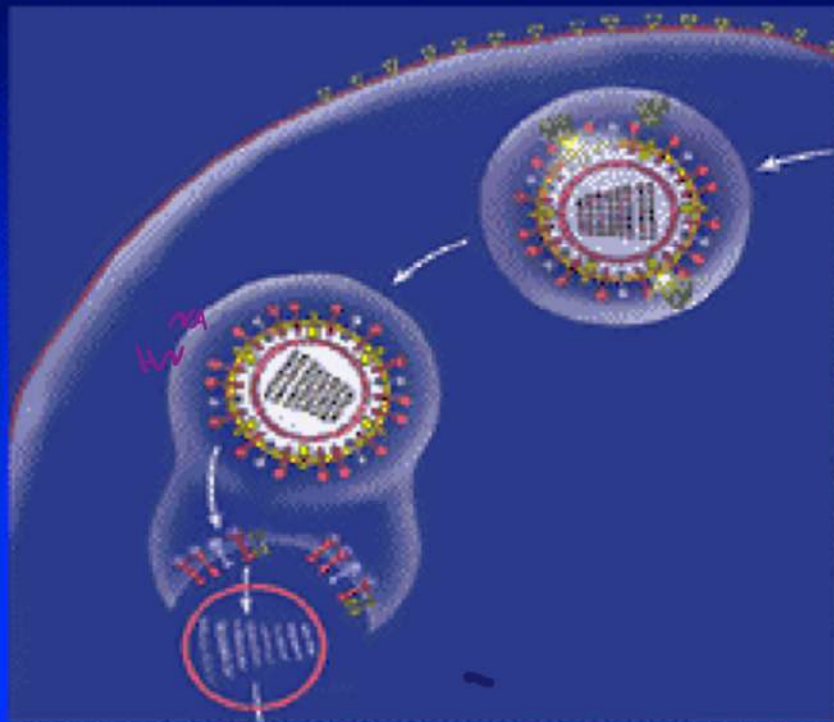
- RSV
- ▶ **Ribavirin** is a guanosine analog. (يتكرر كل سنة)
  - ▶ Requires phosphorylation to mono- di- and triphosphate.

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# Amantadine and Rimantadine: Mechanism of Action

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- Blocks M2 protein channel (type A only)
- Disrupts hydrogen transport, viral uncoating in host cell and therefore viral RNA transcription

# Anti-viral drugs

نظروا بالإنجليزية Interferons وهي نوع من modified form of interferons pegylated interferon  
 once a week تعطى مرة واحدة أسبوعياً  
 or once a month

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acute self limiting infection هو نوع من A type B, C ف هو

hepatitis C to chronic << 85% احتمالية تحول ال

Hepatitis B << 15%

لا ريب

## Hepatic Viral infections :

- Interferons <sup>HBV</sup>  
Part of non specific immunity , Letha ferons are release by infected cell toward nearby cell  
 inhibition of translation of viral protein يعمل

الدكتور طلبو منا واجب، بس هو حكي انه واحد منهم نسبتة ٨٥ و الثاني ١٥، بس فعليا ال hepatitis B نسبتة اقل، تقريبا ١٠٪

- Lamivudine – cytosine analog – HBV

- Entecavir – guanosine analog – HBV

lamivudine resistance strains

viral load  
 NOT complete elimination

- Ribavirin – Hepatitis C (with interferons)

- Sofosbuvir <sup>+ interferons + Ribavirine</sup> – nucleotide analog used in combination with other drugs (Ribavirin and Interferon) for the treatment of hepatitis C virus (HCV) infection. Course of 12 weeks cost 84,000\$.

سوفوبوفير أنا تكلمتني  
 كايبة ريبايرين ال hepatitis C

اللَّهُمَّ صَلِّ وَسَلِّمْ وَبَارِكْ

عَلَى نَبِيِّكَ مُحَمَّدٍ  
صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ

