MICROBIOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 4

laboratory diagnosis and treatment of viral infection (pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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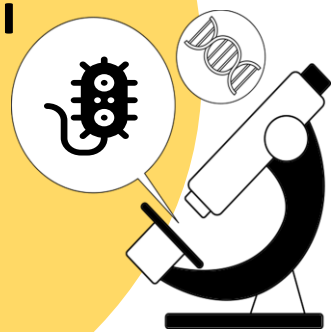
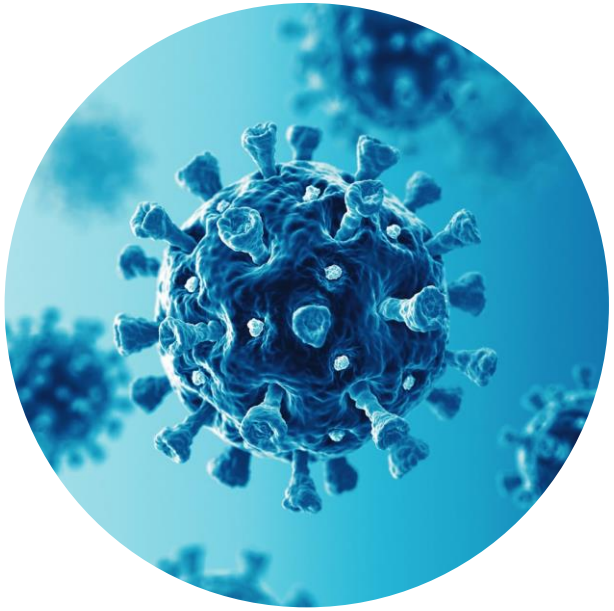


Link for quiz on the previous lecture.



وَتَوَكَّلْ عَلَى الْحَيِّ الَّذِي لَا يَمُوتُ وَسَبِّحْ بِحَمْدِهِ ۚ وَكَفَىٰ بِهِ بِذُنُوبِ عِبَادِهِ خَبِيرًا

3- Laboratory Diagnosis and Treatment of Viral Infection

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Objectives

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

Difficulties

Recall : there's only one condition where the virus can be seen under the light microscope which is → the inclusion body (it's a viral particle that aggregate with proteins and enzymes until it becomes visible under the light microscope).

- Can not be seen under light microscope. Because it's too small.
- Can not be cultivated easily Cultivated = cultured
- Do not grow on culture media because they need living cells to reproduce.
- Treatment was not available

❖ Changed situation

- Rapid techniques
- Screening for Blood transfusion
- Treatment available

Clinical application :
After an acute hemorrhage (نزيف) we need to supply the patient with blood, but we need to check two things:

1) The blood is not carrying infections, by screening for blood borne infections (ex: HIV) , many techniques are used, such as: indication from the antibodies.

2) Blood type (فصيلة الدم).

Specimens

- According to the disease

Place of infection - proper way of taking the sample

  - Respiratory – Throat swab
  - CNS – CSF CSF= cerebrospinal fluid
  - Eyes- Conjunctival scrapings
  - Viremia – Blood
  - GIT and Liver - Stool Stool = waste of the body
  - Skin - Scrapings
  - Lung - the best approach is the mucus
  - Urinary tract infection- urine

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 more stable than enveloped
  why ?

  → Because **envelope** = **phospholipid** bilayer=get **damaged** quickly from the conditions.
  strongest = nucleic acid > protein > lipids
- Viral Transport Medium (tube + ice box)
  - Salt solution – ensures proper ionic concentrations
  - Buffer - maintains pH
  - Protein - for virus stability
  - Antibiotics or antifungals – to prevent contamination

Note: If we leave the virus for >2hrs at the room temperature (20c) it will die, so we need to :

Or

Appropriate store technique

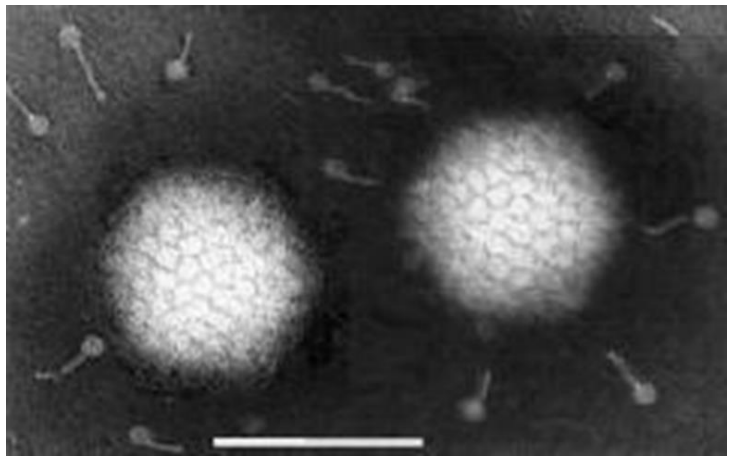
Take it to the lab as soon as possible

It can be stored in refrigerator (4°C) for only about 2-3 hours

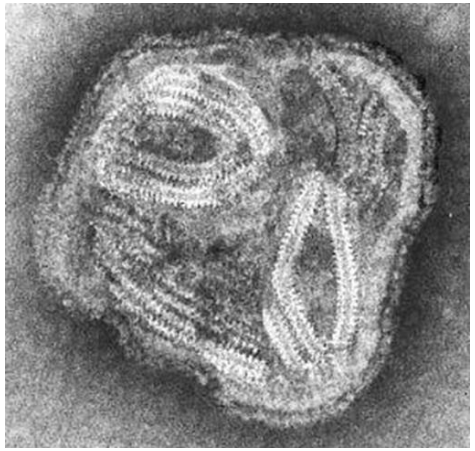
1. Microscopy

- **Electron Microscope** disadvantage : too expensive (only 2 in Jordan) → واحد منهم بالجامعة الأم عزعز
- **Light microscope – Inclusion bodies**
- **Fluorescent Microscope -Fluorescent antibody technique** → addition of specific antibody that emits light

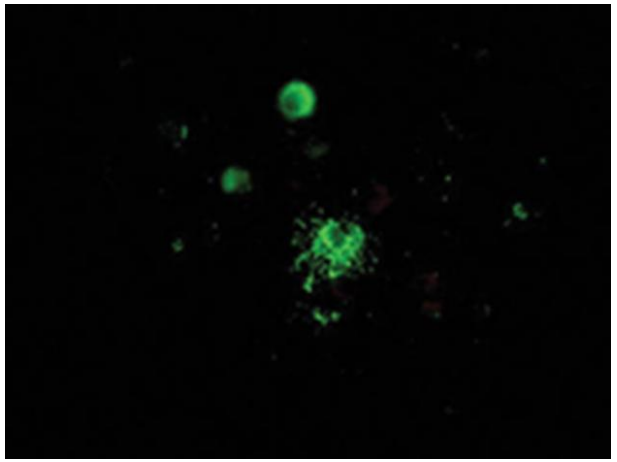
- Electron microscope
- These lines are the spikes
- The virus have an icosahedral shape



Complex virus



Fluorescent Microscope



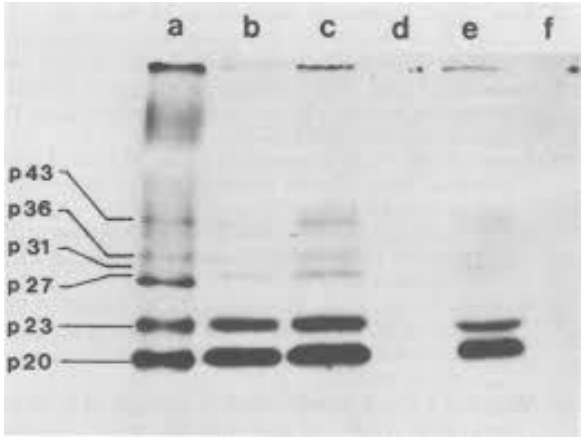
2. Demonstration of Viral Antigens

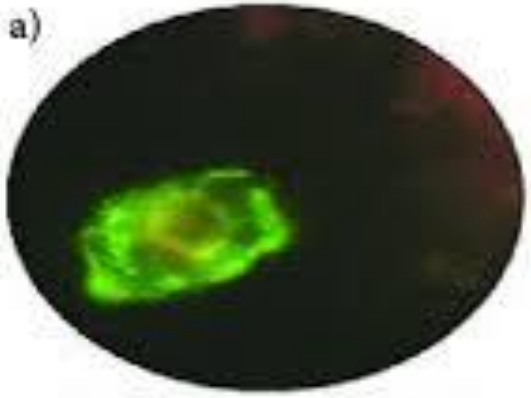
Antigen = any structure that attaches to the antibody

- Foreign
  ex: virus, bacteria
- Self
  ex: carbs, proteins ...

Ways to look for the antigens :

- Precipitation on gel eg: HBsAg
- Immunofluorescence For the antigen not the virus
- Enzyme Linkes Immuno Sorbant Assay (ELISA)



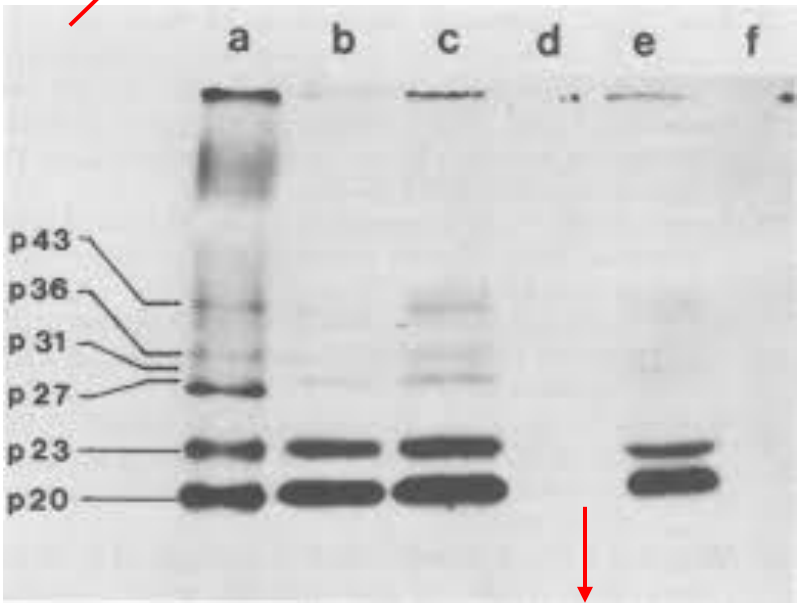


What is the proper viral antigen that is used ?

- *Is it envelope ?* **NO**, because its components looks similar to cells' membranes
- *Is it glycoprotein ?* **YES**, because it's not encoded in human body
  HBsAg= hepatitis b surface antigen

We look for HBsAg in the body , if it's present → this means there is virus

precipitation on gel technique



Indicates that there is no virus (negative)

Immunofluorescence technique

The emitted light tells us that the viral antigen is present (positive)

Enzyme-Linked ImmunoSorbent Assay (ELISA) technique

The colored well means positive test

Blue → positive

Orange → negative

Disadvantages of Viral Antigens test :

However it had developed, and it is not costy.

1) looking for a specific antigen for a specific virus among huge numbers, so the prediction for which antibody must be used is strenuous. (clinicians should put a prediction according to the symptoms they notice on patients, to look for specific antigen)

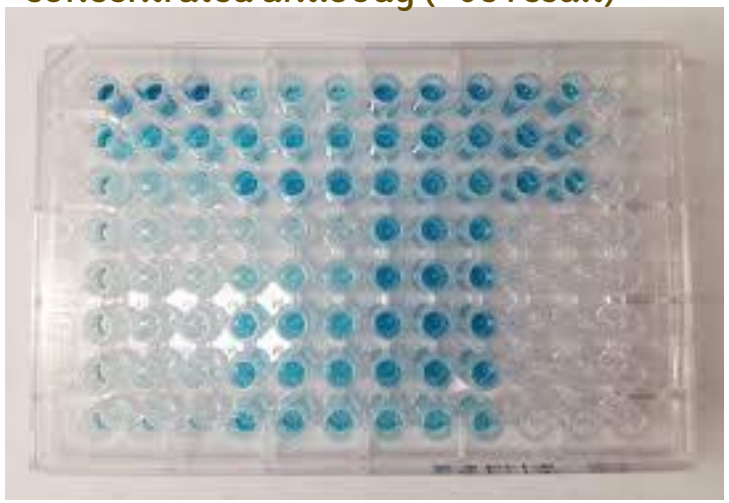
2) we should differentiate between the self and the foreign antigen.

3) Low accuracy.

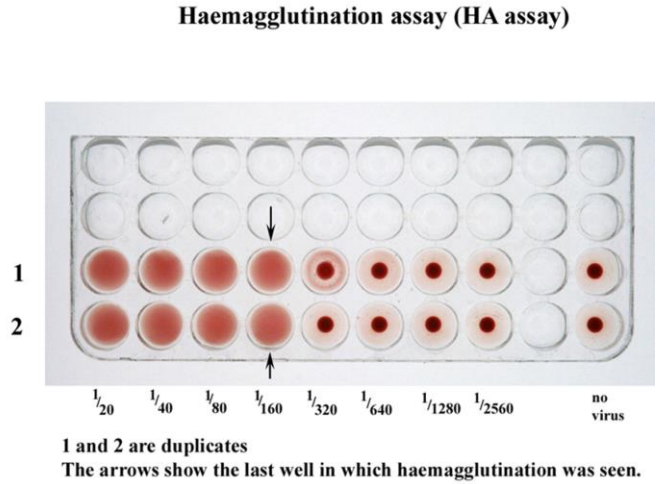
3. Serological Reactions (anti-viral antibodies)

- Rising titre of antibody in paired sample of sera for IgG antibody
  - First sample – At the earliest — With high [IgM], and low [IgG]
  - Second sample – After 2 weeks — With higher [IgG], indicating +ve test result (for acute infection).
- Single sample IgM type of antibody detection
- Techniques –ELISA, Hemagglutination Inhibition (HAI)Test

Same technique as before , notice : darker color means more concentrated antibody (+ve result)



We add red blood cells to the patient sample → if agglutination occurs it indicates positive test



Why does this agglutination happen?
Due to spikes at the surfaces of virus that are hemagglutinating which interact with the RBC and causes clumping.

- The concept behind this is that we use the immune reactions and antibodies produced as a mirror for the virus and its antigen
- antibodies are generally specific for the antigens (99% specific)
- we look for 5 antibodies : IgG, IgA, IgM, IgE, IgD
- IgM (first produced , within 6-7 days) then IgG arises (after few weeks)

- **Disadvantages for this test :**

1)if the patient is immune compromised (weak immune system) he won't produce antibodies, leading to false negative result.
2)it also takes time (weeks-months) for the result.
3) not 100% specific . (overlap could happen, ex: influenzas A1 & A2).

4. Molecular Techniques

Antibodies are specific, but not that useful; so PCR comes to the rescue.

They reach the viral DNA or RNA and provide exactly what the virus is.

- 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 **by the enzyme reverse transcriptase** (converts RNA into cDNA). After this, PCR can be performed.
- Molecular assays are very sensitive**/specific** (able to detect only a few viruses in a clinical sample → **even if there's a few viruses (~5) these tests can detect them**)
- They can also be used to measure the amount of virus (viral load) in a patient's sample, **more DNA means that the patient has more viruses**.

ex. A patient comes with viral load of 10, one week later, he comes with a viral load of 100; meaning that the virus is duplicating fast.

- To identify the specimen, certain questions need to be addressed :
  - Does the specimen contain DNA or RNA?
  - What is the specific sequence of the target primer?

➢ Based on these factors, the **exact** species can be identified.

Ex. Herpes simplex virus 1 strain 3.

❖ Disadvantages:

1. Very expensive.
2. Technical demanding (very specific): any error can lead to false result, as we are dealing with a very small amount of DNA.

Further explanation of the previous slide

- PCR amplifies the DNA in a specimen.
- To target a specific virus, a primer unique to that virus is added.
- ➢ **Remember**: each virus has its own unique DNA or RNA sequence, distinct from all other organisms.
- Since the initial amount of viral DNA is small, PCR amplifies it to a larger amount.
- to detect RNA; DNA must be produced from it, in process known as reverse transcription, then the produced cDNA can be detected by PCR.

5. Viral Isolation and Culture

Viruses must get isolated and cultured (then weakened or killed in case of vaccines) in order to:
1- to study viruses' structure, type of genetic material (DNA or RNA), etc... .
2-to produce a vaccine.

- 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.
  - **Can be used in diagnosis but not that effective.**

1. Using Live Animal Inoculation **(see explanation in the next slide)**
   - Specially bred strains of white mice, rats, hamsters, guinea pigs, and rabbits
   - Animal is exposed to the virus by injection

2. Using Bird Embryos **(see explanation in the next slide)**
   - 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

❖ Disadvantages:
1. Dangerous; as any small mistake can lead to the virus spreading. (as in corona virus in 2019)
2. Demanding and not easy to make.

2. Using Bird Embryos:

Bird's **embryonated eggs**:

- Are covered with a shell.
- The shell covers all proteins the embryo needs.
- The proteins are also needed by the virus.
- Have the embryo with the fast-proliferating cells.
- Proteins and embryo cells provide a good environment to start viral culturing.

Mechanism: getting the egg and inoculating it with the virus.

1. Using Live Animal Inoculation

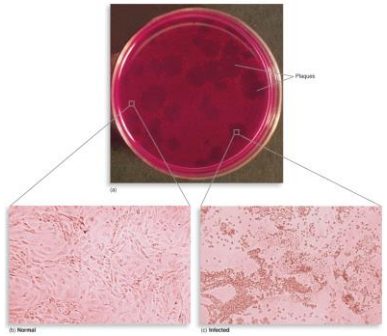
Mechanism: taking the patient specimen (not knowing if it has the virus or not), and the inoculating it into the animal.

How does this affect the Animal?

- The animal's body produces an immune response, producing antibodies.
- The virus starts to replicate; causing clinical symptoms and consequences, and viral load increases causing death for example.

Cell Culture

Similar to bacterial culture but with absolutely different content; the media is **cellular**.



If the virus is there (+ve) → plaques appear
If not (-ve), nothing appears.

Routinely used for growing viruses Classified into 3 types:

- Primary cell culture

✓ Normal cells [1]freshly taken from body & cultured, [2]limited growth:

1. Rhesus monkey kidney.
2. Chick embryo fibroblast.
3. Human amnion cell culture.

[2]Limited growth:

- If the virus is present, it will use these cells for replication.
- Cells die or get affected by viral infections when viruses are introduced.

- Diploid cell strains

✓ cells of single type **with high regeneration capacity** (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

Just to know, The polio vaccine was produced using malignant cells that have been dividing continuously for over 70 years.

Fibroblasts die by the virus, but they rapidly regenerate.

- Continuous cell lines

✓ malignant cells, indefinite subcultivtion

1. HeLa: Human Cancer of cervix cell line
2. HEP-2: Human epithelioma of larynx

Malignant cells can grow and divide indefinitely, even over decades (60-70 years). This continuous cell division means that if a virus is introduced into these cells, it can also keep replicating as the cells divide, allowing the virus to be produced indefinitely.

Detection of Virus Growth in Cell Cultures

cell Morphological changes

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.

Morphological changes (examples):
1- giant cell transformation.
2- multinucleated cell; because the virus needs a genetic material factory.

Addition of the virus to a blood sample, and if Hemagglutination happens, the virus is present.

Introducing the virus to a healthy tissue can cause cancer by transforming healthy/normal cells into cancerous cells (malignant transformation) ex. HPV as we took in pathology!

Reaction to physical and chemical agents

1. **Heat and cold** (Temperature):
   - 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; preserving the virus.
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.

- UV light and X-rays can kill viruses by breaking their DNA.
- UV light is sometimes used to clean certain areas safely, as long as it's controlled to avoid harming people's DNA.
- However, because UV light can be risky for humans, it's used only in specific cases, not everywhere.

5. **Detergents**:
   - Solubilize lipid constituents of viral membranes and disrupt capsids into separated polypeptides, **aiding in the destruction of viruses.**

6. **Formaldehyde**:
   - Formaldehyde destroys viral infectivity by reacting with nucleic acid **and destroying it.**

7. **Quaternary ammonium, organic iodine, low-concentration chlorine, and Alcohols:**
   - Are relatively not effective against viruses as against bacteria; **for example iodine and alcohols (Antiseptics) used to disinfect, kills bacteria but not viruses.**

The previously mentioned methods are used to produce/make vaccines or in sterilization (see next slide)

Common Methods of Inactivating Viruses

especially for surgical instruments and hospital equipment.

- **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

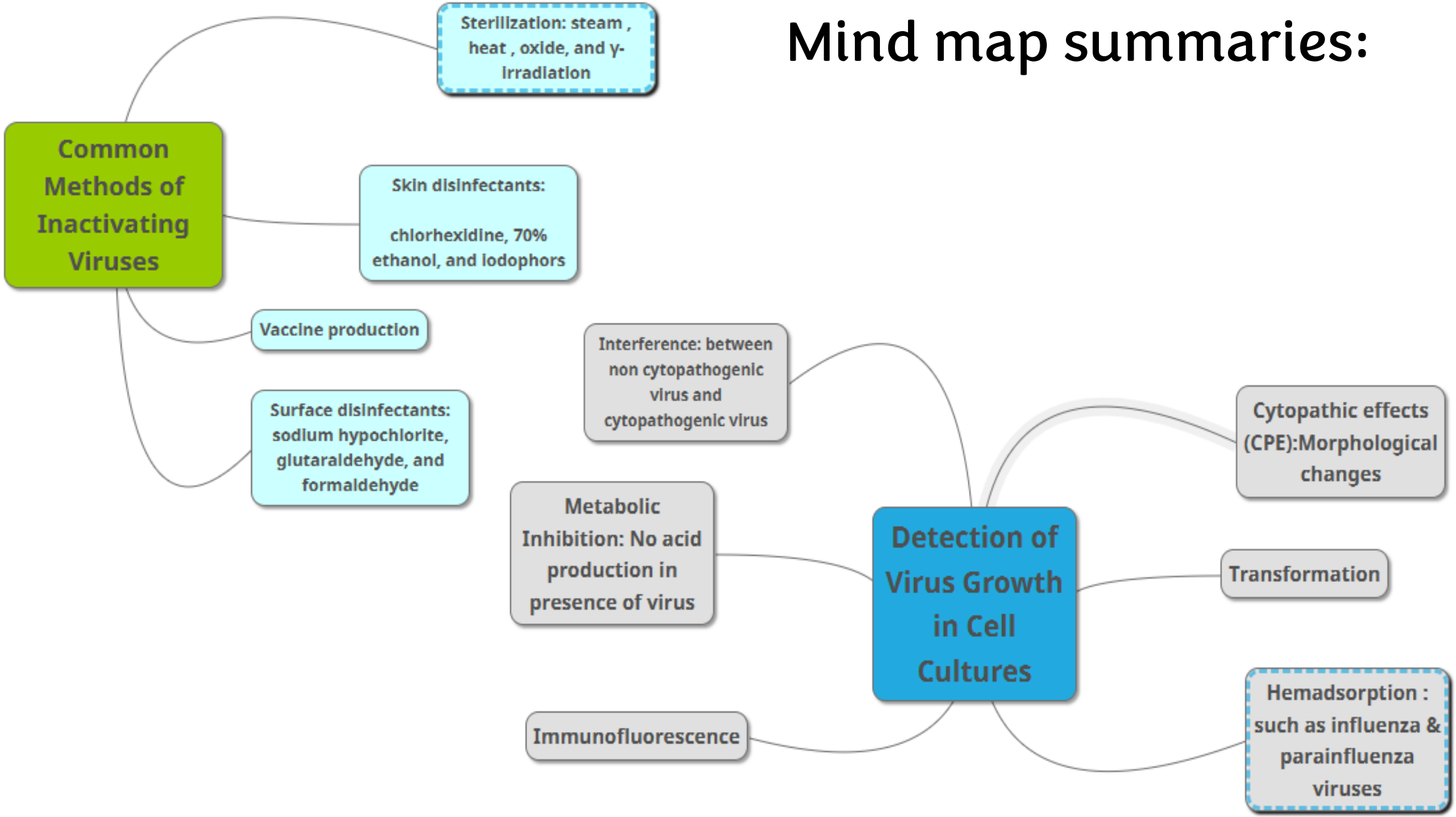
- Autoclave (Moist heat) is the most common method used for sterilization; as It contains moist, heat and pressure.
- Pressure, heat, and moist kill bacteria, spores, and viruses quite effectively.

Summaries for the lecture :

Type of diagnosis :	Microscopy	Demonstration of Viral Antigens	Serologica Reactions (anti-viral antibodies)	Molecular Techniques	Viral Isolation and Culture
Simplified mechanism	Search for the virus under the microscope	Search for the viral antigen	We use the immune reactions and the antibodies produced as a mirror for the virus	Search for the virus through nucleic acid amplification via (PCR)	Culture the virus at specific media and look for it
Extra info	-	-	We take 2 samples (1- at the earliest , 2- after two weeks) for IgM	RNA viruses undergo initial reverse transcription to become DNA - it is very sensitive (able to detect small amount) which is great - Knowing the specific sequence of the target primer + nucleic acid type →identification of the virus	Purposes : 1) isolate and identify viruses 2) Prepare vaccines 3) detailed research on viral structure
Disadvantages	1)too expensive for EM 2) most viruses don't appear under LM	1) Specifity among huge number 2) Require the differentiation between the self and the foreign antigen	1)Immune compromised patient produce false results 2) takes too much time 3) not 100% specific . (overlap could happen)	1)Very expensive 2)Technical demanding(very specific): any error can lead to false result	1) Dangerous 2) Demanding and not easy to make
Types :	EM LM, Fluorescent Microscope	1)Precipitation on gel 2)Immunofluorescence 3) (ELISA)	1)ELISA 2)Hemagglutination Inhibition (HAI)Test	-	1) Using Live Animal Inoculation 2) Using Bird Embryos 3) Cell culture for viral identification

Reaction to physical and chemical agents	Stabilized/ preserved at :	Destroyed by :
1) Temperature	**subfreezing** temperatures	**heating** at 50–60°C
2) Salts	stabilize viruses **in order to resist heat**	
3) pH	**5-9** , some can resist **acidic** conditions also	**alkaline** conditions. (all)
4) Radiation	-	1)Ultraviolet 2) x-ray, 3) high-energy particles
5) Detergents	-	Through **solubilization** of lipid structure and **disruption** of capsids
6) Formaldehyde	-	Through **reacting with nucleic acid**
7) Quaternary ammonium, organic iodine, low-concentration chlorine, and Alcohols:	-	not effective against viruses as against bacteria

Mind map summaries:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	9 11 12	HBsAg= hepatitis b sulfate antigen So that we can know if the IgM concentration increases → which indicates a positive test **so CPR comes to the rescue.**	HBsAg= hepatitis b surface antigen First sample: With high [IgM], and low [IgG] second sample: With higher [IgG], indicating +ve test result (for acute infection). **so PCR comes to the rescue.**
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:


سُورَةُ الإِنسَانِ

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

هَلْ أَتَىٰ عَلَى الْإِنسَانِ حِينٌ مِّنَ الدَّهْرِ لَمْ يَكُن شَيْئًا مَّذْكُورًا ﴿١﴾ إِنَّا خَلَقْنَا
الْإِنسَانَ مِن نُّطْفَةٍ أَمْشَاجٍ نَّبْتَلِيهِ فَجَعَلْنَاهُ سَمِيعًا بَصِيرًا ﴿٢﴾ إِنَّا
هَدَيْنَاهُ السَّبِيلَ إِمَّا شَاكِرًا وَإِمَّا كَفُورًا ﴿٣﴾ إِنَّا أَعْتَدْنَا لِلْكَافِرِينَ سَلَاسِلَا
وَأَغْلَالًا وَسَعِيرًا ﴿٤﴾ إِنَّ الْأَبْرَارَ يَشْرَبُونَ مِن كَأْسٍ كَانَ مِزَاجُهَا كَافُورًا ﴿٥﴾
عَيْنًا يَشْرَبُ بِهَا عِبَادُ اللَّهِ يُفَجِّرُونَهَا تَفْجِيرًا ﴿٦﴾ يُوفُونَ بِالنَّذْرِ وَيَخَافُونَ
يَوْمًا كَانَ شَرُّهُ مُسْتَطِيرًا ﴿٧﴾ وَيُطْعِمُونَ الطَّعَامَ عَلَىٰ حُبِّهِ مِسْكِينًا
وَيَتِيمًا وَأَسِيرًا ﴿٨﴾ إِنَّمَا نُطْعِمُكُمْ لِوَجْهِ اللَّهِ لَا نُرِيدُ مِنكُمْ جَزَاءً وَلَا شُكُورًا
﴿٩﴾ إِنَّا نَخَافُ مِن رَّبِّنَا يَوْمًا عَبُوسًا قَمْطَرِيرًا ﴿١٠﴾ فَوَقَاهُمُ اللَّهُ شَرَّ ذَٰلِكَ
الْيَوْمِ وَلَقَّاهُمْ نَضْرَةً وَسُرُورًا ﴿١١﴾ وَجَزَاهُم بِمَا صَبَرُوا جَنَّةً وَحَرِيرًا ﴿١٢﴾
مُّتَّكِئِينَ فِيهَا عَلَى الْأَرَائِكِ لَا يَرَوْنَ فِيهَا شَمْسًا وَلَا زَمْهَرِيرًا ﴿١٣﴾
وَدَانِيَةً عَلَيْهِمْ ظِلَالُهَا وَذُلِّلَتْ قُطُوفُهَا تَذْلِيلًا ﴿١٤﴾ وَيُطَافُ عَلَيْهِم بِآنِيَةٍ
مِّن فِضَّةٍ وَأَكْوَابٍ كَانَتْ قَوَارِيرَا ﴿١٥﴾ قَوَارِيرَا مِن فِضَّةٍ قَدَّرُوهَا تَقْدِيرًا ﴿١٦﴾
وَيُسْقَوْنَ فِيهَا كَأْسًا كَانَ مِزَاجُهَا زَنجَبِيلًا ﴿١٧﴾ عَيْنًا فِيهَا تُسَمَّىٰ سَلْسَبِيلًا
﴿١٨﴾ وَيَطُوفُ عَلَيْهِمْ وِلْدَانٌ مُّخَلَّدُونَ إِذَا رَأَيْتَهُمْ حَسِبْتَهُمْ لُؤْلُؤًا مَّنثُورًا
﴿١٩﴾ وَإِذَا رَأَيْتَ ثَمَّ رَأَيْتَ نَعِيمًا وَمُلْكًا كَبِيرًا ﴿٢٠﴾ عَالِيَهُمْ ثِيَابُ سُندُسٍ
خُضْرٌ وَإِسْتَبْرَقٌ وَحُلُّوا أَسَاوِرَ مِن فِضَّةٍ وَسَقَاهُمْ رَبُّهُمْ شَرَابًا
طَهُورًا ﴿٢١﴾ إِنَّ هَٰذَا كَانَ لَكُمْ جَزَاءً وَكَانَ سَعْيُكُم مَّشْكُورًا ﴿٢٢﴾ إِنَّا
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خَتْمُ الْقُرْآنِ

نصيحة الشيخ عثمان الخميسس فيما يتعلق بسورة الانسان

لا تحرموا حالكم من الهمة العالية

#ارحموا الافريج(خصوصا الكوميونيتي الله يحميكم)

للمزح فقط-