Microbiology

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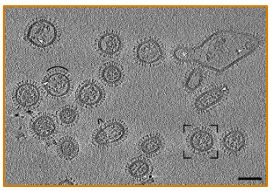
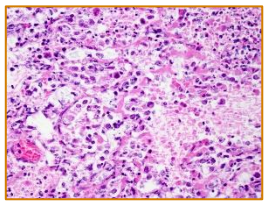
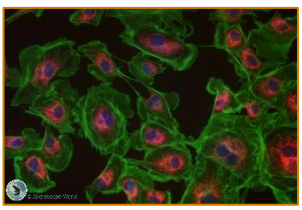
Laboratory Diagnosis and Treatment of Viral Infection

- **Specimens**
  - According to the disease
    - ✓ Respiratory – Throat swab
    - ✓ CNS – CSF
    - ✓ Eyes- Conjunctival scrapings
    - ✓ Viremia – Blood
    - ✓ GIT and Liver - Stool
    - ✓ Skin – Scrapings

- **Specimen Storage and Transport**
  - Keep specimens other than blood at 4oC
  - If delay >24hrs, freeze at -70oC or below.
  - Avoid any storage at -20oC: greater loss in infectivity
  - Non-enveloped viruses more stable than enveloped
  - Viral Transport Medium
    - ✓ Salt solution – ensures proper ionic concentrations
    - ✓ Buffer - maintains pH
    - ✓ Protein - for virus stability
    - ✓ Antibiotics or antifungals – to prevent contamination

- **Methods use**:

1. Microscopy
   - ✓ Electron Microscope
   - ✓ Light microscope – Inclusion bodies
   - ✓ Fluorescent Microscope -Fluorescent antibody technique

2. Demonstration of Viral Antigens
   - ✓ Precipitation on gel eg HBsAg
   - ✓ Immunofluorescence
   - ✓ Enzyme Linkes Immuno Sorbant Assay (ELISA)

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

4. Molecular Techniques

- ✓ Nucleic acid techniques such as polymerase chain reaction (PCR)
- ✓ To detect RNA, (converts RNA into cDNA). After this, PCR can be performed.
- ✓ Advantages: *very sensitive *measure the amount of virus (viral load) in a patient's sample.

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
- ✓ Cells types :

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
   - ✓ Routinely used for growing viruses
   - ✓ Classified into 3 types:
     - Primary cell culture – normal cells freshly taken from body & cultured, limited growth
       a) Rhesus monkey kidney
       b) Chick embryo fibroblast
       c) Human amnion cell culture
     - Diploid cell strains – cells of single type (fibroblast cells) that can be subcultivated for limited number of times, mostly 50
       a) WI-38: human embryonic lung cell
       b) HL-8: Rhesus embryo cell
     - Continuous cell lines – malignant cells, indefinite subcultivtion
       a) HeLa: Human Ca of cervix cell line
       b) 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

- **Anti-viral Targets**
  - ➢ There are several known methods that the makers of Antiviral drugs are looking at, including:
    1. Inhibitors of Attachment
    2. Inhibitors of Cell Penetration and Uncoating
    3. Neuraminidase Inhibitors
    4. Protease Inhibitors
    5. Inhibitors of Nucleic Acid Synthesis
    6. Nucleotide Analogs
    7. Stopping the release of the mature viruses from the host cell

- **Antiviral drug**:
  1. Oseltamivir (Tamiflu)
     - Prevents the mature viruses from leaving the cell
     - It is a neuraminidase inhibitor, it works on both influenza A and B and avian flu

  2. Acyclovir (Zovirax)
     - A widely used antiviral with main implications in the treatment of herpes
     - Inhibits viral DNA polymerase and terminates viral DNA chain growth

  3. Interferons
     - 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 disease

- **Viral Vaccines**
  - General principles types:
    1. Killed-Virus Vaccines
    2. Attenuated Live-Virus Vaccines
    3. Genetic vaccines

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