Edexcel IAL Biology A Level

Topic 6: Microbiology, Immunity and Forensics

Notes

Culturing microorganisms

Culturing microorganisms is an important part of **experimental biology** and understanding how things work at a **molecular level.** To study microorganisms safely, they must first be grown in the lab on one of two types of culture - a **pure culture** containing one type of microorganism only, or a **mixed culture** containing a mix of species.

Growing microorganisms requires **aseptic technique** which means **free from contamination.** Various types of **bacteria and fungal spores** are present in the air and are an unwanted presence when trying to grow a specific microorganism only, since they will compete for **nutrients, space and oxygen** and reduce the yield of the desired culture. Various aseptic techniques have been developed, such as:

- Buying **sterile equipment** or sterilising reusable equipment with a **bunsen burner flame** and **ethanol**.
- Cleaning **surfaces** before and after with **ethanol**.
- Using a **bunsen burner** or other flame to heat the air, causing it to rise and carry away **airborne microorganisms.** The same can be done to bottles or flasks to remove contaminants.

There are 2 different types of culturing - **batch and continuous.** In batch culture, the fermentation is carried out in a closed fermenter. The microorganisms and nutrients are added and then left to grow for a particular period of time. No further nutrients are added, and products are removed at the end of the period. Whereas continuous culture takes place in an **open fermenter**, where nutrients are **continuously added** and products are removed at a steady rate. Even though the batch culture is easier to set up and maintain that the continuous culture, the growth rate isn't as fast. However, in the case of contamination of batch culture, only a single batch is lost whereas in the case of continuous culture, it can lead to huge amount of product lost.

To maximise the yield of product, the **temperature** needs to be maintained at the optimum with a **sufficient nutrient supply** and the **aerobic conditions** to prevent the formation of undesired products through anaerobic respiration. The **pH** needs to be kept constant to ensure that the **enzyme activity** is not altered.

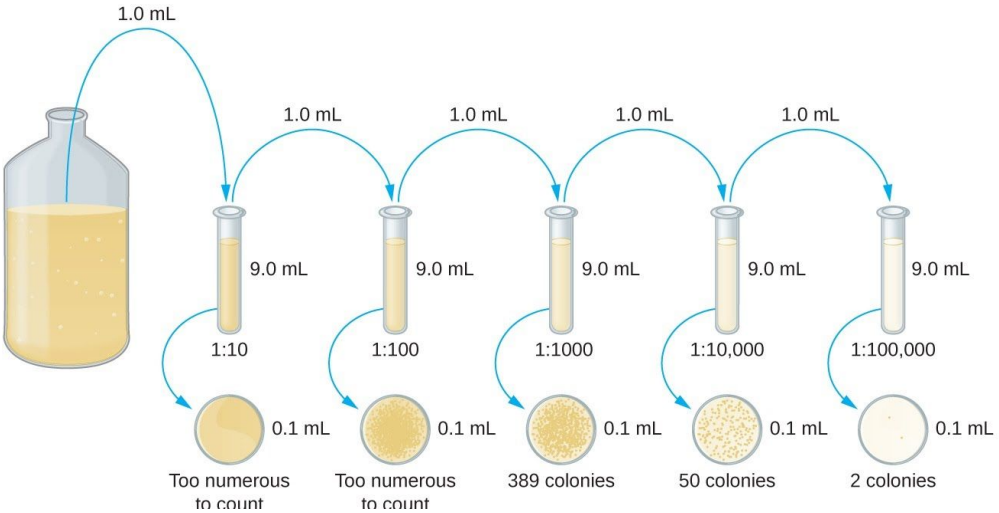
Measuring the growth of cultures

Growth of the culture can be **monitored** once the right environment for the microorganism has been set up. Continual monitoring and recording can be used to plot a **bacterial growth curve.** There are several ways to monitor growth:

- **Cell counts** - This involves pipetting a small sample of the liquid culture onto a slide with a grid on it. The number of cells for a given volume of sample can be **counted and then multiplied up,** to give a prediction for the number of cells in the whole volume of culture.

- **Dilution plating** - This involves mixing known volumes of a culture with a sterile liquid at different ratios to produce a **series of dilutions.** The resulting mixes are left to grow on

agar plates and then counted; some plates will have **too many to count** whereas others will have **no** microorganisms. Using the values counted, you can work back from an **easily-counted plate** using its **dilution factor** to calculate the original number of microorganisms in the sample.

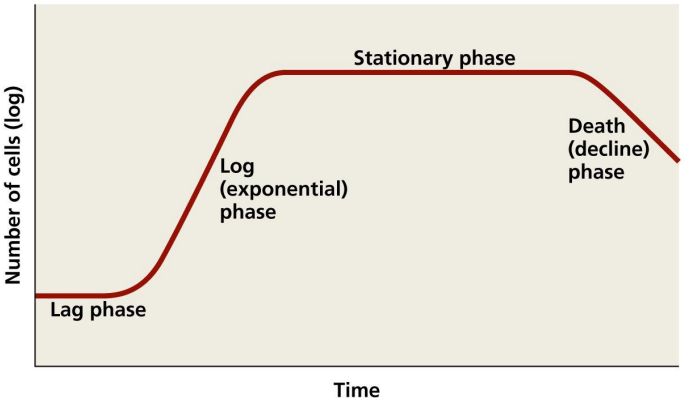


- **Mass** - A **known volume** can be sampled from a liquid culture, placed in a centrifuge and spun till only the **solid mass of cells** remain. They can then be weighed; **the mass then multiplied** to find the total mass of the culture.

- **Turbidity** - The most common method of measuring the growth of cells is this. As cultures grow, the plate becomes more and more **turbid** (cloudy and increasingly opaque), so as more cells grow, **less light can pass through.** Small samples can be taken and placed in a cuvette where a **spectrophotometer** records the amount of light passing through, giving a measurement of size of the culture.

Phases of a bacterial growth curve

1. The first phase of microorganism growth is the **lag phase** where microorganisms are adjusting to the environment before starting to reproduce, thus meaning during the lag phase **the population remains constant**.



2. The next part of the growth curve is the **log phase** where the population size grows **exponentially** meaning that every round of division doubles the population size, so long as the dividing organism has a sufficient amount of nutrients.

3. **The stationary phase** is where the population size reaches its **maximum** due to **decreasing nutrient levels** and build of up toxic substances.

4. The stationary phase is followed by **decline phase** where lack of nutrients and increase in **toxic** products causes **death** of organisms.

Bacteria and viruses

Bacteria and viruses are the main disease causing **pathogens** in humans. Even though they both cause disease, they vary in many ways. Their differences are as following:

- Bacteria can reproduce in **many places,** whereas viruses can only reproduce inside a **host cell**
- Bacteria contain **ribosomes**, viruses do not.
- Bacteria are **living** single-celled microorganisms, while viruses are technically **not living** as they require other living cells to function.
- The structure of viruses is much **simpler** than bacteria.
- Viruses are around **1 hundredth of the size** of bacteria.
- Bacteria reproduce in a process known as **binary fission,** whereas viruses can only replicate their proteins by invading cells known as host cells, which transcribe and translate **the viral genetic information into proteins.**

Viral structure

- **Genetic material** - DNA or RNA
- **Capsid** - A **protein coat** surrounding the genetic material
- **Attachment proteins** - they **stick out from the capsid** and are used to attach to host cells
- **Enzymes** - many viruses contain an enzyme called **reverse transcriptase** which is used to convert their **RNA into DNA,** which can be inserted into the host cell's genome so viral proteins are transcribed.

Life cycle of a virus

There are 2 different life cycles of viruses that infect bacteria, the **lytic cycle** and the **lysogenic cycle.** The lytic cycle is where viruses infect their host cells and immediately insert their genetic information into the host's DNA so the viral proteins are made. As more and more viral proteins are made they eventually burst from the cell in a process known as **lysis,** where the cell membrane is **ruptured and the cell destroyed.**

The second life cycle is the **lysogenic cycle**; during this, the virus enters the host cycle and implants its genetic information again into the genome, but remains **dormant and latent** and instead allows the bacteria to replicate and produce many copies of bacteria containing the viral DNA. In certain conditions, the viral DNA leaves the main genome to form a **plasmid** which is only then translated and transcribed, from which **lysis** later occurs.

Key examples

Ebola virus

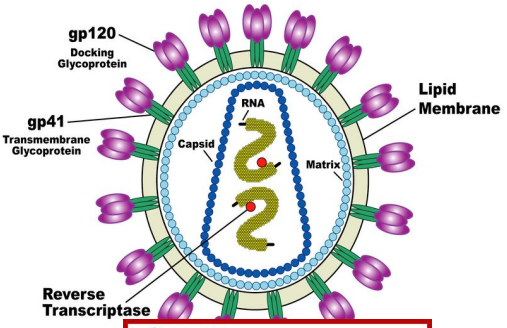
The ebola virus causes a severe and often **fatal fever** and was the cause of a serious epidemic in West Africa between 2013-2015. The virus is transferred via **bodily fluids** and its genetic information is in the form of **RNA**.

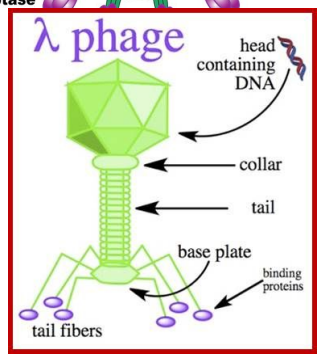
Tobacco mosaic virus (TMV)

This was the first plant virus to be discovered and infects the **chloroplasts**, discolouring the leaves from green to yellow or white forming a **mosaic pattern.** It is spread between plants naturally or through contact from farmers. It also causes the leaves to curl up, reducing their surface area and the leaves' ability to photosynthesise, thus reducing the plant's growth. TMV is a type of **lytic virus.**

Human immunodeficiency virus (HIV)

The HIV virus targets **T helper cells** which are part of the immune system, once the T helper cells are activated the virus replicates itself and eventually **bursts the cells**, causing cell damage and death. This leads to a weakened immune response and is when HIV develops into **AIDS** where the body can't defend against simple infections. Symptoms of AIDS include **weight loss, diarrhoea, dementia, cancers and opportunistic infections such as TB** and can result in death. HIV is a type of latent virus that undergoes the **lysogenic life cycle.**





Lambda phage

A lambda phage is a type of **bacteriophage** - **a virus that infects bacterial cells.** This bacteriophage infects the bacteria **E. coli**, which is a bacteria found in human intestines, normally harmless but can cause food poisoning. The lambda phage structure consists of a **head, tail and tail fibres,** the bacteriophage shape differing from the shapes of viruses that infect humans and mammals.

Tuberculosis

An example of a bacterial disease is **tuberculosis** also known as TB. TB is caused by a bacteria called **Mycobacterium tuberculosis** which infects **phagocytes** in the lungs. The first infection is symptomless as the infected phagocytes are sealed in **tubercles** as a result of **inflammatory response** in the lungs. However, the bacteria lie dormant inside the tubercles as they are not destroyed by the immune system as tubercles are covered with a **thick waxy coat**. When the immune system becomes weakened, the bacteria become active again and slowly destroy the lung tissue thus leading to **breathing problems, coughing, weight loss as well as fever.** TB can potentially lead to death.

Immunity

How pathogens can enter the body

There are many ways disease-causing microorganisms can enter the body but luckily the body has a number of **physical and chemical defences** to stop them entering in the first place and then ways to destroy any that do enter. Ways pathogens could enter include:

- **Inhalation** - Coughing, talking and sneezing are all means through which pathogens can pass into the **respiratory tract** and further.
- **Ingestion** - Eating **food contaminated with pathogens** can cause illnesses like salmonella.
- **Direct contact** - By touching skin to skin or through **bodily fluids**, for example during sexual contact. This is how a most sexually transmitted infections are spread.
- **Vector** - This is an organism that **carries infection,** such as mosquitoes which carry malaria.
- **Fomites** - These are **inanimate objects** like dust that might land on the skin, eyes, mouth etc that are **contaminated with pathogens.**

Physical barriers that protect the body from infection include:

- **Skin** is a tough physical barrier consisting of **keratin**
- **Stomach Acid** (hydrochloric acid) which kills bacteria
- **Gut and skin flora** – natural bacterial flora competes with pathogens for food and space
- **Cough reflex**
- **Mucus** in the respiratory tract that **traps pathogens**

Once a pathogen has got through the **physical barriers** and into the body it has 2 lines of defence - **non-specific** which destroys anything with foreign antigens, and the **specific response** which is specific to the antigens on the pathogen infecting the body. All cells, toxins and viruses have **antigens made of protein** on their surface which enables the body to recognise whether something in the body is supposed to be there or not. If it isn't, it will have foreign antigens that the white blood cells won't recognise, so **an immune response is activated.**

Non-specific response

- **Inflammation** – when a tissue becomes damaged, for instance by bacteria, chemicals including **histamines** are released. This causes blood **vasodilation** of blood vessels which increases the flow of blood to the infected area and increases **permeability of blood vessels.** As a result of that **antibodies, white blood cells and plasma** leak out into the infected tissue and destroy the pathogen.

- **Lysozyme action** – lysozyme is an enzyme found in secretions such as **tears and mucus** which **kills bacterial cells** by damaging their **cell wall.**

- **Interferon** – interferons are **proteins** that **prevent viruses spreading to uninfected cells** by stopping protein synthesis in viruses, so stopping their replication.

- **Phagocytosis** - a process in which a type of white blood cell known as a **phagocyte** engulfs pathogens. The pathogen is isolated in the phagocyte, in a **phagocytic vesicle** which **lysosomes** release **lysozymes** in to digest the pathogen, destroying it.

Specific response

After the pathogen is **engulfed and destroyed,** its chemical markers called **antigens** are then presented on the surface of the **phagocyte**. The phagocyte then becomes an **antigen presenting cell** (known as a **macrophage**) which actives other types of immune systems. There are 2 main types of white blood cells, which are involved in the different responses:

1. **B cells** mature in the bone marrow and are involved in the **humoral response.**
2. **T cells** move from the bone marrow to the **thymus gland** where they mature, they are involved in the **cell mediated response.**

Specific immune response glossary:

- **Memory cells** are cells which replicate themselves when exposed to an invading pathogen and remain in the lymph nodes searching for the same antigen, thus resulting in a much **faster immune response.** They allow **long term immunity.**
- **B effector** cells (also known as plasma cells) are **antibody producing** cells. When the correct antibody is produced to fit the antigen on the pathogen, the antibody divides by mitosis in order to multiply so that the infection can be prevented. This is called **clonal selection** as the antibody clones itself.
- **T helper** cells **stimulate B cells** and **T killer cells to divide.**
- **T killer** cells **destroy pathogen** infected cells by creating holes its cell surface membrane, causing it to burst.
- **Memory T cell** - remembers **antigens** for future infection.

T cell response (cellular response)

1. T cells each have different **receptors**, when a T cell with receptors **complementary** to the antigens is presented by an **antigen-presenting cell**, it becomes **activated**.
2. The T cell divides by **mitosis** rapidly to produce many copies.
3. T helper cells stimulate **T killer cells and B cells**, therefore activating the humoral **B cell response.**

B cell response (humoral response)

1. **Complementary** B cells encounter and **bind to antigens on pathogens** and become **activated**. They digest the pathogen and present its antigens, allowing T cells to be activated.
2. They **proliferate and divide** to produce **plasma cells or memory cells.**
3. These cells produce **antibodies** with a shape complementary to the pathogen's antigens. The antibodies are released into the blood, hence why it is a **humoral response.**

4. The **memory cells** remain in the blood to initiate a **fast response** if the same pathogen infects the body again.

Types of immunity

There are 2 types of immunity - **active and passive** and each can be obtained **naturally or artificially.**

Active immunity is where the body **physically produces antibodies and memory cells.** This means it takes a while for immunity to develop, requires exposure to antigens and the immunity is **long-term** since memory cells will recognise antigens upon future infection.

- **Natural** active immunity - where the person has come across antigens naturally, by **catching the infection**
- **Artificial** active immunity - would be gained after a **vaccination** where antigens otherwise wouldn't naturally enter the body.

Passive immunity occurs when **the body doesn't make antibodies** against a pathogen but has immunity anyway by receiving immunity from another source. It doesn't require exposure to antigens, it is **short-lasting** as no memory cells are made and gives **immediate protection**.

- **Natural** passive immunity - antibodies being passed to a foetus / baby through the **placenta / breast milk.**
- **Artificial** passive immunity - being **injected** with antibodies.

Antibiotics

Antibiotics are **chemicals** that can also be used to **fight infection by killing the bacteria and stopping their growth**. There are two types of antibiotics:

- **Bactericidal** antibiotics **kill bacteria** by destroying their cell wall thus causing them to burst.
- **Bacteriostatic** antibiotics which **inhibit the growth of bacteria by stopping protein synthesis and production of nucleic acids** so the bacteria can't grow and divide.

Evolutionary race between pathogen and host cell

As organisms have **evolved** over time to defend against infection from pathogens, they too have evolved to **evade the immune system.** For instance, the virus HIV has a **high mutation rate** which cause its pathogens to change, meaning every infection requires a **new primary immune response** even if the person has had it before, since the antigens have changed so **aren't recognised by memory cells.**

Furthermore, mutations may arise in bacteria that make them **immune to antibiotics;** antibiotics provide a **selection pressure,** so bacteria who are immune to them have a selective advantage and are more likely to survive, reproduce and pass on immunity to future generations. Over time the **advantageous allele** for immunity would increase in frequency creating a **resistant strain**, this would happen relatively quickly in bacteria as they reproduce up to once every 20 minutes, so advantageous alleles get passed on rapidly.

Resistance in hospitals

Resistance to antibiotics results in antibiotic resistant to bacterial infections in hospitals such as **MRSA.** Hospitals have developed various ways of **controlling the spread of antibiotic resistant infections**, for example:

- New patients are **screened** at arrival, **isolated** and **treated** if they are infected to prevent the spread of bacteria between patients
- Antibiotics are **only used when needed** and their course is **completed** to ensure that all the bacteria are destroyed and to **minimise the selection pressure** on bacteria to prevent resistant strains from forming
- All staff must follow the **code of practice** which includes **strict hygiene regimes,** such as **washing hands** with alcohol based antibacterial gels and **wearing suitable clothing** which minimises the transmission of resistant bacteria

Decomposition and recycling of carbon

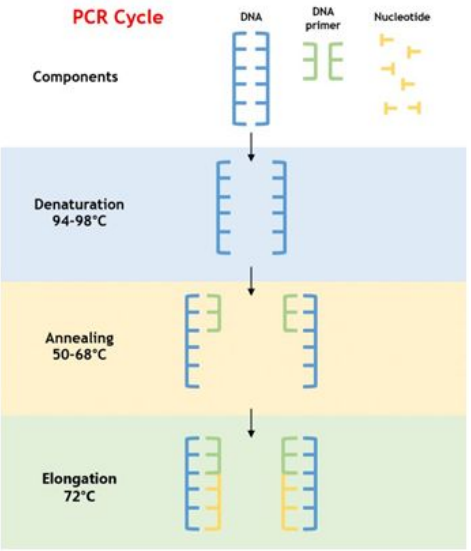
Microorganisms play an important role in **breaking down organic matter** and **returning inorganic ions and carbon to the environment** for use by other organisms. Decomposition consists of 2 main parts - **autolysis** where the body's own enzymes digest and break down tissues and **putrefaction** where microorganisms, such as bacteria and fungi, break down the remaining dead tissue.

Very quickly microorganisms can break down **large amounts of tissue**, forming **gas and decomposition fluid** which leaves the body through **orifices,** like the mouth and nose. The decomposers obtain many nutrients from the body, allowing lots of respiration and leading to their multiplication and further decomposition.

The **inorganic ions** released by the decomposition process are **returned to the soil**, where they are **assimilated** into plants and the carbon taken in by decomposers is released to the atmosphere as they respire. The CO_2 released is then taken in by plants via **photosynthesis** where the carbon is converted into **biomass** in the plant; this then moves through the food chain as the plants are eaten by animals, which are in turn either eaten themselves or die, where the **decomposition cycle** begins again.

Gene technology

Gene technology such as **genome sequencing and genetic engineering** have a lot of potential to advance science and medical treatment in the future, but before DNA can be changed it must first be **amplified** so there is enough of it to work with. A common **in vitro technique** of amplifying DNA is through the **polymerase chain reaction (PCR)**, it occurs as follows:



1. A reaction mixture is set up by mixing the **DNA sample, primers, free nucleotides and DNA polymerase** which is the enzyme involved in creating new DNA strands.
2. The mixture is then heated to **95 degrees** to break the **hydrogen bonds** and to separate the two strands.
3. The mixture is then cooled to a temperature between **50-65 degrees** depending on the type of primers used, so that they can **bind** to the strands.
4. Temperature is increased to about **70 degrees,** as this is the optimum temperature for **DNA polymerase** to work at.
5. **DNA polymerase** creates a copy of the sample by **complementary base pairing** using the free nucleotides and it bonds the nucleotides together, forming the **sugar-phosphate backbone.**
6. This cycle is repeated around **30 times** to produce enough DNA.

Gel electrophoresis

Gel electrophoresis is a process used to **separate the DNA fragments** according to their size using an **electric current**. It occurs as following:

1. The **amplified DNA** is placed in a **slab of gel** and smothered in a **conductive buffer solution** through which a current is passed.
2. DNA is **negatively charged** so moves through the gel towards the **positive cathode** at the other end of the gel.
3. **Shorter fragments of DNA travel faster** and therefore further, so the DNA pieces are **separated according to size.**
4. The position of DNA across the slab is **unique** for each person, giving a **genetic fingerprint.**

Genetic fingerprints are unique because people have different numbers of **variable number tandem repeats** (VNTRs, these are repeats of certain sequences of bases – 1 person may have 10 repeats of CAG over and over, another person may have 50 repeats) at various positions across their genome, making parts of their DNA different lengths. **The chances of 2 individuals having the same VNTRs at all locations is so slim** that the genetic fingerprint produced from electrophoresis and separation of the fragments can be used to **identify people,** such as linking DNA found at a crime scene to a person.

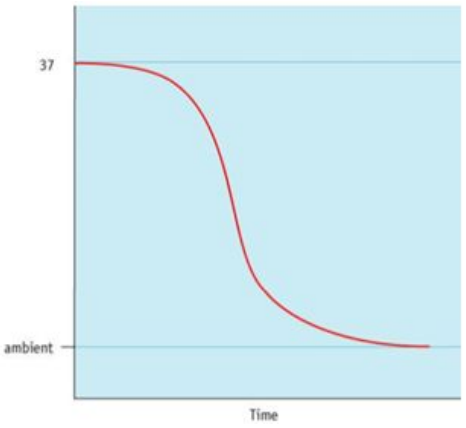
Comparing genetic fingerprints can also be used to determine **genetic relationships**, such as with **paternity tests.** An individual will share roughly **half** of the same VNTRs as their parents or children, with less and less VNTRs in common with more distant relationships, for instance cousins.

Breeding programmes can also compare **genetic fingerprints** to ensure they are not breeding closely-related family members, as this can increase the risk of **genetic disorders** and **limit genetic variation.**

Estimating time of death

How long an organism has been dead can be estimated by looking at the following things:

- **Stage of succession** – There are 5 main stages of decomposition (**fresh, putrefaction, fermentation, dry decay and skeletonization**) that occur after death, each attracting **different organisms** that come and feed on the organism. The different stages can occur over days, weeks and years; meaning analysis of succession can aid estimating the time of death of even **long-dead** organisms.



- **Body temperature** – body temperature decreases over the first **24 hours** in the following shaped curve. The environment the body was found in must also be considered, since if found in **water** or where there is **air movement** it speeds cooling while **clothing** and **being indoors** slows cooling.

- **Degree of muscle contraction** – **rigor mortis** is the process where the body **muscles contract after death.** The myosin and actin cross bridges **can't unbind** as this requires ATP, which is no longer made after death. Small muscles contract first, followed by larger ones. **36 hours after death** the muscles begin to **unstiffen** as muscle fibres are broken down, the smaller muscles are the last to unstiffen.

- **Forensic entomology** – This is **the study of the insects** found in the body. Maggots can also be used – by observing their length and the temperature they're found at, also looking at what stage of their **life cycle** they are and finally by taking some maggots and continuing to keep them in the lab until they **pupate**, from there you can work backwards to estimate when they were born.

- **Extent of decomposition** – The **appearance** of the body can give an estimate to time of death.

Greenish discolouration of abdomen (36hrs)

↓

Spreads across rest of body (36 – 72hrs)

↓

Discolouration darkens to reddish green (36 – 72hrs)

↓

Discolouration darkens to purple-black (72hrs)

↓

Body becomes bloated with gas (one week)

↓

Gas is released, body deflates & shrinks (one week +)