Laboratory Diagnosis

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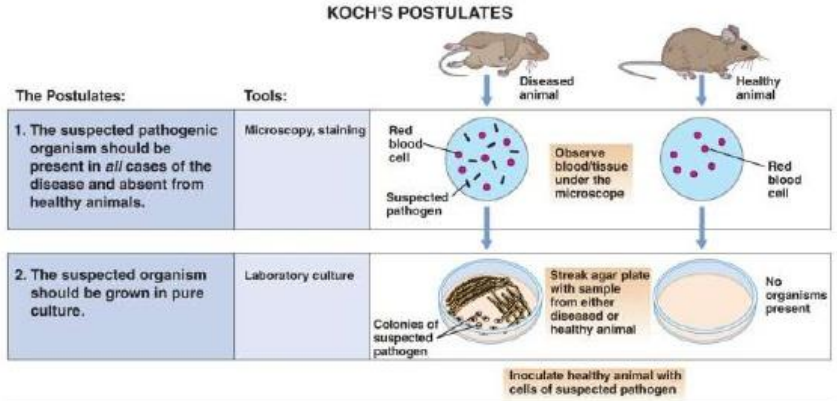
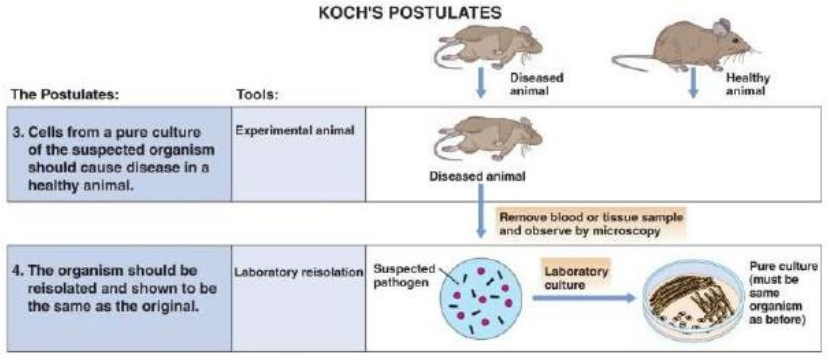
Contents of Teaching in Medical Virology Lecture:

Koch's Postulates
Applicable in many bacterial diseases

1. Organism present only in diseased individuals
2. Organism cultivated in <u>pure culture</u> from diseased individual



3. Organism causes disease when injected into healthy individuals
4. Organism re-isolated from infected individual from point 3.



River's Postulates

- T.M. River, 1937
- Modified from Koch's Postulates (proof of bacterial diseases)

1. Isolate virus from diseased hosts.
2. Cultivation of virus in host cells.
3. Proof of filterability.
4. Production of a comparable disease when the cultivated virus is used to infect experimental animals.
5. Reisolation of the same virus from the infected experimental animal.
6. Detection of a specific immune response to the virus.

Viral Diagnostics in the Clinical Laboratory

- Over 60% of all infectious disease cases seen by a physician are due to viral infections.
- Quality of patient specimens and their transport to the laboratory is important.

Storage and Collection of Biological Specimens for Viral Testing

- What types of specimens are collected to diagnose?
  - *Respiratory tract infections*: Nasal and bronchial washings, throat and nasal swabs, sputum
  - *Eye infections:* throat and Conjunctival swab/scraping
  - *Gastrointestinal tract infections*: stool and rectal swabs
  - *Vesicular rash*: vesicle fluid, skin scrapings
  - *Maculopapular rash*: throat, stool, and rectal swabs
  - *CNS (encephalitis and meningitis cases)*: stool, tissue, saliva, brain biopsy, cerebrospinal fluid
  - *Genital infections*: vesicle fluid or swab
  - *Urinary tract infections*: urine
  - *Blood borne infections*: blood

Three General Approaches for Laboratory Diagnosis of Viral Infections

- Direct detection
  - –Microscopy or staining
- Virus Isolation
  - –PCR
- Serology
  - –Antibodies

Antibody assays

- An **acute or recent infection** may be confirmed by demonstrating the presence of specific **IgM** in a single serum sample, or showing a **sero-conversion** or **rise in titre of specific IgG** in paired sera.
  In general, the presence of IgG and the absence of IgM, is indicative of past infection or immunity.
  These days, antibody assays are usually tested by means of the enzyme-linked immuno-assay (**ELISA) technique.**

Serology

- The development of antibodies to different components of the virus is used in staging the disease. For example in hepatitis B and HIV infections this approach is used.

Serology

Detection of rising titers of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection.

Classical Techniques	Newer Techniques
1. Complement fixation tests (CFT)	1. Radioimmunoassay (RIA)
2. Haemagglutination inhibition tests	2. Enzyme linked immunosorbent assay (EIA)
3. Immunofluorescence techniques (IF)	3. Particle agglutination
4. Neutralization tests	4. Western Blot (WB)
5. Counter-immunoelectrophoresis	5. RIBA, Line immunoassay

ELISA

- Enzyme-Linked Immuno-Sorbant Assays (ELISAs)
  - Enzyme reacts with substrate to produce colored product
  - Very sensitive
    - HIV test
      - If positive twice, Western Blotting is performed next

Viral Serology

– Primary and secondary responses to viral infections
  - IgM (1st exposure)
  - IgG (2nd exposure)

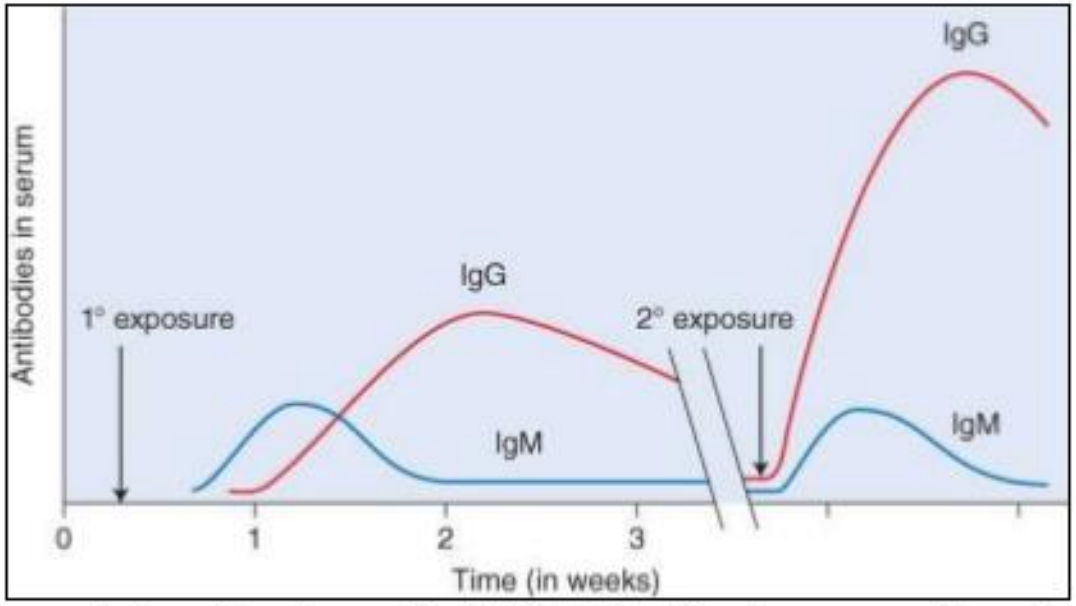


Figure 5.18: Primary (1 degree) and secondary (2 degree) antibody responses toward a viral pathogen.

Serological Diagnosis

Detection of specific antobodies

- Detection of Immunologlublins Ig G. Ig M Ig A
- Raise of titers Ist sample later sample **(convalescent sample)** tested after 10 – 14 days Raise of titer is diagnostic

Micro plate ELISA for HIV antibody: colored wells indicate reactivity

Serology

Criteria for diagnosing Primary Infection

- 4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera
- Presence of IgM
- Seroconversion
- A single high titre of IgG (or total antibody) - very unreliable

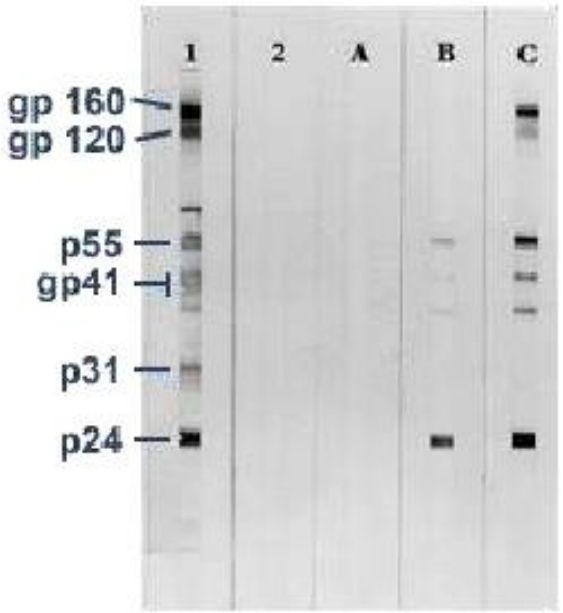
Criteria for diagnosing Reinfection

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

Western Blot

HIV-1 Western Blot

- Lane1: Positive Control
- Lane 2: Negative Control
- Sample A: Negative
- Sample B: Indeterminate
- Sample C: Positive



Western blot testing for HIV-1

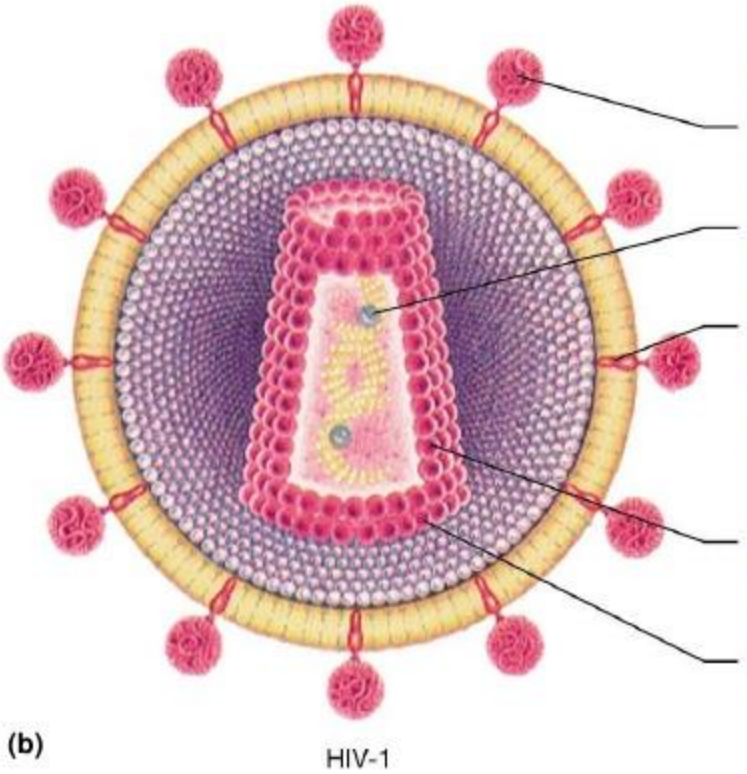


Figure 5.21b: The structure of HIV-1.

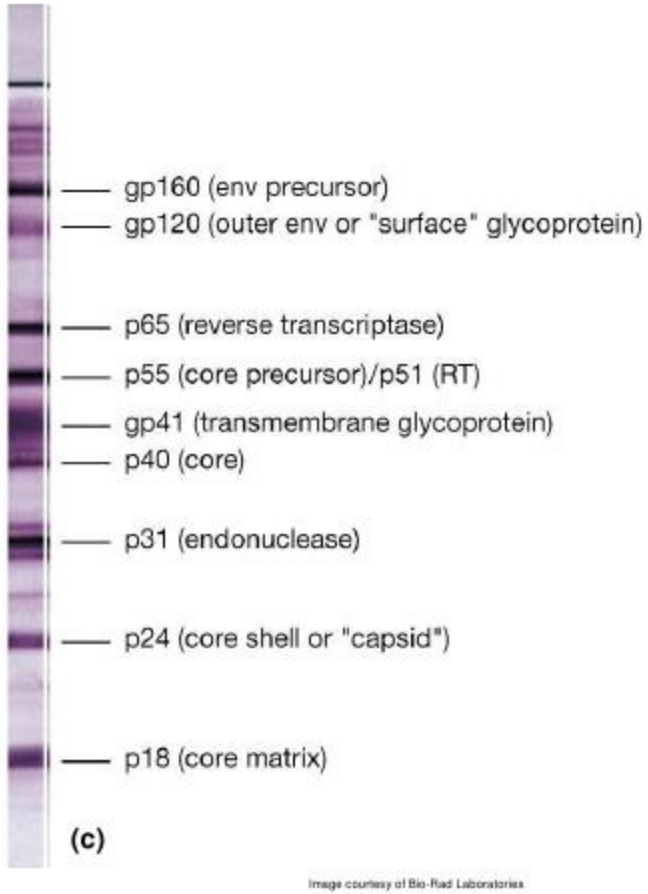


Figure 5.21c: The typical results of a Western blot testing patient serum for HIV-1 antibodies.

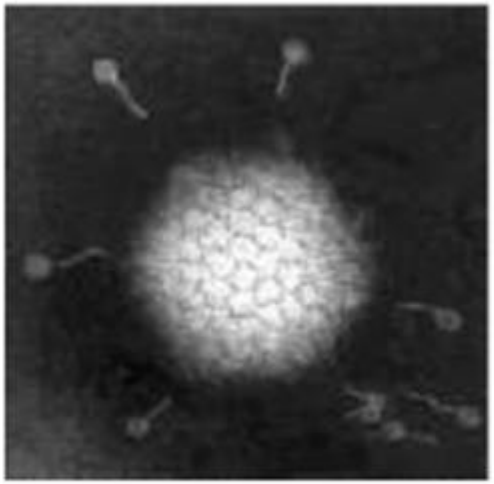
Microscopy

Electron Microscope	• Examine specimen for viruses
Immuno-electron microscopy	• Labeled antibody
Immunofluorescence	• Fluorescent tag bound to Fc region of Ab
Light microscope	• Histological appearance of affected cells • Inclusion bodies

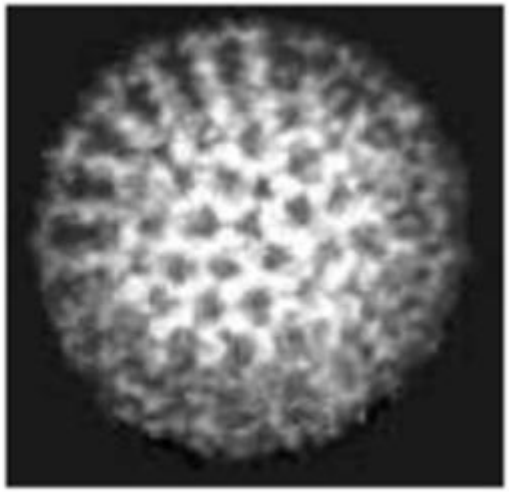
Direct demonstration of virus

- **(a) Electron Microscopy**
- Viruses are very small and cannot be visualized by light microscopy. Historically the electron microscope was very useful in defining the morphology of many human viruses. However, it is **not a tool that is routinely used to identify viruses in a diagnostic setting**. This is because viruses are usually present in very small numbers in clinical specimens and other contaminating material tends to obscure their presence.

Pictures of viruses in E.M



Adenovirus

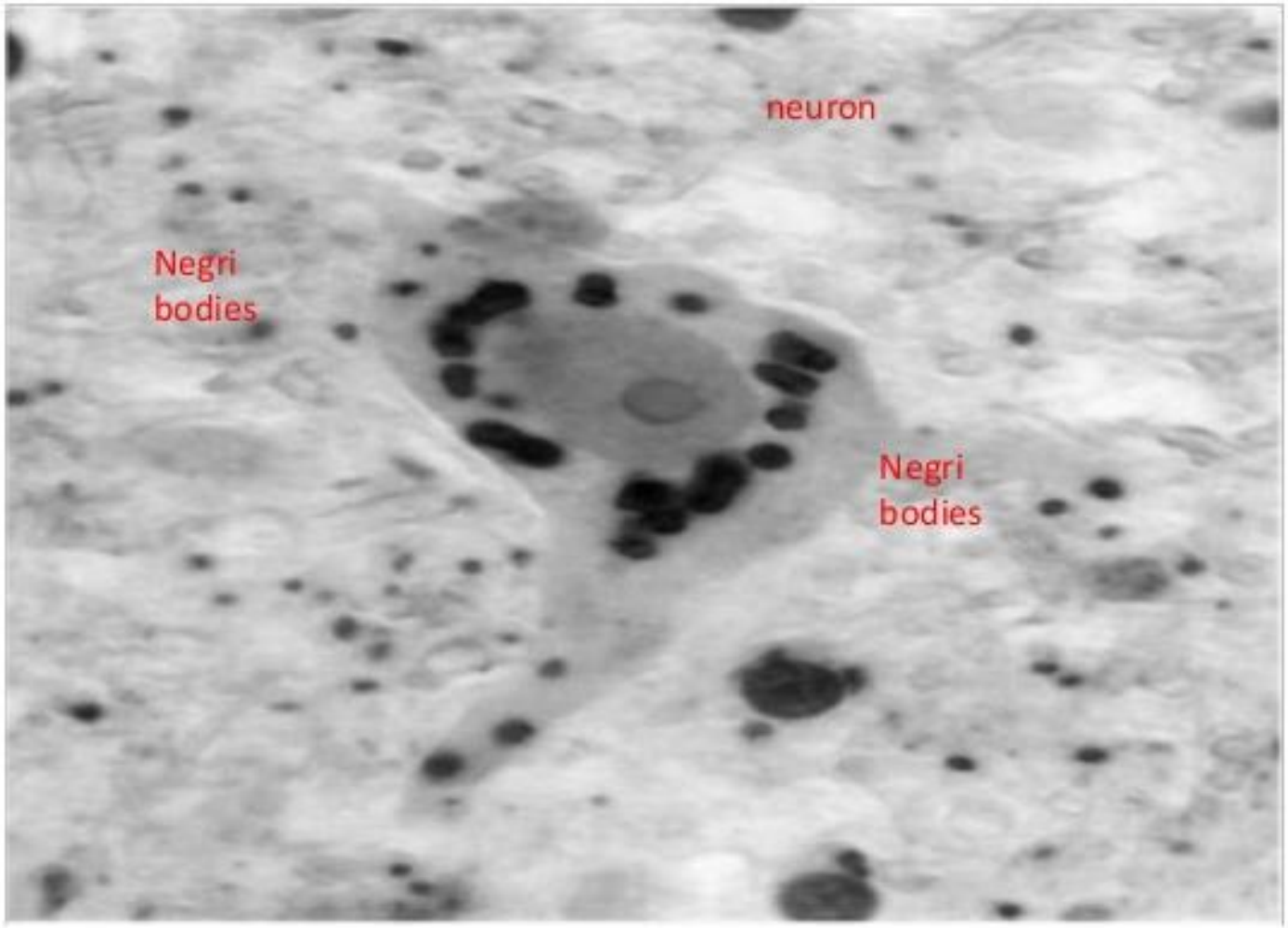


Rotavirus

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
- Eg: Negri bodies in rabies .

Negri bodies in Rabies



Direct demonstration of virus

- **(b)** Demonstration of **virus-infected cells** in clinical samples by labelled antibodies.
- This technique is commonly used to identify the causative agent in a patient with a respiratory infection, caused by viruses such as RSV, Influenza or Adenovirus.
- Infected cells synthesize and express viral proteins (antigens). The presence of these can be detected using specific mono-clonal or poly-clonal antibodies labelled with fluorescene (a green dye). The antibody binds to the cells if they express the corresponding antigen. The cells can then be visualized by examination under a fluorescent microscope. Positive cells fluoresce a bright green colour.
- The limitation of the test is that you have to know what virus you are looking for.
  The advantage is that one can get a very rapid answer as to which virus is causing the problem.

Culture

- Viruses can only replicate in living cells. Therefore to culture them *in vitro* one must provide them with living cells. In the past it was common to use **laboratory animals**, or **chick embryos** to grow viruses, but these have largely been replaced by the use of **cell monolayers.**
- The clinical sample is inoculated into a test tube containing a glass cover slip on which a cell monolayer is growing. Replicating viruses change the appearance of the cells to induce a cytopathic effect. Different viruses cause different types of cytopathic effects. Only some medically important viruses can be cultured.
- **Immunofluorescence:** Another way to identify a virus growing in a cell culture is to add fluorosceine labelled monoclonal antibodies to likely viruses to the cell sheet and examine under a fluorescent microscope.

Indirect Examination

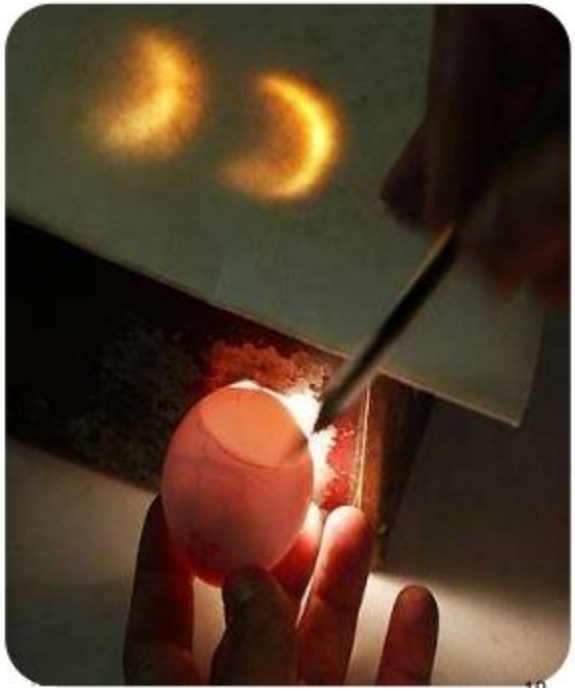
1. Cell Culture	Cytopathic effect (CPE)
	haemabsorption
	immunofluorescence
2. Eggs	pocks on CAM
	haemagglutination
	inclusion bodies
3. Animals	disease or death

Isolation of virus

- Laboratory animals
- Fertilized Hen's Egg
  - Chorioallantoic membrane
  - Allantoic cavity
  - Amniotic cavity
  - Yolk sac
- Organ/Tissue/Cell Culture
- Growth identified by serological method like neutralization.

Regular Methods in Use

- Egg inoculation Pox virus, Influenza
- Into tissue culture

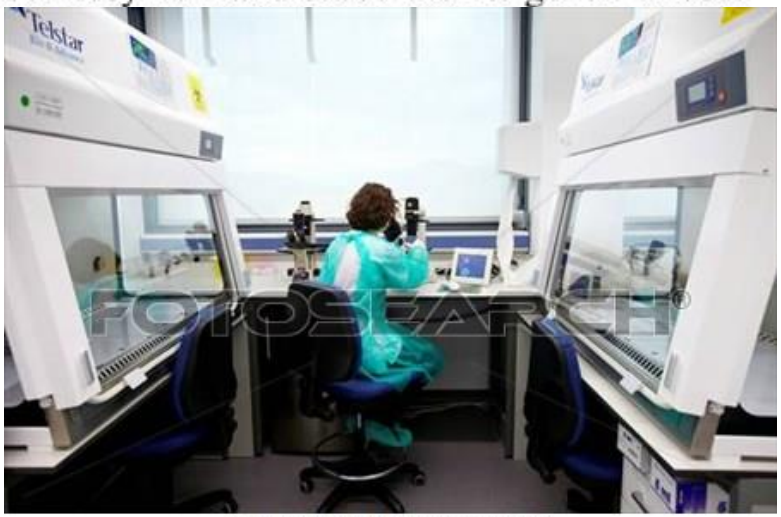


Cell culture

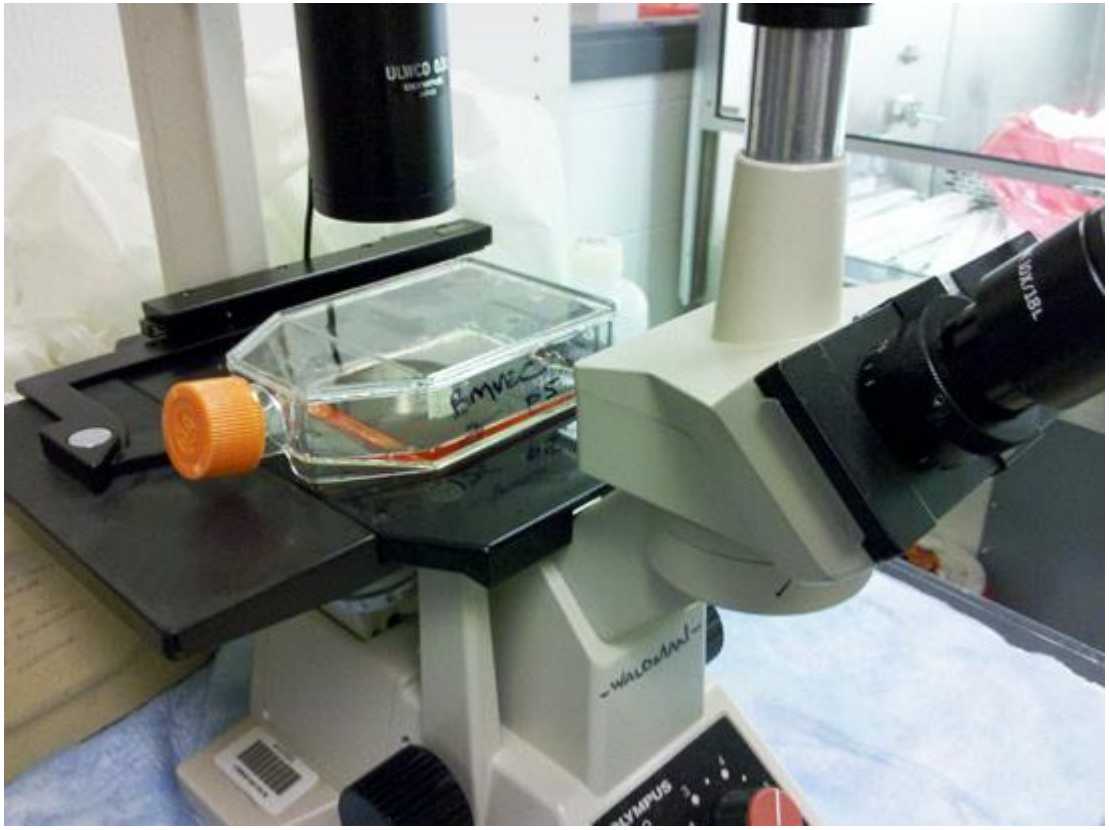
Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

1. Primary cells - Monkey Kidney
2. Semi-continuous cells - Human embryonic kidney and skin fibroblasts
3. Continuous cells - HeLa, Vero, Hep2, LLC-MK2, MDCK

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.



Inverted microscope

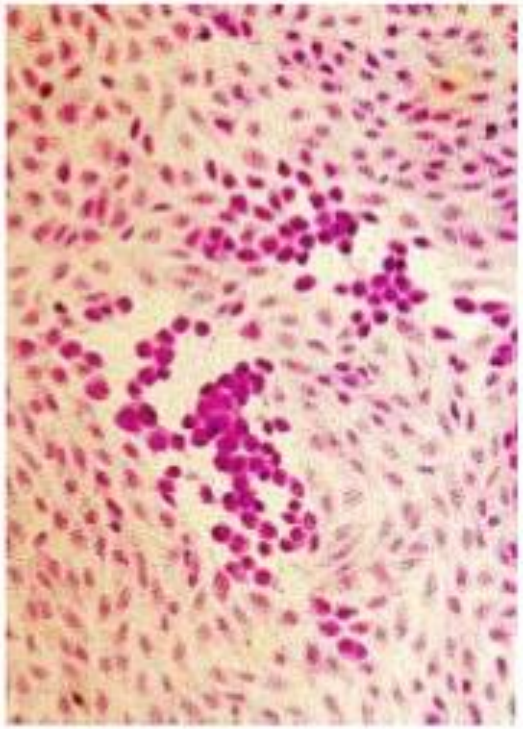


Cytopathic Effects

- Visible results of viral infection
- Cell death by
  - Multiplying viruses
  - Inhibition of DNA, RNA or protein synthesis
  - Effects on permeability of membrane
- Cytopathic effects (CPEs) of infected cells can be observed with inverted light microscopes
  - Rounding/detachment from plastic flask
  - Syncytia/fusion
    - Fusion of cells
  - Shrinkage
  - Increased refractivity
  - Aggregation
  - Loss of adherence
  - Cell lysis/death
- Common observations of CPEs
  - Inclusion body formation
    - Intracellular virus parts (replication or assembly)
  - Hemadsorption assays

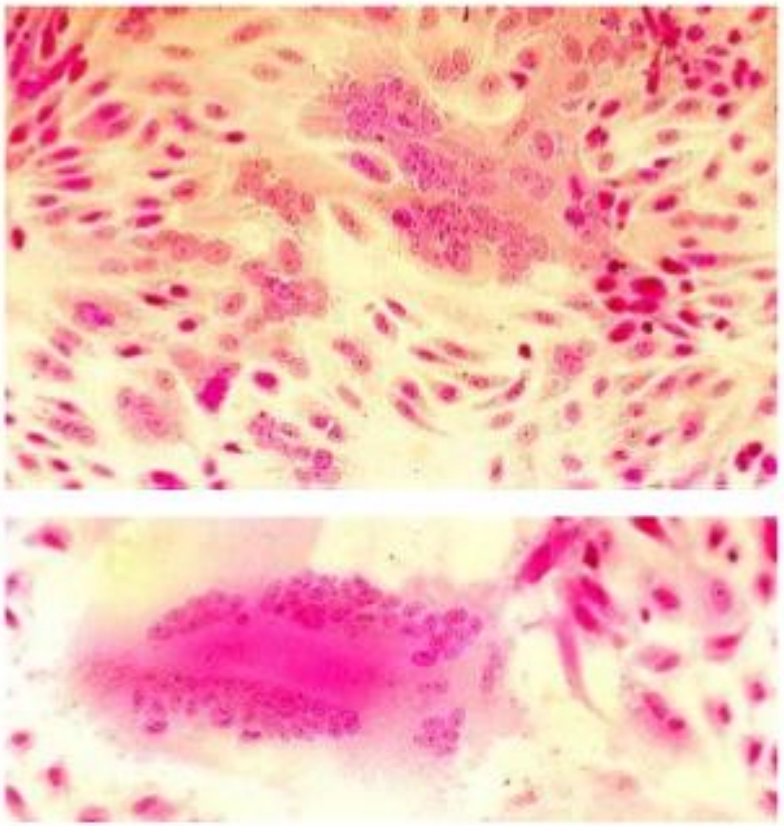
Cytopathic Effect

Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells



Cytopathic effect of enterovirus 71 and HSV in cell culture: note the ballooning of cells.
(Virology Laboratory, Yale-New Haven Hospital, Linda Stannard, University of Cape Town)

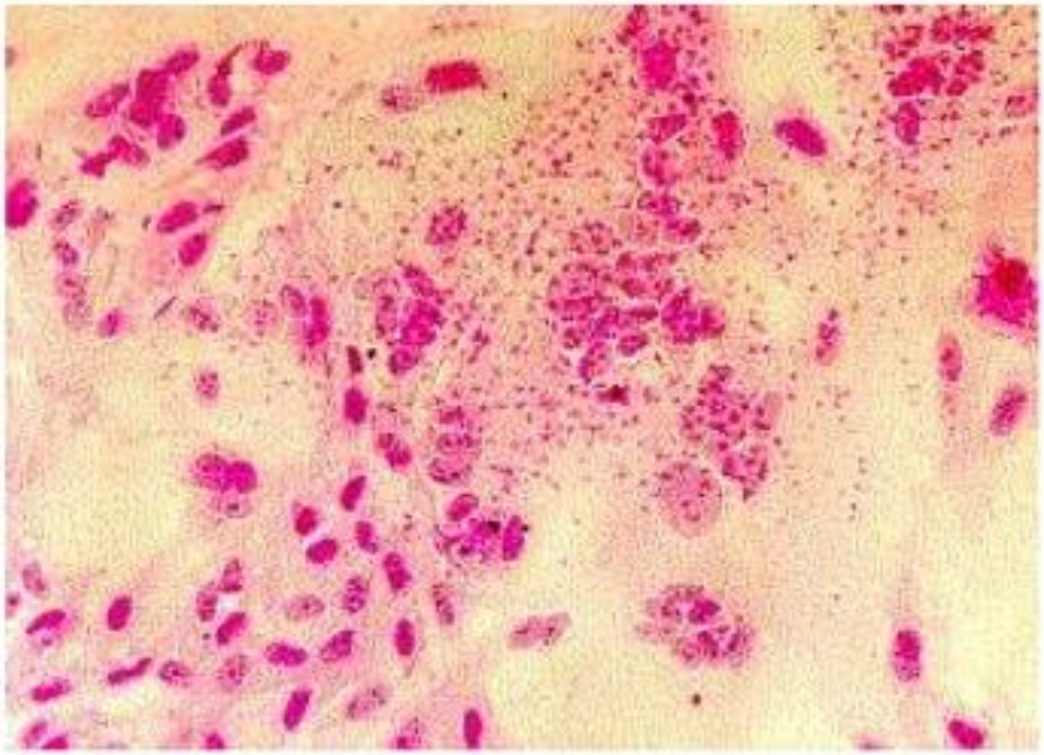
Cytopathic Effect



Syncytium formation in cell culture caused by RSV (top), and measles virus (bottom).

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

Haemadsorption

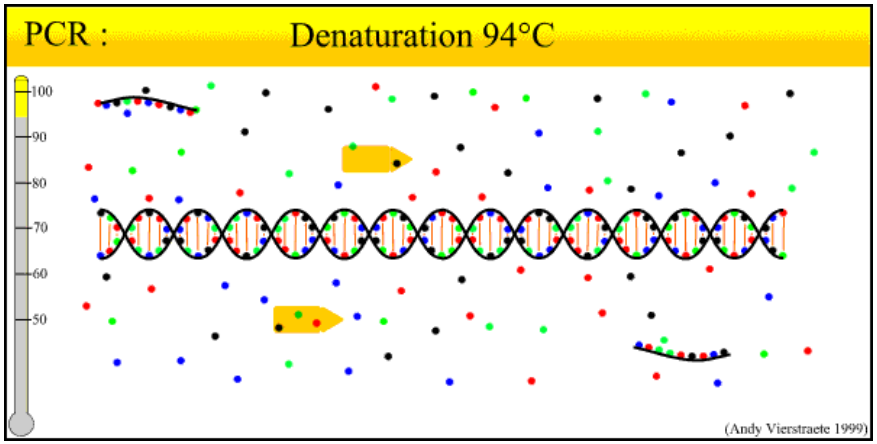


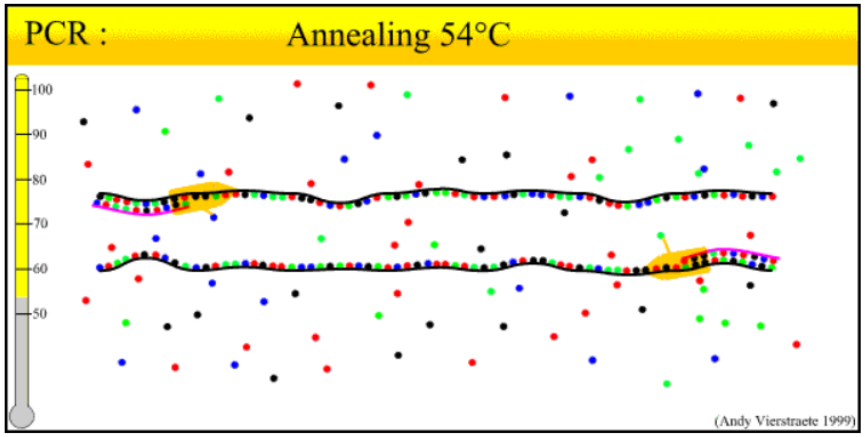
Syncytial formation caused by mumps virus and haemadsorption of erythrocytes onto the surface of the cell sheet.

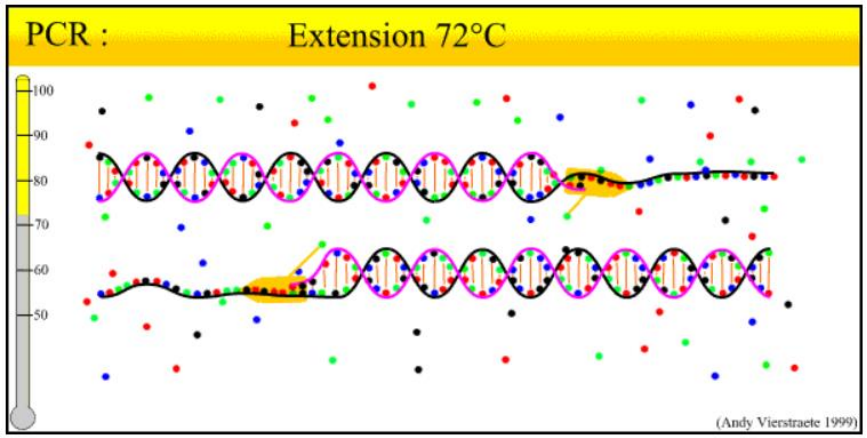
Molecular techniques

- Nucleic acid amplification techniques such as polymerase chain reaction (PCR) can be used to detect viral genomes in clinical material. The same technique can be used to detect any DNA sequence (viral, bacterial or other). 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.

– Nucleic acid methods
  - PCR (DNA), RT-PCR (RNA)
  - Can be used to detect viruses that are noncultivatable
  - Rapid identification (e.g. RT-PCR—4 Corners outbreak of hantavirus or FRET in the field)
  - Can be used to manage patients (e.g. HIV viral load)





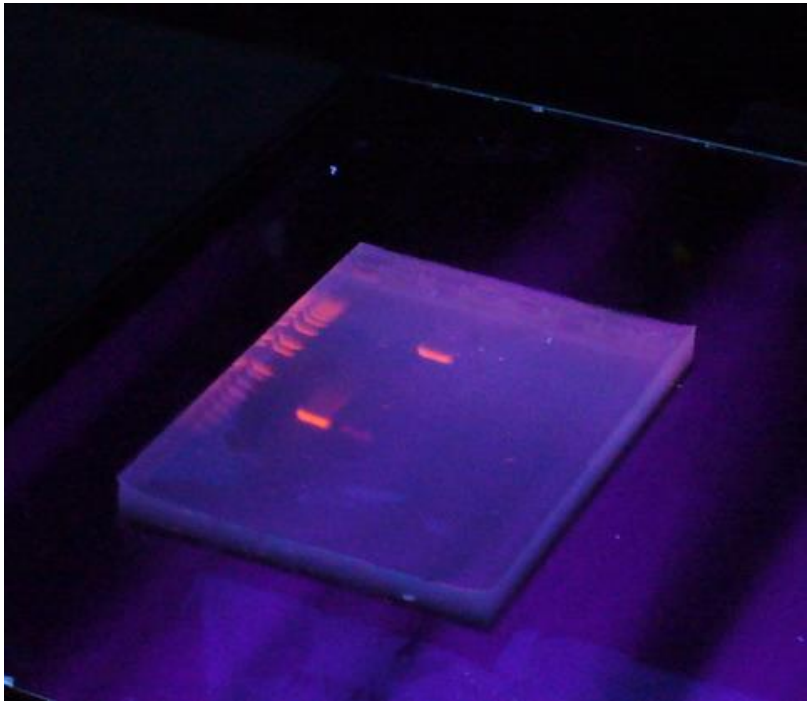


A target DNA sequence can be amplified to the point where it can be readily identified using labelled probes in a hybridisation assay

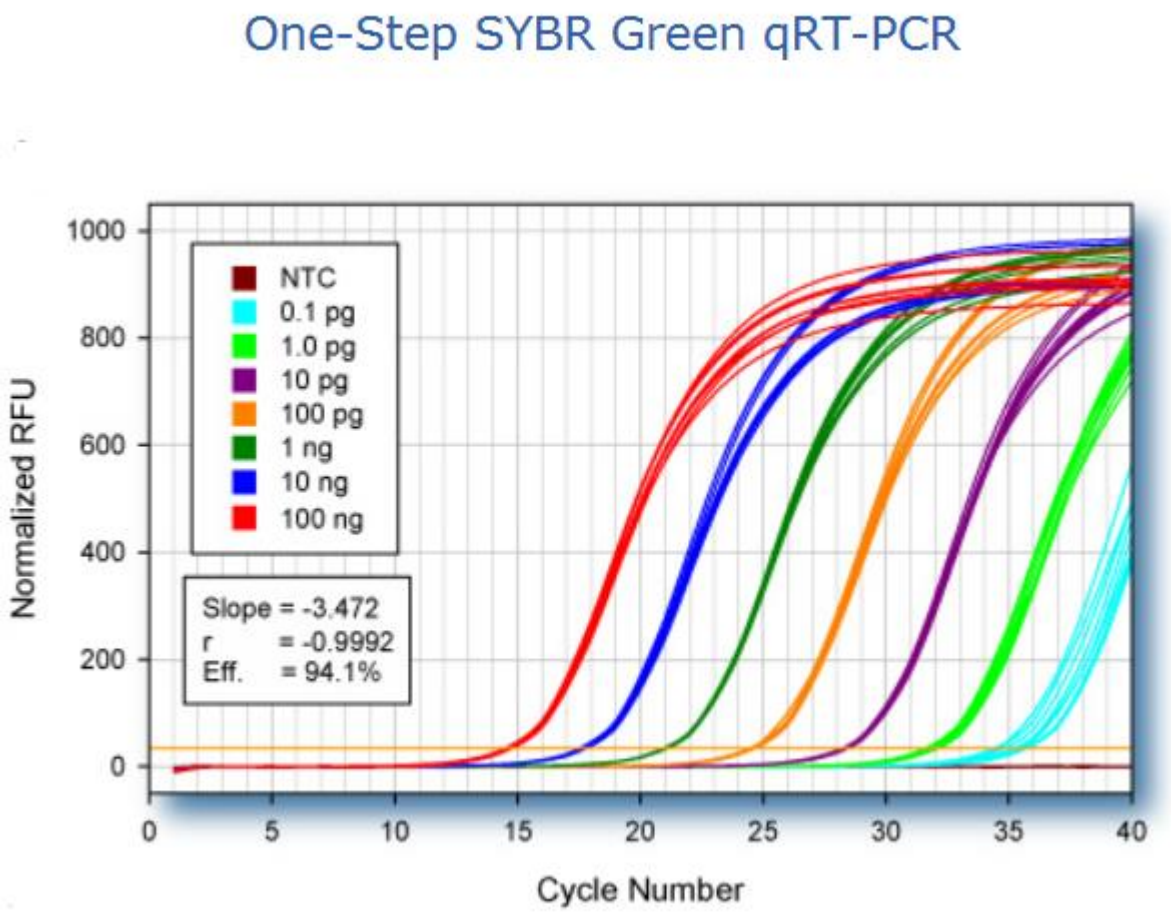
- The technique is used for the diagnosis of infections caused by HIV , HPV , Herpes simplex virus, Hepatitis B & C,Enterovirus,EBV ,Rubella & Rotavirus

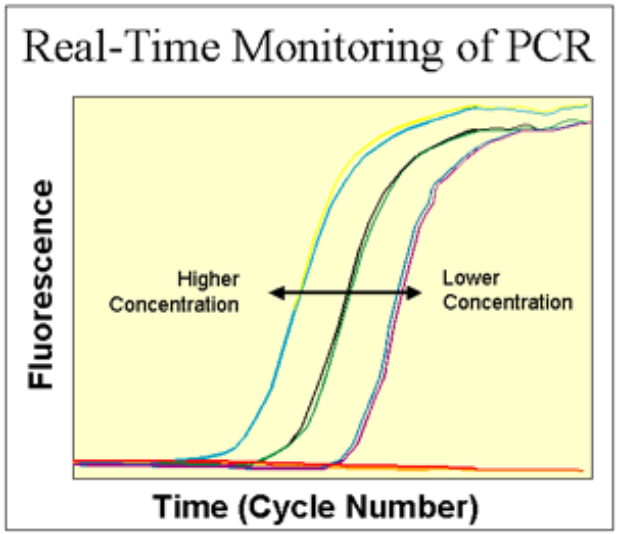
Thermalcycler for PCR technique

In house PCR, agarose jel imagination



Real time PCR, digital curve imagination





CLASSIFICATION ACCORDING TO THE PRINCIPLE