VIRAL GENOME DETECTION TECHNIQUES

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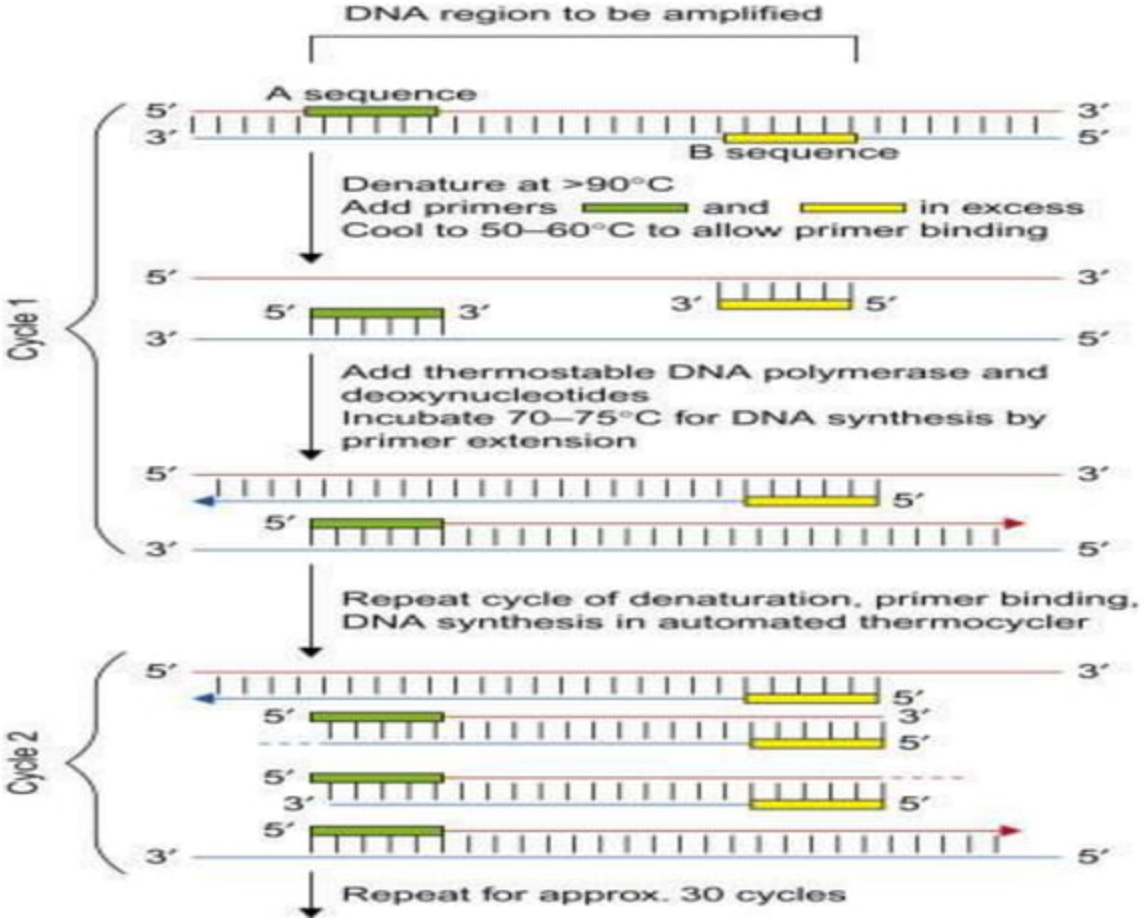
PCR

- The viral DNA extracted from a very small number of virions or infected cells can be amplified to the point where it can be readily identified.
- It involves three basic steps
  - Viral DNA extraction from the specimen.
  - Amplification of specific region of viral DNA.
  - Detection of amplified regions by gel electrophoresis

- **Reverse Transcriptase PCR (RT-PCR)** ; RT-PCR is used for the detection of RNA viruses. After RNA extraction, the viral RNA is reverse transcribed to DNA, which is then subjected to amplification similar to that followed in PCR.
- PCR can also be used to detect viral RNA by including a preliminary step in which reverse transcriptase is used to convert RNA to DNA.

- **Real Time PCR ;** It has the advantage of quantifying viral nucleic acid in the samples

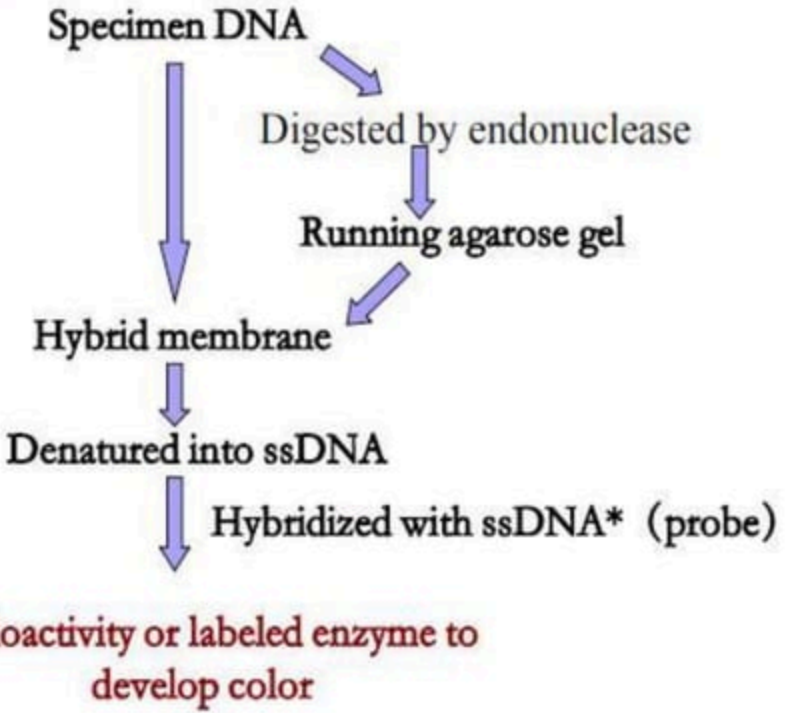

DNA region to be amplified
A sequence
B sequence
5′
3′
Denature at >90°C
Add primers and in excess
Cool to 50–60°C to allow primer binding
Add thermostable DNA polymerase and deoxynucleotides
Incubate 70–75°C for DNA synthesis by primer extension
Repeat cycle of denaturation, primer binding, DNA synthesis in automated thermocycler
Repeat for approx. 30 cycles
Cycle 1
Cycle 2

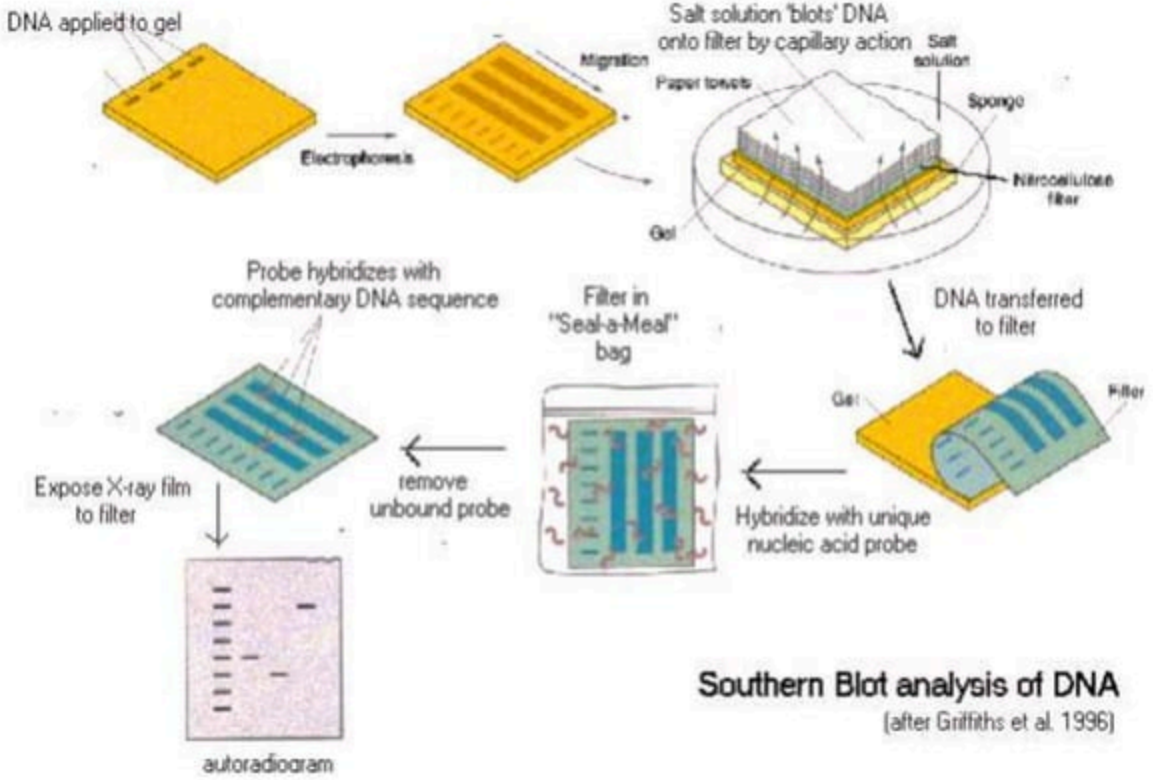
Nucleic Acid Probe HYBRIDIZATION

Nucleic Acid Probe

- Nucleic acid probes have a low sensitivity compared to polymerase chain reaction (PCR) as it directly detects the viral genes in the specimen without amplification
- It is a labelled nucleic acid sequence complementary to a part of nucleic acid sequence of the target virus
- Both DNA and RNA probes are commercially available

Nucleic acid molecular hybridization

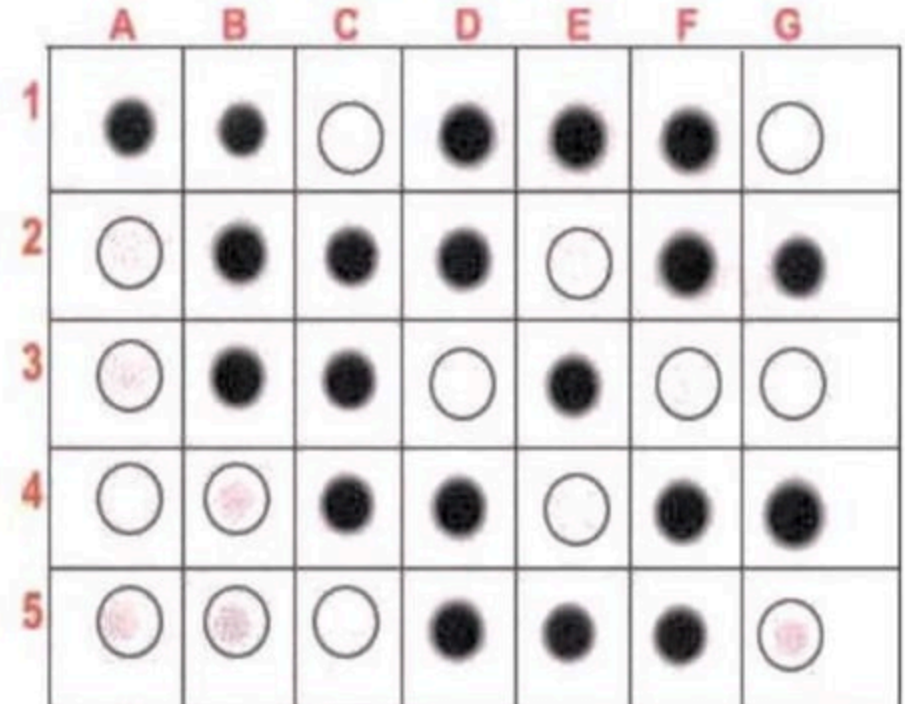




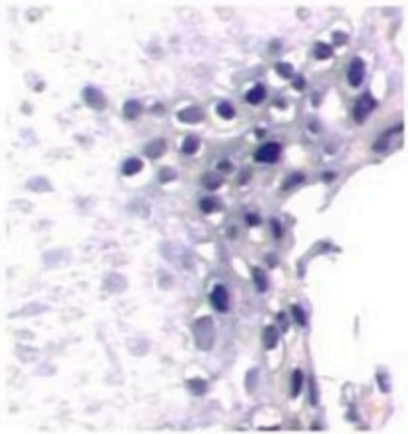
Southern Blot analysis of DNA

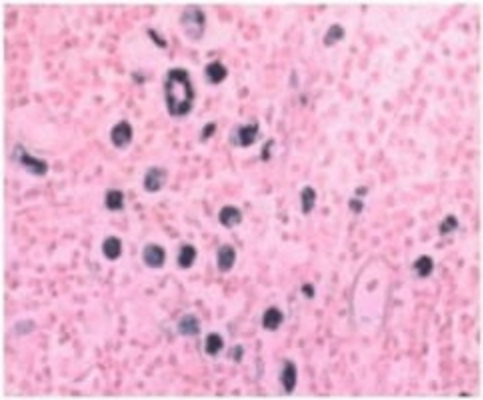
(after Griffiths et al. 1996)

Dot Blot Hybridization



in situ hybridization

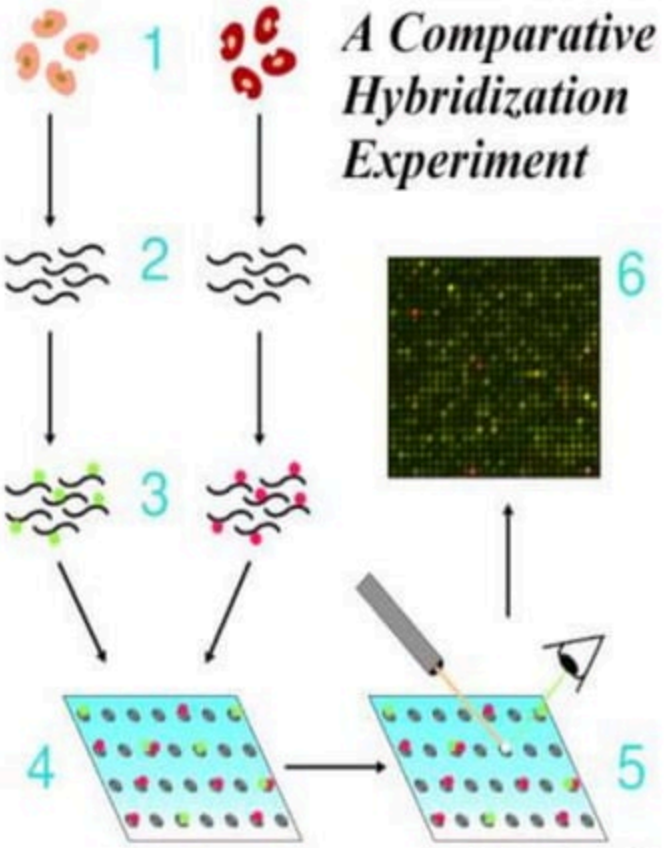




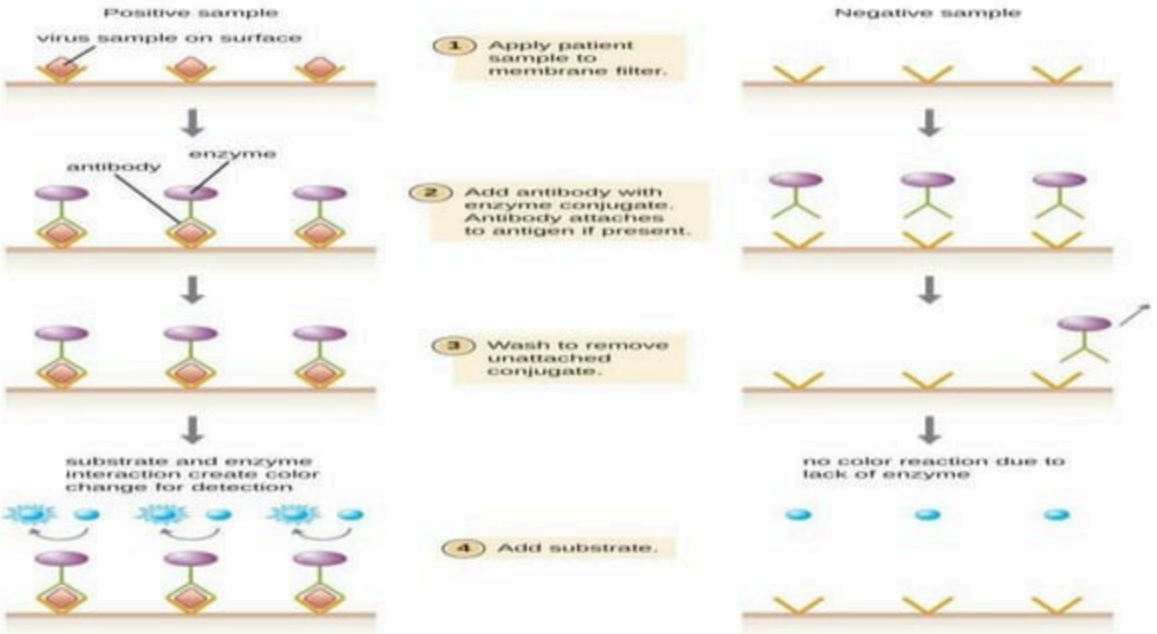
DNA Microarray

- The microchip is a solid support matrix onto which have been "printed" spots, each containing one of several hundred to several thousand unique oligonucleotides. These oligonucleotides can represent conserved sequences from virtually all viruses represented in the various genetic databases, or can be customized to represent only viruses from a given specific disease syndrome, such as acute respiratory disease in children. The basis of the technology is the capture by these oligonucleotides of randomly amplified labeled nucleic acid sequences from clinical specimens. The binding of a labeled sequence is detected by laser scanning of the chip and software programs that assess the strength of the binding. From the map position of the reacting oligonucleotides, the software identifies the virus in the clinical sample. This type of test was used to initially determine that the virus responsible for severe acute respiratory syndrome (SARS) was a coronavirus

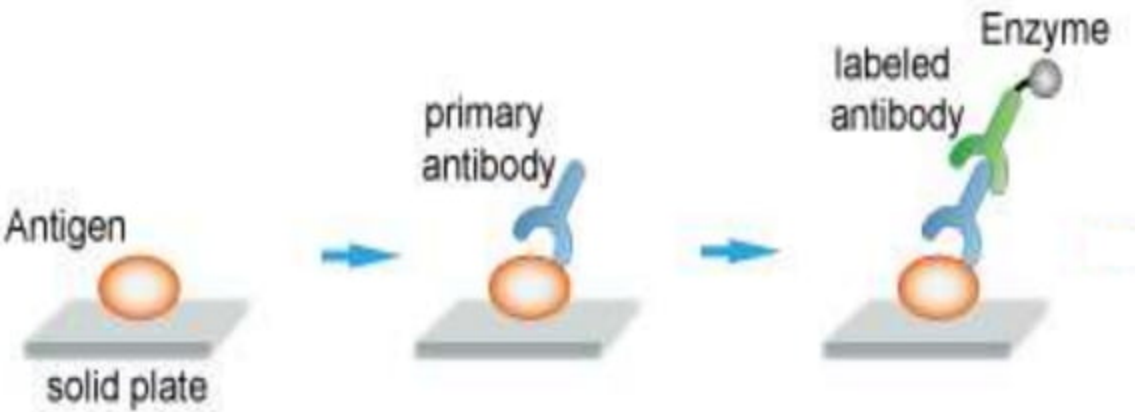
DNA Microarray (Chip assay)



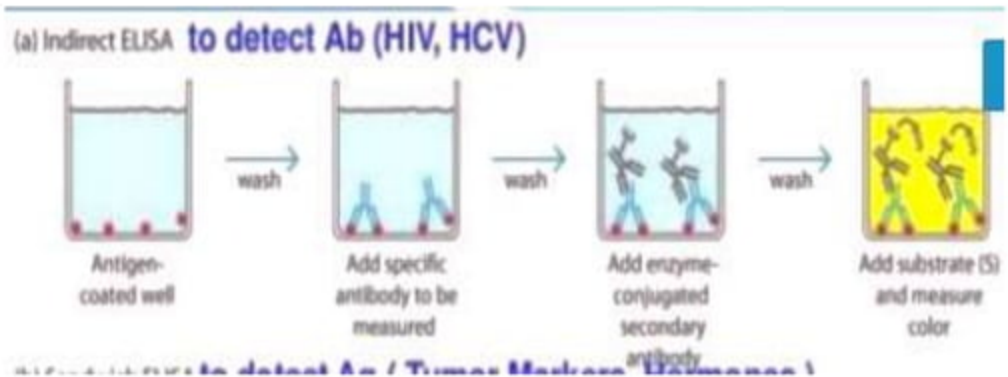
Direct ELISA



INDIRECT ELISA



1. Antigen is coated onto wells by passive adsroption and incubation.
2. Primary antibody is added and incubated with
3. Anti-species antibody conjugated with enzyme is added and incubated.antigen.


(a) Indirect ELISA to detect Ab (HIV, HCV)
wash
wash
wash
Antigen-coated well
Add specific antibody to be measured
Add enzyme-conjugated secondary antibody
Add substrate (S) and measure color

- An ELISA test may be used to diagnose:
- HIV
- rotavirus
- varicella-zoster virus
- Zika virus

Viral Diagnostics in the Clinical Laboratory

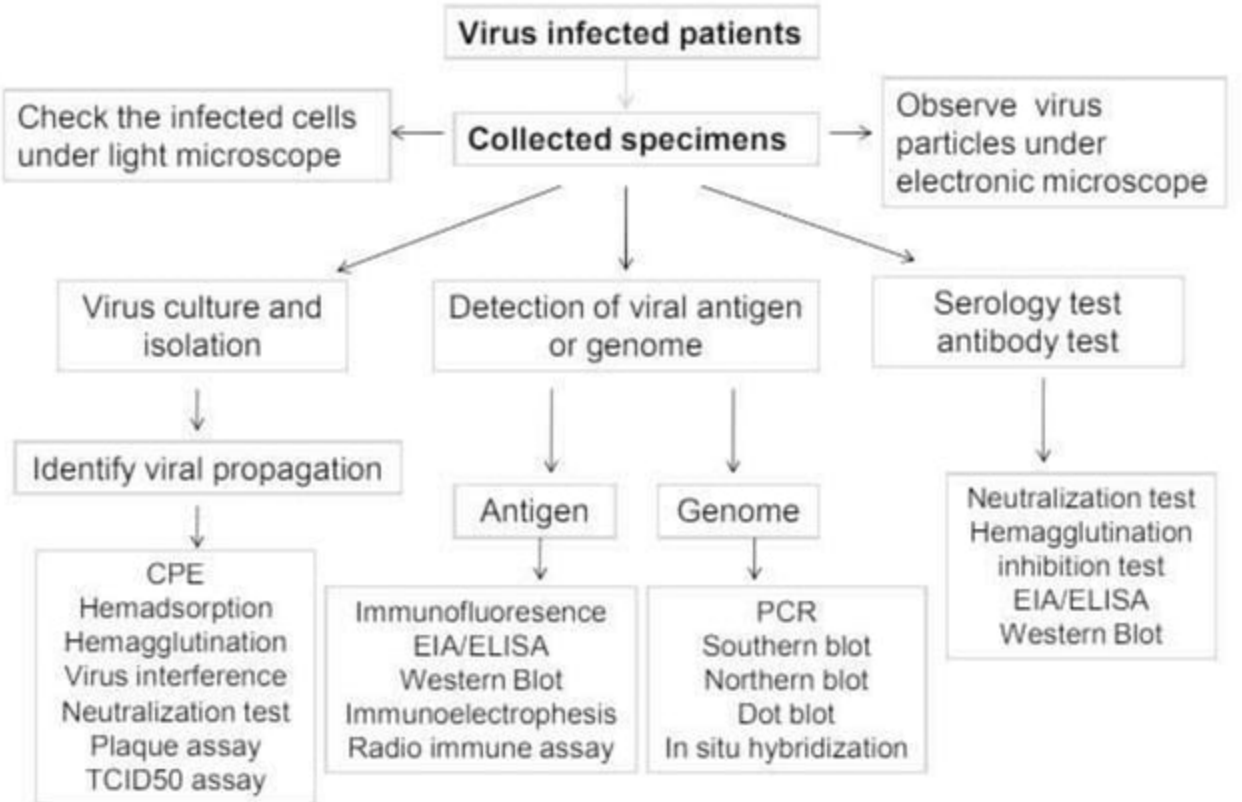
- Over 70% of all infectious disease cases seen by a physician are due to viral infections. ?

- Quality of patient specimens and their transport to the laboratory is important.

Why to diagnose a viral diseases

- Screening of viral infection (blood donors, pregnant mothers,elder women, TB patients)
- For diagnosis of etiological agent of viral infection
- For instituting proper (HIV, Herpetic encephalitis) antiviral therapy
- Detection and prediction of etiological agent of Epidemics
- Identification of antigenic variation of virus- which helps in vaccine preparation, control of out breaks Helps in suspecting a new viral infection

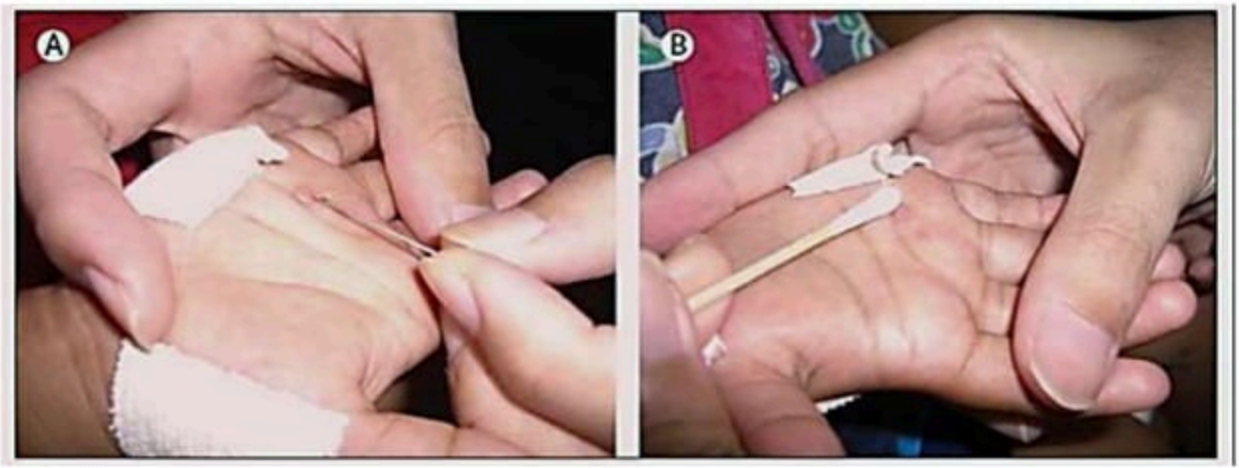
Procedures for laboratory viral diagnosis



Types of specimens collected for viral diagnosis

- Respiratory tract – Nasopharyngeal swab, nasal wash, throat swab
- Gastrointestinal tract – Stool, vomit
- Skin (Rash) – Biopsy
- Eye – Conjunctival swab, corneal swab
- Central nervous system – Cerebral spinal fluid, blood, throatswab
- Genital infections – Cervical swab, vesicle fluid
- Urinary tract infections: urine
- Bloodborne infections: blood

Collection of vesicular fluid from palmar lesions for virological diagnosis of HFMD



Three General Approaches for Laboratory Diagnosis of Viral Infections

- **Direct detection**

  in patient material of virions, viral antigens, or viral nucleic acids
  - Microscopy or staining
  - Detection of nucleic acid, antigens

- **Virus Isolation**

  in cultured cells, followed by identification of the isolate
  - CPE and other characters

- **Serology**

  detection and measurement of antibodies in the patient's serum
  - Antibodies (ELISA

Direct Examination

1. Electron Microscopy

morphology of virus particles immune electron microscopy

2. Light Microscopy

histological appearance inclusion bodies

3. Antigen Detection

immunofluorescence, ELISA etc.

4. Viral Genome Detection

hybridization with specific nucleic acid probes polymerase chain reaction (PCR)

Electron Microscopy

- Examine specimen for viruses

- **Shape:** Viruses can be identified based on their distinct appearances.
    - Adenovirus-space vehicle shaped
    - Astrovirus-star shaped

- **Virus detection from tissue culture:**
- EM can also be used for detection of viral growth in tissue cultures.

Drawbacks

- EM is highlyexpensive
- Has low sensitivity with detection threshold of 107 virions/mL

Light Microscopy

- Histological changes in infected cells

Cytopathic effects

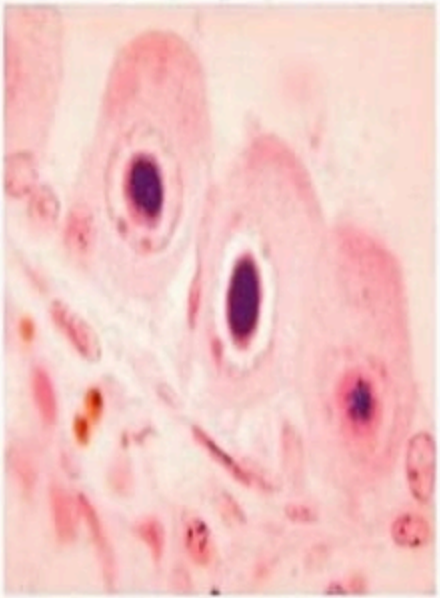
- Cytopathic effect or cytopathogenic effect (CPE) refers to structural changes in host cells that are caused by viral infection

Formation of syncytia

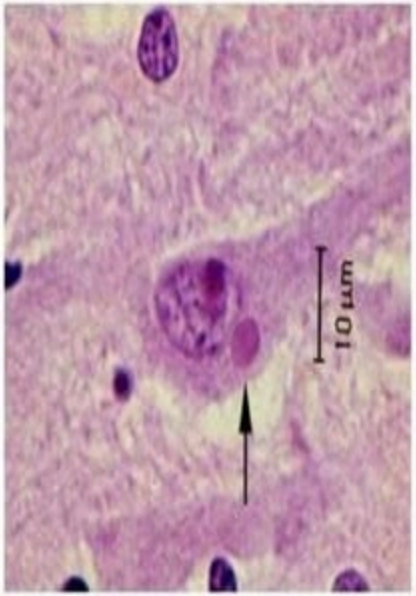
- Syncytia are large cytoplasmic masses that contain many nuclei.
- Herpesvirus characteristically produce cell fusion

- **Grape- like clusters :** characteristic of adenoviruses infections
- **Inclusion bodies**: staining of tissue sections may be useful for detection of inclusion bodies which helps in the diagnosis of certain viral infections.
- Examples of inclusion bodies
- Negri bodies – Rabies
- Cytomegalic inclusion bodies - CMV infections (CMV- Cytomegalovirus)

Cytomegalovirus infection: Owl's eye nuclear inclusion bodies



Rabies infection: cytoplasmic Negri bodies



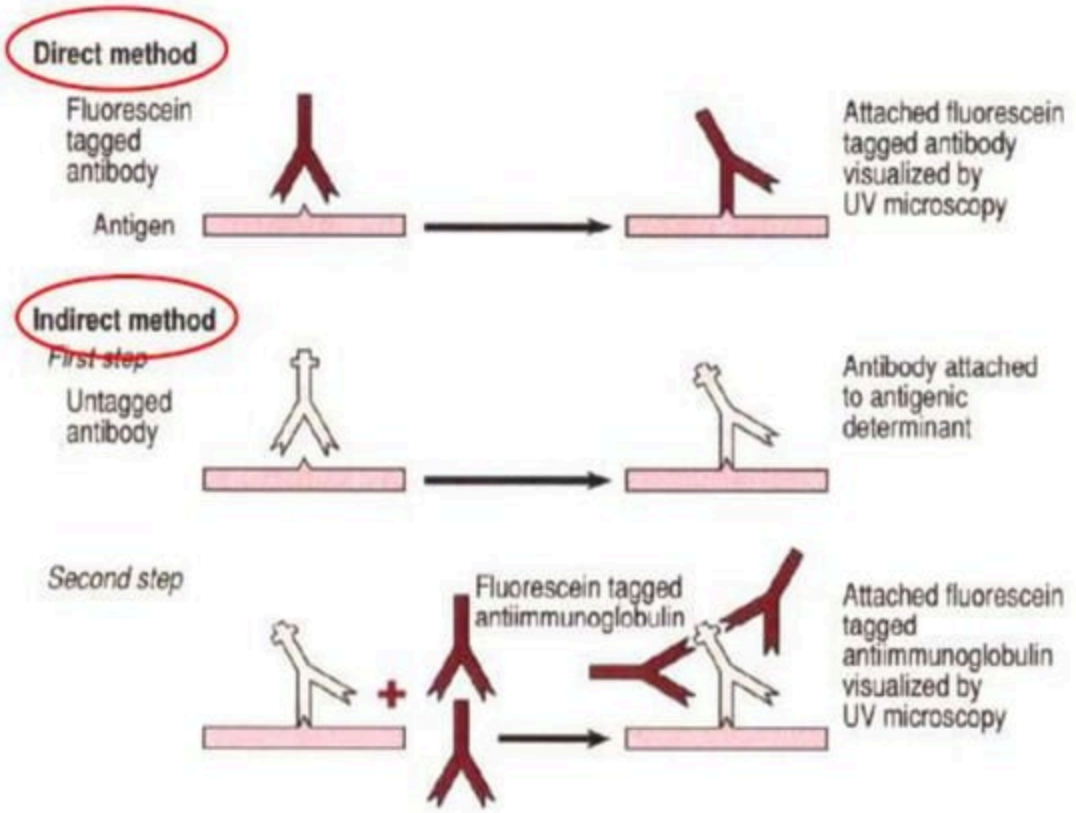
Immunofluorescence

- Direct IF- technique employed to detect viral particles in the clinical samples

- **Procedure:** Specimen is mounted on slide, stained with specific antiviral antibody tagged with fluorescent dye and viewed under fluorescent microscope.

- Diagnosis of rabies virus antigen in skin biopsies

- Rapid diagnosis of respiratory infections caused by influenza virus


Direct method
Fluorescein tagged antibody
Antigen
Attached fluorescein tagged antibody visualized by UV microscopy
Indirect method
First step
Untagged antibody
Antibody attached to antigenic determinant
Second step
Fluorescein tagged antiimmunoglobulin
Attached fluorescein tagged antiimmunoglobulin visualized by UV microscopy

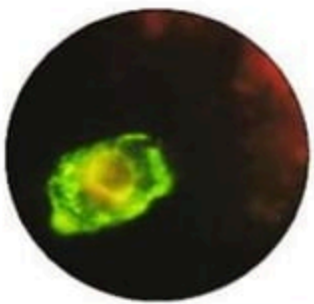
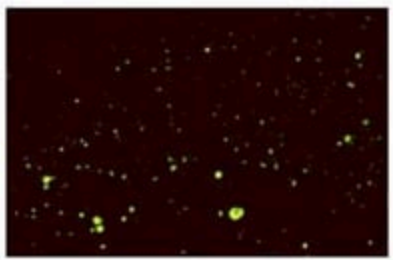


Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)

(Virology Laboratory, Yale-New Haven Hospital)



Positive immunofluorescence test for rabies virus antigen. (Source: CDC)