Advanced Medical virology

Viral Laboratory Diagnosis
Lecture 4

BY
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laboratory diagnosis of viral infections

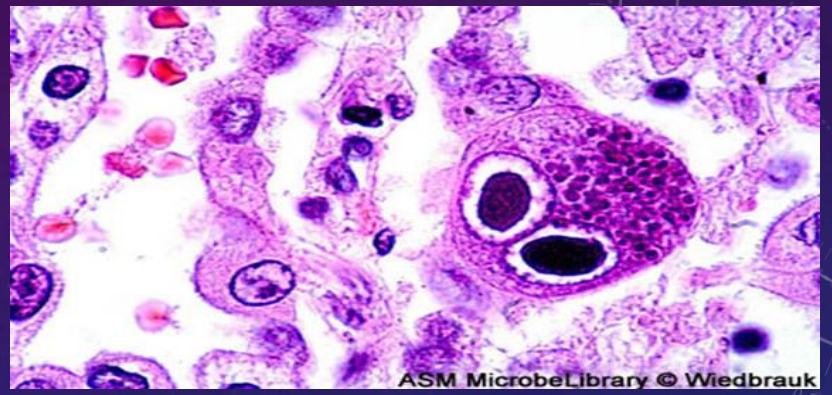
There are five approaches to the diagnosis of viral diseases by the use of clinical specimens:

1- Microscopic examination.
2- Cell culture.
3- Serological tests .
4- Detection of viral Ag.
5- Molecular method .

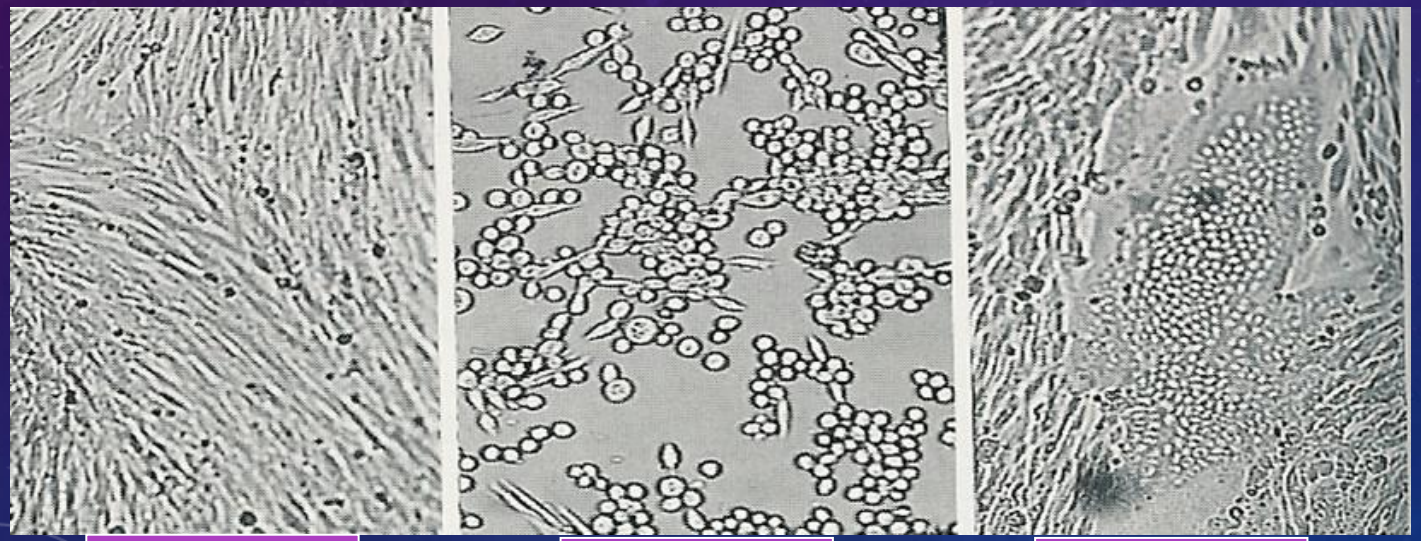
Microscopic examination

Light microscopy:

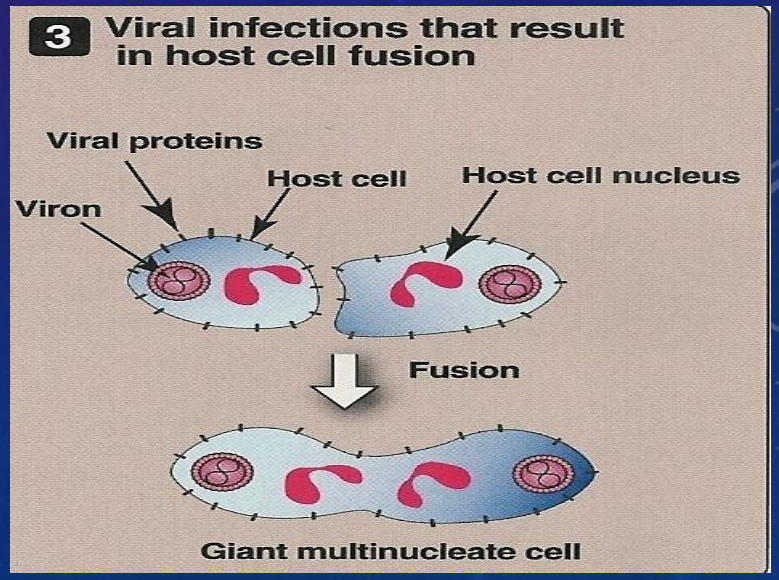
- Histological appearance
  Ex. Inclusion bodies



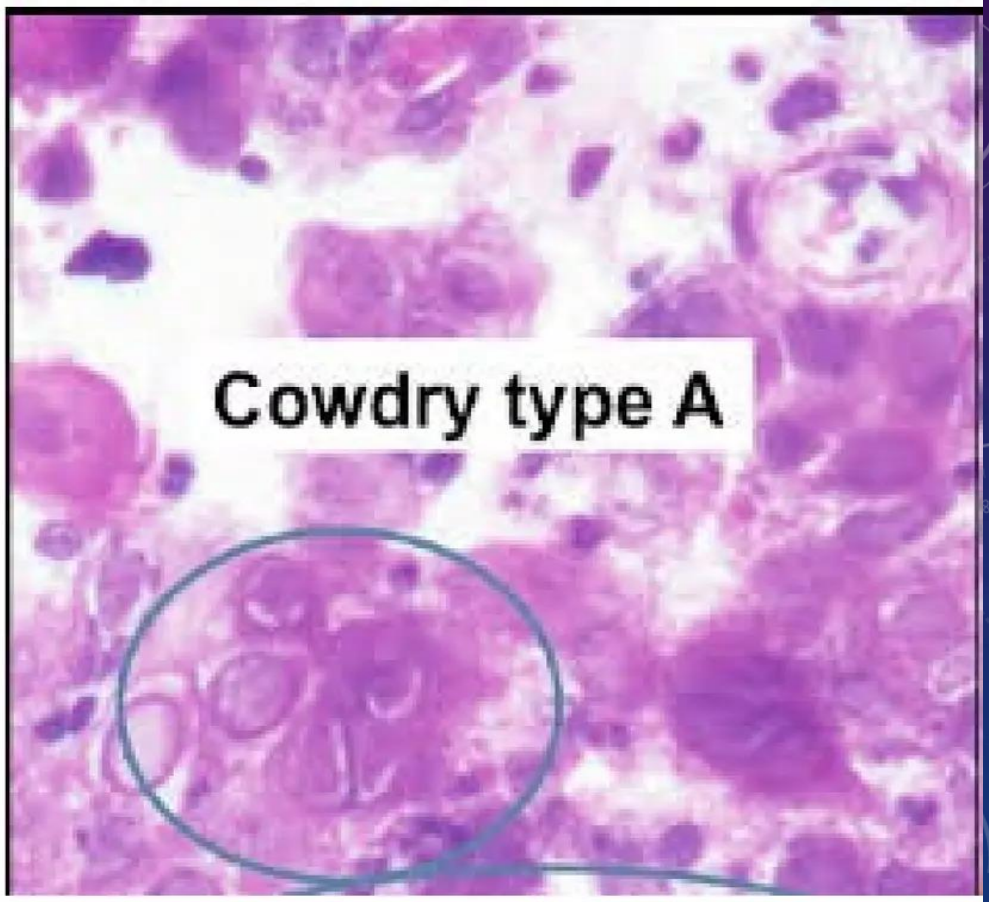
Owl's eye (CMV)



Uninfected cc | Cell rounding | **Syncytium**



- Intranuclear:
- Cowdry Type A:
- Variable in size, granular in appearance
- Seen in Herpes, Yellow fever



Microscopic examination

Electron microscopy

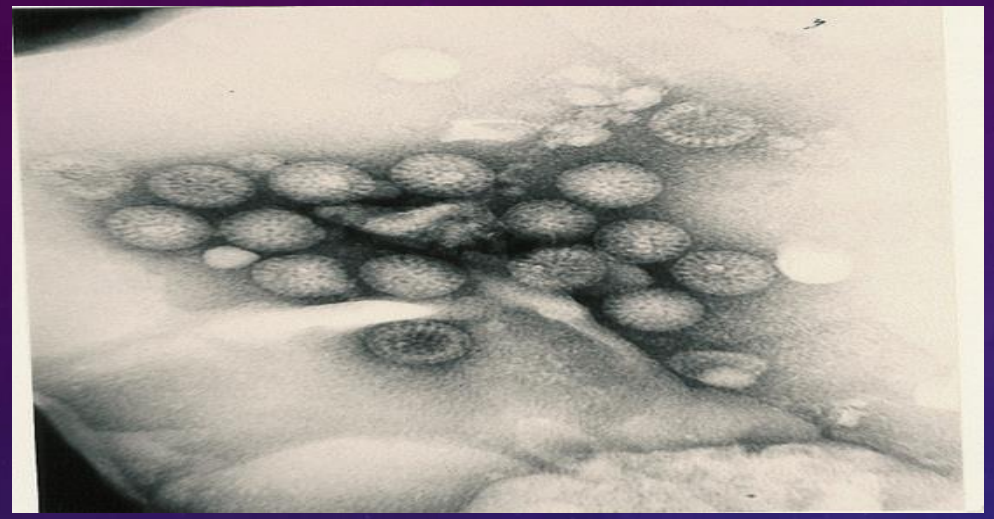
- Morphology& size of virions

Ex. Diagnosis of viruses such as rota, adenoviruses.

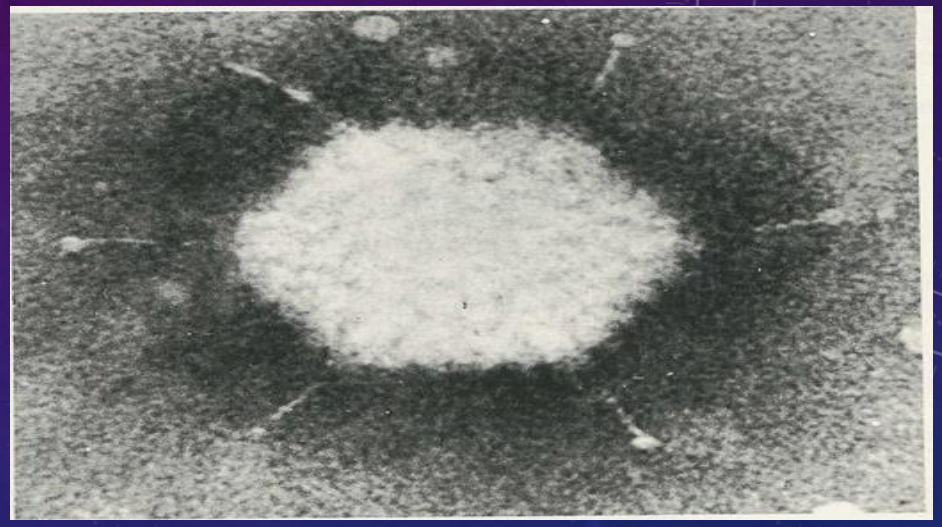
- Diagnosis of skin lesion caused by herpes, or poxviruses.

Electron micrographs

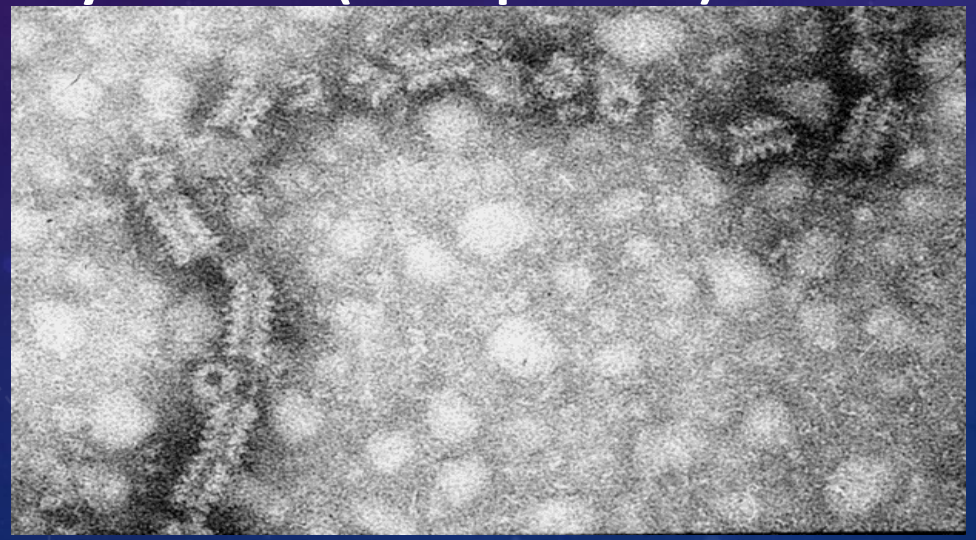
Rotavirus



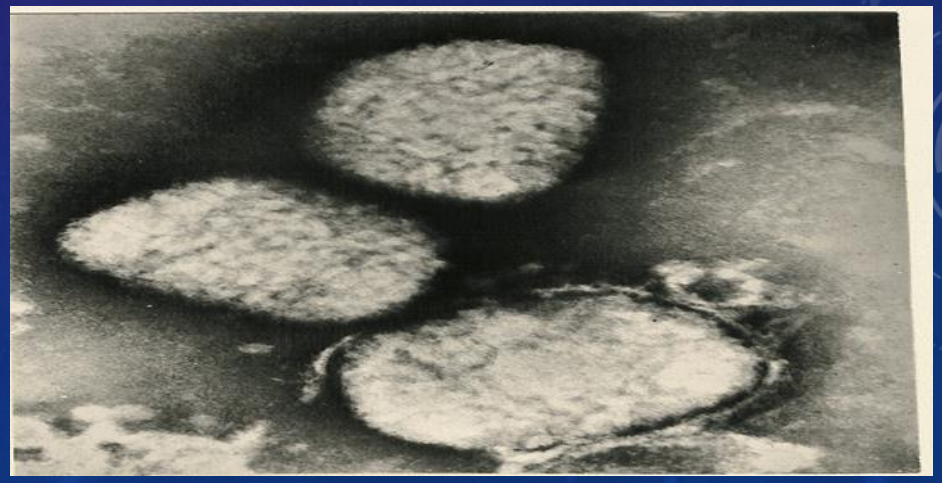
Adenovirus



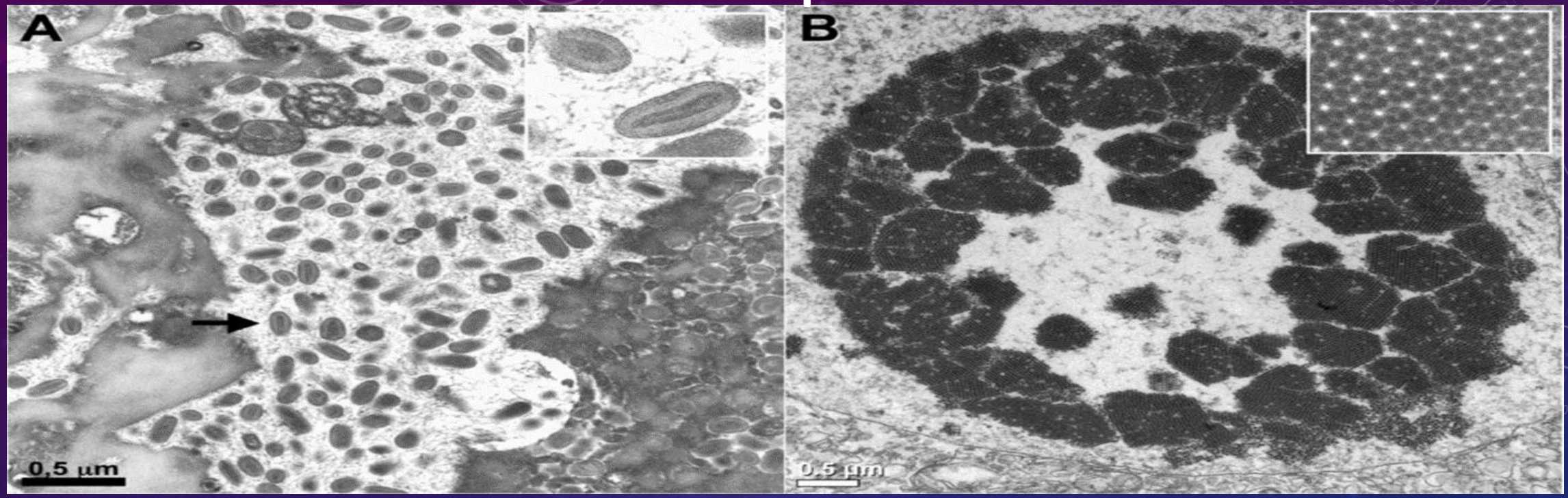
Cylindrical (Mumps virus)



Poxvirus

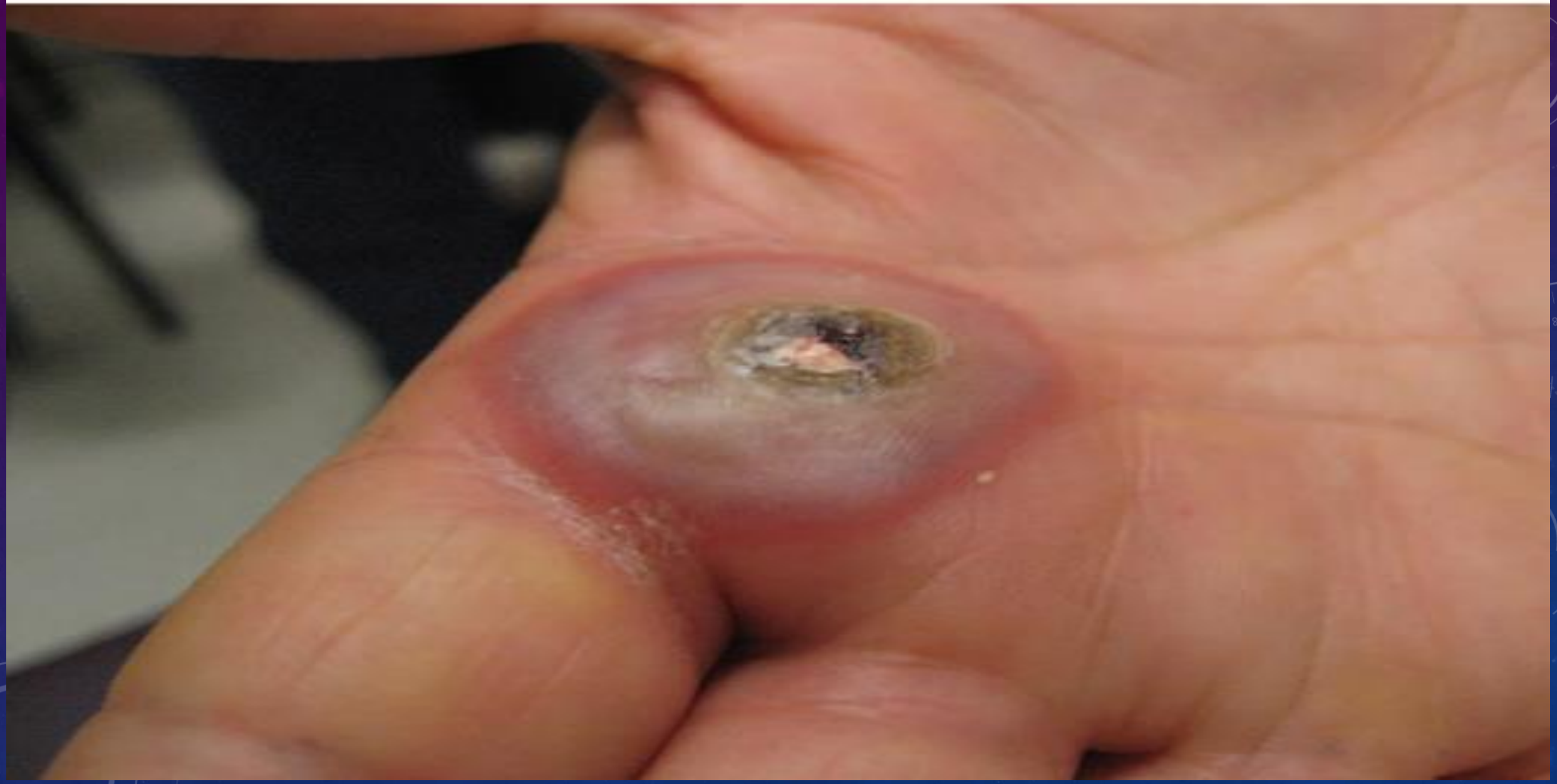


Microscopic examination



Diagnosis of virus infections by examination of ultrathin sections of human tissues or cells
(A) **Parapoxvirus (Orf virus)** infection on a human skin biopsy specimen. Multiple oval viral particles (arrow), comprising a dense core surrounded by an envelope (inset, high magnification), are observed in an infected cell. The Orf virus is a parapoxivirus that causes a common skin disease of sheep and goats, and is occasionally transmitted to human. (B) Polyomavirus (BK virus) infection in cells pelleted from a urine sample taken from an organ-transplant patient. The presence of a large number of viral particles leads to their arrangement into a crystal-like structure (inset, high magnification).

FIGURE. Bulla caused by orf virus infection after puncture by a bone of a recently slaughtered goat — Pennsylvania, 2009



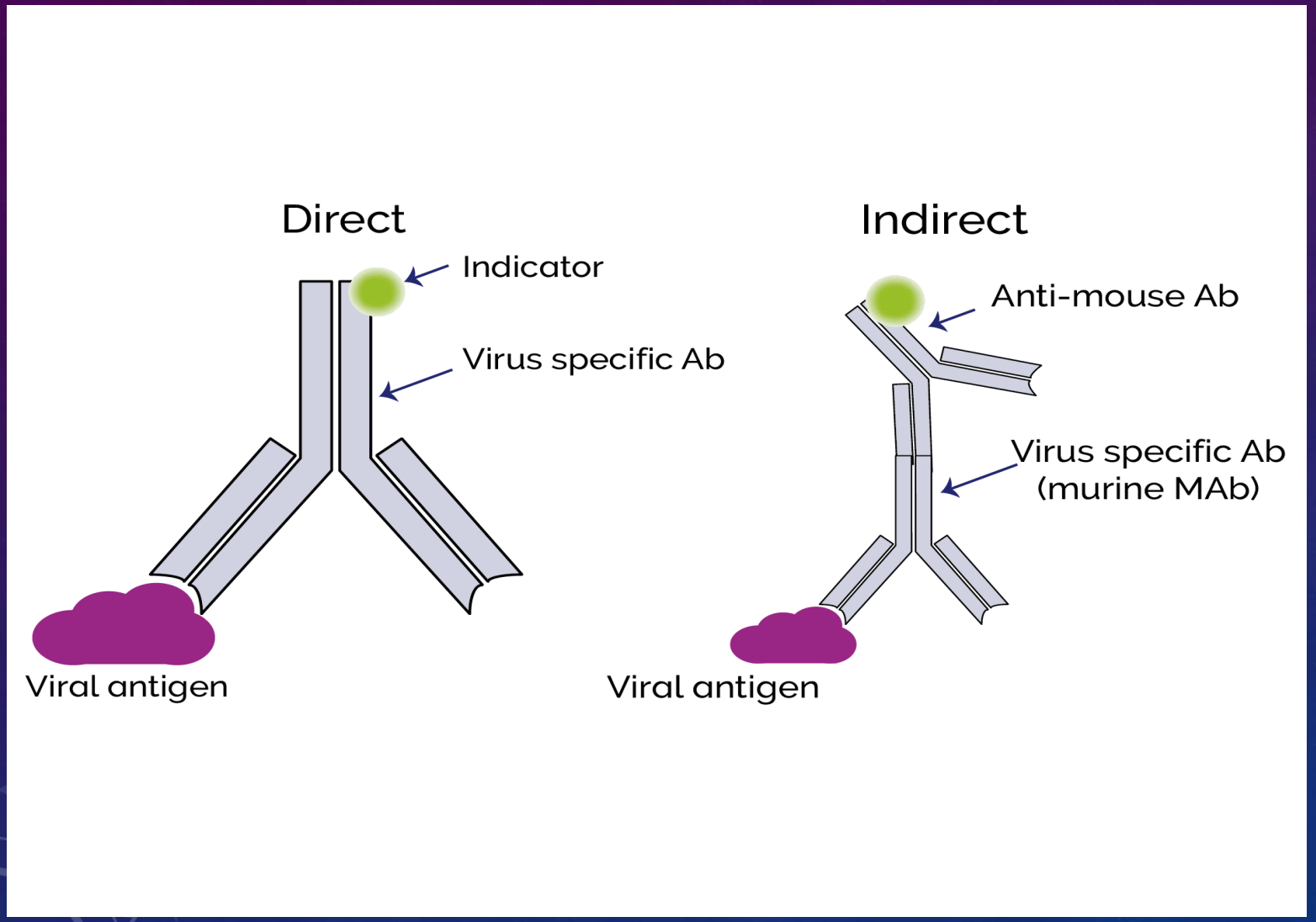
Microscopic examination

UV microscopy:

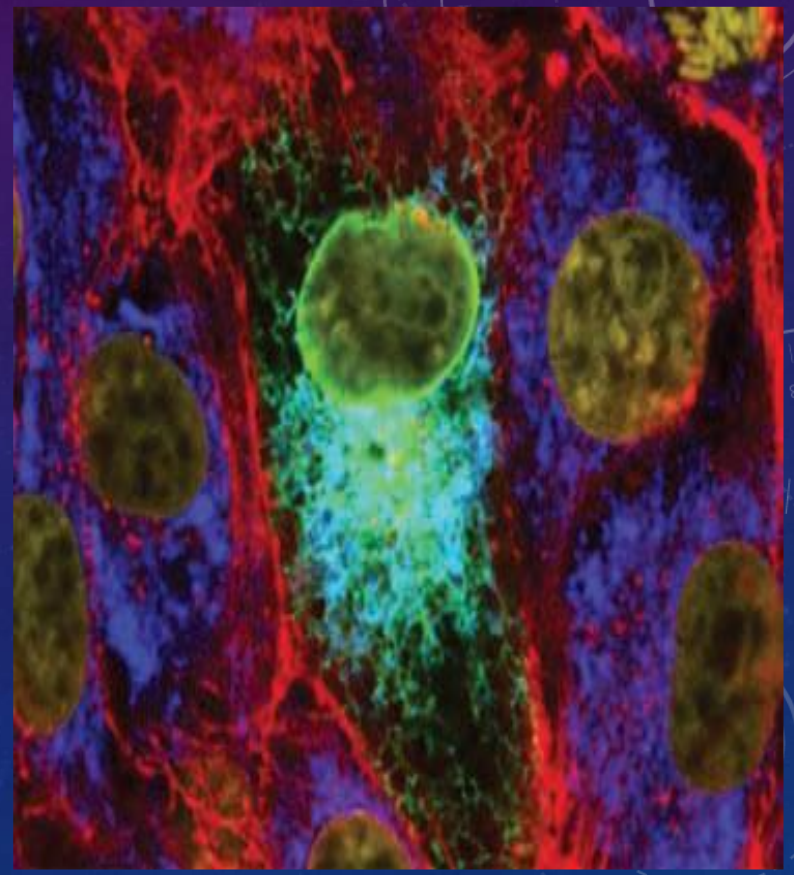
Used for fluorescent antibody staining of the virus in infected cells.

Immunostaining: Antibodies can be used to visualize viral proteins in infected cells or tissues. In direct immunostaining , an antibody that recognizes a viral protein is coupled directly to an indicator such as a fluorescent dye or an enzyme. A more sensitive approach is indirect immunostaining, in which a second antibody is coupled to the indicator. The second antibody recognizes a common region on the virus-specific antibody. Multiple second-antibody molecules bind to the first antibody, resulting in an increased signal from the indicator compared with that obtained with direct immunostaining.

Microscopic examination



Mumps virus



Microscopic examination

Electron microscopy

Problems with Electron Microscopy

- Expensive equipment
- Expensive maintenance
- Require experienced observer

Virus cultivation

The growth of viruses requires cell cultures because viruses replicate only in living cells, not on cell-free media the way most bacteria can. Because many viruses are inactivated at room temperature, it is important to inoculate the specimen into the cell culture as soon as possible; brief transport or storage at 4°C is acceptable.

1- Laboratory animals
2- Embryonated eggs
3- Cell culture

Virus cultivation

1- Laboratory animals

The growth of virus in inoculated animals is indicated by

1- death
2- disease
3-visible lesions

2- Embryonated eggs

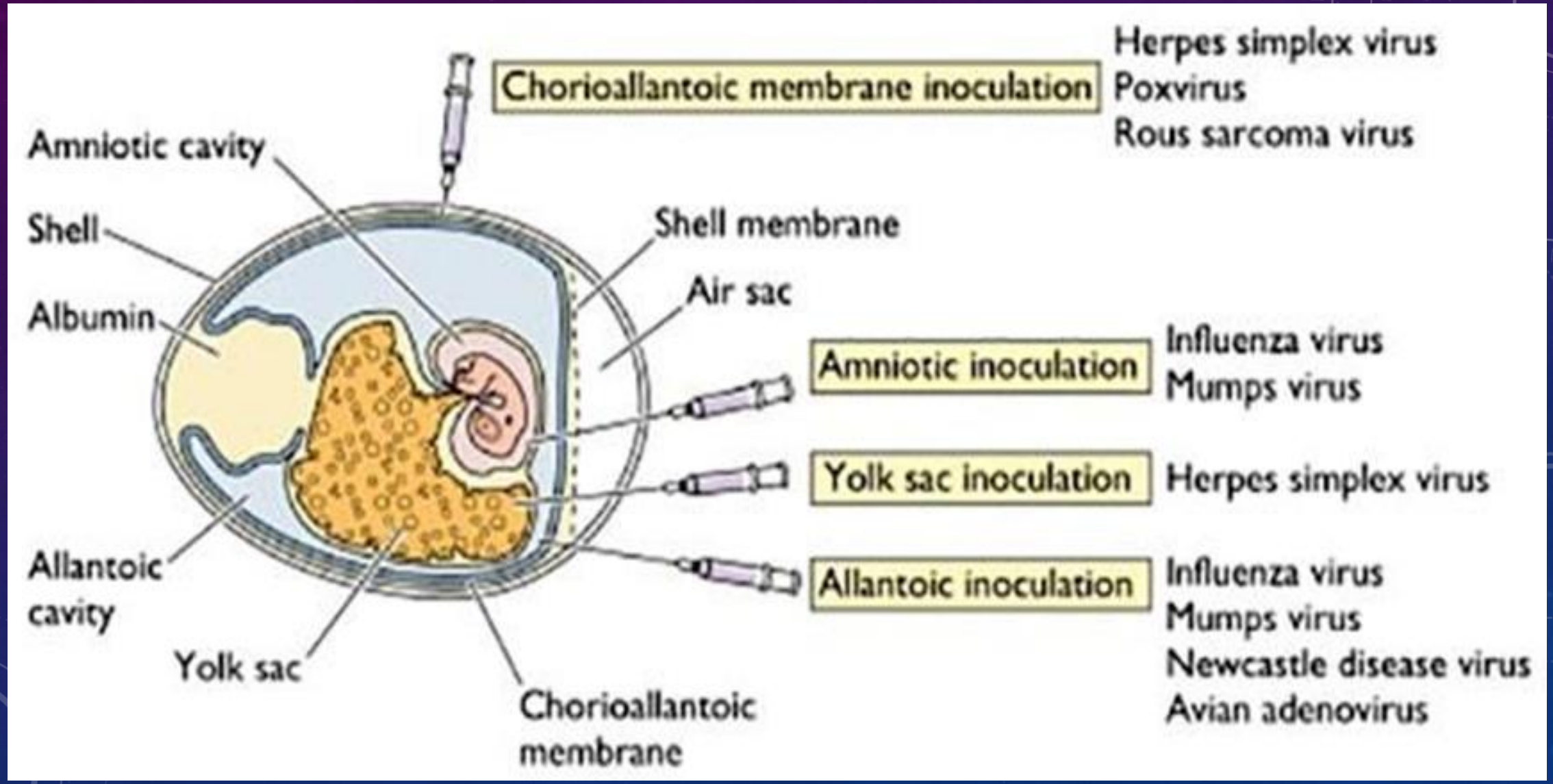
Many viruses were propagated in embryonated chicken eggs. At 5 to 14 day after fertilization, a hole is drilled in the shell and virus is injected into the site appropriate for its replication. This method of virus propagation is now routine only for influenza virus. The robust yield of this virus from chicken eggs has led to their widespread use in research laboratories and for vaccine production.

Embryonated eggs

Signs of viral infection in embryonated egg:

a- Death of embryo as in herpes, mumps.
b- Demonstration of haemagglutination as in influenza, mumps viruses.
c- Demonstration of local lesion called pocks as in vaccinia and small pox.
d- Finding of virus particles in embryo materials by using microscope.

Embryonated eggs



Cell culture: Cell cultures are cells from man or animal origin and grow into a monolayer on the sides of glass or plastic test tubes. Cells are kept moist and supplied with nutrient by keeping them continuously immersed in cell culture medium.

Growth medium is used for cell culture which contain– Minimum Essential Medium (MEM): essential amino acids, vitamins, salts, glucose & sodium bicarbonate, antibiotics & phenol red indicator. Usual antimicrobials added are vancomycin (10 µg/ml), gentamicin (20 µg/ml) and amphotericin (2.5 µg/ml). To counteract the pH decrease, a bicarbonate buffering system is used in the culture medium to keep the cells at physiologic pH(pH 7.2). Phenol red a pH indicator that is red at physiologic, yellow at acidic and purple at alkaline once inoculated with specimen.

Cell Cultures

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

1. **Primary cells:** Are prepared from animal tissues. They have a limited life span, usually no more than 5 to 20 cell divisions. Commonly primary cell cultures are derived from monkey kidneys, human embryonic kidneys, human foreskins and respiratory epithelium. They are used in vaccine production: for example, live attenuated poliovirus vaccine strains may be propagated in primary monkey kidney cells.

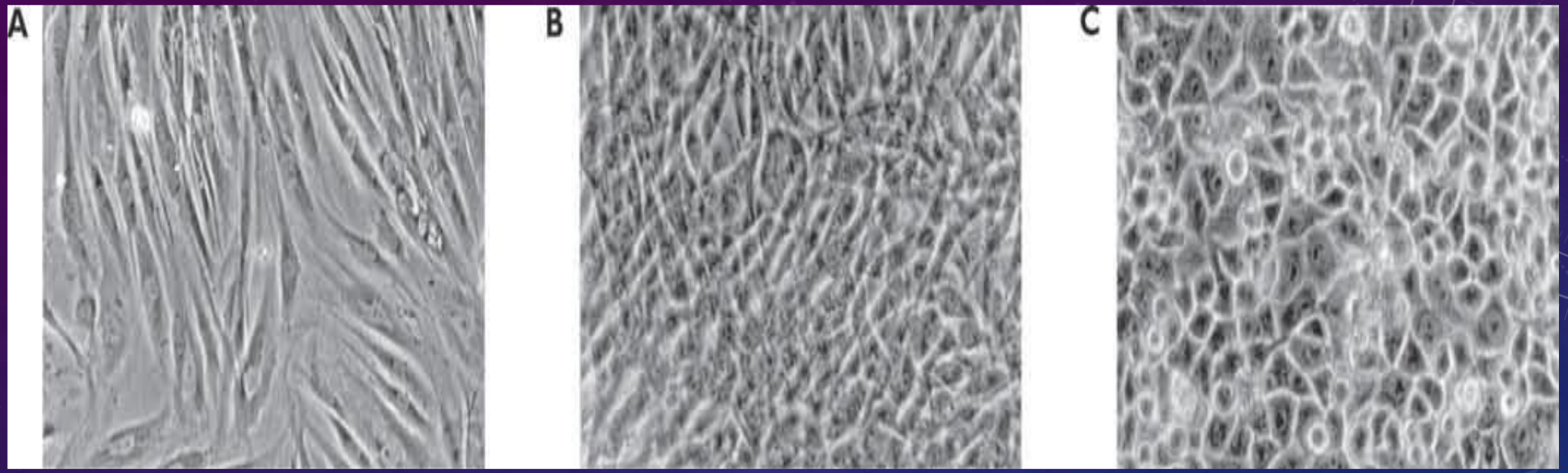
Primary cell cultures were mandated for the growth of viruses to be used as human vaccines to avoid contamination of the product with potentially oncogenic DNA from continuous cell lines.

Cell Cultures

2. Semi-continuous cells: Human embryonic kidney and skin fibroblasts which consist of a homogeneous population of a single type and can divide up to 100 times before dying. The most widely used diploid cells are those established from human embryos, such as the WI-38 strain derived from human embryonic lung.

3. Continuous cells: consist of a single cell type that can be propagated indefinitely in culture. The immortal lines are usually derived from tumor tissue or by treating a primary cell culture or a diploid strain with a mutagenic chemical or a tumor virus. Such cell lines often do not resemble the cell of origin; they are less differentiated (having lost the morphology and biochemical features that they possessed in the organ), and can be tumorigenic (i.e., they produce tumors when inoculated into immunodeficient mice). Examples of commonly used continuous cell lines include those derived from human carcinomas (e.g., HeLa [Henrietta Lacks] cells) , Vero, Hep2, LLC-MK2, MDCK.

Cell Cultures



Different types of cell culture used in virology. Confluent cell monolayers photographed by low-power light microscopy. (A) Primary human foreskin fibroblasts; (B) established line of mouse fibroblasts (3T3);
(C) continuous line of human epithelial cells (HeLa [Box 2.3]). The ability of transformed HeLa cells to overgrow one another is the result of a loss of contact inhibition. Courtesy of R. Gonzalez, Princeton University.

Cell Cultures

PROBLEMS WITH CELL CULTURE

- Long period (up to 4 weeks) required for result.
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.

Cell Cultures

Virus growth in cell culture can be detected by several techniques that can provide **a presumptive identification** :

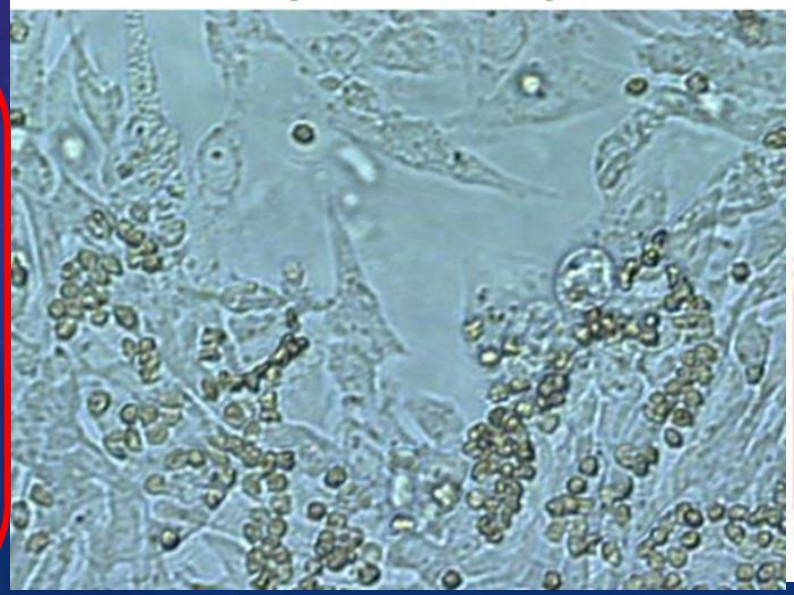
1. **Cytopathic effect (CPE)**
2. **Hemadsorption:** To detect the presence of certain viruses, the hemadsorption test is commonly used. **Influenza and parainfluenza viruses** express a viral hemagglutinin on the surface of infected cells. By the hemadsorption test, the culture medium is removed and replaced with a 0.5% dilute solution of guinea-pig red blood cells.

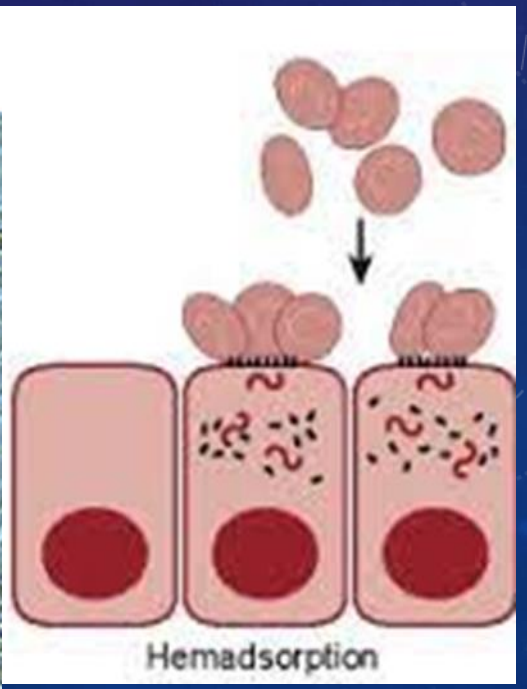
Hemadsorption

Patient serum with suspected Influenza infection + Cultured cells + Red Blood Cells

= aggregation = Positive infection

Microscopic view of hemadsorption





Cell Cultures

3. Interference with the formation of a CPE by a second virus. For example, rubella virus, which does not cause a CPE, can be detected by interference with the formation of a CPE by certain enteroviruses, such as echovirus or Coxsackie virus.

4. A decrease in acid production by infected, dying cells. This can be detected visually by a color change in the phenol red (a pH indicator) in the culture medium. The indicator remains red (alkaline) in the presence of virus-infected cells but turns yellow in the presence of metabolizing normal cells as a result of the acid produced. This technique can be used to detect certain enteroviruses.

Serological tests

A definitive identification of the virus grown in cell culture is made by using known antibody in one of several tests:

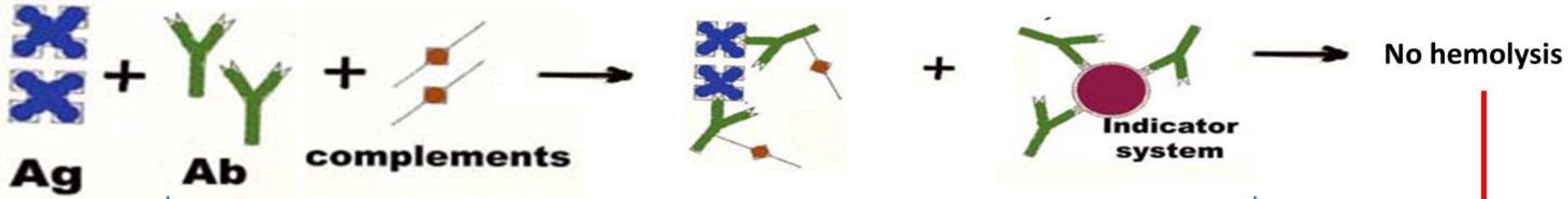
Complement fixation tests (CFT)

If the antigen (the unknown virus in the culture fluid) and the known antibody are homologous, complement will be fixed (bound) to the antigen–antibody complex. This makes it unavailable to lyse the “indicator” system, which is composed of sensitized red blood cells.

Complement fixation test

Procedure

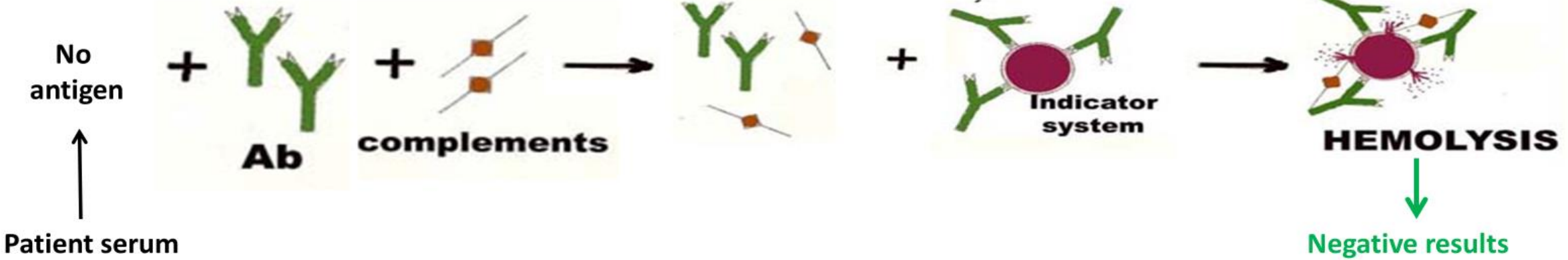
Looking for antigens



Ab , complement, and sRBCs are externally added

Patient serum

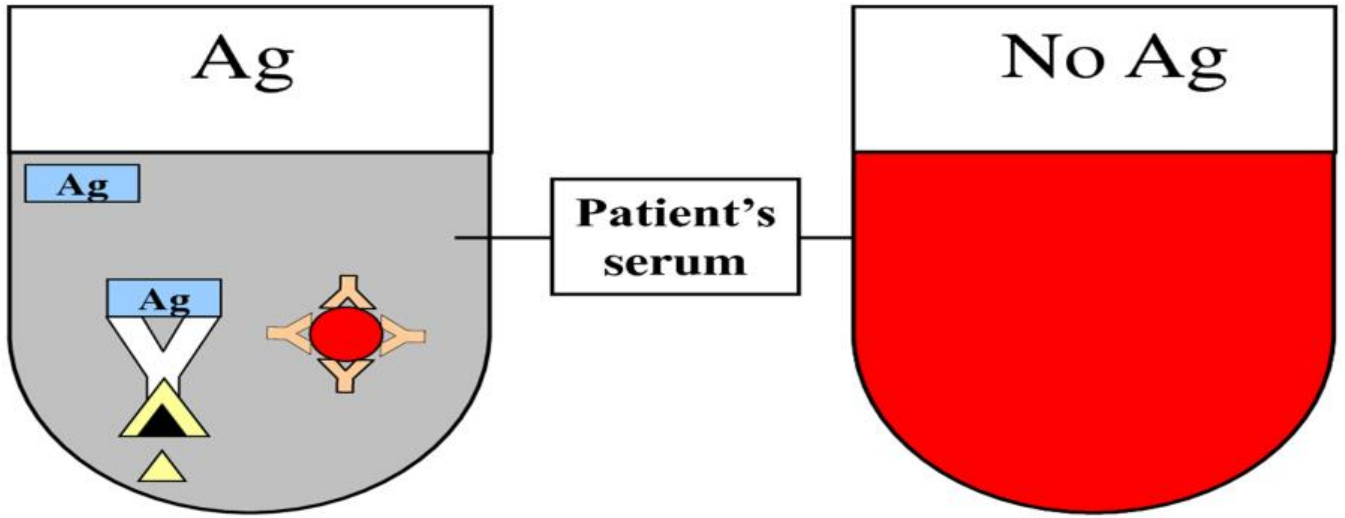
Positive results

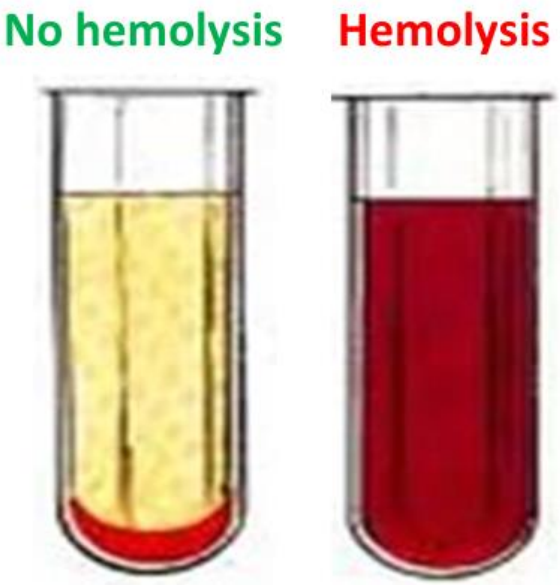


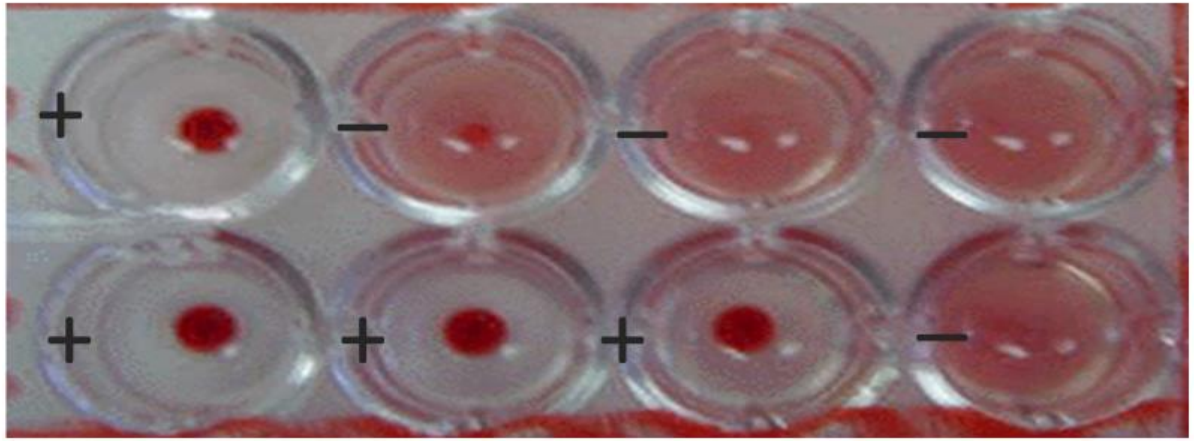
Patient serum

Negative results

Complement fixation test



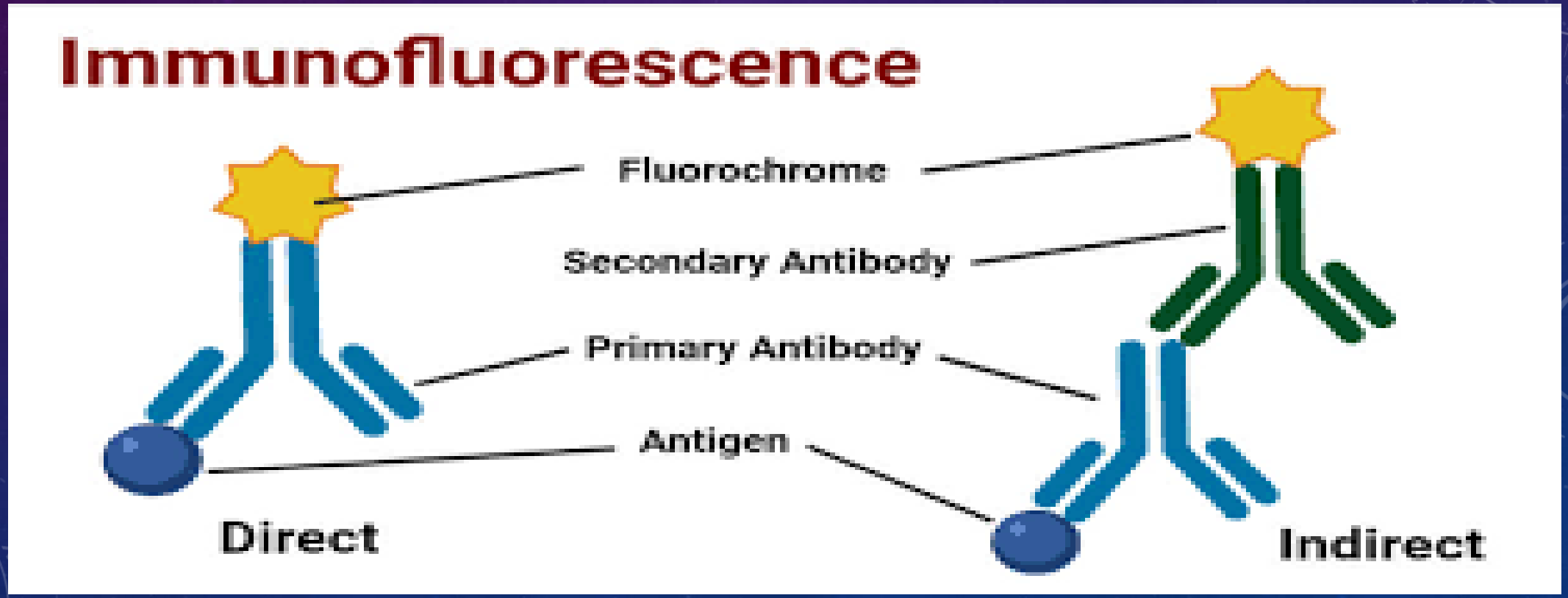




Serological tests

Fluorescent Antibody Assay

If the virus-infected cells and the **fluorescein-tagged antibody** are homologous, the typical apple-green color of fluorescein is seen in the cells by **ultraviolet (UV) microscopy.**



Serological tests

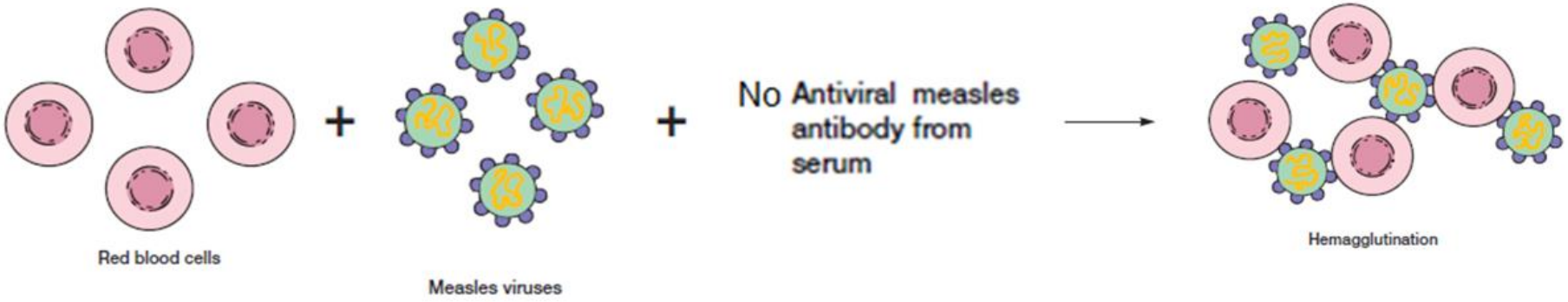
Neutralization tests

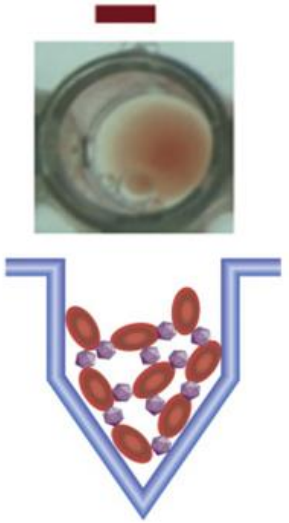
If the virus and antibody are homologous, the antibody bound to the surface of the virus blocks its entry into the cell. This neutralizes viral infectivity because it prevents viral replication and subsequent CPE formation or animal infection.

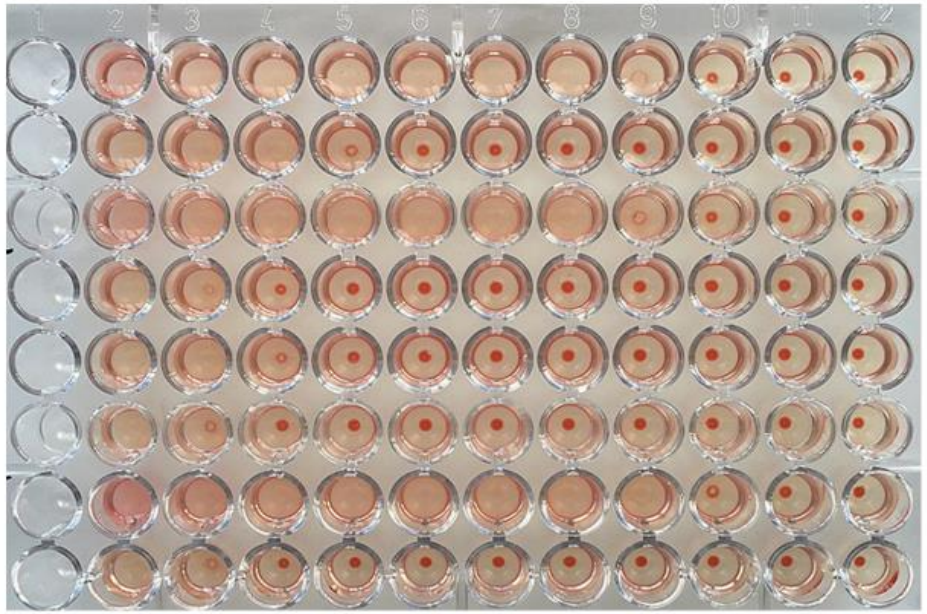
Hemagglutination Inhibition

If the virus and antibody are homologous, the virus is blocked from attaching to the erythrocytes and no hemagglutination occurs. Only viruses that agglutinate red blood cells can be identified by this method.

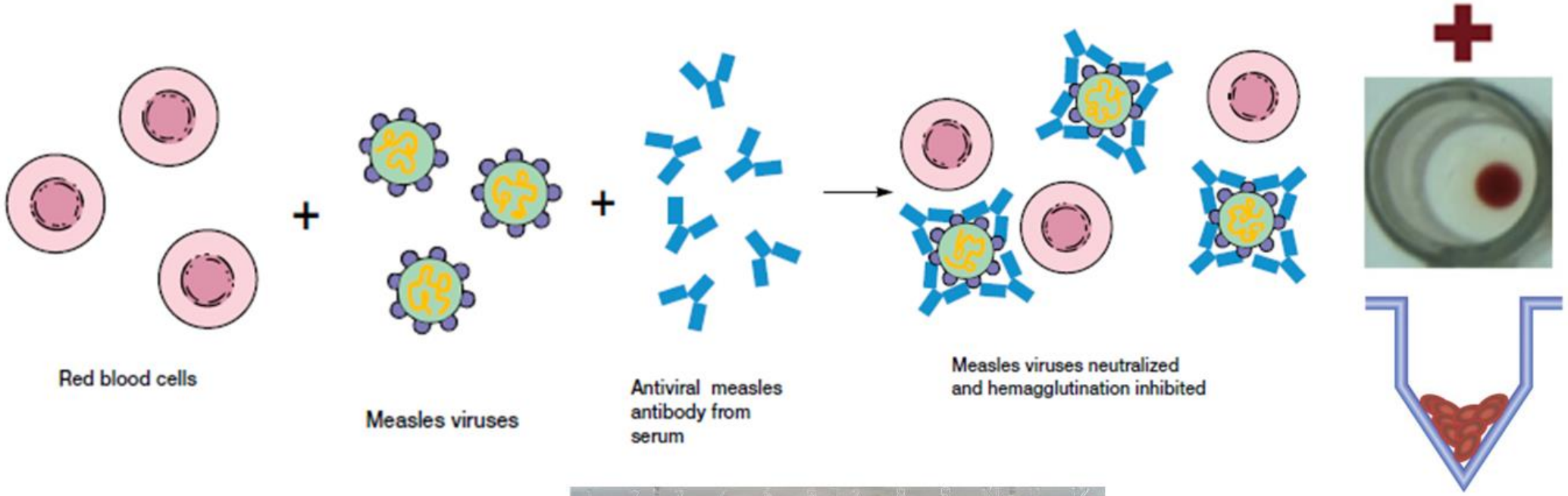
Haemagglutination inhibition test

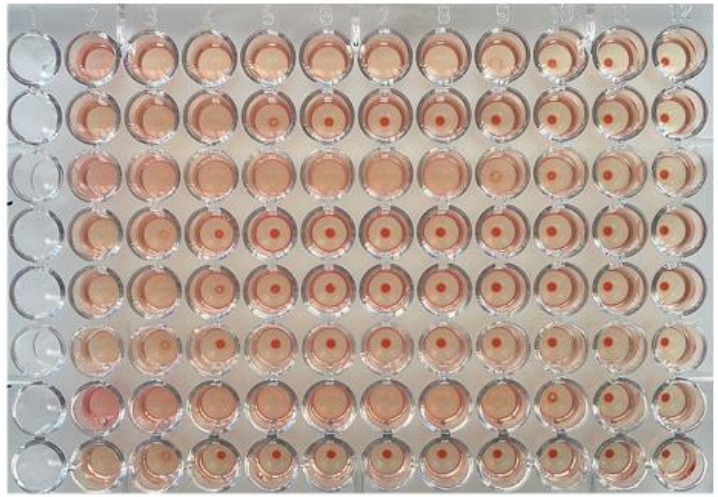






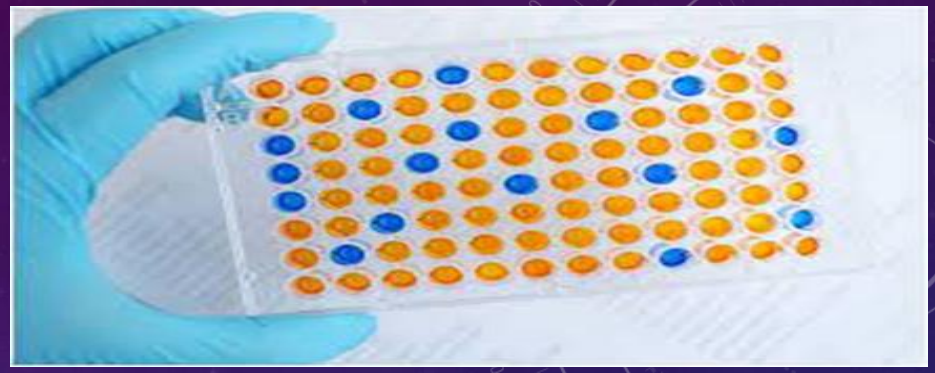
Haemagglutination inhibition test

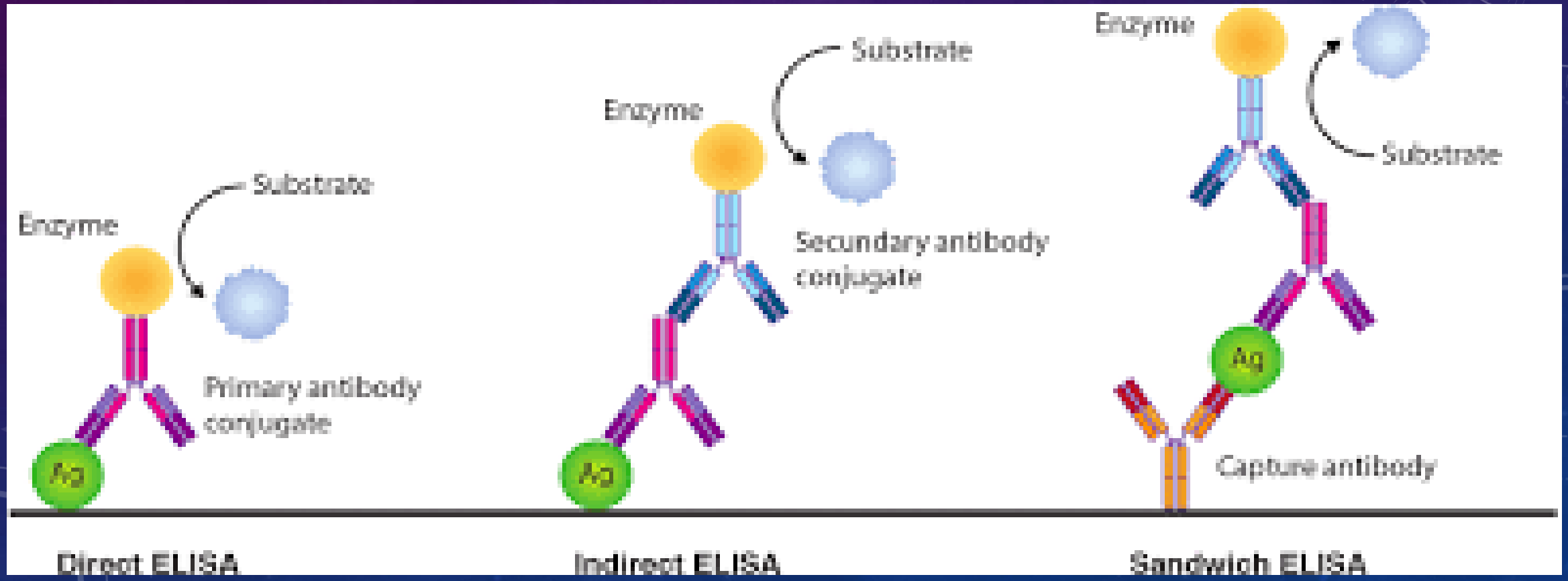




Serological tests

Enzyme Linked Immunosorbent Assay (ELISA).





Serological tests

Problems with Serology:

1- Long period of time required for diagnosis for paired acute and convalescent sera.

2- Mild local infections such may not produce a detectable Abs.

3- Immunocompromised patients often give a reduced or absent Abs.

4- Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result.

Detection of Viral Antigens

- Various formats are available for detection of viral antigens in serum and other samples
- Some important antigen detection tests include:
  - HBsAg and HBeAg antigen detection for hepatitis B infection from serum.
  - NS1 antigen detection for dengue virus infection from serum
  - p24 antigen detection for HIV from serum
  - Rotavirus antigen detection from diarrheic stool
  - CMV specific pp65 antigen detection (serum)

Molecular method .

DETECTION OF VIRAL NUCLEIC ACIDS

Viral nucleic acids (i.e., either the viral genome or viral mRNA) can be detected in the patient's blood or tissues with complementary DNA or RNA (cDNA or cRNA) as a probe. If only small amounts of viral nucleic acids are present in the patient, the polymerase chain reaction can be used to amplify the viral nucleic acids. Assays for the RNA of HIV and hepatitis C virus and the DNA of hepatitis B virus in the patient's blood (viral load) are commonly used to monitor the course of the disease and to evaluate the patient's prognosis.

Molecular method

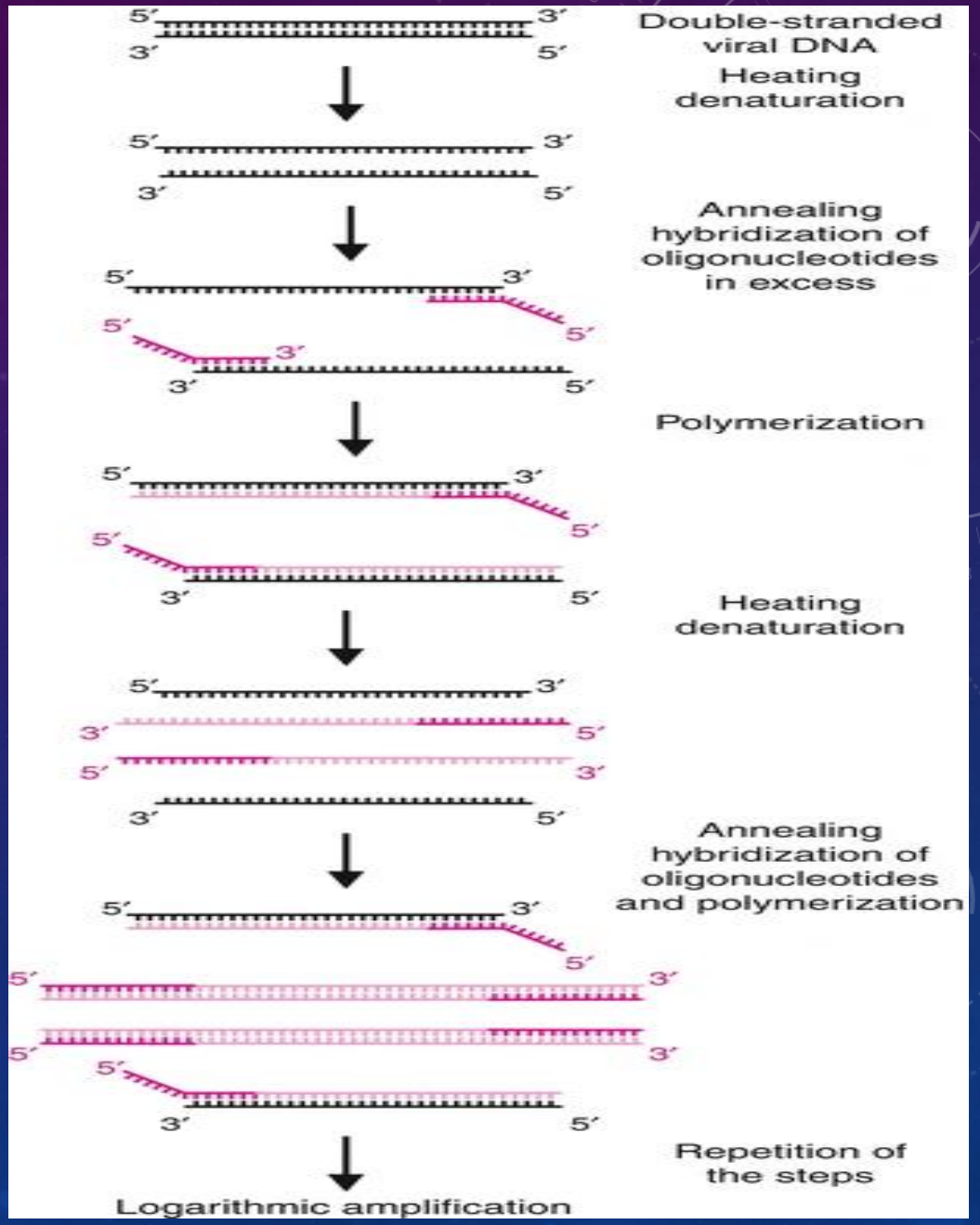
Nucleic acid-based molecular detection techniques have revolutionized diagnostic virology with their faster, highly sensitive, and highly specific diagnosis:

1- Polymerase Chain Reaction (PCR)
2- Reverse Transcription-PCR (RT-PCR)
3- Real-Time PCR (RT-qPCR)
4- Next-Generation Sequencing (NGS)
5- Western Blotting Analysis

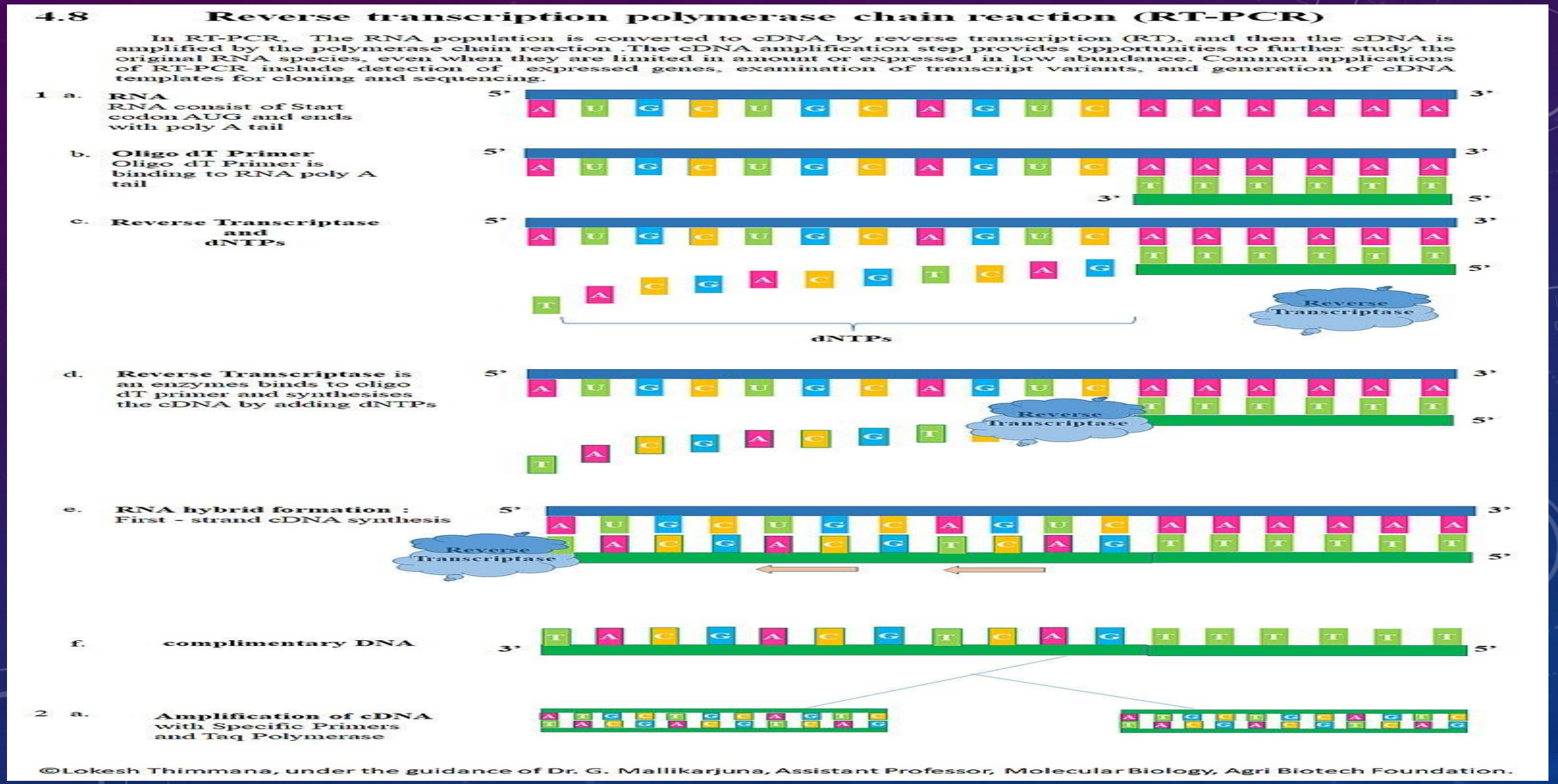
Molecular method

1- Polymerase Chain Reaction (PCR)

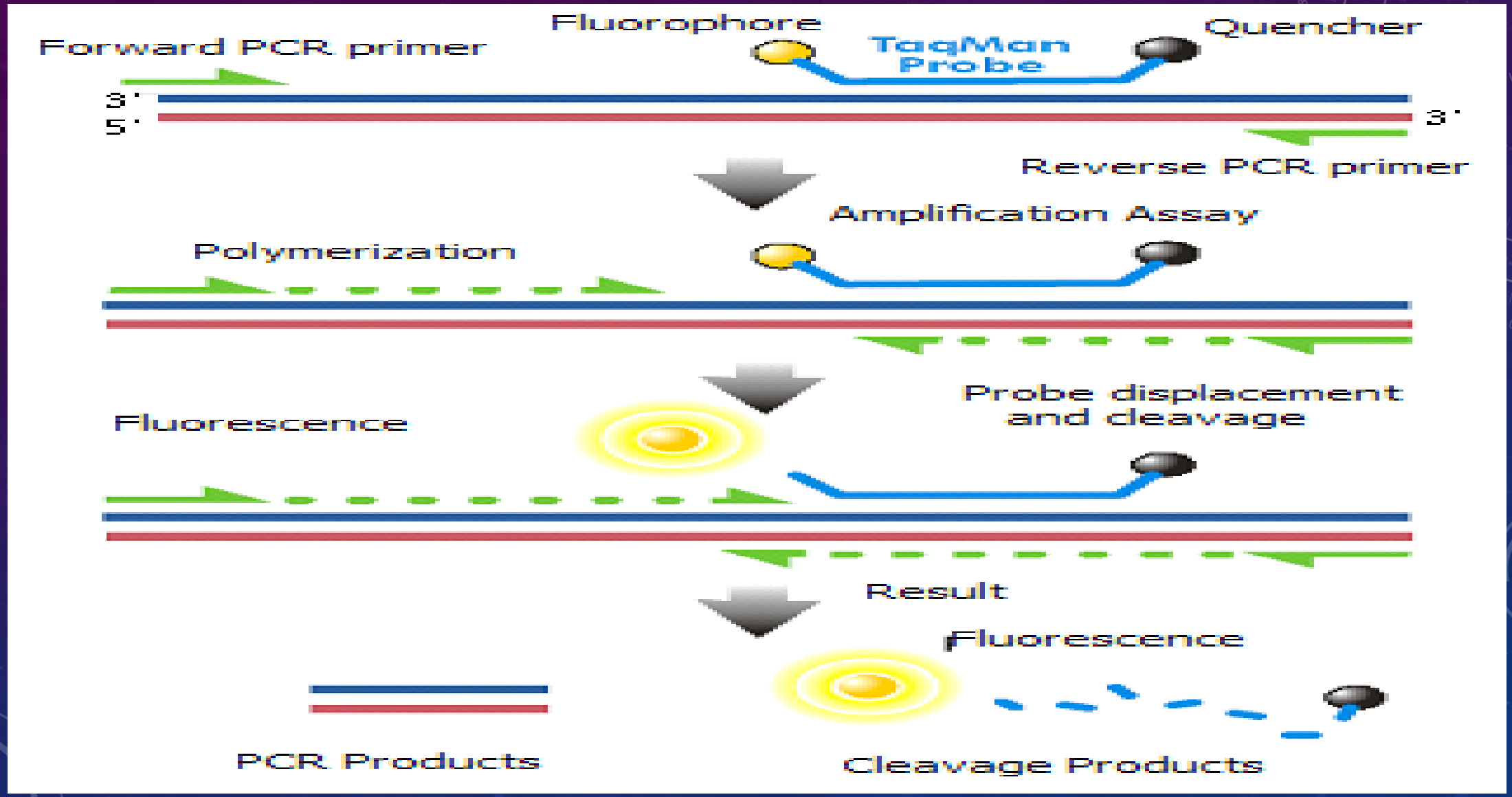
PCR is a typical example of nucleic acid amplification assay. PCR is based on extraction and purification of DNA molecule and exponential amplification of the target sequence, using a thermostable DNA polymerase and two specific oligonucleotide primers. After the PCR reaction, the amplified product can be detected by several techniques, including gel electrophoresis, colorimetric methods, and sequencing.



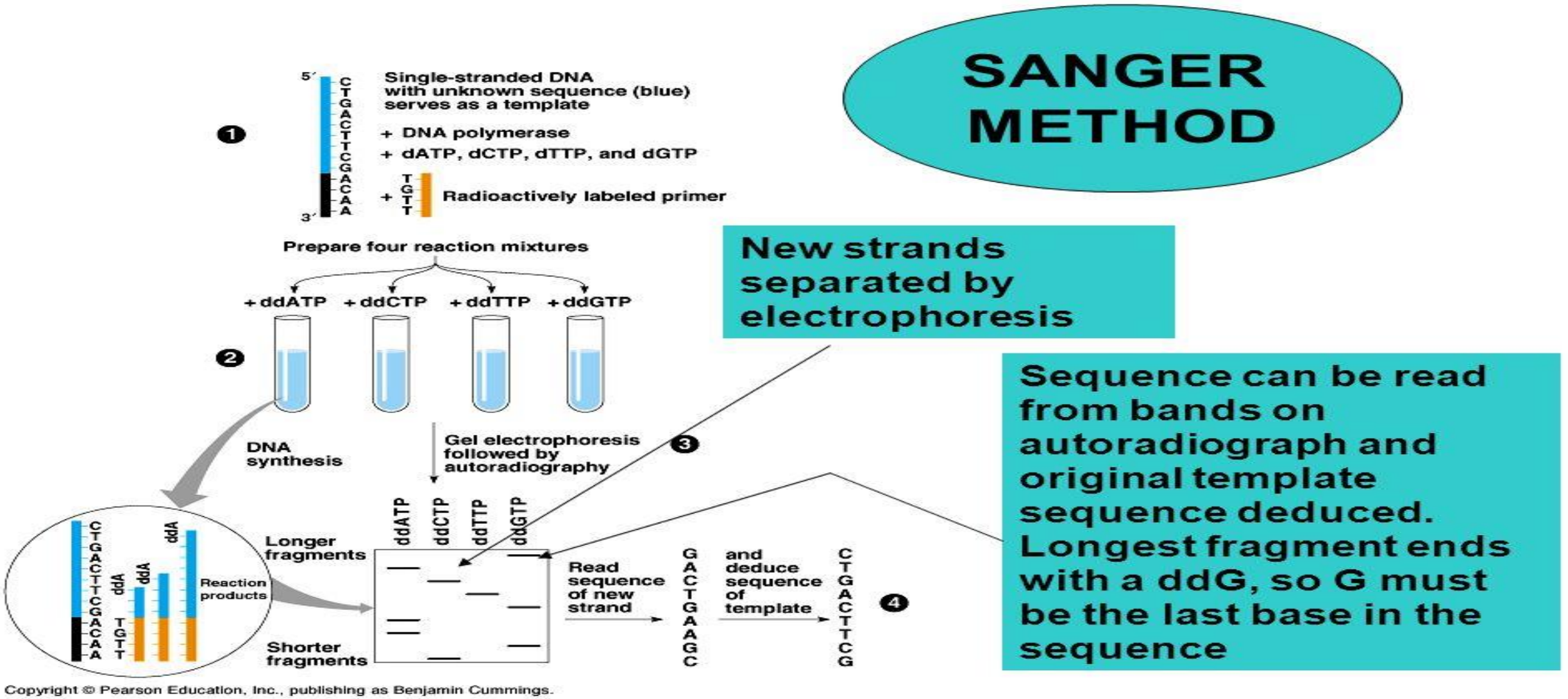
Reverse Transcription-PCR (RT-PCR)



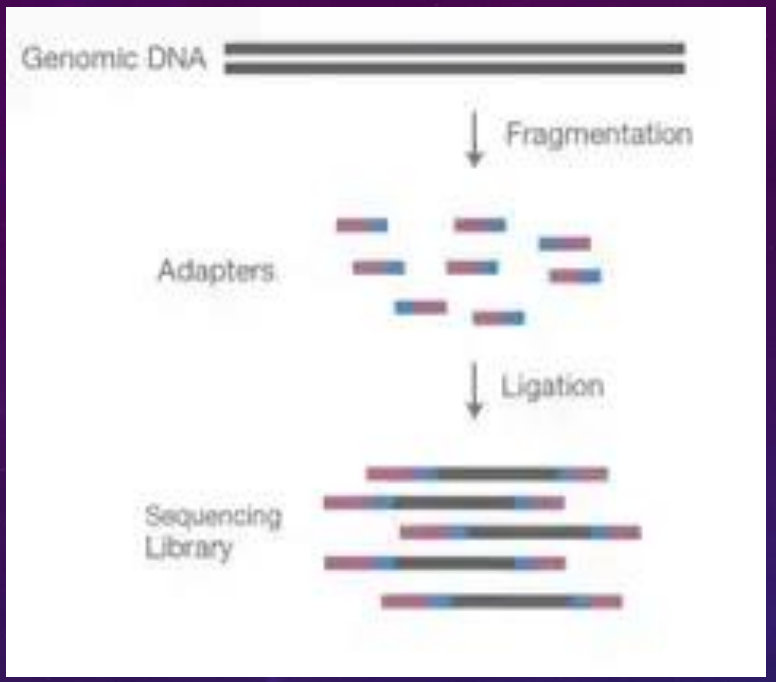
Real-Time PCR (RT-qPCR)

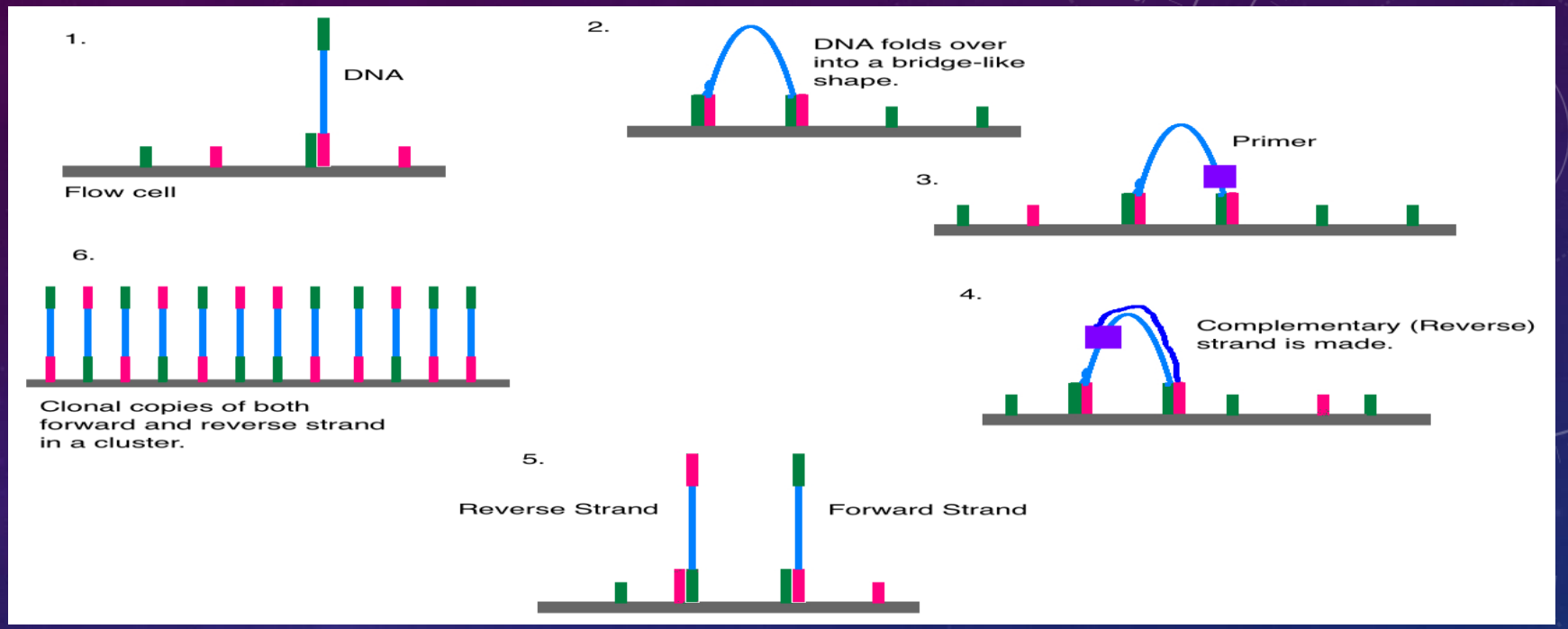


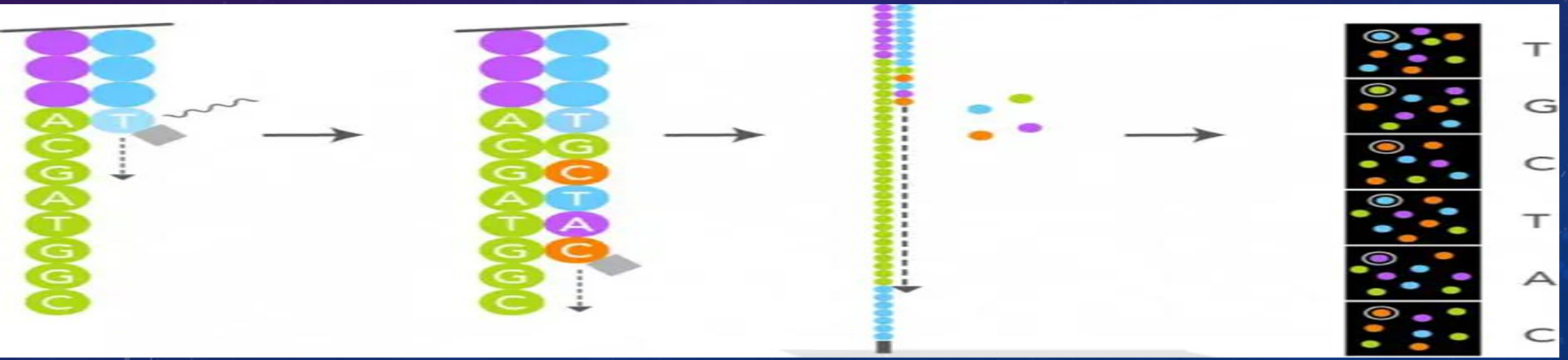
Sanger sequencing



Next-Generation Sequencing (NGS)







Molecular tests

Western Blotting Analysis:The first step is to separate the macromolecules in a sample using gel electrophoresis. Subsequently, are transferred or blotted onto a second matrix, generally a nitrocellulose or polyvinylidene difluoride (PVDF) membrane. Next, the membrane is blocked to prevent any nonspecific binding of antibodies to the surface of the membrane. The transferred protein is then probed with a combination of antibodies: one antibody specific to the protein of interest (primary antibody) and another antibody specific to the host species of the primary antibody (secondary antibody). Often the secondary antibody is complexed with an enzyme, which when combined with an appropriate substrate, will produce a detectable signal. Chromogenic substrates produce a precipitate on the membrane resulting in colorimetric changes visible to the eye.

Western Blotting and Detection