Microbiology

Unit 3 · Viruses and Bacteriophages

Viruses are particles of nucleic acid in a protein coat that copy themselves only inside living cells. After this unit you can describe how a virus is built and classified, trace the lytic and lysogenic cycles of a phage, explain how animal and plant viruses infect and how labs grow them, link certain viruses to cancer, follow the life cycles of HIV and SARS-CoV-2, and calculate a virus titre from a plaque assay or a TCID50 plate.

Taught at 8 of 10 | Usually Sem 3 | 7 chapters

About this unit

This unit is about the smallest agents in microbiology: viruses, which are not cells and can only multiply inside a living host cell. Chapter 3.1 sets out what all viruses share, how a virus particle is built and how viruses are named and grouped. Chapter 3.2 uses bacteriophages, the viruses of bacteria, to show the two basic outcomes of infection: the lytic cycle that kills the cell and the lysogenic cycle that hides inside it. Chapter 3.3 moves to animal viruses and how a laboratory grows them. Chapters 3.4 and 3.5 are shorter: plant viruses, and the viruses that can cause cancer. Chapter 3.6 applies everything to two human viruses every student should know in detail, HIV and SARS-CoV-2. Chapter 3.7 closes with the arithmetic of virology: how a laboratory counts infectious virus.

Chapters

1. General properties, structure and classification of viruses
2. Bacteriophages: structure and lytic and lysogenic cycles
3. Animal viruses: life cycle and cultivation
4. Plant viruses ADVANCED
5. Oncoviruses ADVANCED
6. Human viruses: HIV and SARS-CoV-2
7. Viral quantification assays ADVANCED

How to use this book

- Read the lead paragraph and the figure of each chapter first; they carry the one idea.
- Cover the solution of the worked example and do it yourself with a pencil, then compare step by step.

- Answer each "Check yourself" question in your head or on paper before you open it.

3.1 General properties, structure and classification of viruses

A virus is an infectious particle made of one kind of nucleic acid (DNA or RNA) wrapped in a protein coat, sometimes with a lipid envelope outside. It has no ribosomes and no energy metabolism of its own, so it can multiply only inside a living host cell, using that cell's machinery. Viruses infect every form of life, from bacteria to people, and they matter to a biotechnologist twice over: as causes of disease, and as tools for vaccines, gene therapy and cloning.

You need: the difference between prokaryotic and eukaryotic cells (Unit 1, Prokaryotic and eukaryotic cells compared) and the idea of a microscope's resolving limit (Unit 1, Fluorescence and electron microscopy).

What makes a virus a virus

The French microbiologist André Lwoff summed up the properties that set viruses apart from cells. Learn them as a list; examiners ask for it.

1. **One type of nucleic acid.** A virus particle carries DNA or RNA, never both. Cells carry both.
2. **No independent metabolism.** A virus has no ribosomes and makes no ATP. Outside a cell it is inert.
3. **Obligate intracellular parasite.** It multiplies only inside a living host cell.
4. **Replication from parts, not by division.** A virus does not grow and split. The cell makes many copies of the genome and the proteins separately, and these assemble into new particles.
5. **Small size.** Most viruses are about 20 to 300 nm across, below the resolving limit of a light microscope, so they are seen with an electron microscope. They pass through filters that hold back bacteria.
6. **Not affected by antibacterial antibiotics.** Penicillin and similar drugs act on bacterial walls and ribosomes, which viruses do not have.

The history follows from the size. In 1892 Dmitri Ivanovsky showed that sap from diseased tobacco stayed infectious after passing through a filter that held back bacteria. In 1898 Martinus Beijerinck called the agent a "contagium vivum fluidum", a living infectious fluid, and in the same year Loeffler and Frosch found the first animal virus, of foot-and-mouth disease. In 1935

Wendell Stanley crystallised tobacco mosaic virus (TMV), which showed that a virus could behave like a chemical.

The parts of a virus particle

The complete, infectious particle outside a cell is called a **virion**. Its parts are:

- **Genome:** DNA or RNA, single-stranded (ss) or double-stranded (ds), linear or circular, in one piece or several segments (influenza has eight RNA segments).
- **Capsid:** the protein shell around the genome, built from many copies of one or a few proteins. The visible units of the capsid are called **capsomeres**. The genome plus capsid is the **nucleocapsid**.
- **Envelope (in some viruses):** a lipid bilayer taken from a host membrane as the virus leaves, studded with viral glycoproteins called **spikes** or peplomers. Viruses without one are called **naked**. Enveloped viruses are destroyed by detergents, ether and alcohol, because these dissolve lipid; naked viruses resist them better.
- **Enzymes (in some viruses):** carried inside the virion because the host cannot supply them, for example the reverse transcriptase of HIV or the RNA-dependent RNA polymerase of influenza.

Capsids follow three plans of symmetry. In **helical** symmetry the protein subunits wind around the genome like a spiral staircase, giving a rod (TMV) or a flexible tube. In **icosahedral** symmetry the capsid is a 20-faced solid with 12 vertices and 30 edges (adenovirus, poliovirus); it is the most economical way to close a shell from identical subunits. **Complex** viruses fit neither plan: the poxviruses are brick-shaped, and the T-even phages join an icosahedral head to a helical tail (Chapter 3.2).

Classification: two systems you must know

The official names are set by the **International Committee on Taxonomy of Viruses (ICTV)**. It uses ranks like those of cellular life, from realm down through order (-virales), family (-viridae) and genus (-virus) to species. Family and genus names are written in italics, for example *Retroviridae* and *Lentivirus*. Older books also describe the LHT system of Lwoff, Horne and Tournier (1962), which grouped viruses by nucleic acid, symmetry, envelope and size.

The **Baltimore classification**, proposed by David Baltimore, groups viruses by how they make messenger RNA (mRNA), because every virus must make mRNA for the host ribosome to read. Positive-sense (+) RNA has the same sequence as mRNA and can be translated at once; negative-sense (–) RNA is its complement and must first be copied.

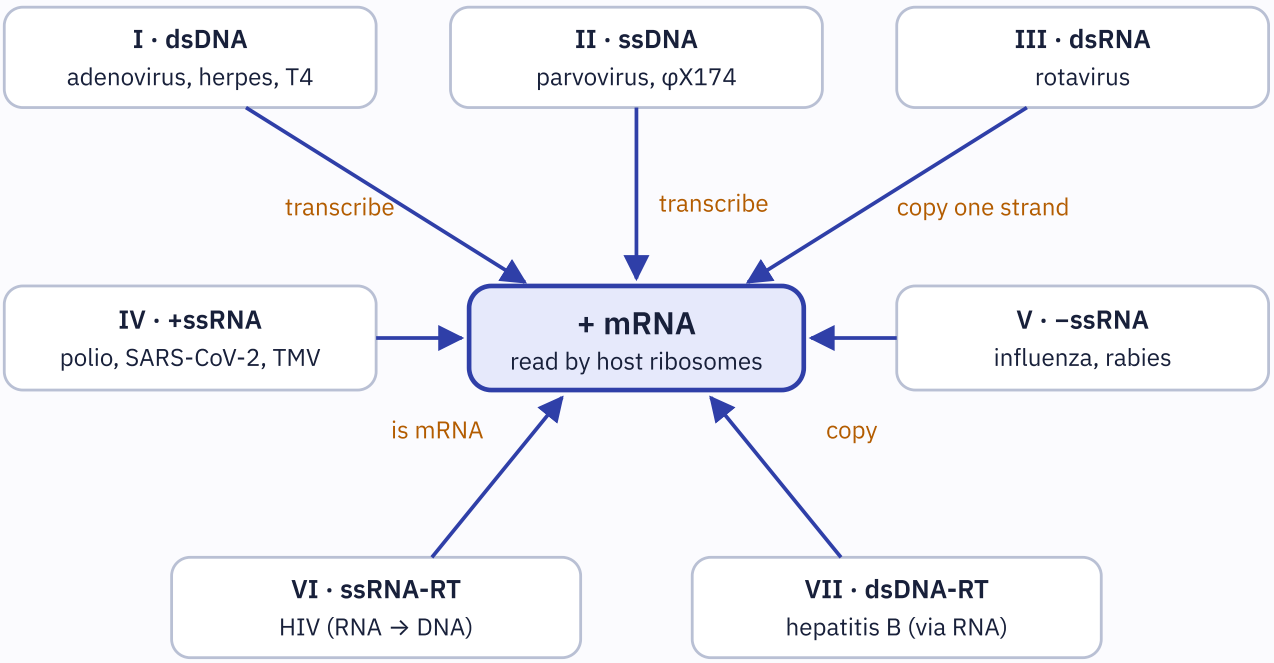


Figure 3.1.1 The Baltimore classification. Seven genome types, each with its own route to + mRNA in the centre. Notice that group IV genomes already are mRNA, group V must copy theirs first (so they carry their own polymerase), and the two RT groups pass through a DNA–RNA switch using reverse transcriptase.

Pathogenicity in brief

A virus causes disease by killing the cells it grows in (the lytic damage seen as cytopathic effect, Chapter 3.3), by making infected cells targets of the immune system, by suppressing immunity (HIV), or by changing cell growth (Chapter 3.5). Its host range and tissue tropism depend mainly on which cell-surface receptor its attachment protein binds. The infections and their spread belong to Unit 6 (Microbial pathogenicity).

Some infectious agents are even simpler than viruses. **Viroids** are short, circular RNA molecules with no capsid that infect plants (Chapter 3.4). **Prions** are misfolded proteins with no nucleic acid at all; they cause slow brain diseases such as Creutzfeldt–Jakob disease. Satellite viruses need a helper virus to multiply.

WORKED EXAMPLE

Question (6 marks). The adenovirus capsid is an icosahedron built from 240 hexons (capsomeres of six protein units) and 12 pentons (capsomeres of five units). (a) Why are there exactly 12 pentons? (b) How many protein units make up the capsid shell? (c) Classify adenovirus in the Baltimore system and say what it needs from the host to make mRNA.

1. **(a)** An icosahedron has 12 vertices. A capsomere at a vertex has five neighbours around it, so it is a pentamer. Every icosahedral capsid therefore has exactly 12 pentons, however many hexons fill the faces.
2. **(b)** Protein units in hexons = 240 × 6 = 1440. Protein units in pentons = 12 × 5 = 60. Total = 1440 + 60 = **1500 units**.
3. **Check with the triangulation rule:** an icosahedral shell holds 60 × T units, where T is the triangulation number. 1500 ÷ 60 = 25, so T = 25, a known value for adenovirus.
4. **(c)** Adenovirus has a linear dsDNA genome, so it is **Baltimore group I**. It needs the host's DNA-dependent RNA polymerase II, which is in the nucleus, so adenovirus DNA must reach the nucleus to be transcribed.

EXAM TRAPS

- **Virion vs virus:** the virion is the complete particle outside the cell; inside the cell, during the eclipse, there is no intact virion at all.
- **Capsid vs capsomere:** the capsid is the whole shell; capsomeres are its visible units. Writing "the capsomere encloses the genome" loses the mark.
- **"Viruses have both DNA and RNA"** is wrong for the virion. The genome is one or the other; an RT virus makes the other form only inside the cell.
- **+ and − sense:** + sense means "same as mRNA", not "double-stranded". Influenza is − ssRNA; polio is + ssRNA.
- The favourite question: "Are viruses living?" Give both sides: they have genes and evolve, but they have no metabolism and cannot reproduce outside a cell.

CHECK YOURSELF

– REMEMBER **Name the three types of capsid symmetry with one example each.** 3 marks

Helical (TMV), icosahedral (adenovirus or poliovirus), complex (poxvirus or T4 phage).

– REMEMBER **Who first showed that a plant disease agent passes through a bacterial filter, and in which year?** 1 mark

Dmitri Ivanovsky, 1892, with tobacco mosaic disease.

– UNDERSTAND **Why does a – ssRNA virus have to carry its own polymerase in the virion?** 3 marks

Its genome cannot be translated, and host cells have no enzyme that copies RNA from an RNA template. Without a packaged RNA-dependent RNA polymerase, no mRNA would be made and the infection could not begin.

– UNDERSTAND **Why is 70% ethanol effective against an enveloped virus but less so against a naked virus?** 2 marks

Alcohol disrupts the lipid envelope, which carries the attachment spikes, so the enveloped virus can no longer bind cells. A naked virus has only a protein capsid, which is more resistant.

– APPLY **An icosahedral capsid has T = 3. How many protein subunits does it contain, and how many of the capsomeres are pentamers?** 3 marks

60 × 3 = **180 subunits**. There are always **12 pentamers**, one at each vertex; the remaining 180 – 60 = 120 subunits form 20 hexamers.

– APPLY **Place hepatitis B virus, rotavirus and rabies virus in Baltimore groups.** 3 marks

Hepatitis B: group VII (dsDNA-RT). Rotavirus: group III (dsRNA). Rabies: group V (– ssRNA).

– ANALYZE **A purified RNA from virus X infects cells when injected; RNA from virus Y does not. What can you conclude about their genomes?** 4 marks

Virus X has + sense RNA: the cell can translate it directly and make the viral polymerase. Virus Y is probably – sense (or dsRNA): its RNA needs a polymerase packed in the virion, which was lost when the RNA was purified.

BEYOND THE SYLLABUS

Virus taxonomy now leans on genome sequences more than on shape. ICTV has added ranks above order, such as the realm *Riboviria* for RNA viruses that use an RNA-dependent RNA polymerase, and has moved species names to a two-word format. Giant viruses such as mimivirus, discovered in amoebae, are larger than some bacteria and carry genes for parts of the translation system, which has reopened the "are viruses alive" debate. Capsid assembly is also engineered: virus-like particles (capsids with no genome) are the basis of the HPV and hepatitis B vaccines. Search "ICTV Master Species List" and "virus-like particle vaccine" to go further. This is not for the exam.

3.2 Bacteriophages: structure and lytic and lysogenic cycles

Bacteriophages ("phages") are viruses that infect bacteria. They were found by Frederick Twort in 1915 and by Félix d'Hérelle in 1917, who named them "bacteria eaters". A phage can follow one of two paths after it enters a cell: the **lytic cycle**, which makes new phages and bursts the cell, or the **lysogenic cycle**, in which the phage genome sleeps inside the bacterial chromosome and is copied with it. Phages taught us much of molecular biology, they are tools for cloning, and they are returning as a treatment for antibiotic-resistant infections.

You need: virion parts and capsid symmetry (Chapter 3.1) and the Gram-negative cell envelope (Unit 2, Bacterial cell wall).

Structure of phage T4

The T-even phages (T2, T4, T6) of *Escherichia coli* are the textbook example of complex symmetry. T4 has about 169 kb of linear dsDNA. Its parts, from top to bottom:

- **Head:** an elongated icosahedron of protein that holds the tightly packed DNA.
- **Collar and neck:** join the head to the tail.
- **Tail:** a hollow core tube surrounded by a **contractile sheath**. When the sheath contracts, the core is driven through the cell envelope like a syringe needle.
- **Baseplate:** a hexagonal plate at the tail tip with short tail pins (spikes) and an enzyme that makes a hole in the peptidoglycan.
- **Tail fibres:** six long, jointed fibres that recognise receptors on the bacterial outer membrane.

Other phages are simpler. Phage λ (lambda) has an icosahedral head and a long, flexible tail that does not contract; its genome is 48.5 kb of dsDNA. φX174 is a small icosahedral phage with circular ssDNA. M13 is a filamentous ssDNA phage that leaves the cell without killing it, and MS2 is a small ssRNA phage. Textbooks group tailed phages into three families by tail type: *Myoviridae* (contractile tail, T4), *Siphoviridae* (long non-contractile tail, λ) and *Podoviridae* (short tail, T7). Know these names for the exam, but note that the ICTV, in its 2022 taxonomy update (published 2023), abolished these three families as not reflecting real ancestry and now places tailed phages in the class *Caudoviricetes*, classified by genome sequence.

The lytic cycle

A **virulent** phage such as T4 can only follow the lytic cycle. The steps are:

1. **Adsorption.** Tail fibres bind specific receptors (for T4, outer-membrane proteins and lipopolysaccharide). The receptor decides the host range.
2. **Penetration.** The baseplate settles on the surface, the sheath contracts, the core pierces the envelope and the DNA is injected. The empty capsid stays outside as a "ghost".
3. **Early gene expression.** The host RNA polymerase reads phage early genes. Their products shut down host transcription and break down host DNA. T4 DNA contains hydroxymethylcytosine in place of cytosine, so phage nucleases cut host DNA and spare the phage's own.
4. **Replication and late genes.** The phage genome is copied many times; late genes make head, tail and fibre proteins.
5. **Assembly (maturation).** Heads, tails and fibres are made separately and join; DNA is pumped into the head by a packaging motor.
6. **Release.** A **holin** makes holes in the inner membrane, letting an **endolysin** reach and digest the peptidoglycan. The cell bursts and releases the progeny.

The **one-step growth curve** (Ellis and Delbrück, 1939) measures this cycle. A culture is infected so that every cell gets a phage, diluted so that new phages cannot infect again, and sampled for plaque-forming units over time. Three numbers come from it. The **eclipse period** is the time when no infectious phage can be found even inside broken cells; the injected genome is not yet a virion. The **latent period** is the time from infection until the burst, when free phages first rise; for T4 at 37 °C it is around 25 minutes. The **burst size** is the average number of phages released per infected cell.

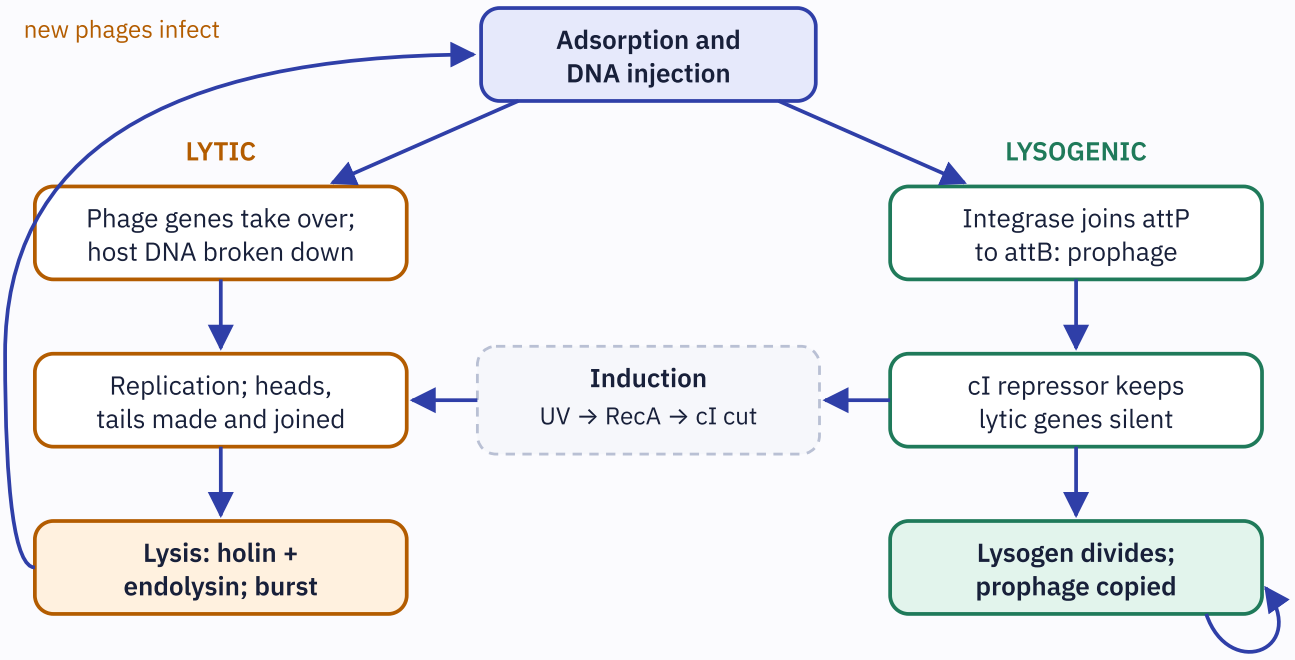


Figure 3.2.1 The two paths of a temperate phage such as λ. Left: the lytic cycle ends in a burst of new phages that infect more cells. Right: in lysogeny the genome integrates as a prophage and is passed to daughter cells; the loop shows many generations. The dashed box is induction: DNA damage switches a lysogen back to the lytic path.

The lysogenic cycle

A **temperate** phage can choose either path. Phage λ is the model. After its linear DNA enters, the two single-stranded **cos** ends pair up and the genome closes into a circle. Then a genetic switch decides the outcome.

- If the λ **cI repressor** wins, it binds the operators that control the lytic genes and keeps them off. The phage enzyme **integrase** joins the phage site *attP* to the bacterial site *attB* (between the *gal* and *bio* genes of *E. coli*). The integrated genome is a **prophage**; the cell carrying it is a **lysogen**.
- If the **Cro** protein wins, it blocks cI synthesis and the lytic genes run.

Conditions push the switch. Many phages per cell (high multiplicity of infection) and poor nutrition favour lysogeny, which makes sense: when hosts are scarce, it is safer to wait inside one.

A lysogen has three special properties. It is **immune to superinfection**: the cI repressor already present also silences any new λ genome that enters. It can be **induced**: UV light or DNA-damaging chemicals activate RecA (part of the SOS response), which triggers self-cleavage of cI; the prophage is cut out by integrase with excisionase (Xis) and enters the lytic cycle. And it may show **lysogenic conversion**: prophage genes change the host's properties. The diphtheria toxin of *Corynebacterium diphtheriae*, the cholera toxin of *Vibrio cholerae* and the Shiga toxins of

some *E. coli* are all coded by phage genes. Phages also move bacterial genes between cells by transduction, which belongs to Unit 4 (Bacterial recombination).

WORKED EXAMPLE

Question (6 marks). In a one-step growth experiment, *E. coli* at 2×10^8 cells/mL is infected with T4. Unadsorbed phages are removed and the culture is diluted 10^4-fold. A plaque assay of the diluted culture just after infection gives 1.8×10^4 PFU/mL (each infected cell forms one plaque). At 60 minutes the diluted culture gives 2.7×10^6 PFU/mL, and the count stops rising. Find the burst size and explain why the infected culture was diluted.

1. **Infective centres before the burst:** 1.8×10^4 per mL of diluted culture. Each is one infected cell.
2. **Phages after the burst:** 2.7×10^6 per mL of the same diluted culture.
3. **Burst size** = phages released ÷ infected cells = $2.7 \times 10^6 \div 1.8 \times 10^4$ = **150 phages per cell**. (Strictly, subtract the 1.8×10^4 starting count from the top; 150 becomes 149, which rounds the same.)
4. **Why dilute:** after a 10^4-fold dilution the bacteria are about 2×10^4/mL, so released phages rarely meet an uninfected cell. Only one cycle is measured; that is what "one-step" means.

EXAM TRAPS

- **Eclipse vs latent period:** the eclipse ends when new virions appear inside the cell; the latent period ends later, when they are released. The eclipse is always the shorter of the two.
- **Virulent vs temperate:** T4 is virulent (lytic only); λ is temperate (lytic or lysogenic). "λ is lysogenic" alone is incomplete.
- **Prophage vs lysogen:** the prophage is the integrated phage DNA; the lysogen is the bacterium carrying it.
- **Immunity:** a lysogen resists the same phage because of the repressor, not because of a missing receptor. The phage still adsorbs and injects.
- Do not say every phage lyses its host: filamentous phages such as M13 leak out through the envelope without killing the cell.

CHECK YOURSELF

– REMEMBER **List the structural parts of phage T4.** 3 marks

Icosahedral head with DNA, collar, tail core inside a contractile sheath, baseplate with tail pins, and six long tail fibres.

– REMEMBER **Which two scientists independently discovered bacteriophages?** 1 mark

Frederick Twort (1915) and Félix d'Hérelle (1917).

– UNDERSTAND **Why does a λ lysogen resist infection by another λ phage?** 3 marks

The prophage keeps making cI repressor. Any new λ genome that enters is bound by this repressor at once, so its lytic genes never switch on and it cannot integrate or multiply. This is immunity to superinfection.

– UNDERSTAND **How do holin and endolysin work together at the end of the lytic cycle?** 2 marks

Endolysin digests peptidoglycan but sits in the cytoplasm. Holin, at a set time, forms holes in the inner membrane so endolysin can reach the wall. The wall breaks and the cell bursts.

– APPLY **A culture with 5×10^5 infected cells/mL releases 4×10^7 PFU/mL after one cycle. What is the burst size?** 2 marks

$4 \times 10^7 \div 5 \times 10^5 =$ **80 phages per infected cell**.

– APPLY **A culture of λ lysogens is exposed to UV light. Predict what you see in the flask over the next hour, and why.** 3 marks

UV damages DNA, RecA becomes active and cI cleaves itself. The prophage is excised and enters the lytic cycle, so after the latent period the culture clears (lyses) and free λ phages appear.

– ANALYZE **A harmless *Vibrio cholerae* strain becomes toxigenic after exposure to a phage. Explain, and say why this matters for public health.** 4 marks

The cholera toxin genes are carried by a phage. When it establishes itself in the harmless strain, the strain gains the toxin genes: lysogenic conversion. Toxigenic strains can therefore arise from non-toxigenic ones in the environment, so surveillance must track the phage as well as the bacterium.

BEYOND THE SYLLABUS

Phage therapy, the use of lytic phages to treat infections, is being tested again for bacteria resistant to all antibiotics, and phage-derived endolysins are being developed as drugs. Phage parts are standard laboratory tools: λ for cloning vectors, M13 for phage display of antibodies (the work behind the 2018 Chemistry Nobel to George Smith and Gregory Winter), and T7 RNA polymerase in protein expression. CRISPR–Cas, the gene-editing tool, began as a bacterial immune system against phages. Search "phage therapy clinical trial" and "phage display" to go further. This is not for the exam.

3.3 Animal viruses: life cycle and cultivation

Animal viruses follow the same plan as phages (attach, enter, copy, assemble, leave), but the details change because an animal cell has no wall, has a nucleus, and takes things in by endocytosis. The whole virion usually enters the cell and must be **uncoated** inside. Because viruses cannot grow on a nutrient medium, a laboratory must supply living cells: a live animal, a fertilised egg, or a cell culture. These cultivation methods are how viruses are isolated from patients, studied and made into vaccines.

You need: envelope, capsid and the Baltimore groups (Chapter 3.1) and the lytic cycle of phages (Chapter 3.2) for comparison.

The life cycle in six steps

1. **Attachment (adsorption).** A viral attachment protein binds a specific receptor on the cell. Influenza haemagglutinin binds sialic acid; HIV gp120 binds CD4; poliovirus binds CD155. The receptor explains host range and tissue tropism: polio infects primates because only primates carry the matching receptor.
2. **Penetration.** Three routes. In **receptor-mediated endocytosis** the cell swallows the bound virion into an endosome (influenza, adenovirus). In **membrane fusion** the envelope fuses with the plasma membrane and the nucleocapsid is released into the cytoplasm (HIV). Some naked viruses **translocate** or open a pore to pass their genome through the membrane (poliovirus).
3. **Uncoating.** The capsid is removed to free the genome. Often the acid pH of the endosome triggers it: in influenza, the M2 ion channel lets protons into the virion, which loosens the core, and haemagglutinin fuses the envelope with the endosome membrane.

4. **Biosynthesis.** Viral mRNA, proteins and genomes are made. A rule of thumb: DNA viruses replicate in the nucleus, where the host's DNA and RNA polymerases are, except poxviruses, which carry their own enzymes and replicate in the cytoplasm. RNA viruses replicate in the cytoplasm, except influenza and the retroviruses, which use the nucleus. Early proteins (enzymes, regulators) come first, late proteins (capsid parts) later.
5. **Assembly (maturation).** Capsid proteins gather round new genomes. Herpesviruses assemble in the nucleus; picornaviruses in the cytoplasm.
6. **Release.** Naked viruses usually leave when the cell lyses. Enveloped viruses **bud**: viral glycoproteins gather in a patch of host membrane, the nucleocapsid pushes against it, and the membrane wraps it. The envelope comes from the plasma membrane (influenza, HIV), the nuclear membrane (herpes) or internal membranes (coronaviruses, Chapter 3.6). Budding need not kill the cell at once.

The result of infection varies. A **lytic** (cytocidal) infection kills the cell. A **persistent** infection releases virus slowly for a long time (hepatitis B). A **latent** infection keeps the genome silent for years and reactivates later, as herpes simplex does in nerve ganglia to cause cold sores, or varicella-zoster to cause shingles. A **transforming** infection changes cell growth (Chapter 3.5).

Cultivation method 1: experimental animals

Animals were the first hosts used in virology. Suckling (newborn) mice injected into the brain or abdomen are still used for viruses that do not grow well in culture, such as coxsackieviruses and some arboviruses (viruses carried by mosquitoes and ticks). The disease, death or antibody response shows that the virus is present. Animal use is costly, slow and raises welfare concerns, so it is now a last resort.

Cultivation method 2: embryonated eggs

Ernest Goodpasture and colleagues showed in the early 1930s that viruses grow in fertilised hen's eggs. The egg is sterile, cheap and has several tissues, so different viruses can be put in different places. A hole is drilled in the shell over the air sac, the virus is injected, the hole is sealed with wax, and the egg is incubated. The four routes are shown in Figure 3.3.1.

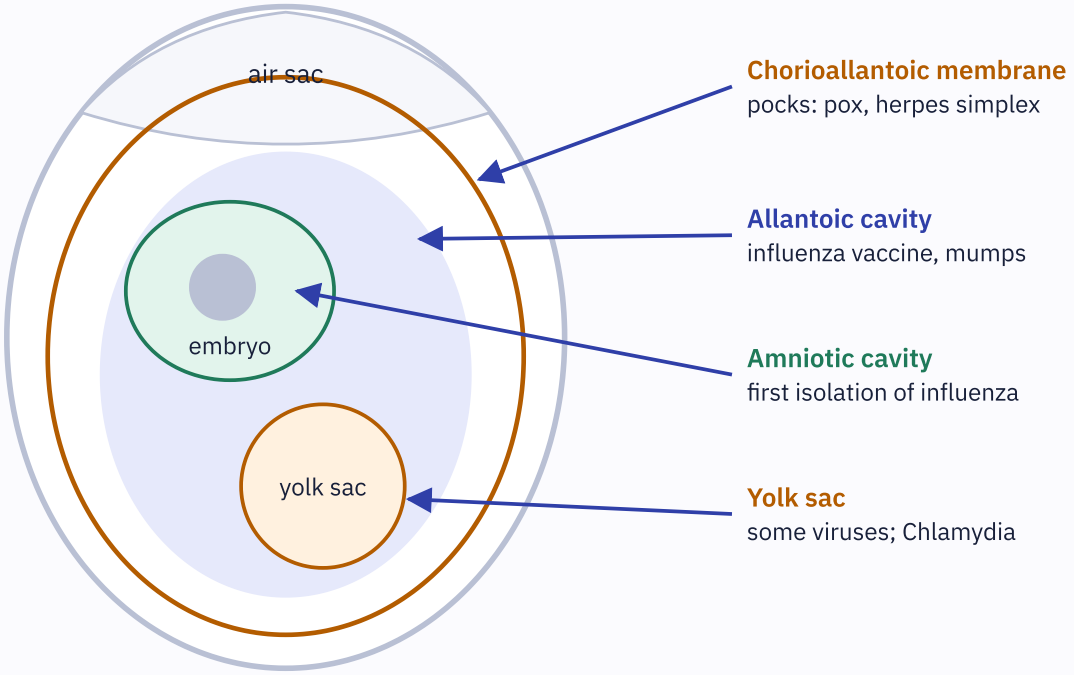


Figure 3.3.1 An embryonated hen's egg (simplified, not to scale) and its four inoculation routes. The orange ring stands for the chorioallantoic membrane, the blue space is the allantoic cavity, the green sac round the embryo is the amnion. Notice that each route matches a group of viruses and a way of reading the result.

- **Chorioallantoic membrane (CAM):** poxviruses and herpes simplex form visible white spots called **pocks**. Counting pocks gives a rough titre, and their look helps identify the virus.
- **Allantoic cavity:** influenza and mumps viruses grow to high yield. Most influenza vaccine has been made this way, using many millions of eggs each year.
- **Amniotic cavity:** used for the first isolation of influenza virus from a patient's sample.
- **Yolk sac:** used for some viruses and for the obligate intracellular bacteria *Chlamydia* and *Rickettsia*.

Growth in an egg is shown by death of the embryo, pocks on the CAM, or haemagglutinin in the harvested fluid (Chapter 3.7).

Cultivation method 3: cell culture

Today most viruses are grown in cells cultured in flasks as a single layer (a **monolayer**) on plastic, fed with a medium containing salts, glucose, amino acids, vitamins and serum. Three kinds are used:

TYPE	WHAT IT IS	EXAMPLES AND USE
Primary culture	Cells taken straight from an organ; survive only a few subcultures	Monkey kidney, chick embryo fibroblasts

TYPE	WHAT IT IS	EXAMPLES AND USE
Diploid cell strain	Normal chromosome number; divide about 50 times, then die (the Hayflick limit)	WI-38, MRC-5 (human foetal lung); used for some human vaccines
Continuous cell line	Grow indefinitely, often from tumours; abnormal chromosomes	HeLa (human cervical cancer), Vero (monkey kidney), HEp-2, BHK-21, MDCK (dog kidney, for influenza)

Virus growth in culture is detected by:

- **Cytopathic effect (CPE):** visible damage seen under the microscope, such as rounding and detachment of cells, giant multinucleate cells (**syncytia**, in measles and respiratory syncytial virus), or **inclusion bodies** (Negri bodies of rabies in nerve cells).
- **Haemadsorption:** red blood cells stick to cells infected by influenza or parainfluenza viruses, because viral haemagglutinin sits on the cell surface before budding. This shows the virus even when there is no CPE.
- **Interference:** a non-cytopathic virus is revealed when it blocks the CPE of a second virus added later.
- **Immunofluorescence or PCR** on the cells, for viral antigen or genome.

WORKED EXAMPLE

Question (8 marks). A throat swab from a child with fever is sent for virus isolation. Influenza is suspected. (a) Describe two ways to grow the virus. (b) After three days the cell culture shows little CPE. How would you still show influenza virus is present? (c) Why is the sample not put on nutrient agar?

1. **(a) Egg:** inoculate the amniotic cavity of an embryonated egg for first isolation; pass the harvested fluid into the allantoic cavity for higher yield. **Cell culture:** inoculate MDCK cells (with trypsin in the medium, which influenza needs to cleave its haemagglutinin).
2. **(b) Haemadsorption:** add guinea pig or chicken red cells to the monolayer; they stick to infected cells. Or test the culture fluid for **haemagglutination**: red cells clump. Confirm the type by immunofluorescence or RT-PCR.
3. **(c)** Viruses have no metabolism and need living cells; on agar they will not multiply at all.

EXAM TRAPS

- **Haemagglutination vs haemadsorption:** haemagglutination is red cells clumping with free virus in a tube; haemadsorption is red cells sticking to infected cells in a monolayer.
- **Cell strain vs cell line:** a diploid strain has a limited lifespan; a continuous line grows forever. HeLa is a line, not a strain.
- **"All DNA viruses replicate in the nucleus"**: poxviruses are the exception. "All RNA viruses replicate in the cytoplasm": influenza and retroviruses are the exceptions.
- Do not write "the animal virus injects its DNA". Only phages with tails inject; animal viruses enter whole.
- The favourite question: "Describe the cultivation of viruses", with a labelled egg diagram. Draw the four routes.

CHECK YOURSELF

– REMEMBER **Name the four routes of inoculation in an embryonated egg.** 2 marks

Chorioallantoic membrane, allantoic cavity, amniotic cavity, yolk sac.

– REMEMBER **What is cytopathic effect? Give two examples.** 2 marks

Visible damage to cultured cells caused by virus growth: cell rounding and detachment, syncytia, inclusion bodies.

– UNDERSTAND **Why does an enveloped virus usually not need to lyse its host cell to leave it?** 3 marks

It leaves by budding: viral glycoproteins gather in the host membrane and the nucleocapsid is wrapped by that membrane as it pushes out. The membrane reseals, so the cell can keep releasing virus.

– UNDERSTAND **Why does the receptor decide which tissues a virus infects?** 2 marks

The first step is binding of the viral attachment protein to a specific receptor. Cells that lack the receptor cannot be entered, so only tissues that carry it are infected.

– APPLY **Choose a cultivation method for (a) vaccinia virus to see pocks, (b) a rabies vaccine, (c) a coxsackievirus that grows poorly in cells.** 3 marks

(a) Chorioallantoic membrane of an egg. (b) A cell culture such as Vero cells or a diploid strain. (c) Suckling mice.

– APPLY **A drug blocks the influenza M2 ion channel. Which step of the life cycle does it stop, and why?** 3 marks

Uncoating. M2 lets protons from the acid endosome into the virion; this loosens the core so the genome can be released. Blocking M2 keeps the genome locked in the capsid. (Amantadine acts this way.)

– ANALYZE **Compare cell culture and embryonated eggs for making a vaccine for millions of people.** 4 marks

Eggs are proven and cheap per egg but need huge numbers of eggs months ahead, are hard to scale fast, and some people are allergic to egg protein. Cell culture in bioreactors scales faster, is closed and sterile, avoids egg protein, but needs certified cell lines and costly media. For a sudden outbreak, cell culture is more flexible.

BEYOND THE SYLLABUS

Vaccine plants now grow viruses in Vero or MDCK cells on microcarrier beads in stirred bioreactors of hundreds or thousands of litres, the same scale-up ideas taught in bioprocess engineering. Organoids, three-dimensional mini-organs grown from stem cells, let researchers grow viruses such as human norovirus that refused to grow in ordinary cell lines. The HeLa line also raises questions of consent, since the cells were taken in 1951 without the patient's permission. Search "Vero cell bioreactor vaccine" and "organoid virology" to go further. This is not for the exam.

3.4 Plant viruses

Plant viruses cause mosaic, leaf curl, yellowing and stunting in crops, and in India they cut the yield of banana, papaya, cotton, tomato and rice. Their biology is shaped by one obstacle: the thick plant cell wall. A plant virus cannot enter a cell by itself, so it needs a wound or a vector, usually an insect, to get in, and special movement proteins to spread from cell to cell. This shorter chapter gives the core that an exam answer needs.

Features and examples

Most plant viruses have + ssRNA genomes. Tobacco mosaic virus (TMV) is a rigid helical rod about 300 nm long and 18 nm wide, with about 2,130 copies of one coat protein wound round an RNA of about 6,400 nucleotides. Cauliflower mosaic virus (CaMV) has dsDNA copied through

RNA (Baltimore group VII), and the geminiviruses (twin icosahedra) have small circular ssDNA genomes. Indian examples worth naming:

- **Banana bunchy top virus**: ssDNA; spread by the banana aphid; leaves bunch at the top.
- **Papaya ringspot virus**: + ssRNA potyvirus; spread by aphids; ring spots on fruit and mosaic on leaves.
- **Cotton leaf curl and tomato leaf curl viruses**: geminiviruses (begomoviruses) spread by the whitefly *Bemisia tabaci*.
- **Rice tungro disease**: two viruses together, spread by green leafhoppers; yellow-orange, stunted plants.

Even smaller are **viroids**: naked circular RNAs of a few hundred nucleotides that code for no protein. Potato spindle tuber viroid, found by Theodor Diener in 1971, has 359 nucleotides.

How plant viruses spread

Between plants, viruses travel by **mechanical contact** (TMV on hands and tools), through **seed** and pollen, by **grafting** and vegetative propagation (banana suckers, potato tubers), and above all by **vectors**: aphids, whiteflies, leafhoppers, thrips, nematodes and soil fungi. Insect transmission is classed by how long the vector stays infective.

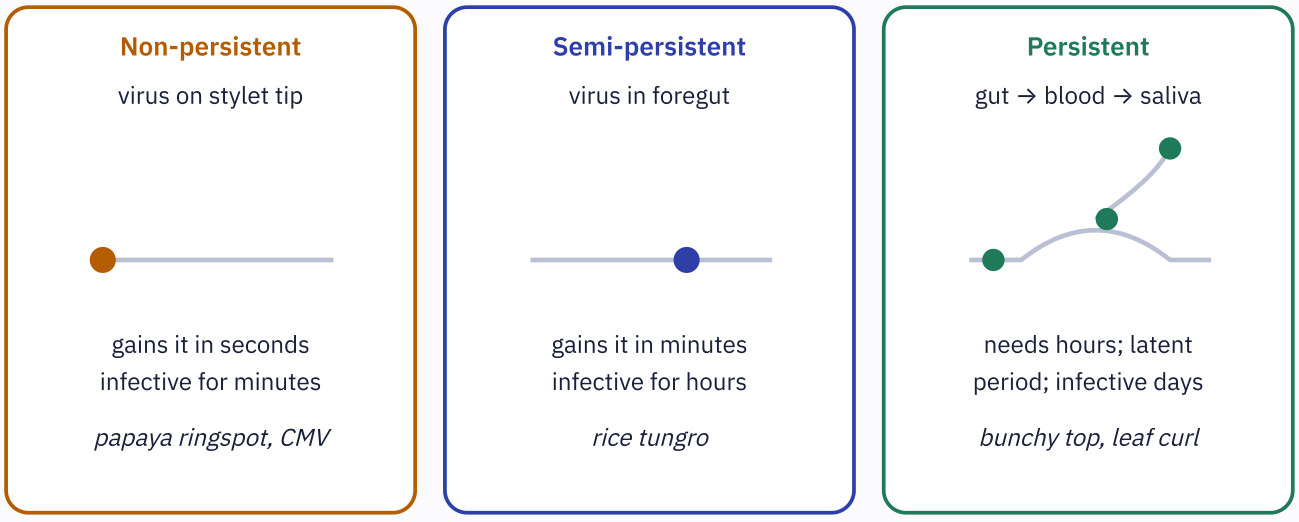


Figure 3.4.1 The three modes of insect transmission. The grey line is the insect's feeding tube from mouthparts (left) inward; the dots show where the virus is held. Notice that the deeper the virus goes into the insect, the longer it takes to pick up and the longer the insect stays infective.

Inside the plant, the virus moves from cell to cell through **plasmodesmata**, the channels that link neighbouring cells. A viral **movement protein** (the 30 kDa protein of TMV is the classic case) widens these channels so the genome can pass. For long-distance spread, the virus enters the

phloem and travels with the sugar flow to young leaves and roots; that is why symptoms often appear first in new leaves.

Diagnosis and control

Plant viruses are detected by symptoms, by **indicator plants** (hosts that show a clear reaction), by ELISA for coat protein, and by PCR or RT-PCR. In the **local lesion assay** (Francis Holmes, 1929), TMV rubbed on leaves of a resistant tobacco such as *Nicotiana glutinosa* gives dead spots, one per infection, which can be counted like plaques (Chapter 3.7).

There are no antiviral sprays for crops, so control is preventive: virus-free planting material (often from **meristem tip culture**, since the growing tip is frequently free of virus), control of vectors, removal of infected plants, resistant varieties, and **cross-protection** (a mild strain protects against a severe one). Genetic engineering added **coat-protein-mediated resistance**: transgenic papaya carrying the papaya ringspot virus coat protein gene has been grown in Hawaii since the late 1990s.

WORKED EXAMPLE

Question (4 marks). Aphids fed on an infected plant for 30 seconds transmit virus P at once, but lose the ability within an hour. Aphids fed for 30 seconds never transmit virus Q; those fed for a day transmit it only after a two-day wait, and then for the rest of their lives. Classify each mode and say which control works better against each.

1. **Virus P:** short acquisition, no waiting, short retention: **non-persistent** (stylet-borne).
2. **Virus Q:** long acquisition, a latent period, long retention: **persistent circulative**.
3. **Control:** insecticides kill too slowly to stop P, because a brief probe is enough; reflective mulches, barrier crops or oil sprays work better. For Q, killing vectors before the latent period ends does help.

EXAM TRAPS

- **Viroid vs virus:** a viroid has no capsid and codes for no protein; do not call it "a small virus".
- Plant viruses cannot "attach to a receptor" on an intact plant cell; the wall blocks them. Entry is through wounds or vectors.
- Do not write that insecticides control all insect-borne plant viruses; they fail for non-persistent ones.

CHECK YOURSELF

- REMEMBER **Name the vector of cotton leaf curl virus.** 1 mark

  The whitefly *Bemisia tabaci*.

- UNDERSTAND **Why do plant viruses need movement proteins?** 2 marks

  The cell wall blocks free passage between cells; movement proteins widen the plasmodesmata so the viral genome can pass to the next cell.

- UNDERSTAND **Why is meristem tip culture used to make virus-free plants?** 2 marks

  The fast-dividing tip has no mature vascular tissue and the virus often has not reached it, so plants grown from it start free of virus.

- APPLY **TMV has about 2,130 coat protein subunits and each covers 3 nucleotides. Estimate the RNA length.** 2 marks

  2,130 × 3 = 6,390 nucleotides, close to the measured length of about 6,400.

- ANALYZE **A farmer sprays insecticide weekly, yet papaya ringspot spreads. Explain and suggest a better plan.** 3 marks

  The virus is non-persistent: an aphid probing for seconds transmits it before the insecticide kills it. Use virus-free seedlings, barrier crops, removal of infected plants, and resistant or transgenic varieties.

BEYOND THE SYLLABUS

The CaMV 35S promoter is one of the most used promoters in plant genetic engineering. Plant viruses are also being used as expression vectors to make vaccines and antibodies in tobacco leaves, and RNA interference sprays that target viral genes are under study. Search "virus-induced gene silencing" and "plant molecular farming". This is not for the exam.

3.5 Oncoviruses

An oncovirus (oncogenic or tumour virus) is a virus that can turn a normal cell into a cancer cell. In 1911 Peyton Rous showed that a cell-free filtrate of a chicken sarcoma caused new tumours in healthy chickens; the agent is now called Rous sarcoma virus. Viruses are linked to a large share of human cancers worldwide, mainly cervical and liver cancers, and some of

these cancers can now be prevented by vaccines. This shorter chapter covers the mechanisms and the human viruses.

How a virus can cause cancer

Cell division is held in balance by two kinds of genes. **Proto-oncogenes** (c-onc) push the cell to divide, like an accelerator. **Tumour suppressor genes**, such as *p53* and *Rb*, stop division or trigger cell death when DNA is damaged, like brakes. A virus causes cancer by pressing the accelerator or cutting the brakes.

1. **Carrying an oncogene.** Acute transforming retroviruses carry a viral oncogene (v-onc), a mutated copy of a host proto-oncogene picked up during evolution. The *src* gene of Rous sarcoma virus was the first. J. Michael Bishop and Harold Varmus showed that *src* came from a normal cell gene, and shared the 1989 Nobel Prize for it.
2. **Insertional activation.** A slow retrovirus with no oncogene integrates its DNA next to a cell proto-oncogene, such as *c-myc*; the strong viral promoter or enhancer then switches that gene on too much.
3. **Viral oncoproteins that block tumour suppressors.** DNA tumour viruses make proteins that bind and disable p53 and Rb. In high-risk human papillomavirus (HPV), the E6 protein marks p53 for destruction and the E7 protein binds Rb, freeing the cell to enter S phase. SV40 large T antigen and adenovirus E1A/E1B act the same way in laboratory cells.
4. **Chronic inflammation.** Hepatitis B and C viruses infect the liver for decades; repeated cell death and regrowth, with inflammation, builds up mutations that lead to liver cancer.

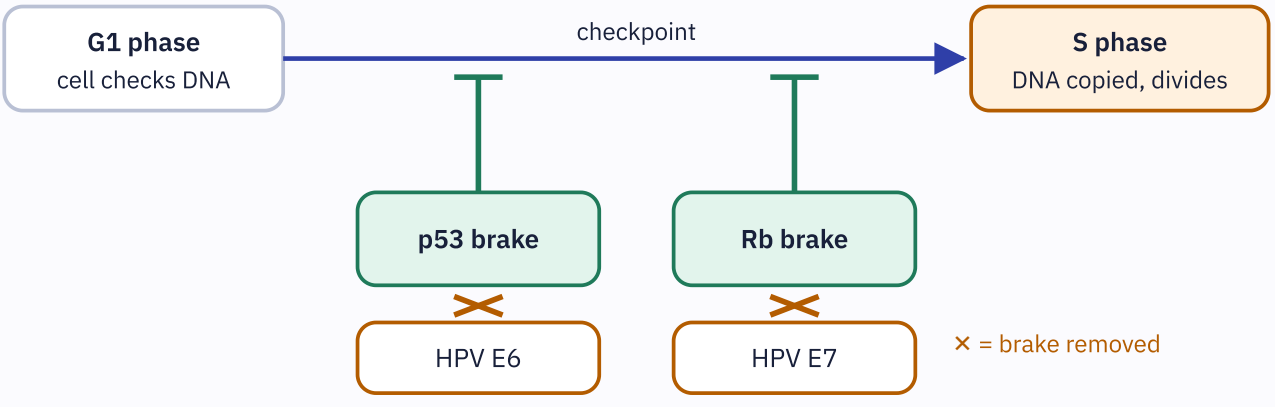


Figure 3.5.1 How high-risk HPV cuts the brakes. p53 and Rb normally block the move from G1 to DNA synthesis (S phase) when something is wrong. E6 removes p53 and E7 disables Rb, so the cell divides with damaged DNA and mutations pile up.

The human oncoviruses

VIRUS	GENOME	MAIN CANCER
Human papillomavirus 16, 18 (HPV)	dsDNA	Cervical cancer; some throat and anal cancers
Hepatitis B virus (HBV)	dsDNA-RT	Hepatocellular carcinoma
Hepatitis C virus (HCV)	+ ssRNA	Hepatocellular carcinoma
Epstein–Barr virus (EBV)	dsDNA (herpes)	Burkitt lymphoma, nasopharyngeal carcinoma
Kaposi sarcoma herpesvirus (HHV-8)	dsDNA (herpes)	Kaposi sarcoma, mainly with HIV
Human T-lymphotropic virus 1 (HTLV-1)	ssRNA-RT	Adult T-cell leukaemia
Merkel cell polyomavirus	dsDNA	Merkel cell carcinoma of skin

Infection alone rarely causes cancer. Most HPV infections clear by themselves; cancer follows only in the few that persist for years, together with other factors. That long gap is what makes screening (Pap smear, HPV DNA test) and vaccination work.

WORKED EXAMPLE

Question (4 marks). Three tumours are studied. Tumour A has the full genome of a retrovirus integrated beside *c-myc*; the virus has no oncogene. Tumour B contains a retrovirus carrying a gene almost identical to a cell kinase gene. Tumour C contains integrated HPV-16 DNA expressing E6 and E7. Name the mechanism in each.

1. **A:** insertional activation; the viral promoter drives *c-myc*.
2. **B:** transduced viral oncogene (v-onc), like *src* in Rous sarcoma virus.
3. **C:** oncoproteins that inactivate tumour suppressors: E6 degrades p53, E7 binds Rb.

EXAM TRAPS

- **v-onc vs c-onc:** c-onc is the normal cell gene (proto-oncogene); v-onc is the viral copy. Normal cells carry c-onc, so having it is not cancer.
- Oncoviruses include RNA and DNA viruses; do not say "only retroviruses cause cancer".
- Write "E6 → p53, E7 → Rb", not the other way round.

CHECK YOURSELF

- REMEMBER **Who first transmitted a tumour by a cell-free filtrate, and when?** 1 mark

  Peyton Rous, 1911, with a chicken sarcoma.

- REMEMBER **Name two viruses linked to liver cancer.** 1 mark

  Hepatitis B virus and hepatitis C virus.

- UNDERSTAND **Why does losing p53 make a cell more likely to become cancerous?** 3 marks

  p53 stops the cell cycle or causes cell death when DNA is damaged. Without it, damaged cells keep dividing and pass on mutations, which build up to cancer.

- APPLY **Explain why an HPV vaccine given at age 10 can prevent a cancer that appears at age 45.** 3 marks

  The vaccine prevents the persistent high-risk HPV infection that must last for years before cancer develops. No persistent infection, no E6/E7 activity, so the chain is broken at the start.

- ANALYZE **Why do acute transforming retroviruses cause tumours in weeks, while slow ones take months?** 3 marks

  Acute viruses carry an oncogene that transforms every infected cell at once. Slow viruses must, by chance, integrate next to a proto-oncogene in some cell, and further mutations are usually needed.

BEYOND THE SYLLABUS

India launched its own quadrivalent HPV vaccine, Cervavac from the Serum Institute of India, in 2023. Oncolytic viruses turn the idea round: engineered viruses that grow in and kill cancer cells, such as the herpes-based talimogene laherparepvec approved for melanoma. Search "oncolytic virotherapy" to go further. This is not for the exam.

3.6 Human viruses: HIV and SARS-CoV-2

Two RNA viruses show every idea of this unit at work. HIV, the human immunodeficiency virus, is a retrovirus that writes its RNA into the DNA of immune cells and slowly destroys the immune system, causing AIDS. SARS-CoV-2, the coronavirus behind COVID-19, is a fast respiratory virus that copies its RNA in the cytoplasm and spreads before symptoms show.

Each step of their life cycles is the target of a drug or a test, which is why examiners love them.

> You need: Baltimore groups IV and VI (Chapter 3.1) and the animal virus life cycle (Chapter 3.3).

HIV: structure

HIV belongs to the family *Retroviridae*, genus *Lentivirus* ("slow virus"). It was identified in 1983 at the Pasteur Institute by Françoise Barré-Sinoussi and Luc Montagnier, who shared the 2008 Nobel Prize. There are two types: HIV-1, the worldwide cause of AIDS, and HIV-2, mainly in West Africa, which progresses more slowly. In India the first cases were found in 1986 in Chennai by Dr Suniti Solomon and her team.

The virion is roughly spherical, about 100 nm across. From outside in:

- A lipid **envelope** from the host cell, carrying spikes of **gp120** (binds the receptor) on **gp41** (anchors in the membrane and drives fusion). The numbers are the protein mass in kilodaltons.
- A **matrix** of p17 protein lining the envelope.
- A cone-shaped **capsid** of p24 protein, holding **two copies** of + ssRNA (about 9.7 kb each) and three enzymes: **reverse transcriptase** (RT), **integrase** and **protease**.

The genome has three main genes, *gag* (core proteins), *pol* (the enzymes) and *env* (envelope glycoproteins), plus regulatory and accessory genes such as *tat*, *rev* and *nef*.

HIV: life cycle

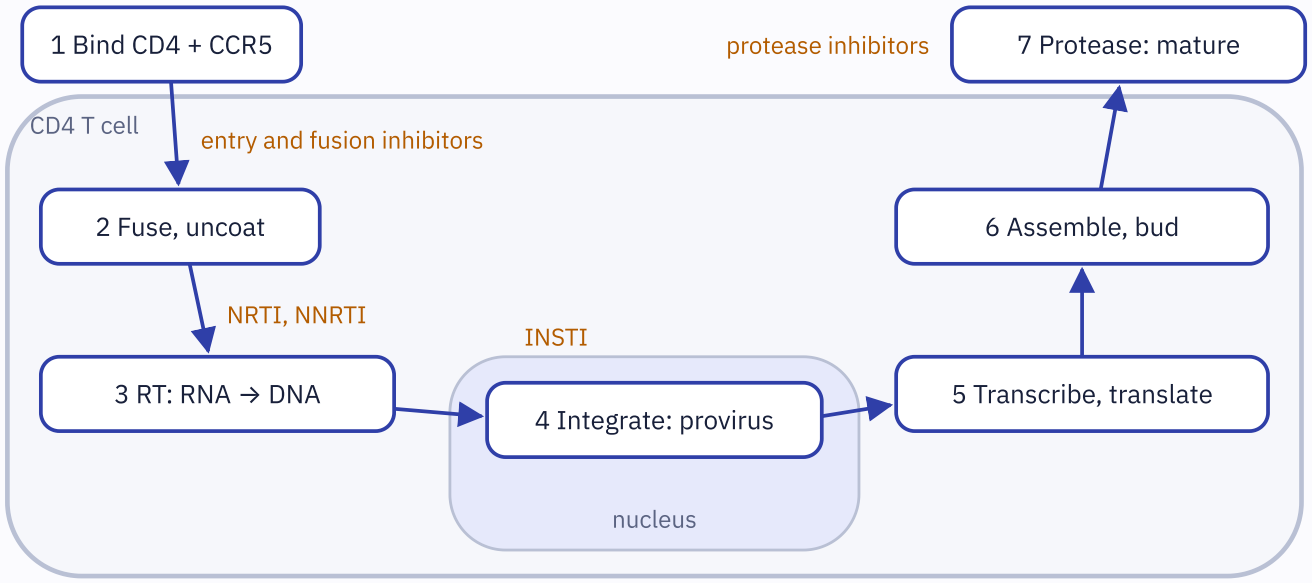


Figure 3.6.1 The HIV life cycle in a CD4 T cell, with the drug class that blocks each step in orange. Notice that the provirus in the nucleus (step 4) is a permanent copy; drugs stop new rounds of infection but cannot remove it, which is why treatment is lifelong.

1. **Binding.** gp120 binds the **CD4** receptor on helper T cells, macrophages and dendritic cells, then a **co-receptor**, CCR5 or CXCR4.
2. **Fusion and uncoating.** gp41 pulls the two membranes together; the core enters the cytoplasm.
3. **Reverse transcription.** RT copies the RNA into dsDNA. RT has no proofreading, so it makes many errors; this is the source of HIV's fast evolution and drug resistance.
4. **Integration.** The DNA enters the nucleus and integrase inserts it into a host chromosome. The integrated DNA is the **provirus**; it can stay silent in resting cells (the latent reservoir).
5. **Expression.** Host RNA polymerase II transcribes the provirus; Tat boosts transcription and Rev exports unspliced RNAs. Gag and Gag-Pol polyproteins are made.
6. **Assembly and budding** at the plasma membrane, taking envelope with gp120/gp41.
7. **Maturation.** Viral protease cuts Gag and Gag-Pol into their final proteins, forming the cone-shaped core. Without this step the particle is not infectious.

Disease and diagnosis. HIV spreads by sexual contact, blood (shared needles, unsafe transfusion) and from mother to child. After an acute flu-like phase, years of clinical latency follow while CD4 T cells fall. AIDS is diagnosed when the CD4 count falls below 200 cells/µL or when opportunistic infections appear, such as tuberculosis, *Pneumocystis* pneumonia and oral candidiasis. Tests detect antibodies (ELISA and rapid tests), antibody plus p24 antigen (fourth-

generation tests) or viral RNA (nucleic acid tests, used for viral load). **Antiretroviral therapy (ART)** combines drugs from different classes, so a virus would need several mutations at once to escape.

SARS-CoV-2: structure and life cycle

The WHO declared COVID-19 a pandemic on 11 March 2020. The virion is enveloped, roughly spherical, about 100 nm, with a crown ("corona") of spikes. Its genome is + ssRNA of 29,903 nucleotides in the reference sequence, among the largest RNA genomes known. Four structural proteins: **S** (spike), **E** (envelope), **M** (membrane) and **N** (nucleocapsid, which coats the RNA).

1. **Attachment:** the receptor-binding domain of the S1 part of the spike binds **ACE2** on cells of the nose, airway and lung.
2. **Entry:** a host protease must cut the spike to expose the fusion machinery of S2: TMPRSS2 at the cell surface, or cathepsins inside endosomes.
3. **Translation:** being + sense, the genome is translated at once into two large polyproteins (pp1a and pp1ab, the second made by a ribosomal frameshift). Two viral proteases, the main protease (Mpro) and the papain-like protease (PLpro), cut them into 16 non-structural proteins.
4. **Replication:** these proteins build a replication complex inside double-membrane vesicles made from the endoplasmic reticulum. The RNA-dependent RNA polymerase (nsp12) makes − strand copies, then new genomes and a set of shorter **subgenomic mRNAs** for the structural proteins. Unusually for an RNA virus, an exonuclease (nsp14) proofreads, which keeps the long genome stable.
5. **Assembly and release:** virions assemble in the ER–Golgi compartment and leave by exocytosis.

Tests target the genome (RT-PCR) or the N protein (rapid antigen tests). Drugs target the polymerase (remdesivir, molnupiravir) and the main protease (nirmatrelvir). India used three kinds of vaccine: Covishield (a chimpanzee adenovirus vector carrying the spike gene), Covaxin (whole inactivated virus, Bharat Biotech with ICMR) and ZyCoV-D (a DNA vaccine).

FEATURE	HIV-1	SARS-COV-2
Baltimore group	VI (ssRNA-RT)	IV (+ ssRNA)
Receptor	CD4 + CCR5 or CXCR4	ACE2 (+ TMPRSS2)
Genome copy made in	Nucleus (as provirus)	Cytoplasm only
Proofreading	No	Yes (nsp14)

FEATURE	HIV-1	SARS-COV-2
Infection	Lifelong	Acute, usually cleared

WORKED EXAMPLE

Question (6 marks). (a) Two RT-PCR samples for SARS-CoV-2 give cycle threshold (Ct) values of 20 and 29. Assuming 100% efficiency (the target doubles every cycle), how many times more viral RNA did the first sample hold? (b) A patient's CD4 count is 150 cells/μL. What does this mean?

1. **(a)** Ct is the cycle at which the signal crosses the threshold; less starting RNA needs more cycles. Difference = 29 − 20 = 9 cycles.
2. Each cycle doubles the target, so the ratio = 2^9 = **512 times more RNA** in the first sample. A *lower* Ct means *more* virus.
3. **(b)** Normal CD4 counts are several hundred to over a thousand cells/μL. Below 200 cells/μL defines AIDS in a person with HIV, with high risk of opportunistic infections; ART and preventive drugs (such as co-trimoxazole) are urgent.

EXAM TRAPS

- HIV is **+ sense** ssRNA but is Baltimore group VI, not IV: its RNA is copied to DNA, not translated directly as the first step.
- **gp120 vs gp41:** gp120 binds CD4; gp41 drives fusion. Swapping them is the commonest slip.
- "ART cures HIV" is wrong: it suppresses replication; the provirus stays.
- **Ct value:** high Ct = little RNA. Students often reverse it.
- ACE2 is the receptor; TMPRSS2 is a protease, not a receptor.

CHECK YOURSELF

– REMEMBER **Name the three enzymes packed in the HIV virion.** 3 marks

Reverse transcriptase, integrase, protease.

– REMEMBER **Name the four structural proteins of SARS-CoV-2.** 2 marks

Spike (S), envelope (E), membrane (M), nucleocapsid (N).

– UNDERSTAND **Why does HIV develop drug resistance quickly, and how does combination ART counter it?** 4 marks

RT has no proofreading, so each round makes mutants; huge numbers of virions are made daily. Some mutants resist one drug. With three drugs of different classes, a virus would need several resistance mutations at once, which is very unlikely.

– UNDERSTAND **Why are people with two copies of a broken CCR5 gene (CCR5-Δ32) highly resistant to most HIV-1?** 2 marks

Most transmitted HIV-1 uses CCR5 as its co-receptor. Without CCR5 on their cells, gp120 cannot complete entry.

– APPLY **Match each drug to its target: dolutegravir, nirmatrelvir, maraviroc, tenofovir.** 4 marks

Dolutegravir: HIV integrase. Nirmatrelvir: SARS-CoV-2 main protease. Maraviroc: CCR5 (blocks HIV entry). Tenofovir: HIV reverse transcriptase (nucleotide analogue).

– APPLY **Ct values of 18 and 24 are seen in two patients. Find the fold difference in RNA.** 2 marks

24 – 18 = 6 cycles; 2^6 = **64-fold** more RNA in the Ct 18 patient.

– ANALYZE **HIV infection lasts for life, while most SARS-CoV-2 infections clear in weeks. Explain using their life cycles.** 4 marks

HIV integrates as a provirus in long-lived resting cells, hiding from immunity and drugs, and it destroys the CD4 T cells that coordinate immunity. SARS-CoV-2 stays in the cytoplasm, makes no DNA copy, and infected airway cells die or are cleared by an intact immune response.

BEYOND THE SYLLABUS

A few people have been cured of HIV after stem cell transplants from CCR5-Δ32 donors, done to treat their cancer; gene editing of CCR5 is being studied as a safer route. Long-acting injectable drugs such as cabotegravir and lenacapavir are changing HIV prevention. For coronaviruses, genomic surveillance (in India, the INSACOG network) tracked variants such as Delta and Omicron, and mRNA vaccine technology won the 2023 Nobel Prize in Physiology or Medicine. Search "HIV latent reservoir" and "SARS-CoV-2 variant surveillance". This is not for the exam.

3.7 Viral quantification assays

To make a vaccine, test an antiviral drug or follow a patient, you must know how much virus a sample holds: its **titre**. Viruses are too small to count under a light microscope, so virologists count what they *do*: holes in a lawn of cells, damaged wells, clumped red cells, or copies of their genome. This chapter gives the main assays and the arithmetic an exam expects.

You need: serial dilution and plate counts (Unit 4, Measuring growth), plaques from Chapter 3.2 and cell culture from Chapter 3.3.

Infectivity assays: plaques and TCID50

Plaque assay. A virus sample is serially diluted, usually tenfold. For phages, each dilution is mixed with host bacteria in soft top agar and poured over a nutrient agar plate (the double-agar overlay). For animal viruses, it is added to a cell monolayer, then covered with agarose or methylcellulose so new virus can only reach neighbouring cells. Each infectious particle starts a round of infection that spreads outward and leaves a clear zone, a **plaque**, in the lawn or monolayer (stained with crystal violet or neutral red). One plaque comes from one infectious unit, so:

```
titre (PFU/mL) = number of plaques ÷ (dilution × volume plated in mL)
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Here PFU means plaque-forming units, the dilution is the fraction (10^{-6} for a millionfold dilution), and only plates with about 30 to 300 plaques are counted: fewer gives large random error, more and the plaques merge.

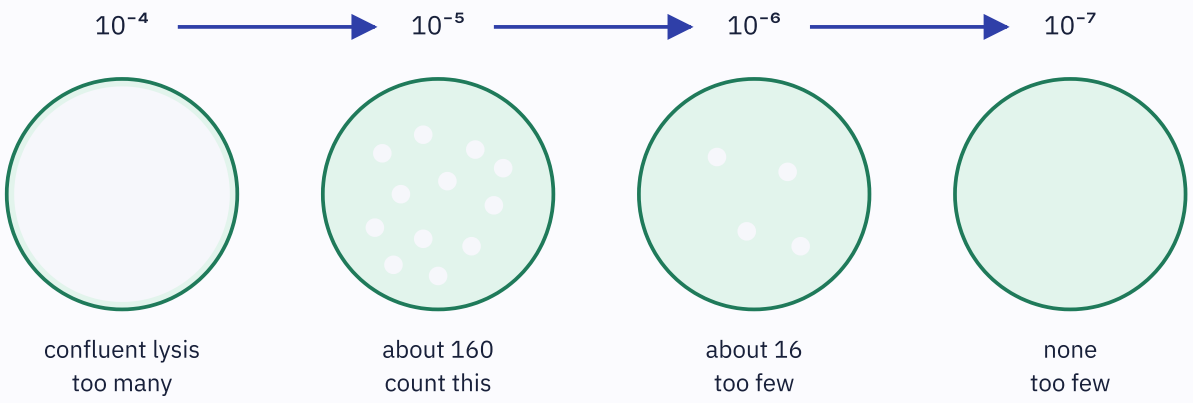


Figure 3.7.1 A plaque assay series (dots stand for plaques; not every plaque drawn). Each tenfold dilution cuts the plaque count tenfold. Only the plate in the 30–300 range is used for the titre; notice that the plate before it is fully cleared.

$TCID_{50}$ (50% tissue culture infectious dose). Some viruses do not form clear plaques. Instead, several wells of cells are inoculated with each dilution, and each well is scored as infected (shows CPE) or not. The $TCID_{50}$ is the dilution that infects half the wells, found by the **Reed–Muench** or the **Spearman–Kärber** method. The same idea in animals gives the LD_{50} (lethal dose) or ID_{50} (infectious dose), and in eggs the EID_{50}. From the Poisson distribution, 1 $TCID_{50}$ holds on average about 0.69 infectious units (ln 2), so PFU ≈ 0.7 × $TCID_{50}$ when both work on the same cells. The **focus-forming assay** is a plaque assay read by antibody staining, for viruses that do not kill cells.

Particle and genome assays

- **Haemagglutination (HA) assay.** Viruses such as influenza clump red blood cells. Twofold dilutions of virus are mixed with red cells in a U-bottom plate: a mat of cells means agglutination; a tight button means none. The **HA titre** is the reciprocal of the highest dilution with full agglutination. It measures particles, infectious or not. Its variant, **haemagglutination inhibition**, measures antibodies.
- **Quantitative PCR (qPCR or RT-qPCR).** Counts genome copies against a standard curve. Fast and sensitive, used for viral load in HIV and hepatitis patients, but it counts dead virus too.
- **Electron microscopy** counts particles directly; **ELISA** measures a viral protein, such as HIV p24.

The **particle-to-PFU ratio** compares total particles with infectious ones. It is usually well above 1, because many particles are defective or never start an infection. Dividing the number of infectious units added by the number of cells gives the **multiplicity of infection** (MOI). With MOI = m, the fraction of cells that get no virus is e^{-m} (Poisson); at m = 1 that is 37%.

WORKED EXAMPLE 1: PLAQUE ASSAY

Question (4 marks). A phage stock is diluted to 10^{-6} and 0.1 mL is plated. Three plates give 148, 162 and 155 plaques. Find the titre.

1. Mean plaques = (148 + 162 + 155) ÷ 3 = 465 ÷ 3 = 155. This is within 30–300, so it is valid.
2. Titre = 155 ÷ (10^{-6} × 0.1 mL) = 155 ÷ 10^{-7} mL = **1.55×10^{9} PFU/mL**.

WORKED EXAMPLE 2: $TCID_{50}$ BY SPEARMAN–KÄRBER

Question (6 marks). Tenfold dilutions 10^{-1} to 10^{-6} of a virus are each put into 8 wells, 0.1 mL per well. Wells with CPE: 8, 8, 8, 6, 2, 0. Find the titre in $TCID_{50}$/mL.

1. Proportions infected: 1, 1, 1, 0.75, 0.25, 0. Their sum S = 4.0.
2. Spearman–Kärber: $\log_{10}$(endpoint dilution) = $x_0 - d(S - 0.5)$, where x_0 = log of the lowest dilution (all wells infected) = −1, and d = log step = 1.
3. = −1 − 1 × (4.0 − 0.5) = −4.5. The 50% endpoint is $10^{-4.5}$: it lies between 10^{-4} (75%) and 10^{-5} (25%), as expected.
4. So 0.1 mL of $10^{-4.5}$ dilution holds 1 $TCID_{50}$; the undiluted stock holds $10^{4.5}$ $TCID_{50}$ per 0.1 mL = $10^{5.5}$ $TCID_{50}$/mL ≈ **3.2×10^{5} $TCID_{50}$/mL**.

EXAM TRAPS

- Divide by the volume plated: forgetting 0.1 mL makes the answer ten times too small.
- PFU counts **infectious** units; HA, qPCR and EM count **particles** or genomes. They do not match.
- $TCID_{50}$ is not a number of particles; it is a dose that infects half the wells.
- Do not use a plate with 5 or 600 plaques for the titre.

CHECK YOURSELF

– REMEMBER **What does PFU stand for, and what is the countable plaque range?** 1 mark

Plaque-forming units; about 30 to 300 plaques per plate.

– UNDERSTAND **Why is an overlay of agarose put on cells in a plaque assay?** 2 marks

It stops new virus spreading through the liquid, so infection can only pass to neighbouring cells and each infectious unit forms one separate plaque.

– APPLY **0.2 mL of a 10^{-5} dilution gives 84 plaques. Find the titre.** 2 marks

$84 \div (10^{-5} \times 0.2) = 84 \div 2 \times 10^{-6} =$ **4.2×10^{7} PFU/mL**.

– APPLY **10^{6} cells are infected with 3×10^{6} PFU. What fraction of cells stay uninfected?** 2 marks

MOI = 3; fraction uninfected = $e^{-3} \approx$ **0.05 (5%)**.

– ANALYZE **A stock has 10^{11} particles/mL by EM but 10^{8} PFU/mL. Interpret this, and say which number a vaccine dose should be based on.** 3 marks

Particle-to-PFU ratio = 1000: most particles are defective or fail to infect. A live vaccine dose is set by infectivity (PFU or $TCID_{50}$), since only infectious units replicate; particle count matters for safety and for non-replicating vaccines.

BEYOND THE SYLLABUS

Digital PCR splits a sample into thousands of droplets and counts positive ones, giving absolute genome numbers without a standard curve, using the same Poisson logic as MOI. Wastewater surveillance used RT-qPCR on city sewage to follow COVID-19 trends, and is now used for other viruses. Search "digital droplet PCR" and "wastewater-based epidemiology". This is not for the exam.

Unit summary

KEY TERMS AND FORMULAS

TERM	MEANING OR FORMULA	CHAPTER
Virion	Complete infectious particle: genome + capsid (+ envelope)	3.1
Icosahedral capsid	20 faces, 12 vertices (pentons), 60 × T subunits	3.1
Baltimore groups	I dsDNA, II ssDNA, III dsRNA, IV +RNA, V –RNA, VI RNA-RT, VII DNA-RT	3.1
Burst size	phages released ÷ infected cells	3.2
Prophage / lysogen	Integrated phage DNA / the bacterium carrying it	3.2
Cytopathic effect	Visible damage to cultured cells by virus	3.3
Movement protein	Widens plasmodesmata for cell-to-cell spread	3.4
v-onc / c-onc	Viral oncogene / normal cell proto-oncogene	3.5
Provirus	HIV DNA integrated in the host chromosome	3.6
Ct fold change	$2^{\Delta Ct}$ (at 100% PCR efficiency)	3.6
Plaque titre	PFU/mL = plaques ÷ (dilution × mL plated)	3.7
Spearman–Kärber	log endpoint = $x_0 - d(S - 0.5)$	3.7
Uninfected fraction	e^{-MOI}	3.7

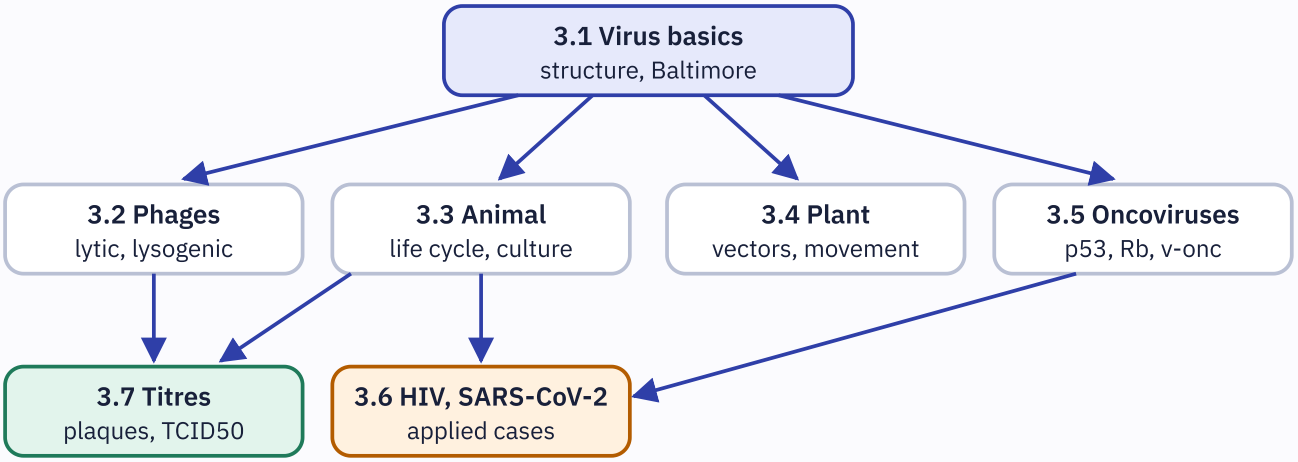


Figure 3.S.1 How the chapters connect. Chapter 3.1 feeds all the others; phages and animal-cell culture both lead to counting virus (3.7); the animal virus life cycle and oncoviruses (via HIV-related cancers such as Kaposi sarcoma) lead to the two human case studies (3.6).

1. A virus is one kind of nucleic acid in a protein capsid, sometimes enveloped; it multiplies only inside living cells.
2. Capsids are helical, icosahedral or complex; every icosahedral capsid has 12 pentons.
3. The Baltimore classification sorts viruses into seven groups by the route from genome to mRNA.
4. Virulent phages are lytic only; temperate phages such as λ can integrate as a prophage, controlled by the cI repressor and induced by DNA damage.
5. Animal viruses enter whole and must uncoat; enveloped ones leave by budding.
6. Viruses are grown in animals, embryonated eggs (four routes) or cell cultures, and detected by CPE, haemadsorption or molecular tests.
7. Plant viruses need wounds or vectors to enter and movement proteins to spread; control is preventive.
8. Oncoviruses act by carrying oncogenes, activating proto-oncogenes, or disabling p53 and Rb.
9. HIV integrates as a provirus in CD4 cells; SARS-CoV-2 replicates in the cytoplasm after binding ACE2.
10. Titres come from plaques (PFU), $TCID_{50}$, haemagglutination or genome copies; only infectivity assays count live virus.

Exam question bank

Remember and understand

▼ 1. Describe the general properties of viruses and the structure of a virion, with a labelled diagram. (10 marks)

Lwoff's properties (one nucleic acid, no metabolism, obligate parasite, assembly from parts, size 20–300 nm, filterable, not affected by antibiotics); virion parts: genome, capsid, capsomeres, nucleocapsid, envelope and spikes, enzymes; three symmetries with examples; diagram of an enveloped icosahedral virus.

▼ 2. Explain the Baltimore classification with an example for each group. (10 marks)

Basis: route to mRNA; seven groups with examples; + vs – sense; why – RNA viruses carry polymerase; RT groups; diagram as in Figure 3.1.1; compare briefly with ICTV ranks.

▼ 3. Describe the structure of bacteriophage T4 and its lytic cycle. (12 marks)

Labelled diagram (head, collar, sheath, core, baseplate, pins, fibres); adsorption, penetration, early genes and host DNA breakdown, replication, late proteins, assembly, holin–endolysin lysis; one-step growth curve with eclipse, latent period and burst size.

▼ 4. Explain lysogeny in phage λ. What are prophage induction, immunity and lysogenic conversion? (12 marks)

Temperate phage; cos circularisation; cI vs Cro switch; integrase, attP × attB; prophage, lysogen; immunity to superinfection; UV, RecA, cI cleavage, excision with Xis; conversion examples: diphtheria, cholera and Shiga toxins.

▼ 5. Describe the methods of cultivating animal viruses. (12 marks)

Animals (suckling mice); embryonated egg with four routes and uses, labelled diagram; cell culture: primary, diploid strain, continuous line with examples; detection by CPE, haemadsorption, interference, immunofluorescence; merits and limits of each.

Apply

▼ 6. Describe the structure and life cycle of HIV and relate each step to a class of antiretroviral drug. (14 marks)

Virion parts and genes; seven steps; drug classes: entry/CCR5 inhibitors, fusion inhibitors, NRTI and NNRTI, INSTI, protease inhibitors; why combination therapy; course of infection, CD4 count, diagnosis.

▼ 7. Explain the entry and replication of SARS-CoV-2 and how its tests, drugs and vaccines target these steps. (12 marks)

Structure, S/E/M/N; ACE2 and TMPRSS2; polyproteins, Mpro and PLpro; replication complex, subgenomic mRNAs, proofreading; assembly and exocytosis; RT-PCR and Ct, antigen tests; remdesivir, nirmatrelvir; Covishield, Covaxin, ZyCoV-D.

▼ 8. A phage stock diluted 10^{-7} gives 210 plaques from 0.1 mL; an animal virus gives the $TCID_{50}$ data 8, 8, 7, 3, 0 wells of 8 at 10^{-2} to 10^{-6} (0.1 mL per well). Calculate both titres and explain each assay. (10 marks)

Phage: $210 \div (10^{-7} \times 0.1) = 2.1 \times 10^{10}$ PFU/mL. $TCID_{50}$: proportions 1, 1, 0.875, 0.375, 0; S = 3.25; log endpoint = −2 − (3.25 − 0.5) = −4.75; titre = $10^{5.75}$ $TCID_{50}$/mL ≈ 5.6×10^5. Explain overlay, countable range, CPE scoring and the 50% endpoint.

▼ 9. Describe the transmission of plant viruses by insect vectors and suggest control measures for a whitefly-borne leaf curl disease of tomato. (10 marks)

Non-persistent, semi-persistent, persistent (circulative, propagative) with features and examples; whitefly–begomovirus is persistent; control: virus-free seedlings in insect-proof nurseries, vector control, resistant varieties, removal of infected plants, crop-free periods.

Analyze and evaluate

▼ 10. Compare the multiplication of a bacteriophage with that of an enveloped animal virus. (10 marks)

Spans Chapters 3.2, 3.3 and 3.6. Attachment (tail fibres vs glycoprotein spikes); entry (injection vs endocytosis or fusion); uncoating (not needed vs needed); site of replication (cytoplasm vs nucleus or cytoplasm); release (lysis by holin–endolysin vs budding); latency (lysogeny vs provirus or episome); a table makes this clear.

▼ 11. Compare lysogeny in phage λ with HIV latency. (10 marks)

Spans Chapters 3.2 and 3.6. Both integrate (integrase, specific site vs near-random site); both silent and inherited by daughter cells; λ kept off by a repressor, HIV by the resting state of the cell; induction by DNA damage vs T-cell activation; consequences: immunity to superinfection vs lifelong infection and need for lifelong ART.

▼ 12. Discuss how viruses cause cancer, with examples of human oncoviruses, and evaluate vaccination as cancer prevention. (12 marks)

Proto-oncogenes and tumour suppressors; v-onc (Rous, *src*), insertional activation, E6/E7 vs p53/Rb, chronic inflammation; table of human oncoviruses; HPV and HBV vaccines prevent infection years before cancer; limits: needs coverage before exposure, does not treat existing infection.

▼ 13. A sample of influenza virus has an HA titre of 1024, 10^9 genome copies/mL by RT-qPCR and 10^7 PFU/mL. Explain why the three numbers differ and which one you would use for (a) a vaccine potency test, (b) infecting cells at MOI 1. (10 marks)

HA counts particles that bind red cells, qPCR counts genomes including defective and dead particles, PFU counts infectious units; particle-to-PFU ratio above 1. (a) For an inactivated vaccine, antigen content (HA protein) matters; for live vaccines, infectivity. (b) Use PFU to set MOI.

▼ 14. "Viruses are at the border of living and non-living." Evaluate this statement. (8 marks)

For life: genes, mutation and evolution, replication, host specificity. Against: no metabolism or ribosomes, inert and crystallisable (Stanley's TMV), no division. Viroids and prions push the border further; giant viruses blur it from the other side. A reasoned conclusion.

Glossary

ACE2	Angiotensin-converting enzyme 2; the cell receptor for SARS-CoV-2.
Burst size	Average number of virions released from one infected cell.
Capsid	Protein shell around the viral genome.
Capsomere	Visible structural unit of a capsid.
CD4	Surface protein of helper T cells; the main receptor for HIV.
Cytopathic effect	Visible damage to cells caused by virus growth.
Eclipse period	Time after infection when no infectious virions exist inside the cell.
Embryonated egg	Fertilised hen's egg with a developing embryo, used to grow viruses.
Envelope	Lipid membrane round some virions, taken from the host cell.
Haemadsorption	Red cells sticking to virus-infected cells in culture.
Haemagglutination	Clumping of red cells by virus particles.
Induction	Switching a prophage from lysogeny into the lytic cycle.
Latent period	Time from infection until progeny virus is released.
Lysogen	A bacterium carrying a prophage.
Lysogenic conversion	Change in a host's properties caused by prophage genes.
MOI	Multiplicity of infection: infectious units added per cell.
Movement protein	Plant virus protein that lets the genome pass through plasmodesmata.
Oncovirus	A virus that can cause cancer.
Plaque	Clear zone in a cell lawn or monolayer made by virus from one infectious unit.
Prion	Misfolded infectious protein with no nucleic acid.
Prophage	Phage genome integrated into the bacterial chromosome.

Provirus	Retroviral DNA integrated into the host chromosome.
Reverse transcriptase	Enzyme that makes DNA from an RNA template.
Syncytium	Giant cell with many nuclei, formed by fusion of infected cells.
$TCID_{50}$	Dose that infects 50% of inoculated cell cultures.
Temperate phage	Phage that can follow either the lytic or the lysogenic cycle.
Viroid	Small naked circular RNA that infects plants and codes for no protein.
Virion	Complete, infectious virus particle.

Sources and further reading

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