Laboratory Diagnosis of Viral Infections & Viral Genetics

Summer Term
Lecture no. 5

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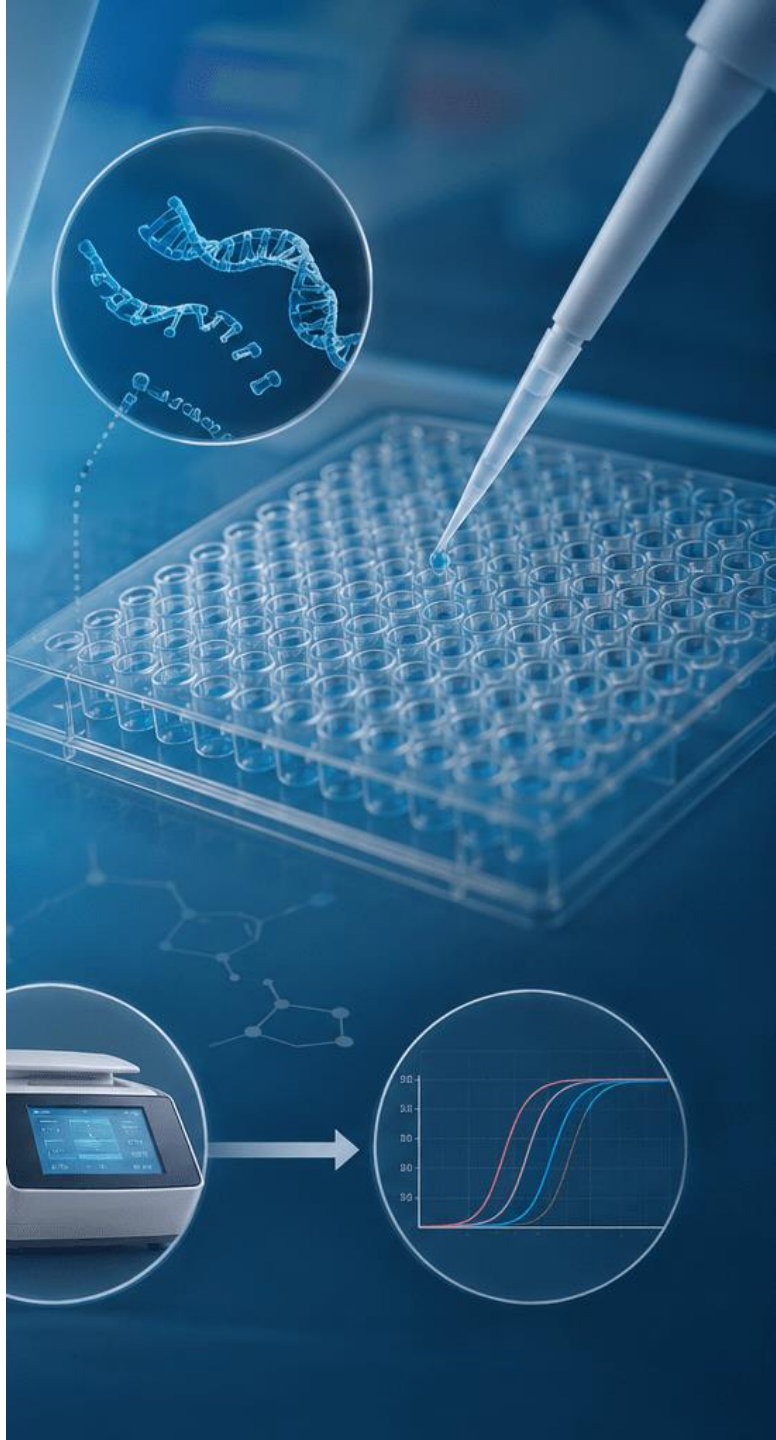
Learning Objectives:

1. Master pre-analytical variables in viral specimen collection, handling, and transport.

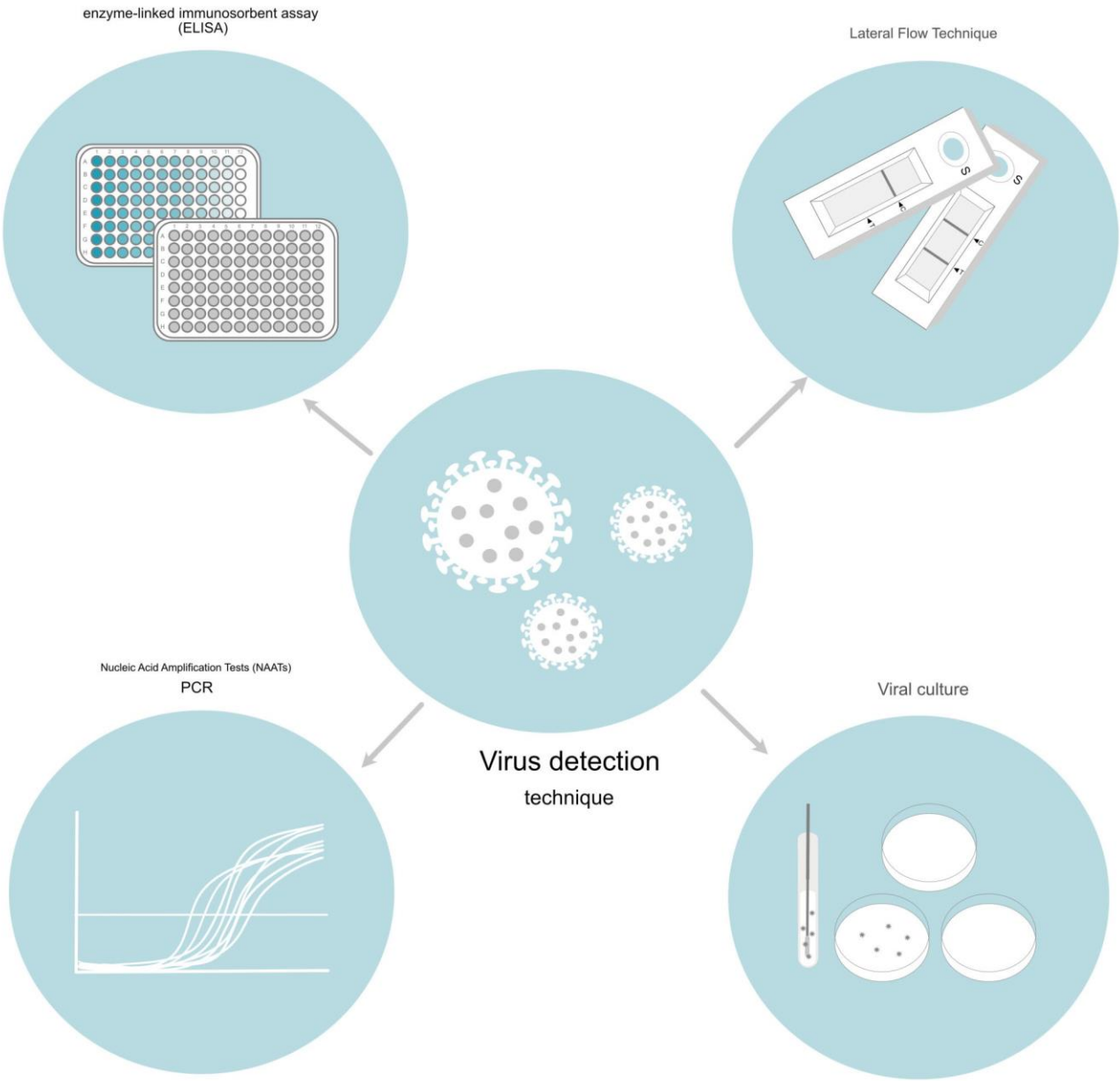
2. Differentiate **classical** diagnostic methods (culture, microscopy) from **modern** molecular assays.

3. Compare **performance** characteristics and utility of Nucleic Acid Amplification Test (NAATs), serology, and rapid diagnostic tests (RDTs).

4. Evaluate complex laboratory profiles to **accurately interpret** false positives, false negatives, and clinical relevance.



→ Overview of Diagnostic Modalities in Clinical Virology.



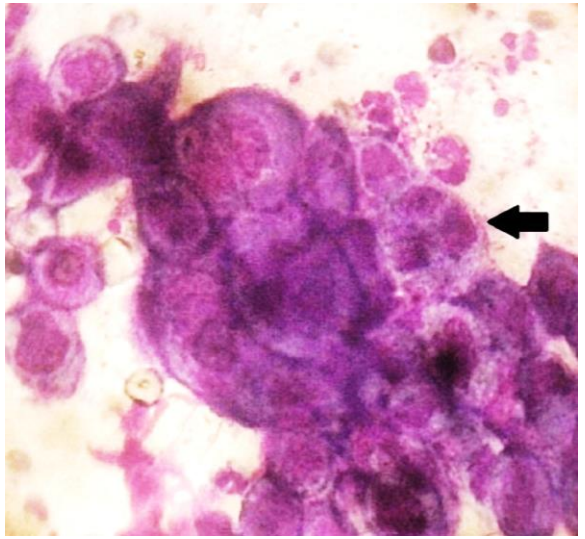
Pre-Analytic Considerations & Classical Diagnostic Modalities

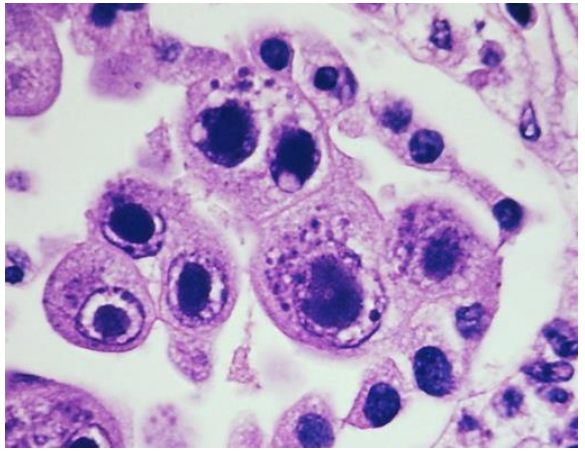
1. Specimen Collection, Handling & Transport_

- ❑ **<u>Timing of Collection</u>:** Peak viral shedding occurs early in acute illness (first 3–5 days). Late collection severely diminishes diagnostic yield.
- ❑ **<u>Swab & Transport Media Selection</u>:**
  - **Swabs:** Synthetic tips (Dacron/Polyester, Rayon, Flocked Nylon) on plastic or aluminum shafts. ***Avoid calcium alginate swabs or wooden shafts*** **(contain substances that inhibit PCR and coat viral particles).**
  - **Viral Transport Medium (VTM) or UTM (Universal Transport Medium):** Buffered salt solution containing proteins (BSA or gelatin) to stabilize viral capsids/envelopes and antibiotics/antifungals to inhibit bacterial and fungal overgrowth.
- ❑ **<u>Storage & Transport Conditions</u>:**
  - **Refrigeration (2–8°C):** Safe for short-term processing (up to 48–72 hours).
  - **Freezing (-70°C or lower):** Required for long-term storage. ***<u>Avoid</u>*** *standard -20°C freezers* due to ice crystal formation during defrost cycles, which damages enveloped viruses.

2. Microscopic Techniques

- **Light Microscopy & Cytopathology:**
  - Detection of **characteristic** Cytopathic Effects (CPE) in host tissue samples.
  - **Classic Examples:** Tzanck smear showing multinucleated giant cells and Cowdry A inclusion bodies in HSV/VZV; "Owl's eye" intranuclear inclusions in Cytomegalovirus (CMV).
- **Direct Immunofluorescence (DFA):**
  - Fluorescent-tagged antibodies directly bind viral antigens in patient cell scrapings (e.g., RSV or Influenza nasal washes).
- **Transmission Electron Microscopy (TEM):**
  - Direct visualization of viral morphology independent of cultural growth.
  - *Limitations:* Low sensitivity (requires >10_6 particles/mL), labor-intensive, requires expert operators. Used primarily in outbreak investigations for novel pathogen discovery.





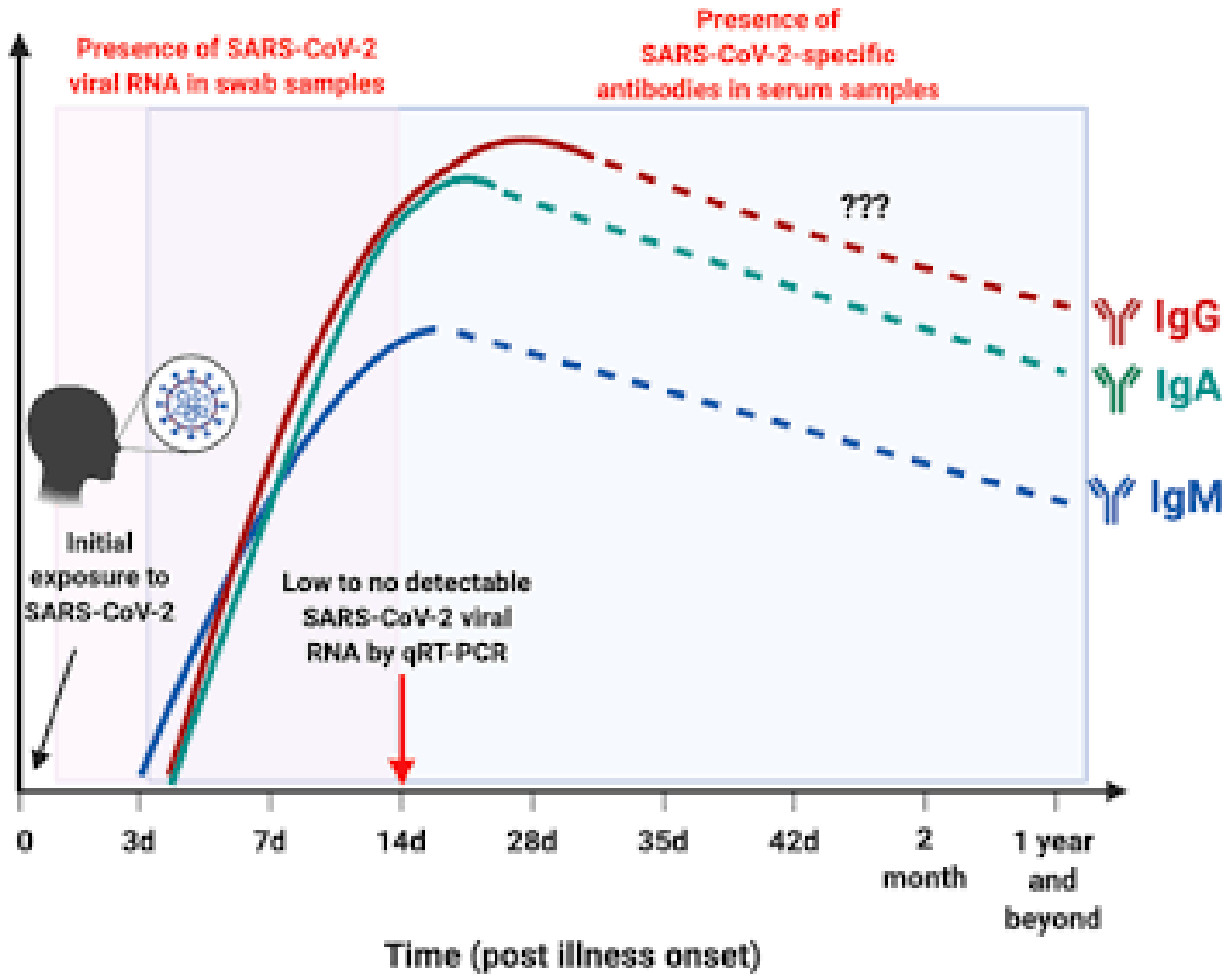
3. Viral Culture & Isolation

- **Cell Culture Systems:**
  - **Primary Cell Lines:** Derived directly from tissue (e.g., Rhesus monkey kidney); limited passages.
  - **Continuous Cell Lines:** Transformed/immortalized cells (e.g., HeLa, Vero, Hep-2); infinite passages.
- **Detection Methods:**
  - **Observation of CPE:** Cell lysis, syncytia, rounding, or vacuolation over days to weeks.
  - **Shell Vial Technique:** Centrifugation-enhanced culture where viral entry is accelerated onto a monolayer, followed by early antigen detection (24–48 hours) via immunofluorescence.

4. Antigen Detection & Rapid Diagnostic Tests (RDTs)

- **Antigen Assays (ELISA/EIA_ Enzyme Immunoassay):** Direct capture of viral proteins (e.g., HBV HBsAg, Rotavirus capsid, HIV p24).
- **Lateral Flow Immunoassays (LFIA):** Point-of-Care (POC) formats.
  - **Mechanisms:** Capillary action drives sample across membrane conjugated with gold nanoparticles or fluorescent beads.
  - **Trade-offs:** High specificity, low-to-moderate sensitivity relative to PCR. Critical for rapid triage during respiratory or gastrointestinal outbreaks.

→ The HIV p24 antigen is a **viral capsid protein** that appears in the blood during early HIV infection, making it a key marker for detecting the virus before antibodies develop.



Diagnostic Timeline: Nucleic Acid *vs.* Serological Markers.

Molecular Diagnostics, Serology & Clinical Result Interpretation

1. Molecular Diagnostics: PCR, RT-PCR & Sequencing

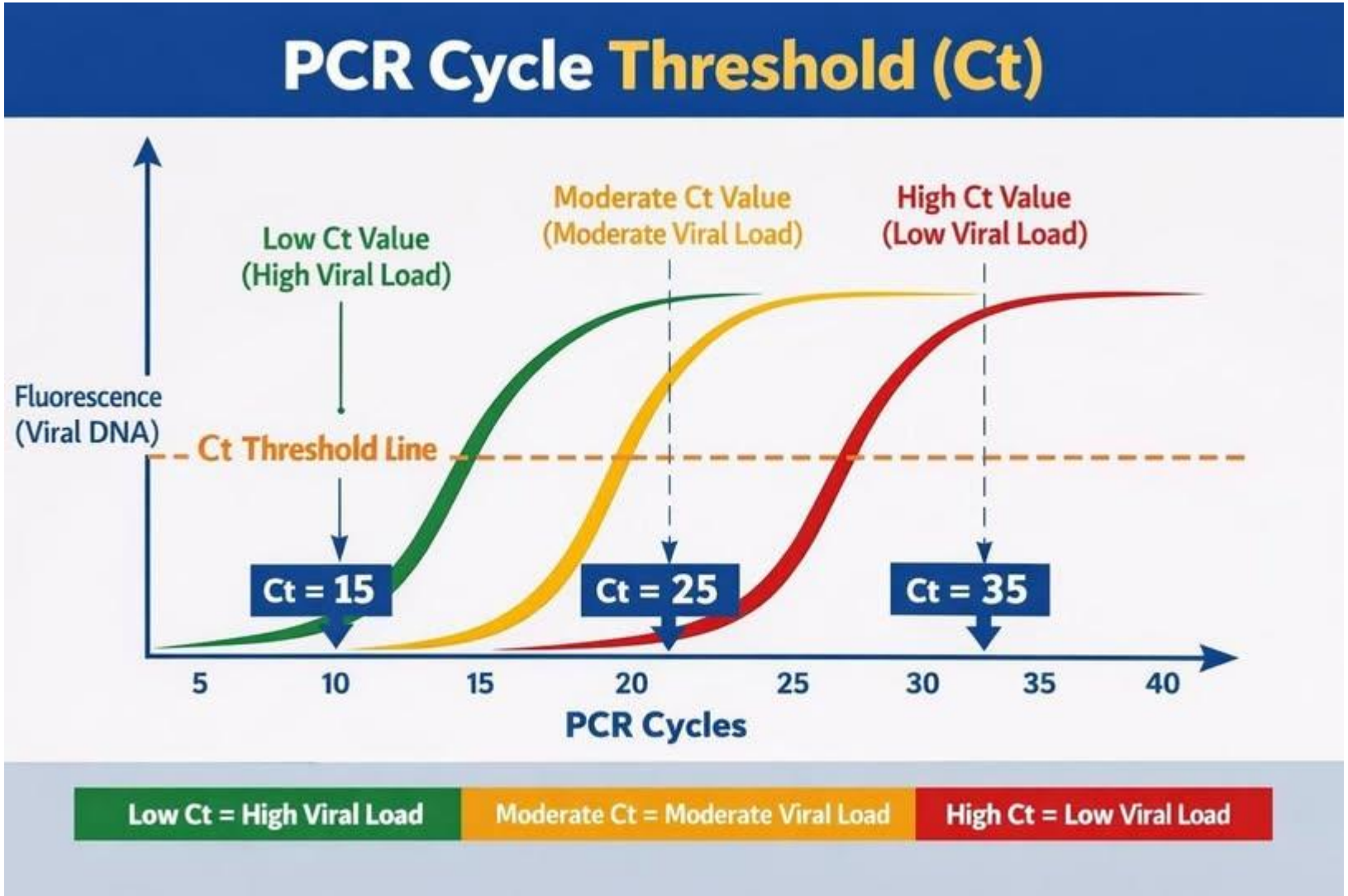
❑ Nucleic Acid Amplification Tests (NAATs):

- **PCR *vs.* RT-PCR:** Standard PCR for DNA viruses (HSV, EBV, Adenovirus); Reverse-Transcription PCR (RT-PCR) converts RNA genomes to cDNA first (Influenza, SARS-CoV-2, HCV, HIV).
- **Real-Time / Quantitative PCR (qPCR):** Monitors fluorescence signal during **exponential amplification phase**. Cycle threshold (Ct) values are <u>inversely</u> proportional to viral load (lower Ct = higher target nucleic acid concentration).
- **Multiplex Molecular Panels:** Simultaneous target amplification of dozens of pathogens in single syndromic panels (e.g., upper respiratory, meningitis/encephalitis, gastrointestinal panels).

- **Multiplex molecular panels** are advanced laboratory tests that detect multiple pathogens or genetic targets from a single clinical specimen at the same time.

- **PCR Cycle Threshold (Ct):**

❑ What Ct is: The cycle threshold (Ct) is the PCR assay's amplification cycle at which fluorescence crosses a predefined threshold. Lower Ct → more target nucleic acid in the sample; higher Ct → less.



❑ Sanger *vs.* Next-Generation Sequencing (NGS):

- **Sanger Sequencing:** Target-specific sequencing used to <u>assess specific gene resistance mutations</u> (e.g., HIV antiretroviral resistance mutations).
- **Next-Generation Sequencing (NGS / Metagenomics):** Unbiased sequencing without prior targeted primers. Essential for tracking lineage evolution, variant surveillance, and unknown pathogen discovery.

→ **<u>Sequencing</u>** is the process of putting items in a specific order or finding the exact order of components in a system.

→ In **biology** and science, it most commonly refers to DNA sequencing, which determines the exact order of chemical bases_ adenine (A), thymine (T), cytosine (C), and guanine (G), in a genetic code.

→ A **primer** is a short single-stranded nucleic acid (RNA or DNA) that serves as a starting point for DNA synthesis.

→ Essential for laboratory processes like Polymerase Chain Reaction (PCR) to amplify specific genetic sequences.

2. Viral Serology & Immunoassays

- **Antibody Kinetics:**
  - **IgM:** Appears early (days post-symptom beginning), signals primary/acute infection, declines over months.
  - **IgG:** Appears later, indicates past exposure, recovery, or immunity.

- **Serological Diagnostic Samples:**
  - **Seroconversion:** Shift from negative to positive antibody status.
  - **Paired Sera Analysis:** Demonstrating a ≥ 4-fold rise in specific IgG antibody titer between acute (week 1) and recovering (weeks 2–4) samples.
  - **IgG Avidity Testing:** High-avidity IgG reflects long-standing immunity; low-avidity IgG indicates recent primary infection.

3. Interpretation of Results & Clinical Pitfalls

Diagnostic Profile	Clinical Interpretation	Common Pitfall [challenge] / Action
▪ **PCR positive / Serology negative**	▪ **Hyper-acute infection**	▪ **High infectivity**; re-test serology in 2 weeks if confirmation needed.
▪ **PCR negative / Serology positive (IgG only)**	▪ **Past infection** or **post-vaccination** immunity	▪ Does not explain acute symptoms; **look for alternate etiology** [reasons].
▪ **High Ct value (low viral load) on PCR**	▪ **Residual** viral RNA shedding or late recovery	▪ **May not represent active infectious virus**; interpret in clinical context.
▪ **IgM positive in high-risk context**	▪ **Acute** primary infection	▪ **Beware of false positives** due to rheumatoid factor or cross-reacting flaviviruses.

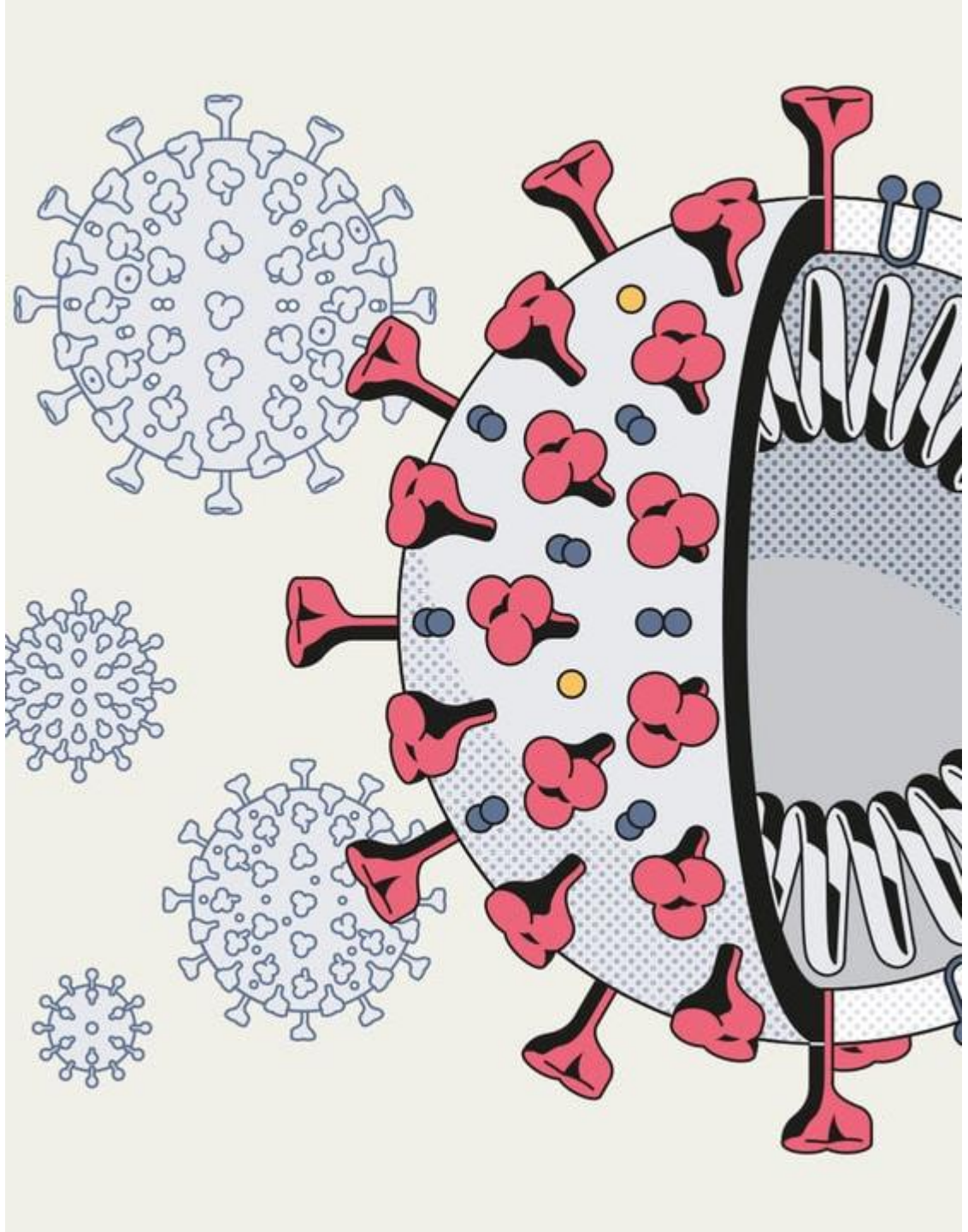
- A **serology test** is a blood test that looks for specific **antibodies or antigens** to see if your immune system has reacted to an infection, virus, or foreign substance.

Syllabus

- Introduction to Virology
- Viral Structure and Classification
- Viral Replication
- Pathogenesis and **Viral Genetics**
- Viruses and Cancer
- Viral Vectors and Gene Therapy
- Antiviral Drugs and Vaccines

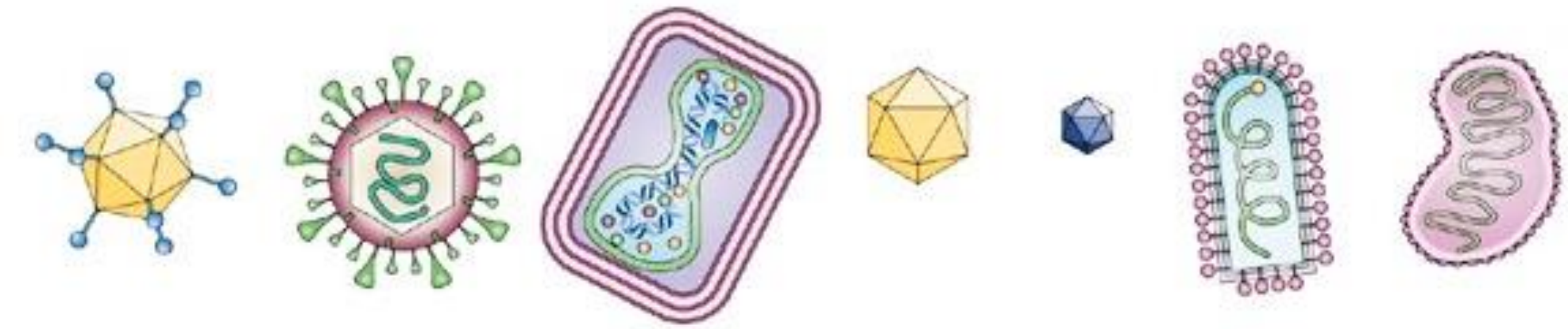
Viral Genetics

- **Viral genetics** is the study of the **genetic material of viruses**, how viral genomes are organized and replicated, and how they change over time.
- Viruses can contain either **DNA or RNA** as their genetic material, which may be single-stranded or double-stranded.
- Their genomes encode **proteins** needed for [1] replication, formation of [2] new virus particles, and [3] interactions with host cells.



Core Genome Types

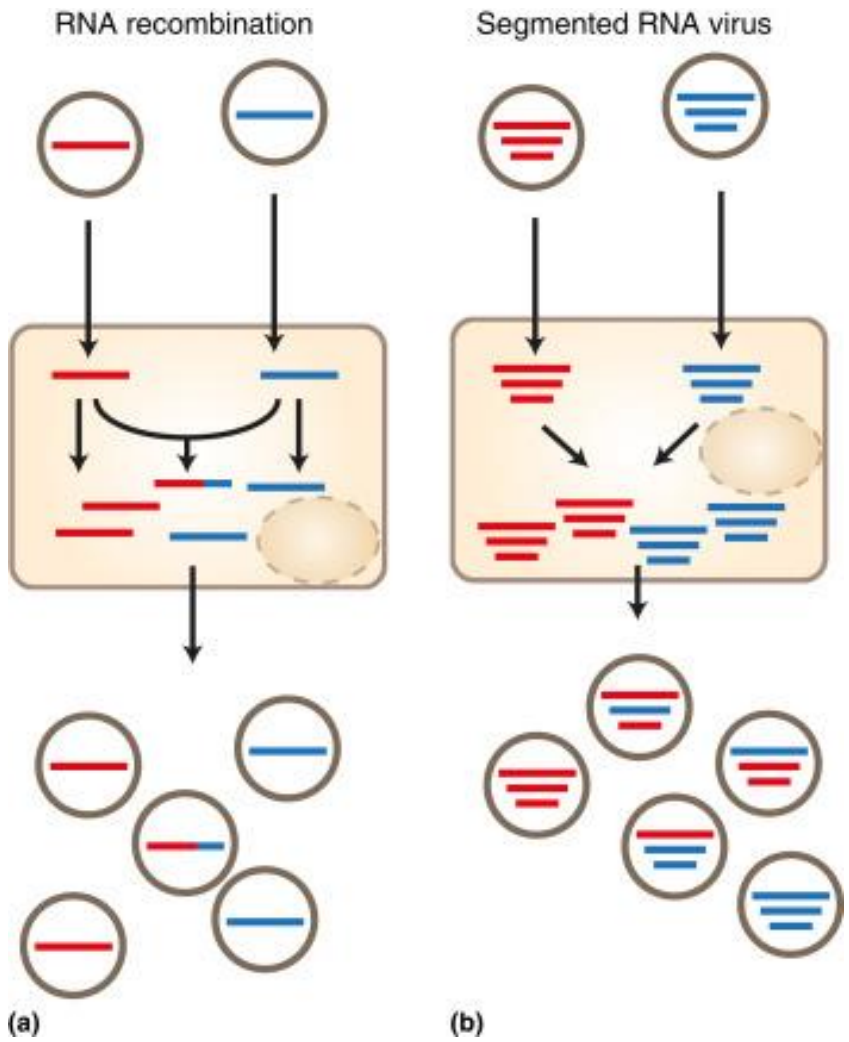
- **DNA or RNA:** Viral genomes can be single-stranded or double-stranded, linear, circular, or segmented.
- **Host dependency:** Viruses are **parasites** that **must use a host cell** to copy their genes and make proteins.



Important aspects of viral genetics include:

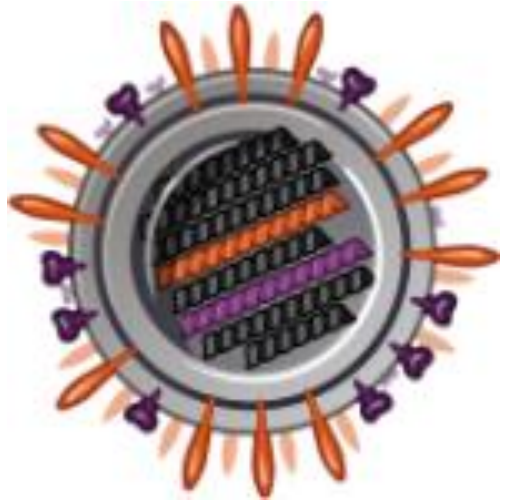
- **Mutation**: Viral genomes acquire genetic changes during replication. RNA viruses often mutate relatively rapidly because many RNA-replicating enzymes have → limited proofreading.

- **Recombination**: Two related viral genomes can exchange genetic material when they infect the same cell.



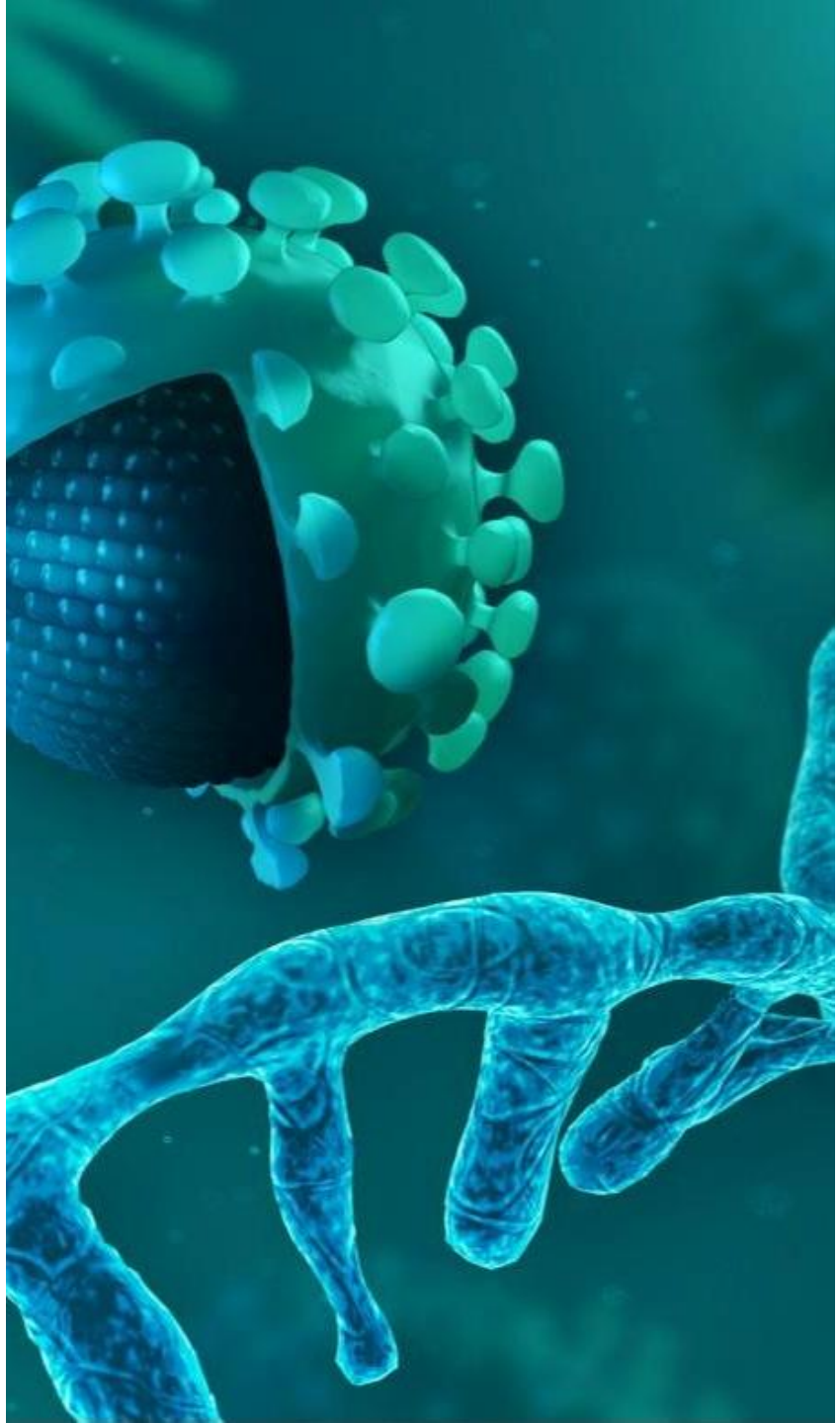
- **Reassortment:** In viruses with **segmented genomes**, such as influenza viruses, → entire genome segments can be exchanged during coinfection.

- **Selection:** Mutations that improve viral survival or transmission can become more common in a population.



- **Genetic drift**: → Random changes can also alter the frequencies of viral variants.

- **Host–virus interactions**: Viral genes influence which cells or species a virus can [1] infect and how it [2] interacts with host defenses.



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