3- Laboratory Diagnosis and Treatment of Viral Infection

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Objectives

Part 1

1. Understand principles of laboratory diagnosis of viral infections
2. Differentiate methods of viral detection and isolation
3. Describe viral reaction to physical and chemical agents
4. Describe and apply common methods of Inactivating viruses

Part 2

5. Understand principles and classes of anti-viral agents
6. Understand principles, types, and application of viral vaccines

Difficulties

- Can not be seen under light microscope
- Can not be cultivated easily
- Do not grow on culture media
- Treatment was not available

Changed situation

- Rapid techniques
- Screening for Blood transfusion
- Treatment available

العينات Specimens

- According to the disease
  - Respiratory – Throat swab , Nose Swap , Lung او من
  - CNS – CSF Needle Between Lu & L5
  - Eyes- Conjunctival scrapings
  - Viremia – Blood (Virus في الدم)
  - GIT and Liver - Stool
  - Skin - Scrapings

لـ لما الواحد يكح بتطلع سوائل

Specimen Storage and Transport

tube
فيحمولد بتحافظ على الـ Virus Stability
لازم نخلي الفايروس حي
لازم اخلي بمكان الـ Biohazard

Storage

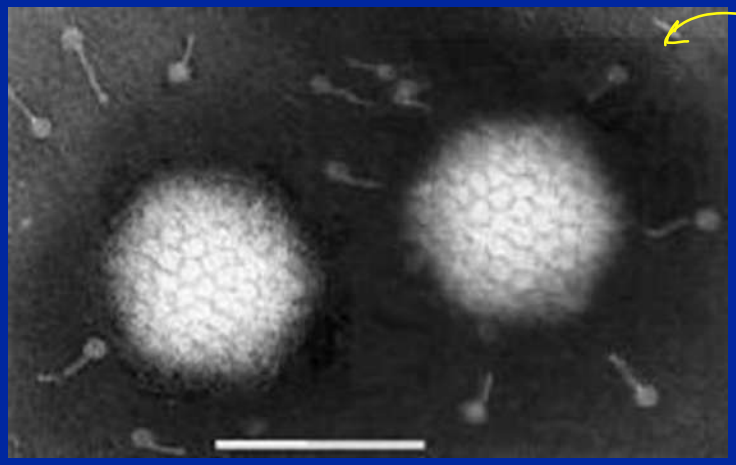
- Keep specimens other than blood at 4ºC ⇒ مباشرة : دقائق – ساعتين Room T
  لحد عدة ساعات ⇐ اكثر على درجة حرارة 4°
- If delay >24hrs, freeze at -70ºC or below.
- Avoid any storage at -20ºC: greater loss in infectivity
  freezer عادي
- Nonenveloped viruses more stable than enveloped
  يعني بتتأثر بسرعة بالحرارة وبتتكسر وبتتنوب

Transport

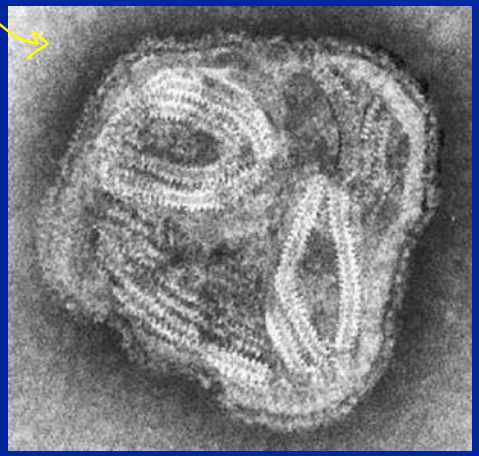
- Viral Transport Medium
  - Salt solution – ensures proper ionic concentrations
  - Buffer - maintains pH
  - Protein - for virus stability
  - Antibiotics or antifungals – to prevent contamination

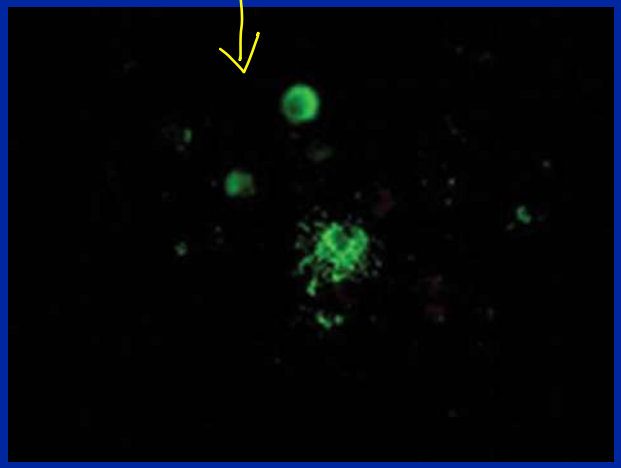
1. Microscopy

- Electron Microscope ⇒ too small بالوضع الطبيعي لانه (very expensive وغير متوفر)
- Light microscope – Inclusion bodies (اذا عمل light micro لكن بقدر اشوفه بال)
- Fluorescent Microscope -Fluorescent antibody technique



electron



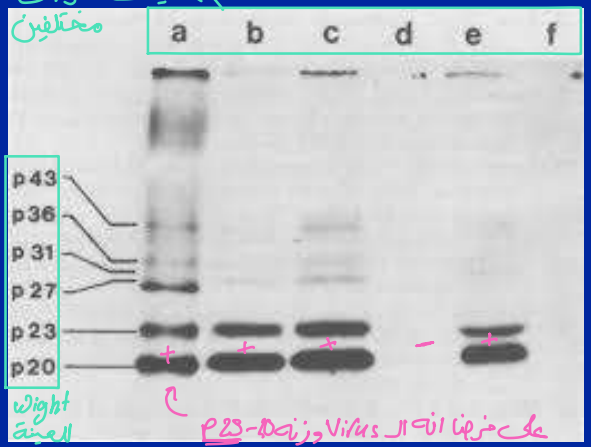


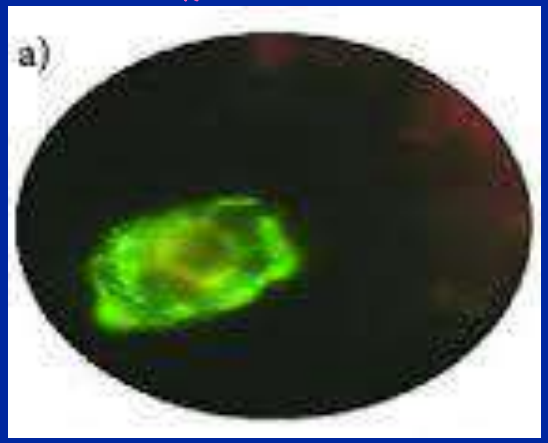
2. Demonstration of Viral Antigens

Glycoprotien هو envelope الـ Virus كل خاصية فيه او عنه Protien ← Capsid نستخدم

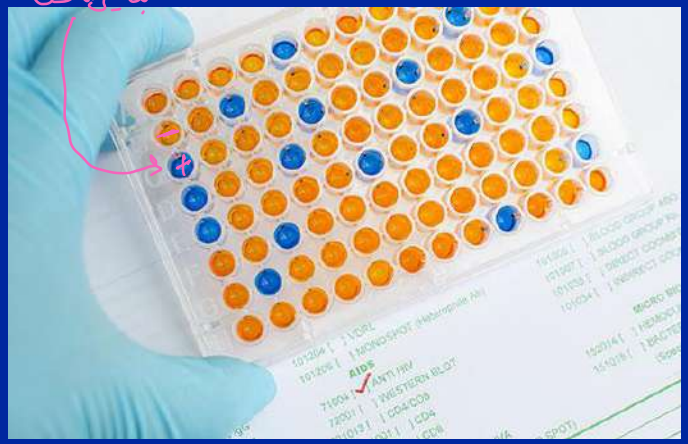
Antigens ← Virus خاصية بتميز كل

- Precipitation on gel eg HBsAg ← Hepatitis Surface Antigen موجود على سطح الفايروس
- Immunofluorescence
- Enzyme Linkes Immuno Sorbant Assay (ELISA)



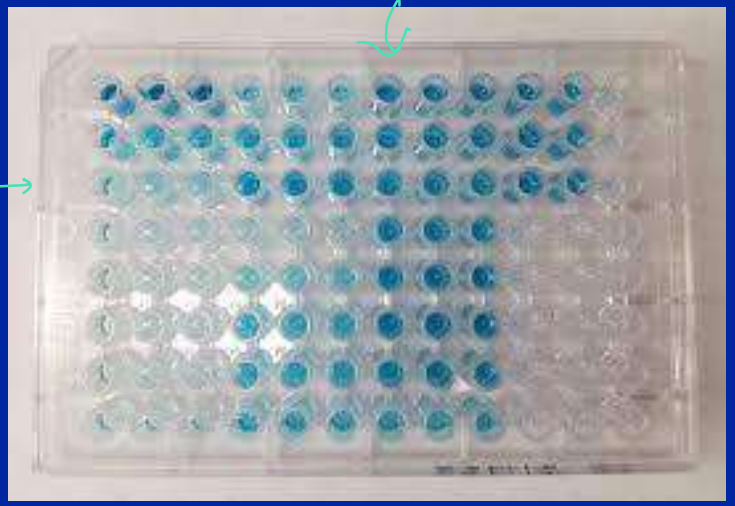


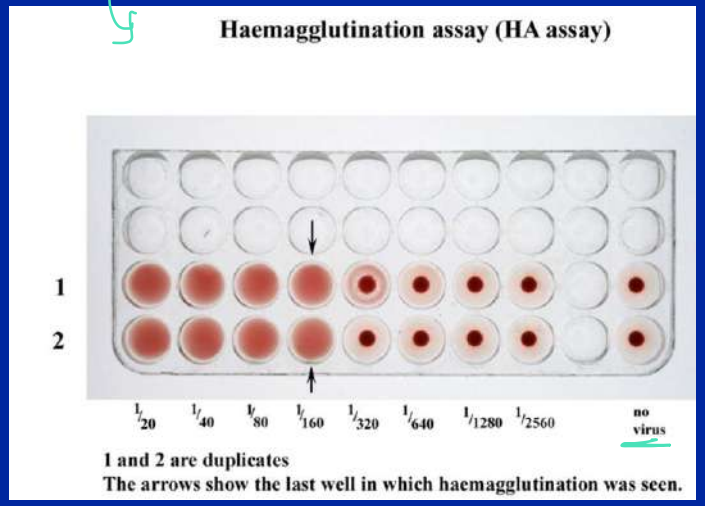
اذا احتوى على Ag لـ بتغير اللون



3. Serological Reactions (anti-viral antibodies)

- Rising titre of antibody in paired sample of sera for IgG antibody
  - First sample – At the earliest
  - Second sample – After 2 weeks
- Single sample IgM type of antibody detection
- Techniques –ELISA, Haemagglutination Inhibition (HAI)Test





بعاي الخطوة
Genome المعلم هو الـ

4. Molecular Techniques

- Nucleic acid amplification techniques such as polymerase chain reaction (PCR) can be used to detect viral genomes in clinical material.
- To detect RNA, an initial reverse transcription step is performed (converts RNA into cDNA). After this, PCR can be performed.
- Molecular assays are very sensitive (able to detect only a few viruses in a clinical sample.)
- They can also be used to measure the amount of virus (viral load) in a patient's sample.

بكشف المادة الوراثية عن طريق DNA لو كان نووي
RNA → DNA
بعد خطوة التكثير الـ Genome بدي اعرف نووي Virus
اما عن طريق Sequance ← يكون في Programme هو اي بيطلع نووي Virus
Primer او ← إذا كان موجود فالفيروس موجود / إذا لا منافي Virus
VL كم في Viruses
لو كان VL = 100 بالبداية
وبعد العلاج صار VL = 20 ← يعني انه العلاج مفيد
لـ بعدر بعاي الطريقة اتابع حالة المريض
كانت غالية لكن صارت أرخص
وبعض الفايروسات قادرة على تغير الـ Genome تلقيها

5. Viral Isolation and Culture

- Primary purposes of viral cultivation
  - To isolate and identify viruses in clinical specimens
  - To prepare viruses for vaccines
  - To do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

1. Using Live Animal Inoculation
   - Specially bred strains of white mice, rats, hamsters, guinea pigs, and rabbits
   - Animal is exposed to the virus by injection

2. Using Bird Embryos
   - Enclosed in an egg- nearly perfect conditions for viral propagation
   - Chicken, duck, and turkey are most common
   - Egg is injected through the shell using sterile techniques

3. Cell culture for viral identification

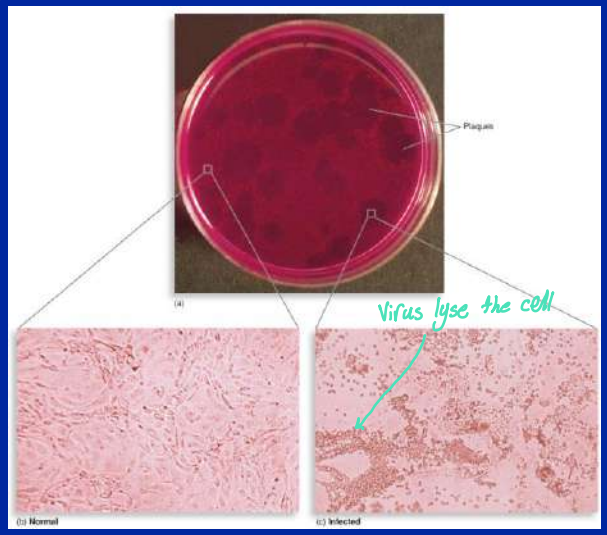
Cell Culture

Routinely used for growing viruses
Classified into 3 types:

1) **Primary cell culture** – normal cells freshly taken from body & cultured, limited growth
   1. Rhesus monkey kidney
   2. Chick embryo fibroblast
   3. Human amnion cell culture

2) **Diploid cell strains** – cells of single type (fibroblast cells) that can be subcultivated for limited number of times, mostly 50
   1. WI-38: human embryonic lung cell
   2. HL-8: Rhesus embryo cell

3) **Continuous cell lines** – malignant cells, indefinite subcultivtion
   1. HeLa: Human Ca of cervix cell line
   2. HEP-2: Human epithelioma of larynx



Detection of Virus Growth in Cell Cultures

1. Cytopathic effects (CPE) – morphological changes in cultured cells, seen under microscope, characteristic CPE for different groups of viruses
2. Metabolic Inhibition – no acid production in presence of virus
3. Hemadsorption – influenza & parainfluenza viruses, by adding guinea pig erythrocytes to the culture
4. Interference – growth of a non cytopathogenic virus can be tested by inoculating a known cytopathogenic virus: growth of first virus will inhibit the infection by second
5. Transformation – oncogenic viruses induce malignant transformation
6. Immunofluorescence – test for viral Ag in cells from viral infected cultures.

Reaction to physical and chemical agents

1. Heat and cold:

- Icosahedral viruses tend to be stable, while Enveloped viruses are much more heat labile
- Viral infectivity is generally destroyed by heating at 50–60°C for 30 minutes
- Viruses can be preserved by storage at subfreezing temperatures

2. Salts:

- Many viruses can be stabilized by salts in order to resist heat inactivation

3. pH:

Viruses are usually stable between pH values of 5.0 and 9.0. Some viruses (eg, enteroviruses) are resistant to acidic conditions. All viruses are destroyed by alkaline conditions.

4. Radiation:

Ultraviolet, x-ray, and high-energy particles inactivate viruses

5. Detergents:

Solubilize lipid constituents of viral membranes and disrupt capsids into separated polypeptides

6. Formaldehyde:

Formaldehyde destroys viral infectivity by reacting with nucleic acid

7. Quaternary ammonium, organic iodine, low-concentration chlorine, and Alcohols are relatively not effective against viruses

Common Methods of Inactivating Viruses

- Sterilization may be accomplished by steam under pressure, dry heat, ethylene oxide, and γ-irradiation
- Surface disinfectants include sodium hypochlorite, glutaraldehyde, and formaldehyde
- Skin disinfectants include chlorhexidine, 70% ethanol, and iodophors
- Vaccine production may involve the use of formaldehyde, ultraviolet irradiation, or detergents to inactivate the vaccine

Part 2 ↓

Treatment and Prevention of Viral Infections

micro-organism

- As bacteria and protozoa do not rely on host cellular machinery for replication, processes specific to these organisms provide ready targets for developing antibacterial and antiprotozoal drugs.
- However, because viruses are obligate intracellular parasites, antiviral drugs must be capable of selectively inhibiting viral functions without damaging the host, making the development of such drugs very difficult.
- Furthermore an ideal drug would reduce disease symptoms without modifying the viral infection so much as to prevent an immune response in the host.
- There is a need for antiviral drugs active against viruses, for which vaccines are not available or are not highly effective.

Anti-viral Development

- Viruses are now becoming better understood and several viral genomes have been properly mapped. Scientists are now looking for the best drug targets

يتعرف على الـ Virus وهو داخل الخلية وما يدمر الخلايا للـ Host ← Ideal Drug

- The main point of interest is any viral protein that the host organism does not normally produce

إذا في بروتين ينتجه الـ Virus والجسم ما بنتجه بكون تطوير الدوا عليه ← Target

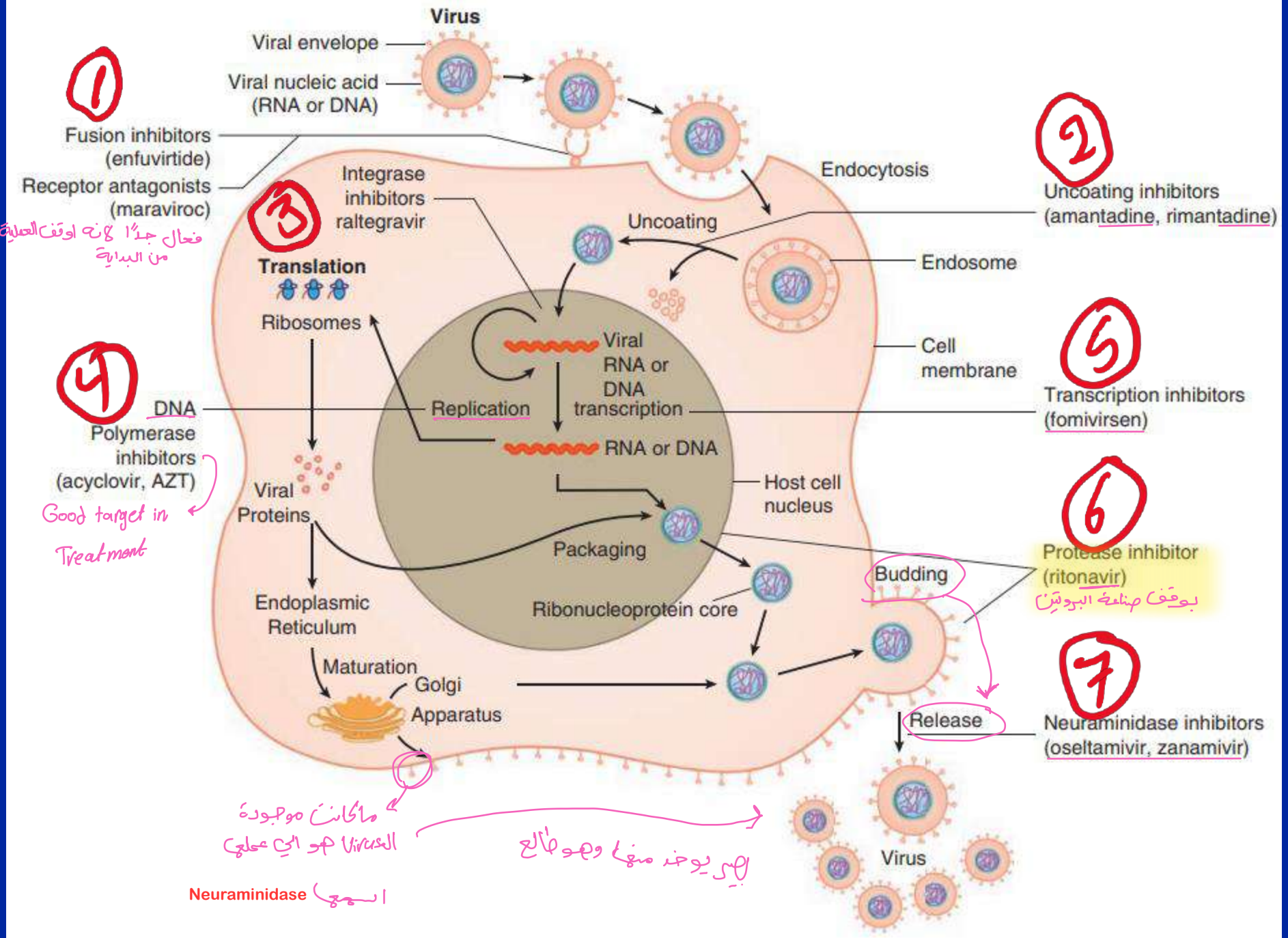
- Once these viral proteins are identified they are tested using a large-scale screening process to test for effectiveness

بزرع الـ Virus و بستخدم الأدوية المختلفة وبشوف فعاليتهم

Anti-viral Targets

Viral Replication Steps مطابقة لـ

- There are several known methods that the makers of Antiviral drugs are looking at, including:
  1. Inhibitors of Attachment (maraviroc)
  2. Inhibitors of Cell Penetration and Uncoating (enfuvirtide) amantadine, rimantadine
  3. Neuraminidase Inhibitors (oseltamivir, zanamivir)
  4. Protease Inhibitors (ritonavir)
  5. Inhibitors of Nucleic Acid Synthesis
  6. Nucleotide Analogs
  7. Stopping the release of the mature viruses from the host cell


Virus
Viral envelope
Viral nucleic acid (RNA or DNA)
1
Fusion inhibitors (enfuvirtide)
Receptor antagonists (maraviroc)
فعال جدًا لأنه أوقف العملية من البداية
3
Integrase inhibitors raltegravir
Translation
Ribosomes
Endocytosis
2
Uncoating inhibitors (amantadine, rimantadine)
Uncoating
Endosome
Cell membrane
Viral RNA or DNA transcription
5
Transcription inhibitors (fomivirsen)
4
DNA Polymerase inhibitors (acyclovir, AZT)
Good target in Treatment
Replication
RNA or DNA
Host cell nucleus
Viral Proteins
Packaging
6
Protease inhibitor (ritonavir)
بوقف صناعة البروتين
Budding
Endoplasmic Reticulum
Ribonucleoprotein core
Maturation
Golgi Apparatus
Release
7
Neuraminidase inhibitors (oseltamivir, zanamivir)
ماكانت موجودة الـVirus هو اللي عملها
هي يوخذ منها وهو طالع
Neuraminidase اسمها
Virus

1. Oseltamivir (Tamiflu)

- Prevents the mature viruses from leaving the cell
- It is a neuraminidase inhibitor, it works on both influenza A and B
- Neuraminidase is an enzyme found on the virus which cleaves sialic acid from cell membrane, leading to a more effective release of viruses
- Used to battle avian flu and influenza

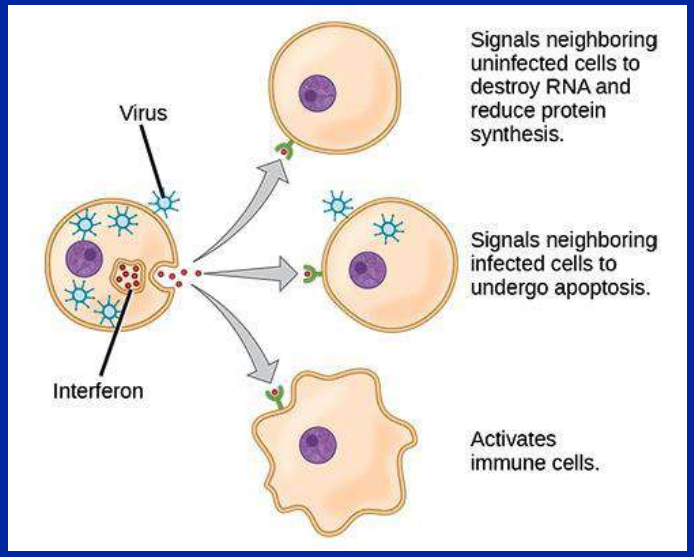
2. Acyclovir (Zovirax)

- A widely used antiviral with main implications in the treatment of herpes
- Seen as a "new age" in antiviral therapy, Gertrude Elion, its creator, was given the Nobel prize for medicine in 1988
- It is a nucleoside analogue and prevents viral replication in infected cells
- Inhibits viral DNA polymerase and terminates viral DNA chain growth

3. Interferons INF (αβγ)

as a drug لـ عملنا منها نسخ وصرنا نعطيها

- α and β interferons are produced by all the cells in response to viral infections
- γ interferons are produced only by T lymphocyte and NK cells in response to cytokines
- The action of interferons leads to an inhibition of translation
- Pegylated interferon-α (Peg-IFα) is given for 6 to 12 months to treat chronic hepatitis C & B disease بكونوا فعالين 30-50٪ خصوصًا



في بداية الموضوع

Viral Vaccines

- General Principles

Types:

1. Killed-Virus Vaccines
2. Attenuated Live-Virus Vaccines
3. Genetic vaccines

- Proper Use of Vaccines
- Vaccine development and future direction

Very safe → Short term effect & less effective

احسن → Disease يرجع يعمل Virus لكن ممكن الـ
فالحوامل والناس اللي مناعتهم ضعيفة ممكن يأثر عليهم الطعوم