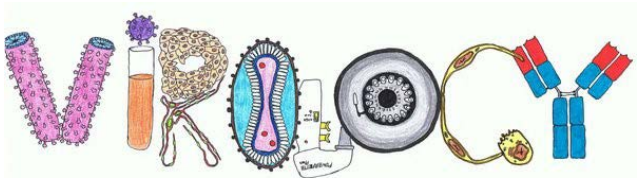
Lab 2:
Diagnostic Tests in Clinical  Laboratories

450 MIC

PRACTICAL PART

SECTION (30397)

Diagnostic Virology

Virus Isolation and Cultivation

Laboratoy Animals

Live animals such as monkeys, mice and rabbits.

After inoculation, the animal is observed for signs of disease like visible lesions or death. so that infected tissues can be examined for virus.

Chicken embroy technique.

A hole is drilled in the shell of the embryonated egg, and a viral suspension or suspected virus-containing tissue is injected into the fluid of the egg.

A-Chorioallantoic Membrane(CAM).
B- Allantoic Cavity.
C- Amniotic Cavity.
D- Yolk Sac.

Cell culture

Cell Cultures are most widely used for virus isolation in-vitro using monolayer cells cultures.

1- Primary cells.
2- Semi-continuous cells.
3- Continuous cells.

CPE: is the visible result of viral replication within cells and morphological changes occurring in cells due to viral infection is called cytopathic effects.

Viral Antigen Detection

Enzyme Linked Immunosorbent Assay (ELISA) detect either antigen (as a direct test)

Nasopharyngeal Aspirate = Influenza A and B
Skin = HSV
Blood = CMV (pp65 antigenaemia test)
Faeces = Adenoviruses

Serologic (Antibody Detection) Methods

Complement fixation test (CFT)

Hemagglutination Inhibition Test (HAI)

Immunofluorescense

Mircoscpy:

Morphology of virus particles. Immune electron microscopy.

Histological appearance of affected cells e.g: Inclusion bodies

Enzyme Linked Immunosorbent Assay (ELISA) detect AB

Nucleic Acid Extraction

Nucleic Acid Amplification

Real-Time PCR

Allows us to measure minute amounts of DNA sequences in a sample!

Polymerase Chain Reaction (PCR)

allows the in vitro amplification of specific target DNA sequences by a factor of 106 and is thus an extremely sensitive technique

1-Virus Isolation and Cultivation

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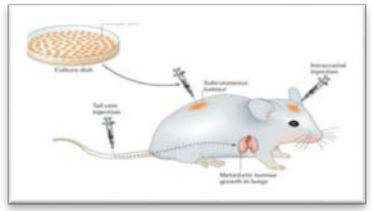

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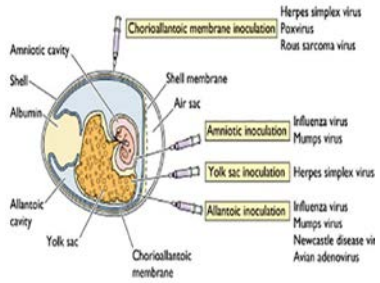

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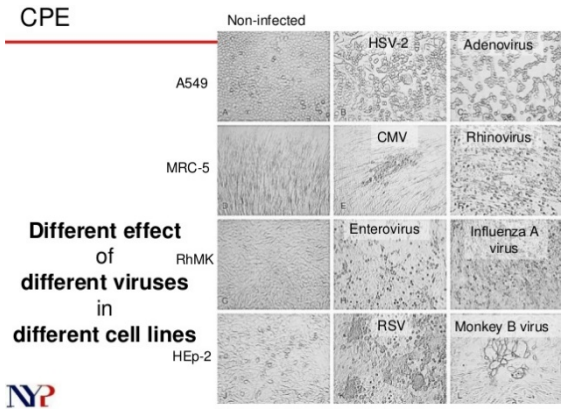

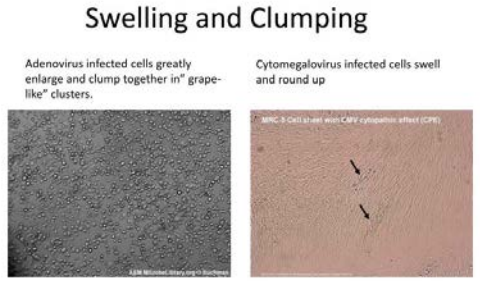

2- Viral Antigen and Antibody Detection

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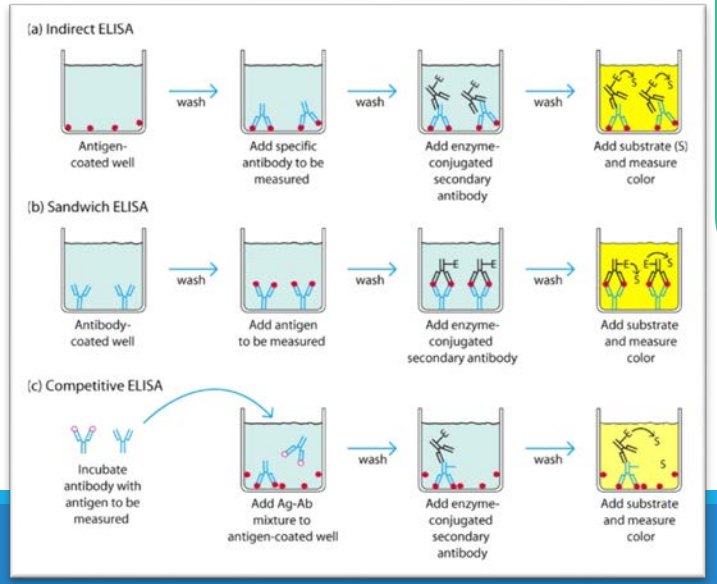
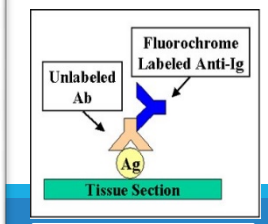
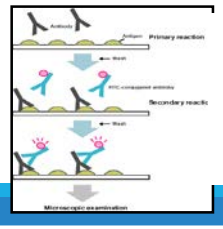


B-Immunofluorescense (IF)

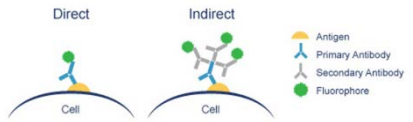
utilizes fluorescent-labeled antibodies to detect specific target antigens.
Fluorescein is a dye such as fluorescein isothiocyante (FITC) witch emits greenish fluorecence under UV light.
Imunofluorescent labeled tissue sections are studied using a fluorescence microscope



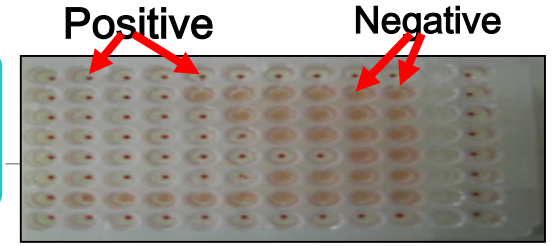
Direct IF

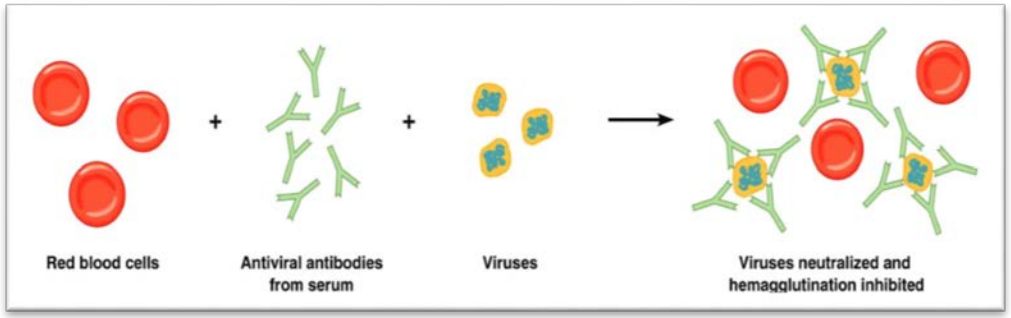


Indirect IF

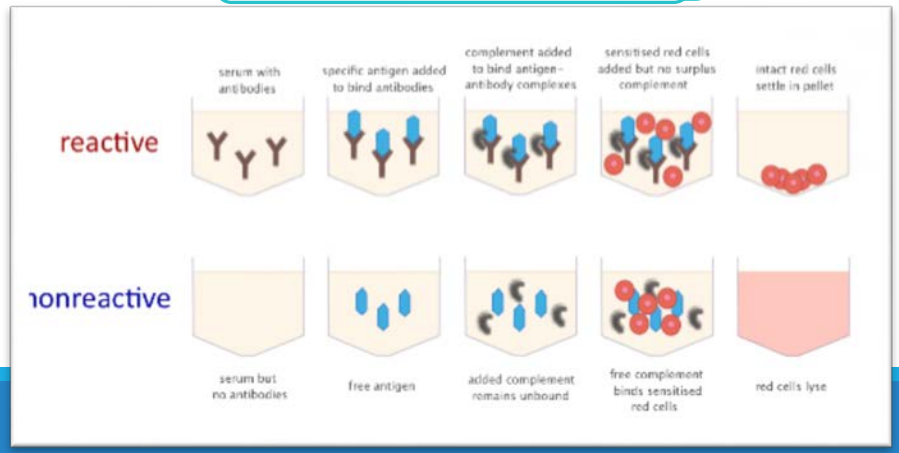


C-Complement fixation (CFT)





D-Hemagglutination Inhibition Test (HAI)



3- Microscopy:

A- Electron Microscopy:

Morphology of virus particles.

Immune electron microscopy:
There are two variants:

1- Classical Immune electron microscopy (IEM): the sample is treated with specific anti-sera before being put up for EM. Viral particles present will be agglutinated and thus congregate together by the antibody.

2- Solid phase immune electron microscopy (SPIEM): the grid is coated with specific anti-sera. Virus particles present in the sample will be absorbed onto the grid by the antibody.

B- Light Microscope:

Histological appearance of affected cells
e.g. Inclusion bodies

❖ **Inclusion bodies:**

•Inclusion bodies are virus-specific intracellular globular masses which are produced during replication of virus in host cells.

• They can be demonstrated in virus infected cells under light microscope after fixation & staining

e.g. Negri bodies in rabies .

4- Nucleic Acid Amplification

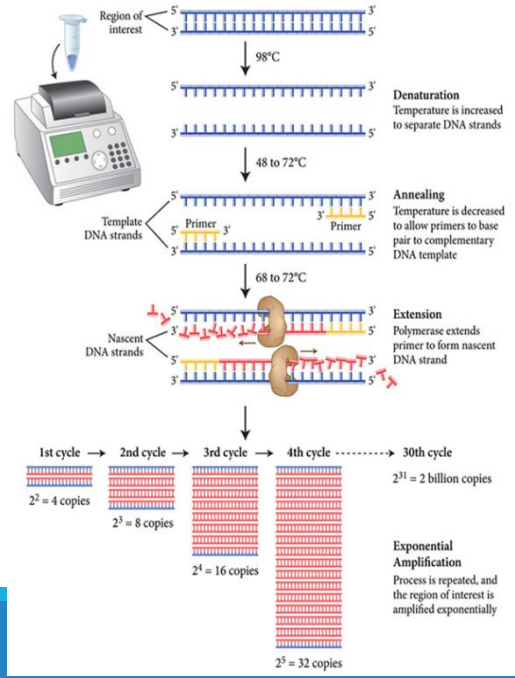
Detection of viral genome (molecular methods). Detection and identification of the PCR product is usually carried out by agarose gel electrophoresis, hybridization with a specific oligonucleotide probe, restriction enzyme analysis, or DNA sequencing.

Classical Molecular Techniques:

Southern blot, in-situ hybridization are examples of classical techniques.
They depend on the use of specific DNA/RNA probes for hybridization.

Polymerase Chain Reaction (PCR)

allows in vitro amplification of specific target DNA sequences by a factor of 10^6 and is thus an extremely sensitive technique.



Real-Time PCR

Allows a PCR reaction to be visualized "in real time" as the reaction progresses **to measure minute amounts of DNA sequences in a sample!**