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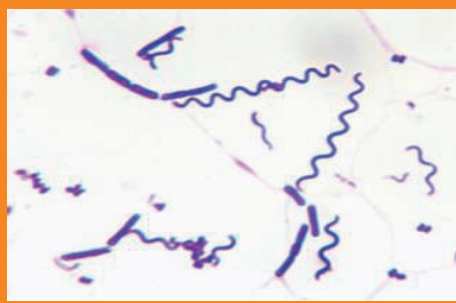
Pearson
BTEC Level 3 National
Extended Certificate in

Applied Human Biology



Unit 2

Practical Microbiology and Infectious Diseases

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Table of Contents

How to use this document

Welcome to your Applied Human Biology course.

A BTEC National in Applied Human Biology will give you the opportunity to develop a range of skills that will prepare you for the world of work, or for continued study at a higher level.

The number of units in your BTEC National qualification varies depending on the size of qualification you are doing.

This document supports the specification and associated assessment guidance, it does not replace them and should not be used in their place. Teachers should use their expertise and judgement regarding the teaching and delivery of this course and ensure that learners are taught all areas of content in sufficient depth in preparation for the external assessment. Every effort has been made to cover as much of the specification as possible. This document **does not** indicate topics, question types or activities that may come up in the external assessment and no member of the examination team has been involved in its creation.

Features of this document

There are a number of different features throughout the document, designed to help you learn about the topics in your course in different ways and understand it from multiple perspectives. Together these features:

- explain what your learning is about
- help to build your knowledge
- help you to reflect on and evaluate your learning
- make you think beyond what you are reading about
- help you make connections between your learning and real-world workplace environments.

In addition, each feature has a specific purpose designed to support your learning.

Features that explain what your learning is about

Getting to know your unit
This section introduces the unit and explains how you will be assessed. It gives an overview of what will be covered throughout the unit.

Features that help you build your knowledge

Worked example

The worked examples show the process you need to follow to solve a problem, such as a maths or science equation. This will also help you to develop your understanding and your numeracy and literacy skills.

Key points

Concise and simple definitions are provided for key words, phrases and concepts, allowing you to have, at a glance, a clear understanding of the key ideas in the unit.

Features connected to your assessment

Assessment practice

These features give you the opportunity to practise some of the skills you will need when you are assessed on the unit. They do not fully reflect the actual assessment tasks but will help you prepare for them.

Features to help you reflect on and evaluate your learning

Pause point

Pause points give you the opportunity to review and reflect on your own learning. The ability to reflect on your learning is a key skill you will need to develop and use throughout your life.

Hint and Expand
These points also give you suggestions to help cement your knowledge and indicate other areas you can look at to expand it.

Case studies are used in the unit to allow you to apply the learning and knowledge from the unit to a scenario from the workplace or industry. Case studies include questions to help you consider the wider context of a topic.

Think future skills

This section includes a case study of someone working in the industry. They talk about their job role and the skills they need. This comes with a Focusing your skills section, which gives suggestions for how you can begin to develop the employability skills and experiences needed to be successful in a career in your chosen sector. This is an excellent opportunity to build up your employability skills.

Getting to know your unit

Assessment You will be assessed by a series of assignments set by your teacher/tutor.	When studying human biology, it is important that you have a good understanding of how microorganisms can cause infectious diseases in humans. You will study how infectious diseases can be transmitted from person to person and explore the different treatments that can be given. You will also learn how to culture and identify microorganisms safely in the laboratory and carry out investigations into how different substances affect their growth.

How you will be assessed

In this unit, you will be assessed by a series of internally assessed tasks set by the unit teacher. Throughout the unit, you will find assessment activities that may help you work towards your assessment. Completing these activities will not mean you have achieved a particular grade, but the research you carry out for them will be relevant and useful when you come to carry out your final assessment.

It is important to check that you have met the Pass grading criteria, shown in the table below, as you work your way through the assignments.

To achieve a Merit or Distinction, you need to present your work in such a way that you met the criteria for those grades.

The assignments set by your tutor will consist of several tasks designed to meet the criteria in the table below.

<table>
<tr><th colspan="3">Assessment criteria</th></tr>
<tr><td colspan="3">This table shows what you must do in order to achieve a Pass, Merit or Distinction grade, and where you can find activities to help you.</td></tr>
<tr><th>Pass</th><th>Merit</th><th>Distinction</th></tr>
<tr><td colspan="3">Learning aim A: Understand the classification and nature of microorganisms</td></tr>
<tr><td>A.P1

Explain the methods used to classify microorganisms.</td><td rowspan="2">A.M1

Analyse the virulence mechanisms of microorganisms that cause infectious diseases and the methods used to classify microorganisms.</td><td rowspan="5">AB.D1

Evaluate the treatments of the types of infectious disease and the current issues in the development of these treatments.</td></tr>
<tr><td>A.P2

Explain the role of the structures found in microorganisms and the factors affecting their growth and virulence that cause infectious disease.</td></tr>
<tr><td colspan="2">Learning aim B: Examine the transmission and treatments of infectious disease</td></tr>
<tr><td>B.P3

Describe the development of different types of disease.</td><td rowspan="2">B.M2

Discuss the development of infectious diseases and their associated prevention and treatment strategies.</td></tr>
<tr><td>B.P4

Explain the prevention and treatment strategies for the different types of infectious disease,</td></tr>
</table>

Learning aim C: Explore the application of techniques to culture and identify microorganisms		
C.P5 Carry out morphological studies, microscopy and staining techniques to identify microorganisms.	**C.M3** Compare the techniques used to identify and cultivate microorganisms in terms of the quality of results obtained.	**C.D2** Correctly use aseptic enumeration techniques and make judgements on the accuracy of the procedure used.
C.P6 Correctly select and use aseptic technique to cultivate microorganisms.	**C.M4** Correctly select and use aseptic technique to grow and measure the growth of microorganisms, including the use of serial dilutions.	
Learning aim D: Investigate the effect of antimicrobial agents on the growth of microorganisms		
D.P7 Plan and carry out an investigation independently, selecting an appropriate method, into the effects of antimicrobials on the growth of organisms.	**D.M5** Plan and carry out an investigation independently, selecting an appropriate method, into the effects of antimicrobials on the growth of organisms with little or no contamination of results.	**D.D3** Evaluate the methods, techniques and data collected to determine the effect of antimicrobials on the growth of microorganisms and the wider impact on the functioning of an organism.
D.P8 Interpret data collected in order to reach a conclusion, considering the impact on prevention and treatment of disease.	**D.M6** Analyse the growth of microorganisms using data collected and in relation to the factors investigated in order to reach valid conclusion, making links to the impact on the prevention and treatment of disease.	

Getting started

Microorganisms can cause disease in humans. Diseases causing microorganisms are called pathogens and include some bacteria, fungi, protists, viruses and prions. Write down a list of different human diseases caused by each of these pathogens. When you have completed this unit, add more diseases to your list. Write down the different ways in which diseases can be transmitted from person to person. When you have completed this unit, see if you can add more to your list.

A Understand the classification and nature of microorganisms

Infectious diseases such as tuberculosis (TB), HIV and Ebola are caused by **microorganisms**. Microorganisms are organisms that cannot be seen with the naked eye; you need to use a microscope to be able to see them. Microorganisms include bacteria, viruses, prions, protists and fungi. Not all microorganisms cause disease but those that do are referred to as **pathogens**.

All cells can be classified into one of six kingdoms of life. The six kingdoms are:

- Animals – these cells are eukaryotic with no cell wall
- Plants – these cells are eukaryotic with a cell wall made of cellulose
- Fungi – these cells are eukaryotic with a cell wall made of chitin
- Protists -these cells are single-celled eukaryotes that do not fit into one of the above kingdoms
- Eubacteria – these cells are prokaryotic with cell walls made of peptidoglycan
- Archaea – these cells are prokaryotic and can live in extreme conditions.

In this section, you will learn about the differences between **eukaryotic cells** and **prokaryotic cells** and the characteristic features of the kingdoms containing disease-causing pathogens.

Key points

Microorganism – microscopic organisms including bacteria, fungi, viruses, prions and protists.

Pathogen – a microorganism that causes disease.

Eukaryotic cell – a cell that contains a nucleus and other membrane bound organelles such as mitochondria.

Prokaryotic cell – a cell that does not contain a nucleus or other membrane bound organelles.

A1 Characteristics of different microorganisms

Prokaryotes

Physical characteristics

A prokaryotic cell is a cell with certain characteristic features, this group of cells includes bacteria. Bacterial cells have:

- no nucleus, their DNA is found as a single loop in the cytoplasm in a region called the nucleoid
- no membrane-bound organelles
- a cell wall made of peptidoglycan
- a cell surface membrane
- ribosomes that are smaller than those found in eukaryotic cells
- small additional rings of DNA called plasmids.

They sometimes have:

- a protective capsule (slime) layer around the cell wall which prevents the cell from drying out
- flagellum that allows the cell to move
- hair-like structures called pili that allow the cell to attach to surfaces or other cells.

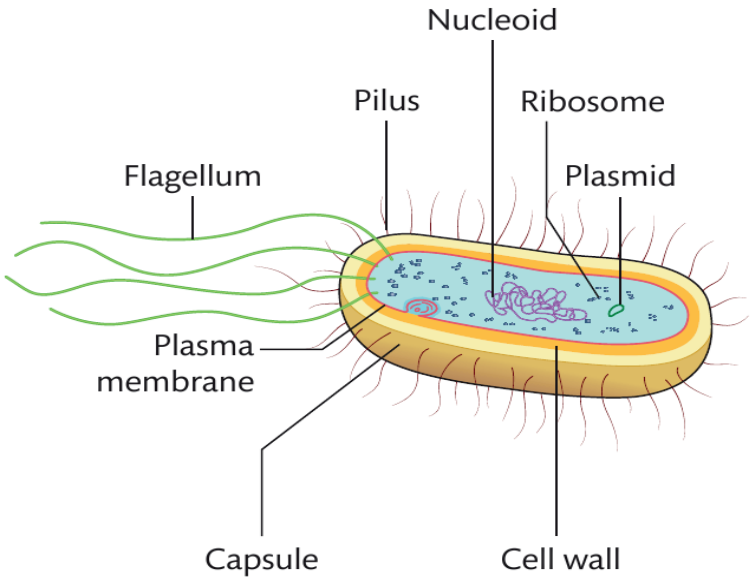


Figure 2.1: Generalised structure of a bacterial cell

The cell wall

Bacterial cells are surrounded by a cell wall composed of peptidoglycan. Bacteria can be sorted into two different groups (classified) based on the structure of their cell wall. Gram-positive bacteria have a thick layer of peptidoglycan in their cell wall, whereas gram-negative bacteria cell walls have a thinner layer of peptidoglycan. Most species of bacteria can be classified as Gram-positive or Gram-negative using a practical technique called Gram staining. You will learn about this procedure later in this unit.

Growth characteristics

Reproduction

Bacteria reproduce using a process called binary fission. Bacteria are prokaryotes, which means that their genome consists of a single loop of DNA, which lies in a region of the cytoplasm called the nucleoid. Binary fission produces two new bacterial cells that are genetically identical to the parent cell. Binary fission is asexual, this means that it does not involve the joining of gametes (sex cells).

The process of binary fission

1. The loop of DNA uncoils and is replicated to produce a second identical loop of DNA.
2. The cell increases in size and other structures are replicated including plasmid DNA and ribosomes.
3. The two loops of DNA are pulled to opposite sides of the cell.
4. The cell surface membrane begins to divide and a cross wall forms around each loop of DNA.
5. The cells separate to form two genetically identical daughter cells.

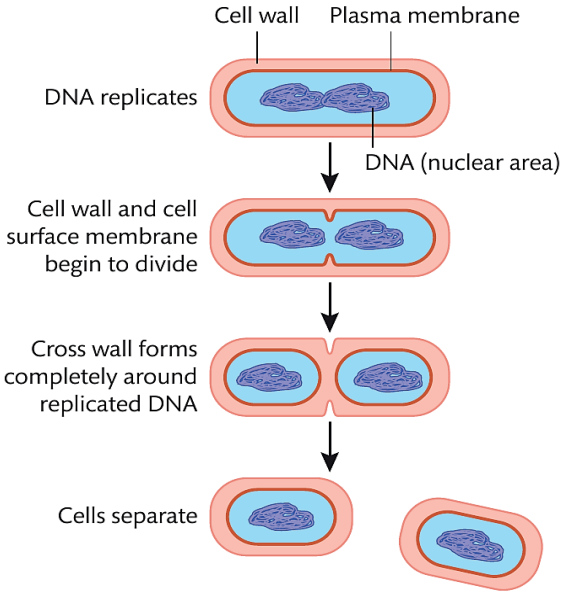


Figure 2.2: Bacterial reproduction by binary fission

Temperature

Bacteria can be classified by their optimum growth temperature. Each microorganism has a minimum, optimum and maximum growth temperature.

Psychrophilic bacteria are capable of growth and reproduction at extremely low temperatures between -5°C and 20°C. The enzymes contained within psychrophiles have adapted to be optimally active at low temperatures.

Mesophilic bacteria thrive in temperatures between 20°C and 45°C. Normal body temperature is approximately 37°C and, therefore, the bacteria that colonise the human body are mesophiles.

Thermophilic bacteria are heat-loving, they have an optimum growth temperature of between 50°C and 70°C but can survive in very high temperatures. The enzymes within thermophilic bacteria are thermostable, i.e. resistant to irreversible denaturing at high temperatures.

Growth curves

When bacteria are grown in a laboratory, the population produces a typical growth curve. In a closed system, like a test tube, the population growth will follow a specific pattern as seen in Figure 2.3.

Growth curves have four characteristic phases.

1. Lag phase – the bacteria cells added to the growth medium increase in size and start to produce the enzymes needed to use the nutrients present.
2. Exponential (log) phase – the bacteria are dividing by binary fission at a constant rate so that the population size doubles with each generation time.
3. Stationary phase – the rate of binary fission decreases as population growth is limited by lack of nutrients, build-up of waste or lack of space. In this phase, the reproduction rate is equal to the death rate.
4. Death (decline phase) – the size of the bacterial population decreases because conditions are no longer able to support reproduction of the cells.

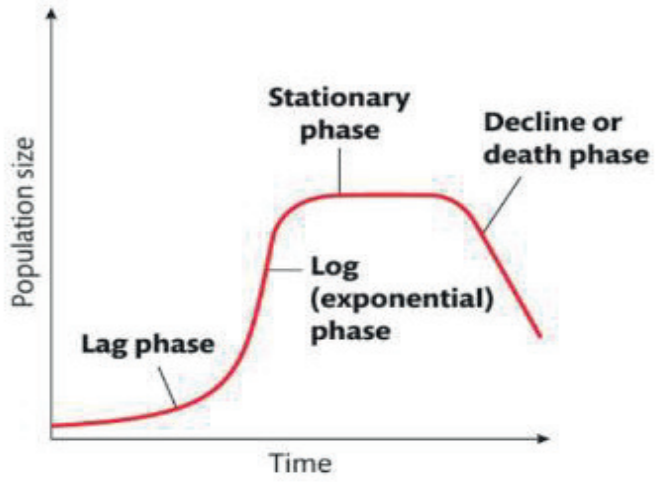


Figure 2.3: Typical bacterial growth curve

Key points

Ribosome – organelle responsible for protein synthesis.

Genome – the genetic information (genes) found in a cell.

Growth medium – the substance used to grow a microorganism containing all the nutrients required for growth. Can be solid, liquid or semi-solid.

Binary fission – a method of asexual reproduction used by prokaryotes.

Species – a group of organisms that share similar characteristics and can interbreed to produce fertile offspring.

Eukaryotes

A eukaryotic cell has certain characteristic features. Animal and plant cells are eukaryotic. Eukaryotic microorganisms include fungi and protists some of which can be human pathogens.

Eukaryotic cells have:

- a nucleus
- membrane-bound organelles
- a cell surface membrane
- ribosomes which are larger than those found in prokaryotic cells
- cell walls made of cellulose (in plant cells) and chitin (in fungi).

Protists

Protists are single-celled eukaryotic organisms; they have their DNA enclosed in a nucleus and have membrane bound organelles. The features of protists are extremely broad with lots of variation between different species. Some species reproduce asexually, and some reproduce sexually. Some species have cell walls while others have flexible cell membranes. Some protists obtain their nutrition by being predators, some are parasites, and some synthesise their own nutrients. Most protists live in a watery or moist environment and some species can live inside the human body and cause disease. Protozoa are a type of protist that obtain their nutrition through the ingestion of organic matter, they are referred to as heterotrophs. Other protists can carry out photosynthesis and so are not called protozoa. An example of a non-protozoan protist is algae. Malaria is a disease caused by a protist such as Plasmodium falciparum, which can be transmitted by female Anopheles mosquitoes. Dysentery is also caused by a protist (an amoeba) that lives in freshwater ponds.

Many protists are motile – they can move.

- *Amoeba* are a group of protists that move using specialised structures called pseudopodia. The amoeba changes the structure of its cell to produce pseudopodia that then enable the cell to drag itself along the environment.
- Some have one or more flagella, which they can rotate or whip to move, e.g. *Euglena*.
- Some protists such as *Paramecium* have a surface covered in rows of cilia that can beat in a synchronised manner to coordinate movement through water.
- Some protists move towards (or away) from a stimulus. Algae, for example, show phototaxis, they move towards sources of light.

Fungi

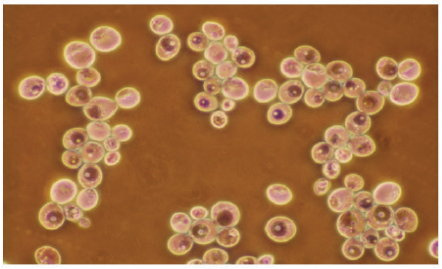
Fungi are eukaryotic organisms; the group includes yeasts, moulds and mushrooms. There are more than 100 species of fungi that can cause disease in humans. As they are eukaryotic cells, fungi contain a nucleus and membrane-bound organelles. Other features of fungi include:

- Can be single-celled such as yeast or multi-cellular such as mushrooms.
- Have cell walls that contain chitin.
- Obtain nutrition by feeding on and breaking down dead organic matter in the ecosystem.
- Most respire anaerobically but some fungi, such as yeast, can use both aerobic and anaerobic respiration.
- Have hyphae which grow from the tips.
- Can be parasites (such as *Tinea* fungi which causes athlete's foot).
- Use either asexual or sexual reproduction or by spores (asexual or sexual).

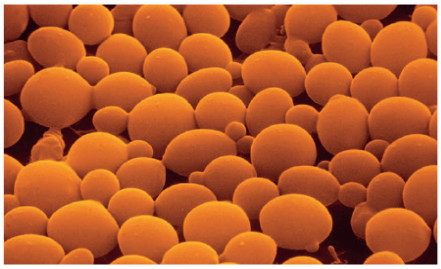
Asexual reproduction in yeast

Yeast cells are single-celled fungi that reproduce by a process called budding.

1. The parent cell forms a bud.
2. The bud grows as the DNA in the nucleus of the parent cell replicates.
3. The nucleus splits into two and one nucleus moves to the bud.
4. A wall forms between the bud and the parent cell.
5. The bud may remain attached to the parent cell or it may break away.



(a)



(b)

Figure 2.4: (a) Light micrograph of yeast cells *Saccharomyces cerevisiae*. Mag x340 (b) Colour enhanced SEM of yeast cells *Saccharomyces cerevisiae* budding

Pause point

Distinguish between prokaryotic and eukaryotic cells.

Hint

Explain the similarities and differences between eukaryotic and prokaryotic cells referring to specific examples.

Extend

Discuss how you could identify if a cell in an image is prokaryotic and eukaryotic.

Viruses and prions

Viruses and prions are not eukaryotic or prokaryotic. This is because viruses and prions are not living and are not cells. They make up a third group of organisms known as the akaryotes. Akaryotes have no cell structure, no cytoplasm or organelles.

Viruses

Viruses can infect all types of cell, there are viruses that can infect animals, plants, bacteria, protists and fungi. Viruses:

- Consist of a capsid (protein coat) made of smaller units, called capsomeres.
- The capsid surrounds the nucleic acid, which can be DNA or RNA.
- Can have a lipid membrane called an envelope surrounding the capsid.
- Are small (smaller than bacteria) with diameters of 20-300nm.
- Can only reproduce inside a host cell.
- Can have a variety of shapes including helical, spherical and polyhedral.

Viruses can only reproduce inside a host cell. To reproduce, viruses attach to cells, insert their genetic material into the host cell, take over the host cell machinery to replicate the nucleic acid and build new viral particles. The viral particles then burst out of the host cell, destroying it. Each new virus particle produced can infect other host cells. There are two types of viral reproduction cycle – lytic and lysogenic.

Table 2.1: Lytic and lysogenic life cycles of viruses

Lytic	Lysogenic
More common	Rarer method of reproduction
Host cell is completely destroyed	Host cell is not destroyed straight away, virus lies dormant letting the cell multiply first
Viral nucleic acid replicates separately from host cell DNA	Viral nucleic acid replicates within the host DNA
One virus destroys one host cell	One virus affects many cells as the virus is passed along to new cells formed during cell division

Retroviruses

HIV (Human Immunodeficiency Virus) is a virus that can spread in bodily fluids such as blood and semen. It infects cells of the immune system called CD4 (T cells). Over time, the destruction of these cells causes the development of AIDS (Acquired Immunodeficiency Syndrome). This weakens the immune system and makes it difficult to fight off other infections. Opportunistic infections take advantage of this and can lead to death.

HIV is an example of a type of virus called a retrovirus. HIV contains glycoprotein spikes on the envelope, which attach to antigens on the surface of the T cells that it infects. Retroviruses such as HIV have RNA as their genetic material and contain an enzyme called reverse transcriptase. When these viruses replicate inside a host cell, the enzyme is used to produce complementary DNA (cDNA) from the RNA nucleic acid. The DNA is incorporated into the host genome and may remain dormant there for several years. When activated, the virus directs the synthesis of new viral particles that use part of the host cell's membrane to form their envelopes. This destroys the host cell.

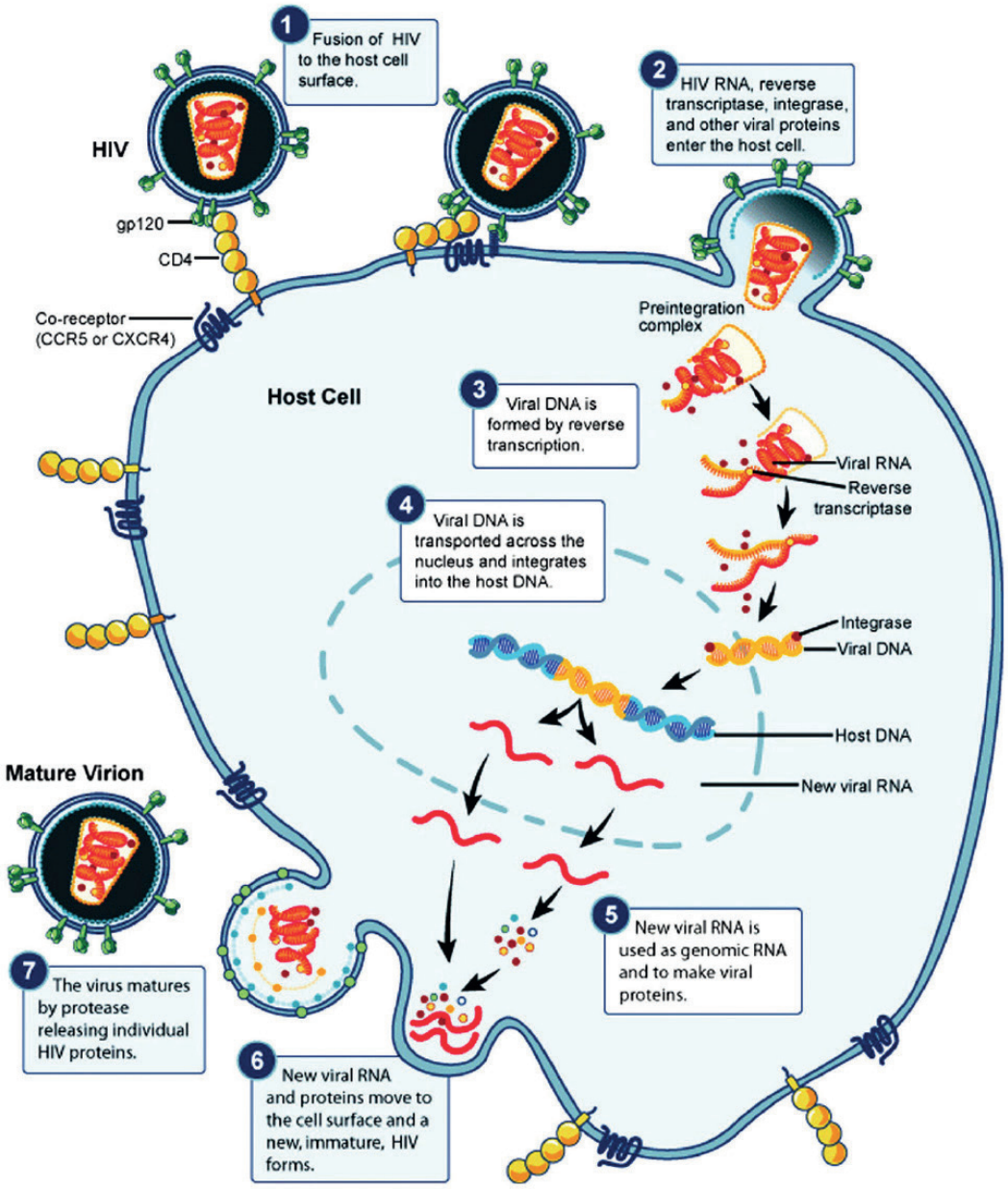


Figure 2.5: Replication of retroviruses such as HIV

Prions

Prions can also cause disease. Disease caused by prions is extremely rare and causes the nervous system to lose function over time. Prion diseases have no known treatment and are always fatal. An example of a neurodegenerative disease caused by a prion is variant Creutzfeldt-Jakob Disease (vCJD). Prions are not living cells; they are proteins that are not folded correctly. Prions are stable and not denatured by heat. The abnormal structure of proteins causes the protein to have infectious properties and protects them from being broken down by normal cellular enzymes. When prions are transmitted to a new organism, the prions cause proteins in that organism to misfold and aggregate (clump together). These aggregates build up in the brain and nervous tissue. causing tissue damage. The damage gets progressively worse over time.

Pause point

Discuss why viral and prionic diseases are more difficult to treat.

Hint
Link to the characteristic features of each group of microorganisms.

Extend
Discuss why prionic diseases are always fatal.

A2 Methods of pathogenicity

Key points

Pathogen – an infectious microorganism or agent that can cause disease.

Pathogenicity – the ability to cause disease.

Transmission – the spread of a pathogen.

Virulence –the degree of infection and damage caused by a pathogen.

Quantitative – measurements made where numerical data is collected.

Pathogenicity is used to describe the ability to cause disease. Whether **transmission** of the pathogen to a host actually leads to the host developing disease depends on the **virulence** of the pathogen. Virulence is the extent to which a pathogen is able to damage host cells and cause disease. A pathogen can be classified as pathogenic but depending on conditions, may produce different levels of virulence. Virulence can be measured on a scale from avirulent (not harmful) to highly virulent.

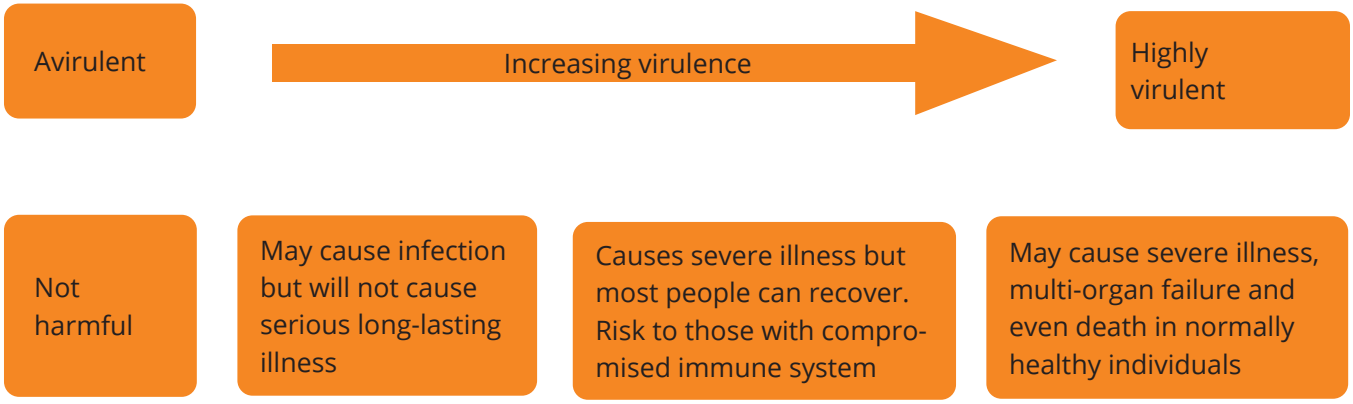


Figure 2.6: Scale of virulence related to the properties of the diseases caused

Quantitative measures are typically used to measure virulence. The two most common measures of virulence are the median infectious dose and median lethal dose. These measurements are carried out in laboratories, often using animal models or simulations. The median infectious dose is the number of pathogens required to cause infection in 50% of organisms infected with the pathogen. The median lethal dose is the number of pathogens required to cause death in 50% of infected organisms. The lower both measurements are, the more virulent the pathogen is.

Factors affecting the virulence of bacteria

The virulence of bacteria depends on several factors: These include

- access to the host (exposure)
- adhesion to the host cell
- production of endotoxins and exotoxins leading to damage to host cells and tissues
- evasion of the immune system
- incubation periods.

Access to the host (exposure)

For a bacterial pathogen to cause disease in a host organism such as a person, the person, must first be exposed to the bacteria. This means that the person must come into contact with the bacteria from another source. We are exposed to thousands of potential pathogens each day, they are present in the air we breathe, the food we eat, the objects and surfaces we touch and in the other people and organisms we interact with every day. The vast majority of these potential pathogens will not cause disease. For a bacterium to cause disease it must first enter the body. There are several places in the human body where pathogens can enter because cells or bodily fluids are in direct contact with the external environment. These include:

- mucosal surfaces at the eyes, nose, mouth, anus and urethra
- the skin (especially when it is broken due to cuts or grazes)
- the placenta in pregnant women can act as entry to the unborn foetus.

Adhesion to the host cells

If the bacteria are successful in making entry into the host organism, they must then attach to the cells of the body tissue. This process of attaching to cells is called adhesion. Bacteria have protein or carbohydrate molecules on their cell surface membrane, which they can use to bind to complementary shaped receptors on body cells. These molecules are called adhesins. Some bacteria also use their capsules to attach to host cells. They do this by producing a biofilm. A biofilm is formed from a group of bacteria with slime and capsule layers, this biofilm can then attach to a surface such as a host cell. Biofilms make it difficult for the bacteria to be removed from the host cell, giving the bacteria time for the next stage in pathogenicity, invasion. This also protects the bacteria against the immune system and the action of antibiotics.

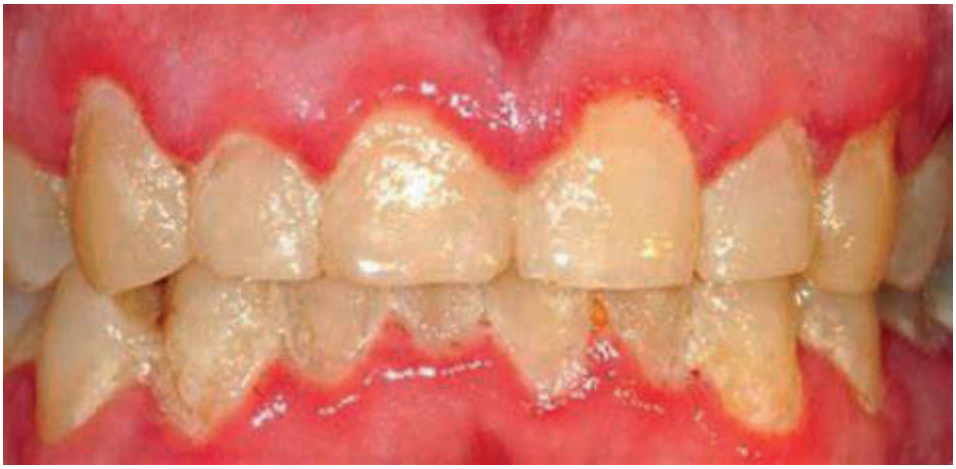


Figure 2.7: Biofilms can also form on the surface of teeth causing gum infections (gingivitis) and tooth decay

Production of endotoxins and exotoxins leading to damage to host cells and tissues

Once bacteria have adhered to the host cell, the next step is invasion. This involves the growth and spread of the bacteria. Bacteria may release toxins as they grow, which will damage the host tissues, cause disease symptoms and allow the bacteria to evade the immune system. There are two types of toxin: endotoxins and exotoxins. Endotoxins are lipopolysaccharides, which form part of Gram-negative bacterial cell walls. When the bacterial cell is destroyed, either by the host's immune system or through self-destruction, endotoxins are released.

Endotoxins cause fever (high temperature) in the host organism. A large amount of endotoxin must be released by the destruction (lysis) of bacteria to cause disease. Examples of bacteria that release endotoxins include *Neisseria gonorrhoea,* the bacteria responsible for the sexually transmitted infection gonorrhoea and *Escherichia coli,* which causes diarrhoea and vomiting.

Exotoxins are proteins that can be secreted by both Gram-negative and Gram-positive bacteria as they grow. These toxins then travel around the host organism and interfere with normal cellular function, they may also help the bacteria to spread around the host organism. Exotoxins do not cause fever but can trigger an immune response as they are recognised by the immune system. Only a small amount of exotoxin is needed to cause disease, these types of toxin are described as potent.

Examples of bacteria that release exotoxins include *Vibrio cholerae*, the bacteria that causes the disease cholera and *Clostridium tetani,* which causes the tetanus. Different exotoxins target different cells, which leads to symptoms and effects that are specific to that disease. The toxin produced by *Clostridium tetani* works by inhibiting the release of acetylcholine neurotransmitter at the synapses of neuromuscular junctions. This results in permanent muscle contraction causing symptoms that begin as stiffness of the jaw (lockjaw) and then progress to violent muscle spasms in other parts of the body. As the bacteria reproduce and spread, they produce more exotoxin, which can cause paralysis of the muscles that control breathing, leading to death. The toxin produced by *Vibrio cholerae* acts on cells of the intestine causing these cells to secrete fluid and electrolytes (salts) out of the cell. This causes diarrhoea, which can lead to dehydration and death.

Table 2.2: Comparison of endotoxins and exotoxins produced by bacteria

Endotoxins	Exotoxins
Lipopolysaccharides	Proteins
Part of the bacterial cell wall	Secreted by the bacteria
Produced by certain gram-negative bacteria only	Produced by certain Gram-negative and positive bacteria
Released when the bacteria cell is destroyed	Released while the bacteria are growing and reproducing
Not very potent (lots needed to cause disease)	Potent (only small amount needed to cause disease)
Cause fever in the host	Do not cause fever in the host
Are not recognised by the host immune system	Are recognised by the host immune system and cause an immune response

Evasion of the immune system

The purpose of the immune response is to detect any substances entering the body that are detected as non-self. Cells contain protein molecules called antigens on their cell surface membrane. The immune system can recognise when a foreign antigen is present in the body and trigger an immune response to get rid of the invader. Bacterial antigens will trigger the immune system, which will lead to the production of antibodies and phagocytosis of the bacteria to remove the infection. Bacteria can use strategies to evade the immune system and prevent phagocytosis, giving time to for adhesion to cells and reproduction.

Strategies for evasion

- Capsule – the bacterial capsule prevents immune cells from attaching to the bacteria and destroying it by phagocytosis. For example, strains of *Streptococcus pneumoniae* with a capsule are more likely to cause infections, such as pneumonia and meningitis, (are more virulent) compared to the bacterial strains with no capsule.
- Proteases – some bacteria produce and release protease enzymes that protect them from phagocytosis. Phagocytosis is triggered when antibodies produced by cells of the immune system bind to proteins on the invading bacteria. Proteases produced by the bacteria bind to and destroy antibodies and stop them from binding to the bacteria thus protecting the bacteria from phagocytosis.
- Mycolic acid – the bacterium *Mycobacterium tuberculosis*, which causes TB, produces a waxy substance called mycolic acid. This substance protects the bacteria and stops it being broken down by phagocytes.
- Antigenic variation – some bacteria can change the shape of antigens on their cell surface membrane. The first time the body is invaded by a particular antigen memory cells are produced, which remain in the body for many years and can quickly produce an immune response should the same antigen enter the body again. This means that the immune system can deal with future infections much more effectively and stop the bacteria before the population has chance to grow. Some bacteria such as *Neisseria gonorrhoeae* change the antigens on the cell surface membrane. This allows secondary infections to evade the host immune system for longer.

Pause point

Discuss why having strategies for evading the immune system increases the virulence of a bacterial species.

Hint

Recall the steps that take place to create an infection.

Extend

Distinguish between the effect of the production of endotoxins and that of exotoxins.

Factors affecting the virulence of eukaryotes

You will remember that some eukaryotic organisms can also cause disease in humans. For example, the fungus *Candida albicans* can cause oral and vaginal thrush infections and fungal **meningitis** is caused by various species of the fungus *Cryptococcus*. Protists are also eukaryotic and some species of these can be pathogenic. *Plasmodium falciparum* causes malaria while another protists called *Trypanosoma brucei* are responsible for African sleeping sickness. These eukaryotic pathogens also have factors that affect how virulent they are.

The virulence factors for eukaryotic pathogens include:

- access to the host
- use of adhesins and toxins
- antigenic variation
- the ability to survive inside phagocytic vesicles.

Access to the host

Like bacteria, fungi and protists must gain entry to the host organism before they can start to reproduce and cause infection. The host must be exposed to the organism and the organism must bypass the host's first line of defence such as the skin, tears and mucus produced by mucosal surfaces.

Use of adhesins and toxins

Like bacteria, fungal pathogens have strategies that allow them to attach to and invade host cells. Some fungi such as *Candida* have glycoproteins on their surface, which bond to the phospholipids of **epithelial** cells in the skin. These fungi then produce enzymes to break down a structural protein in epithelial cells called **keratin**. This helps the fungus to invade the cells. Some fungi can also produce exotoxins called mycotoxins. Some fungal toxins can have serious consequences in humans. A toxin called aflatoxin, a potent **carcinogen**, is produced by *Aspergillus* fungus. Aflatoxin is a **mutagen** and can cause DNA mutations in the cells of the host, leading to the development of cancer in a person who has inhaled the fungus. *Aspergillus* also produces a toxin called gliotoxin that causes host cells to self-destruct. The toxin can cause destruction of **phagocytes,** allowing the fungus to stay in the body longer without being destroyed by the immune system.

Protists have unusual, unique mechanisms for attaching to host cells. *Giardia lamblia,* which causes a disease of the intestines called giardia, use a disc made of microtubules to attach to the lining of the intestine. This pathogen causes the intestinal cells to become inflamed, which stops them working as efficiently to absorb the products of digestion. *Plasmodium falciparum* produces a protein called erythrocyte membrane protein 1 (PfEMP1), which attaches to the surface of the red blood cells it invades. This protein causes the red blood cells to stick together and cause blood clots.

Key points

Meningitis – an infection of the meninges membranes that cover the brain and spinal cord.

Epithelial cells – cells of one of the four types of animal tissue. Epithelial tissue lines the cavities and surfaces of organs and blood vessels.

Keratin – a structural protein that is a component of hair, nails and skin.

Carcinogen – substance capable of causing cancer.

Mutagen – substance that causes changes in the genetics of a cell.

Phagocyte – a type of white blood cell that is capable of engulfing and digesting bacteria and other small cells.

Antigenic variation

Like bacteria, fungi can evade the immune system using antigenic variation. In antigenic variation the proteins on the cell surface membrane of the organism that are recognised by the immune system change from strain to strain. This means that strains of the same fungus or protist may not be recognised by the immune system as a pathogen that has been in the body before as it does not have the same antigens. The organism with the new antigens will not be destroyed by phagocytosis as quickly as it would be should it have the same antigens; antibodies will take longer to be produced. The toxin produced, *Plasmodium falciparum,* PfEMP1, as described earlier, can be recognised by the host's immune system but as the plasmodium replicates over time inside the host, antigenic variation occurs and the structure of the protein changes. This allows the organism to go unrecognised by the immune system and cause chronic infection.

The ability to survive inside phagocytic vesicles

Some fungi and protists have strategies that allow them to withstand phagocytosis.

The process of phagocytosis

1. White blood cells cause phagocytes to bind to the antigens on the pathogen's cell surface membrane. This can also be helped by antibodies binding to the pathogen's antigens first.
2. The cell membrane of the phagocyte expands and surrounds the foreign cell.
3. The cell membrane forms a phagocytic vesicle around the pathogen.
4. Lysosomes in the phagocyte release digestive enzymes to digest the phagocytic vesicle thus destroying the pathogen.
5. Some phagocytes release chemicals called cytokines, which attract new phagocytes to the area to further target other pathogens present.

A fungus called *Cryptococcus* causes meningitis and pneumonia and has a capsule made of a polysaccharide called glucuronoxylomannan. The capsule gives the fungus better resistance towards phagocytosis, increasing its virulence. The protist that causes African sleeping sickness, *Trypanosoma brucei*, resists phagocytosis by producing a glycoprotein capsule that can also change over time to prevent recognition by the immune system.

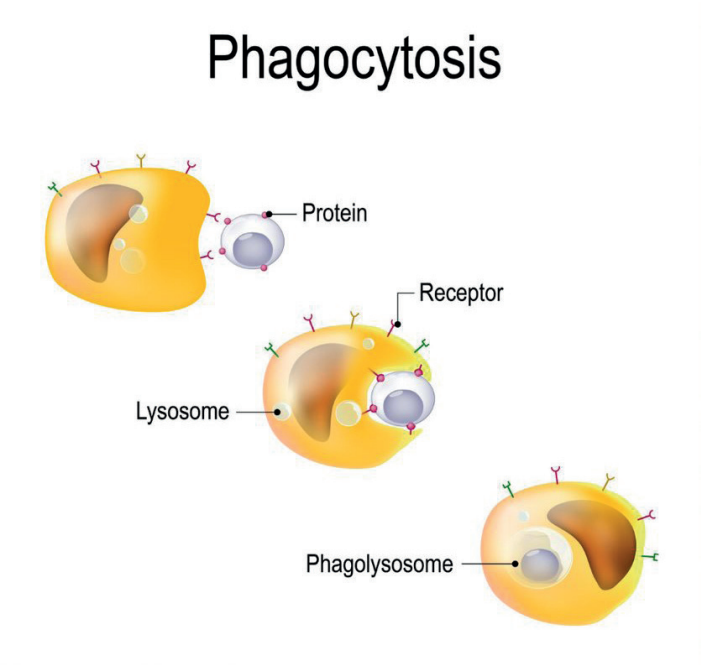


Figure 2.8: The process of phagocytosis

Virulence mechanisms of viruses and prions

Like bacteria and fungi, viruses have factors that determine their virulence and therefore their ability to cause diseases in humans.

These factors include:

- access to the host
- ability to cause direct cell damage
- latency.

Access to the host

In the first step in viral infection, the virus that must gain access to the host organism and then attach to host cells. Viruses like all pathogens can enter the body through cavities and mucosal membranes. Once inside the body, viruses must attach to their target cells to begin the process of replication. Viruses are not living and can only replicate inside host cells. As we have seen earlier, viruses have capsids or envelopes surrounding their genetic material. These structures contain molecules called adhesins. Adhesins can bind to receptors on the membranes of host cells. This then allows the virus to insert its genetic material, enzymes and/or other viral proteins that may be needed for the virus to replicate, into the host cell. Viruses have specific shaped adhesins, which allow them to attach to the target cell they infect. For example, the protein hemagglutinin is found in the influenza viruses that causes flu. Hemagglutinin acts as an adhesin and can bind to glycoprotein receptors on cells of the respiratory system and intestines. It is only these cells in the body that have the glycoproteins of the correct shape for hemagglutinin to bind to and so these cells are the host cells of this specific virus. The *herpes simplex* virus can cause oral or genital herpes (depending on the strain of virus). These viruses have different adhesins to those found on the influenza virus. The adhesins present on *herpes simplex* are known as glycoproteins gB, gC and gD, they have complementary shapes to bind to receptors called heparan sulphate, which is found on the cells of the mucosal surfaces of the mouth and genitals.

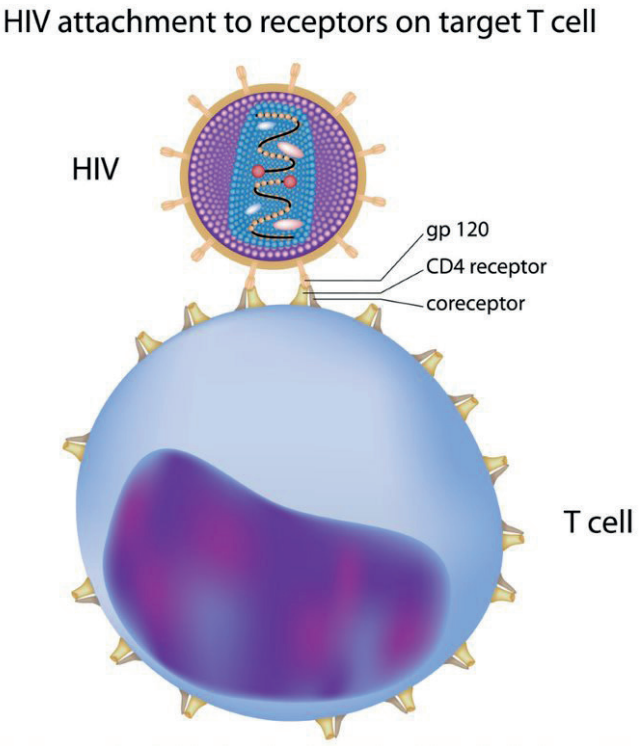


Figure: 2.9: HIV using adhesin glycoprotein gp120 to attach to CD4 receptors of T cells of the immune system

Ability to cause direct cell damage

A virus replicates using the host cell organelles. DNA viruses use proteins and enzymes to make their DNA, which is then used to produce new virus particles through protein synthesis. RNA viruses use host cell ribosomes to make new viral enzymes and proteins to build virus proteins. Replication of viruses can cause direct damage to the host cell by causing major biochemical and structural changes. The changes that happen are referred to as cytopathic – causing cell damage. Cytopathic changes can cause a host cell to die through bursting (lysis) or through programmed cell death (apoptosis). Both of these cause the new virus particles to be released into the surrounding fluid, making them free to infect other cells. Rhinovirus works this way, by causing lysis of respiratory cells leading to the symptoms of the common cold. Some animal viruses leave cells by a process called budding. In budding, the host cell is not killed immediately but cytopathic changes disrupt the normal functioning of cells, so they are unable to carry out their normal role.

Key points

Adhesins – molecules on the capsid or envelopes of viruses that are used to attach to receptors on host cells.

Glycoprotein – proteins with carbohydrate groups attached to the polypeptide chain.

Complementary shape – a relationship between two structures where each structure fits with the other like a lock and key.

Cytopathic – can cause cell damage.

Lysis – bursting of cells.

Apoptosis – programmed cell death.

Latency

Some viruses have the ability to remain dormant (not active) inside host cells for a period of time. For example, herpes simplex virus 1 (HSV 1) which causes cold sores can lie latent inside certain nerve cells. The genome of these viruses can remain latent until activated. Infection with herpes simplex virus occurs in early life and lifelong latency can occur. This explains why people can suffer from recurrent cold sores throughout their lifetime. Human Immunodeficiency Virus (HIV) is another example of a virus that has viral latency, Latent HIV is not affected by the treatments used to slow down infection and prevent the development of AIDS. When a person is undergoing treatment for HIV, they will never be completely free of infection. Latent viruses do not trigger an immune response as no new viral particles are made, this makes It difficult to develop vaccines against latent viral infections.

Prions

There are no specific factors affecting the virulence of prions. This is because prions are not cells but consist only of misfolded proteins. These proteins can evade destruction by the immune system as they are not recognised as foreign antigens. The human body may contain these proteins in their normal form and so the presence of a misfolded protein (prion) can remain undetected and build up in body tissue. The disease variant Creutzfeldt-Jakob Disease (vCJD) is caused by a mutated prion, a misfolded form of the protein PrP. When the misfolded PrP protein comes into contact with a correctly folded form of PrP, it causes the normal PrP to misfold. This process repeats over and over. The number of prions increases dramatically and can build up in brain tissue causing brain damage and degeneration over time.

Evolution and mutation of pathogens

As we have seen, pathogens can evade the immune system by changing their surface antigens. Bacteria may also develop more virulent strains through mechanisms of evolution. Bacteria contain small loops of DNA called plasmids. Plasmids can carry genes that benefit the bacteria in certain situations. For example, genes that give the bacteria resistance to certain antibiotics can be found on the plasmid. These beneficial genes arise by chance mutations. Bacteria containing the plasmid with the beneficial gene can survive the selection pressure (i.e. the presence of the antibiotic). They can then reproduce and pass on the plasmid to the next generation of bacteria. Bacteria can also copy and transfer plasmids to other bacterial cells of the same generation. This is called horizontal gene transfer and can increase the spread of antibiotic resistance in a bacterial population. Multi-drug resistant tuberculosis is a form of tuberculosis infection caused by *Mycobacterium tuberculosis* bacteria that have developed resistance to the antibiotics used to treat the infection. *Mycobacterium tuberculosis* does not have the ability to transfer plasmids by horizontal gene transfer, but instead advantageous chance mutations of the bacterial genome have been passed from generation to generation. These chance mutations in the bacterial DNA have caused the cell wall of the bacteria to become a more effective barrier against the drugs used to treat it and have strains of the bacteria to develop molecular systems that pump drug molecules out of the bacterial cells. In some TB bacteria, the chance mutations that increase the bacterial virulence can increase the overall mutation rate and allow more mutations to build up, increasing the chances of further drug resistance occurring.

The virulence of viruses can also be affected by evolution. HIV is one of the fastest evolving viruses due to its reproduction rate. When HIV reproduces using the body's T cells, it can accumulate a lot of mutations. Some of these mutations might increase the virulence of the virus by making it more resistance to treatments but the mutations may also decrease virulence by altering the virus's ability to attach to host cells. When a person begins taking HIV medication, it will be effective in controlling the reproduction of the virus. However, as the virus replicates, chance mutations may occur and cause new viral particles to be produced that can survive the drugs being taken. Viruses without the advantageous mutation will not survive the medication taken by the patient. However, viral particles with the mutation can survive, infect more T cells and produce more viral particles. The new viral particles may also contain the genes to give them resistance to medication. Over time, the medication will stop working and will need to be changed to prevent the person developing AIDS. This can take anything from a few weeks, or a few years and the patient will have their condition monitored by professionals. To reduce the chances of resistance happening, patients are often prescribed several different antiviral drugs at the same time. This will reduce the chance that viruses with advantageous mutations survive to reproduce. The use of "drug cocktails" thus increases the time between HIV infection and the development of AIDS.

Pause point

Discuss the impact of the evolution of pathogens in causing disease.

Hint

Consider the different methods a pathogen may have for evolving and the impact evolution has on the host.

Extend

How might scientists try to counteract evolution of pathogens in order to prevent and fight disease?

A3 Classification strategies

Classification is the process of sorting things into groups based on common characteristics. Classification helps us to see connections between groups of organisms and cells with similar properties and structures and allows us to study evolutionary relationships.

Classifying bacteria

Bacteria share common features that allow them to be classified as prokaryotic cells. However, within this kingdom, bacteria have other differences allowing them to be classified further.

Shapes

Phenotype refers to the visible characteristics of an organism and includes the shape of the bacterial cells. In order for bacterial cells to be seen, they are stained and viewed under a microscope. Bacterial cells can be different shapes and can therefore be classified by their shape.

Table 2.3: Phenotypic shapes of some human pathogenic bacteria

Shape of bacteria	Description	Examples
Cocci	Round shaped bacteria can be: singular: cocci in pairs: diplococci in chains: streptococci in bunches: staphylococci	*Streptococcus mutans* *Staphylococcus aureus*
Bacilli	Rod-shaped bacteria	*Bacillus cereus* *Escherichia coli*
Spirilla	Corkscrew or spiral shaped bacteria	*Helicobacter pylori* *Treponema pallidum*
Vibrio	Curved rod-shaped or comma-shaped bacteria	*Vibrio cholerae*

Gram staining

Bacteria can also be classified by the structure of their cell wall. Bacteria can be stained by a procedure called Gram staining. This technique is usually one of the first steps taken in a laboratory to identify unknown bacteria. Bacteria can be classified as either Gram-positive or Gram-negative. The process involves staining bacteria so that they can be seen using stains. The first stain, called crystal violet, is a purple stain t retained by the Gram-positive bacteria as they have thick walls made of peptidoglycan. These bacteria will appear purple when viewed with a microscope (Figure 2.10). Gram-negative bacteria have a thinner peptidoglycan wall with an inner and outer membrane, they do not retain the purple stain. These bacteria cannot be seen using a light microscope without staining so a counterstain is used. This is usually a red stain called safranin. Most species of bacteria can be classified as either Gram-positive or Gram-negative, but some bacteria are Gram-variable or Gram-indeterminate. Some bacteria such as *Mycobacterium tuberculosis* do not stain by either stain during Gram staining and are described as acid-fast.

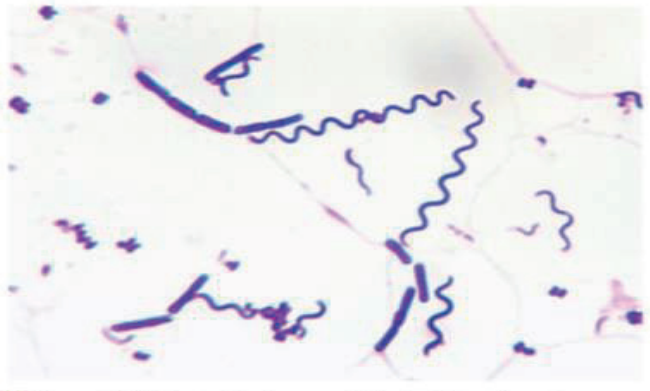


Figure 2.10: Shapes of Gram-positive bacteria: spirilla, bacilli and cocci

Oxygen requirements

Bacteria can also be classified into groups based on their need for oxygen.

- Facultatively anaerobic bacteria are versatile. They can survive in high or low oxygen concentrations. The bacteria *Staphylococcus aureus, Escherichia coli* and *Streptococcus* spp. are facultative anaerobes.
- Obligate anaerobic bacteria only grow where there is no or very little oxygen and are killed by the presence of oxygen. The bacteria *Bacteroides spp*. and *Clostridium spp*. are examples of obligate anaerobes. *S*pecies of *Bacteroides* bacteria living in the colon are obligate anaerobes.
- Obligate aerobe bacteria can only grow where oxygen is in plentiful supply. *Mycobacterium tuberculosis,* which causes TB, Is an obligate aerobe.
- Aerotolerant bacteria do not require oxygen but can survive in its presence. The bacteria that causes tetanus, *Clostridium tetani,* are aerotolerant.

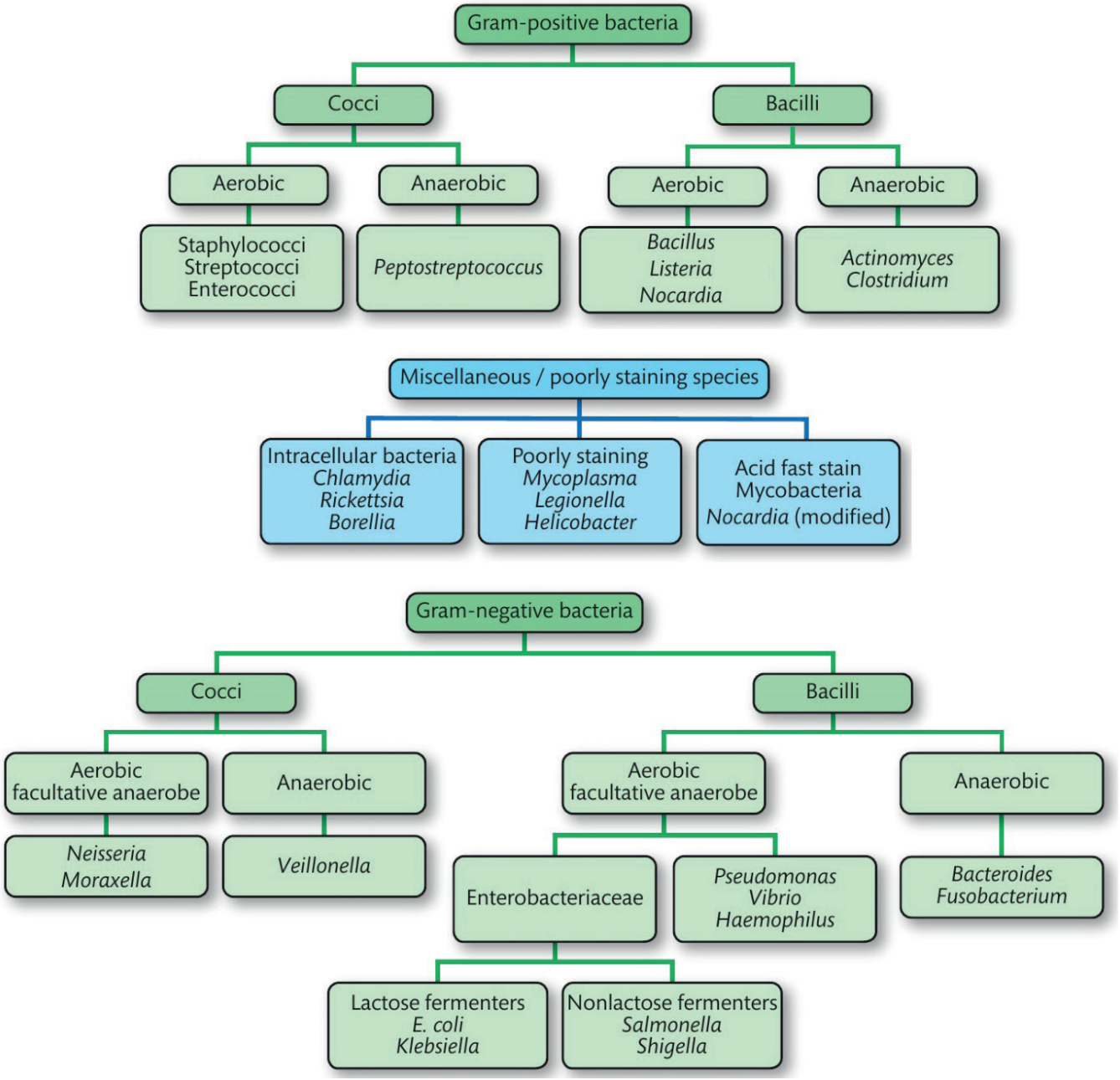


Figure 2.11: Classification of some bacteria

Classification of viruses

The typical features used to classify organisms do not apply to bacteria as they are not living cells. The classification of viruses is based on:

- size
- capsid shape
- presence of an envelope
- type of nucleic acid
- mechanism of replication
- host organism (bacteria, fungi, protist, animal or plant)
- pathology (type of disease they cause).

The International Committee on Taxonomy of Viruses (ICTV) began its classification of viruses in the 1970s. More than 200 000 species of viruses have been identified and scientists believe that there are many more waiting to be discovered.

The Baltimore classification classifies viruses into seven groups (I-VII), depending on their type of nucleic acid. Viral nucleic acid can exist as DNA and RNA and in the following forms:

- double-stranded DNA with a coding and template strand (ds DNA)
- single-stranded coding strand of DNA (ss -DNA)
- positive-sense single stranded RNA, similar to messenger RNA (mRNA: a copy of the coding strand of DNA) (ss +RNA)
- negative-sense single stranded RNA complementary to mRNA (ss -RNA)
- double-stranded RNA; one strand is mRNA and the other strand is complementary to it (ds +/- RNA).

Some viruses (Group VII) have gapped nucleic acid. Table 2.4 shows examples of viruses from each group, their genomes and examples of the diseases caused by these viruses.

Table 2.4: Examples of viruses from each group

Group	Genome	Examples of virus	Examples of disease caused
I	ds DNA	Adenoviruses, herpesviruses, poxviruses	Meningitis, chickenpox, smallpox
II	ss +DNA	Parvovirus	Parvovirus infection
III	ss -DNA	Reoviruses	Gastroenteritis
IV	ss +RNA	Picornaviruses, togaviruses	Hepatitis A, polio, SARs, foot and mouth disease, yellow fever, hepatitis C, rubella
V	ss -RNA	Orthomyxoviruses, paramyxoviruses, rhabdovirus	Influenza, measles, mumps, Ebola, Marburg disease, rabies
VI	ss +/-RNA	Retroviruses	HIV/AIDs
VII	Gapped nucleic acids	Hepadnaviruses	Hepatitis B

Case study

Giant viruses with cell-like features

In 1957, a scientific paper defined viruses as potentially pathogenic, having only one type of nucleic acid, DNA or RNA, unable to grow and replicate by binary fission, and lacking their own metabolic machinery. In 1992, in Bradford, UK, researchers trying to find the organism responsible for an outbreak of pneumonia found an organism similar to a Gram-positive coccus bacterium. They isolated it from a culture of *Acanthamnoeba polyphaga* in a water sample from a hospital cooling tower. The researchers named it *Bradfordcoccus*, but they found it impossible to get meaningful results from the usual tests to identify a bacterium.

The organism was stored and in 2003 it was taken to the University of Marseilles, France. In France, a team used transmission electron microscopy to examine the organism. To their great surprise, the French researchers found it was an enormous virus and renamed it *Acanthamnoeba polyphaga mimivirus* (APMV), in reference to the host organism from which it was first isolated and because it could mimic a bacterium.

APMV is about the same size as the bacterium *Staphylococcus aureus*. Since then, other giant viruses have been discovered and a new viral family, *Mimiviridae*, has been defined. Mimiviridae are generally nucleocytoplasmic large DNA viruses.

Inside the capsid of APMV is a lipid membrane and the virus contains fibrils of peptidoglycan. It has double-stranded DNA and contains 1.2 million base pairs, encoding about 1000 genes, which include some genes for protein translation apparatus, and enzymes related to DNA repair, RNA modification and carbohydrate metabolism. These large viruses can be infected by other smaller viruses.

The discovery of these giant viruses has reopened the debate 'are viruses living organisms?' In the future, the entire classification system for living organisms could change and all living organisms on Earth could be placed into one of two groups: ribosome-encoding organisms (cellular organisms) and capsid-encoding organisms (viral organisms), dispensing with the idea of domains.

Check your knowledge

1. Which aspect of the APMV's structure was responsible for it being thought of as a Gram-positive bacterium?
2. In what ways is APMV different from other viruses?
3. How have viruses played a key role in evolution on Earth?

Protists

Protists are difficult to classify into smaller groups because the kingdom is diverse. This means that different species of protist may not share common features. Protists can differ in their cellular structure, sources of nutrition and metabolism. Protists may be single-celled or multi-celled. They can also be free-living, or they may live with other organisms. Protists can act as parasites where the protist damages the host organism (**parasitism**) or both organisms can benefit from the **symbiotic** relationship (**mutualism**). Many protists are motile, which means they can move around in their watery environments. They may use cilia or flagella to move, some protists even have **pseudopodia** to help them move. Some protists produce their own nutrition from sunlight (**autotrophs**) or require a source of chemicals for their nutrition (**heterotrophs**).

Pause point

Explain why different characteristics are used to classify different microorganisms.

Hint

Start by listing the similar and different characteristics used to classify the different groups.

Extend

Add examples to your work.

Key points

Parasitism – a type of symbiosis where one organism (the parasite) benefits while the other organism (the host) is damaged.

Symbiotic – an interaction between two different organisms that live in close association.

Mutualism – a type of symbiosis where both organisms benefit from the association.

Pseudopodia – a temporary bulge from a cell used for movement and feeding.

Autotrophs – an organism that can form nutritional organic substances from simple inorganic substances.

Heterotrophs – an organism that ingests organic substances for its nutrition.

Assessment activity 2.1 A.P1 A.P2 A.M1

1. Produce a poster of the main groups of microorganisms. For each group include a labelled cell diagram and functions of the cellular components.
2. Explain how the structures and characteristics of microorganisms are used to classify them.
3. Produce flash cards to explain the key terms used in describing the virulence of a micro-organism.
4. Explain the structure and function of collagen.
5. Use examples of different diseases to analyse why some microorganisms are more virulent than others. Try to cover bacterial, viral, fungal and protozoan diseases.

B Examine the transmission and treatments of infectious disease

Infectious diseases are caused when pathogens enter the body and reproduce causing damage to host cells. To do this successfully, the pathogen may have strategies to evade the body's defence systems.

More than 1200 different human infectious diseases are known to date. It is important to be able to classify diseases into similar groups to allow scientists to study the similarities and differences between groups of diseases. This helps us to gain a better understanding of the mechanisms of disease, the spread of disease, prevention measures and possible treatments. The study of infectious diseases and their spread is known as epidemiology.

B1 Classification overview of infectious disease

Diseases can be classified by:

- target organs
- causative agent
- source.

Target organs

Infectious diseases can also be grouped according to the organs they affect. Pathogens cause damage to target organs by binding to cells that make up the tissues of that organ.

Some infectious agents target cells that make up the tissues of organs of the intestinal tract. Examples of intestinal diseases include salmonella, cholera and typhoid fever. Salmonella is caused by Gram-negative, bacillus bacteria that belong to the *Enterobacteriaceae* family. Salmonella infection can cause food poisoning symptoms such as diarrhoea and vomiting. Salmonella infection can be caused by a person eating food that contains the bacteria. Usually these bacteria are killed by cooking, but ingestion of poorly cooked meat such as chicken can lead to salmonella infection. Infection occurs when the bacteria reach the small intestine and reproduce in the tissues of the intestine. As they reproduce, the bacteria release endotoxins that cause gastroenteritis (diarrhoea and vomiting). Most people can recover from salmonella infection without treatment from a doctor but young children, the elderly and those with a weakened immune system may need treatment to prevent dehydration.

Other infectious diseases can target the respiratory tract. The respiratory tract refers to the organs responsible for breathing. The respiratory system allows us to take in oxygen from the air and expel carbon dioxide from the body. The respiratory tract consists of the trachea, bronchi, bronchioles, alveoli and lungs. The respiratory system structure can be seen in Figure 2.12. Infectious diseases that affect the sinuses and throat are called upper respiratory infections. Examples of upper respiratory tract infections include the common cold and laryngitis. Infectious diseases that affect the bronchi, bronchioles, alveoli and lung tissue are called lower respiratory infections. Examples of lower respiratory infections include bronchitis, pneumonia and tuberculosis. Upper respiratory tract infections usually clear up by themselves and do not require treatment, but lower respiratory tract infections can be more serious. Bacterial lower respiratory infections may need antibiotic treatment prescribed by a doctor.

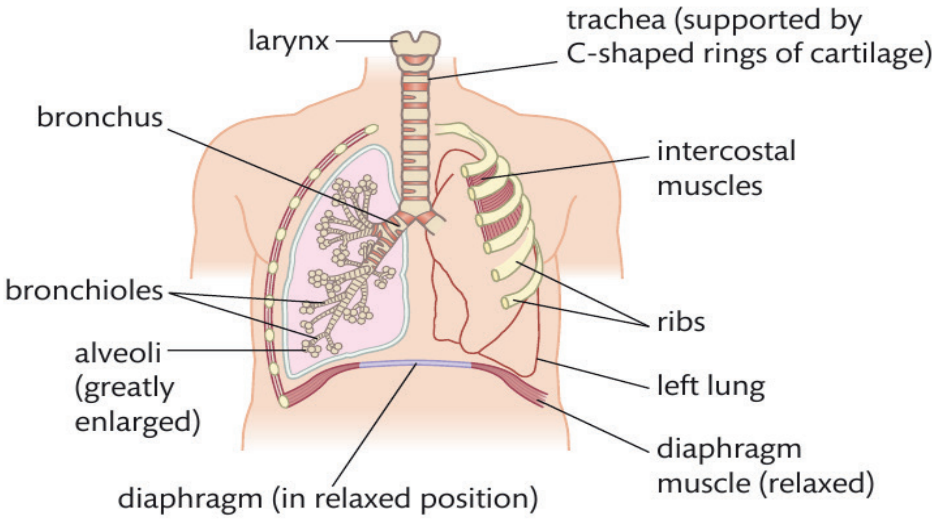


Figure 2.12: The structure of the respiratory system

Infectious diseases can also target the blood stream. The blood stream consists of red blood cells, white blood cells, platelets and plasma protein suspended in a fluid called plasma. Certain infectious pathogens can infect the cells of the blood stream.

Examples of blood stream infections are:

- Human Immunodeficiency Virus (HIV)
- Ebola
- Dengue fever
- Malaria.

HIV enters and destroys specific types of white blood cell called macrophages and CD4 T cells.

Ebola is caused by a virus and is found in Central and West Africa, Infection with this virus causes severe haemorrhaging (blood loss) and is fatal in 60% of infections. The virus infects another type of white blood cell called dendritic cells. Malaria is caused by eukaryotic organisms such as *Plasmodium falciparum* and *Plasmodium vivax*. Stages in the life cycle of this organism are carried in the saliva of the female *Anopheles* mosquito. When a person is bitten by a mosquito carrying the *Plasmodium,* the parasite enters and replicates inside red blood cells. Infected red blood cells are lysed (destroyed) which reduces the person's red blood cell count. This can lead to high fever, chills, extreme fatigue and flu-like symptoms.

The urinary system can also be a target for infectious diseases. The function of the urinary system is to filter waste products from the blood to produce urine. Urine is produced by the kidneys. Urine is then stored in the bladder until it is ready to be released from the body through a tube called the urethra. Urinary tract infections (UTIs) usually occur when bacteria enters the urinary tract but in rare cases they can be caused by fungi. Lower urinary tract infections, infections of the bladder, are known as cystitis. Infection of the upper urinary tract is known as pyelonephritis (kidney infection). The most common bacterial UTI is caused by *Escherichia coli* and is more common in women than men. The urethra that carry urine out of the body is much shorter in women than in men, which means that infectious occur more easily. Symptoms of UTIs include pain on urination, frequent urination, the feeling of needing to urinate despite an empty bladder and lower back pain. In most cases urinary tract infections can be treated by a short course of antibiotics. The following factors can increase the chances of developing a UTI: sexual intercourse, diabetes and obesity.

Some infectious diseases are classified as systemic. This means that they affect the whole body rather than just one organ, organ system or body part. Systemic infectious diseases usually start off localised to one particular part of the body but then spread over time to affect the whole body. This can lead to a wide variety of symptoms. Influenza or "flu" is an example of a systemic infection. Influenza is caused by an influenza virus. Symptoms include fever and chills, cough, runny nose, blocked nose, sore throat, headache, earache, tiredness, muscle pain, watering eyes and a rash. Most people can recover fully from flu with rest and fluids. For those who are vulnerable, such as the young, elderly, immune compromised or those with underlying illnesses, flu can be more serious. These groups of people are encouraged to get the flu vaccination each year to reduce their chances of getting flu. Sometimes very severe flu infections are treated with anti-viral medication.

Pneumonia is a condition that affects the alveoli in the lungs causing them to become inflamed. This produces symptoms including coughing, chest pain, breathing difficulties and fever. Pneumonia can be caused by various viruses or bacteria although bacteria are the most common cause. Pneumonia cases usually clear up within a few weeks, but bacterial pneumonia may require antibiotic treatment. The elderly or people with other lung conditions may take much longer to recover and may need further help from doctors. In those who are vulnerable, pneumonia can become systemic and the patient can develop sepsis. Sepsis (blood poisoning) is life-threatening and occurs when the body responds to infection by triggering an inflammatory immune response. Sepsis can cause fever, rapid breathing, increased heart rate, confusion, swelling and high blood sugar readings. Sepsis, if left untreated, can lead to septic shock. Septic shock is characterised by dangerously low blood pressure and abnormal cellular metabolism. Septic shock can lead to multiple organ dysfunction syndrome (multiple organ failure) and death occurs in around 50% of cases. Patients with sepsis or septic shock are treated by intravenous antibiotics and fluids. Sepsis can also be caused by other bacterial infections.

Pause point

Using a diagram of the human body, identify and describe the different target organs for infectious diseases. Give an example of a disease that targets each organ you include.

Hint

Start by reading over the section and summarising the key target organs.

Extend

Improve your work by explaining how infections can become systemic.

Causative agent

Infectious diseases can also be classified by the type of organism that causes the disease. The organism that causes the disease is also known as the causative agent. Infectious diseases can be bacterial, viral, protozoan, fungal, prionic, helminthic or ectoparasitic.

Table 2.5: Classification of infectious disease by causative agent

Causative agent	Description	Examples of infectious disease
Bacterial	Infectious disease caused by bacteria	Cholera, salmonella, staphylococcus infections
Viral	Infectious disease caused by virus	Measles, HIV, influenza
Protozoan	Infectious disease caused by protist	Malaria, balantidiasis
Fungal	Infectious disease caused by fungi	Athlete's foot, candidiasis (thrush), aspergillosis
Prionic	Infectious disease caused by prion	Variant Creutzfeldt-Jakob disease (vCJD), fatal familial insomnia
Helminthic	Infectious disease caused by parasitic worm	Tapeworm, roundworm, whipworm
Ectoparasitic	Infectious disease caused by a parasite that lives on the surface of the body	Scabies, crab lice (pubic lice), pediculosis (head lice)

Figure 2.13: Scanning electron micrograph (SEM) with colour added of prion proteins taken from the brain of an infected hamster Mag x135 000

Sources of disease

Infectious diseases are spread through the passing of the pathogen from source to host. Once the pathogen causes disease in the host, the host also becomes a source. The host can pass on the infectious microorganism to others causing the disease to spread.

A disease can be classified as an **anthroponosis**, **zoonosis** or **sapronosis**. The ending of each of these words, "-nosis" comes from the Greek word for disease.

Anthroponoses (plural of anthroponosis) are diseases that can pass from human to human. This word comes from the Greek work for man, "anthropos" and the Greek word for disease, "nosos". Examples of anthroponotic diseases include smallpox, gonorrhoea and rubella.

Zoonoses (plural of zoonosis) are diseases that can pass from living animals to humans. This word comes from the Greek word for animal, "zoon" and the Greek word for disease, "noses". Examples of zoonotic diseases include rabies, cat scratch disease and yellow fever. Zoonotic diseases rarely cause extensive outbreaks of disease, but high death rates are usually associated with the presence of disease.

Sapronoses (plural of sapronosis) are diseases that can pass from the non-living environment to humans. This word comes from the Greek work for decaying, "sapros" and the Greek word for disease, "nosos". Soil, water, decaying plants, dead animals and faeces are environments from which the pathogens causing sapronotic diseases can spread to humans. Examples of sapronotic diseases include legionnaires disease, anthrax and cholera.

Key points

Anthroponosis – diseases that can pass from human to human.

Zoonosis – diseases that can pass from living animals to humans.

Sapronosis – diseases that can pass from the non-living environment to humans.

Case study

Giant viruses with cell-like features

Paul works at a local visitor's farm. There is a herd of sheep at the farm, Paul must take special precautions to protect the farm's visitors from zoonotic infections. One of these infections is called ovine chlamydiosis. In healthy humans, this infection can cause mild flu-like symptoms, so visitors are reminded to wash their hands after visiting the farm. This infection can however be life-threatening to pregnant women and can cause stillbirth or miscarriage. Paul has to make sure there are signs up to warn pregnant woman of the risks of contact with sheep while they are at the farm.

Check your knowledge

1 What is meant by a zoonotic infection?
2 Why is it important that Paul puts up these signs?

B2 Transmission of infectious disease

Transmission of infectious disease refers to the passing of the pathogen from one infected source to another. This includes the passing of pathogenic bacteria, viruses, fungi, protozoa or prions to individuals who may or may not have had the infection before. The source of the infection, as we have seen earlier, can be living, e.g. animal, or non-living, e.g. soil. Transmission of disease is by one of two main methods, direct or indirect.

Direct transmission

Direct transmission is when the pathogen is transferred to an individual through physical contact.

An example of direct contact transmission is the transmission of pathogens during pregnancy, at birth or through breast feeding. When a foetus (unborn baby) is developing inside the womb, a temporary organ called the placenta forms. The placenta connects the developing foetus to the uterus using the umbilical cord. The placenta allows for the foetus to take up oxygen and nutrients required for growth and allows waste produced by the foetus to enter the mother's blood stream to be excreted. The placenta is expelled during childbirth after the baby has been born.

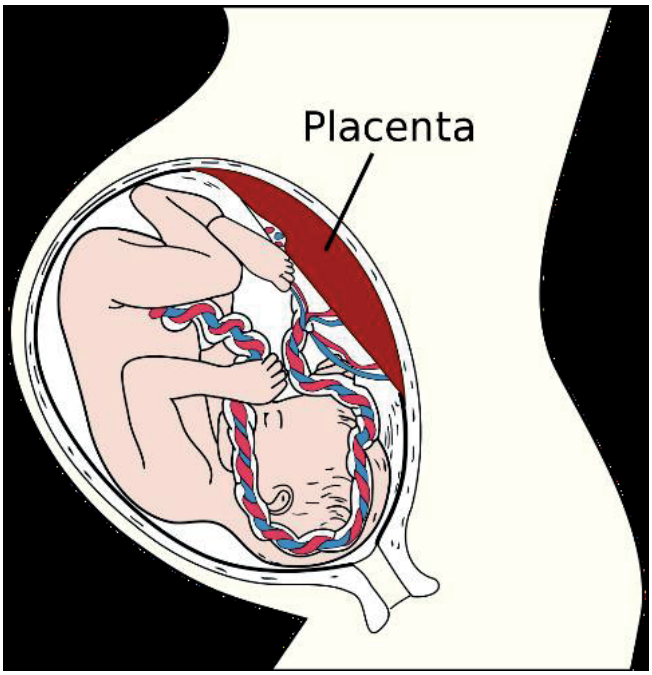


Figure 2.14: The placenta provides a surface for exchange of nutrients, oxygen and waste between the developing foetus and the mother

In 1995 Ford-Jones and Kellner further developed an acronym used to list the most severe infections that can be passed from mother to child. The acronym is CHEAPTORCHES.

It stands for:

- C – chickenpox and shingles
- H – hepatitis C, D and E
- E – enteroviruses
- A – AIDS (HIV infection)
- P - parvovirus B19
- T – yoxoplasmosis
- O – other (Group B streptococcus, Lyme disease)
- R – Rubella
- C – cytomegalovirus
- H – herpes simplex
- E – everything else sexually transmitted (E.g. chlamydia, gonorrhoea)
- S – syphilis.

If the infection is present in the mother's blood stream, it can be transmitted across the placenta to the unborn baby. This can happen in the case of HIV infection. As routine, all pregnant women in the UK are given the opportunity to be screened for HIV, hepatitis B and syphilis in the early stages of pregnancy. If HIV infection is found, anti-viral medication can be given to reduce the replication of the virus and prevent it being passed from the mother to the developing foetus. If syphilis infection is found, the mother can begin antibiotic treatment to prevent the bacteria from spreading to the foetus. Infections can also be spread to the baby during childbirth, as blood from the mother comes into contact with the baby. Sexually transmitted pathogens such as *Neisseria gonorrhoeae* bacteria and *Herpes simplex* virus can be transferred to the baby as it passes through the birth canal. If it is known that the mother has either of these active infections, the baby could be delivered using an operation called a caesarean section to prevent the baby having contact with the pathogens. There are only a small number of infections that produce enough pathogens in breast milk to cause infection in a breast-feeding baby. These infections include HIV, chicken pox that developed five days before or two days after childbirth, cytomegalovirus and Ebola. In these cases, it is best that the baby is fed formula milk rather than breast feeding or using expressed milk.

Infections can spread when an infected person has direct physical contact with a non-infected person. Interactions such as touching, kissing, sexual contact or contact with skin wounds can lead to the transmission of pathogens from one person to another. During this type of transmission, the body fluids that contain the infectious pathogens come into contact with the body fluids or mucous membranes of an un-infected person. Some infections can be spread in saliva, e.g., Epstein-Barr virus (glandular fever) and *Streptococcus* (sore throat). The most common viruses that can passed by contact with infected blood are hepatitis B, hepatitis C and HIV. A person can become infected with these viruses if the skin is broken as they come into contact with infected blood. This could happen if they use a needle that has infected blood on it (intravenous drug users) or if they accidently injure themselves on an infected needle (needle-stick injury). There is a lower risk of infection if infected blood comes into contact with broken skin, eyes, the mouth or nose. Hepatitis B, hepatitis C and HIV can also be spread by unprotected sexual intercourse between an infected person and an uninfected person. A person with HIV (if not undergoing effective treatment) will have the virus in some of their bodily fluids. Semen and vaginal fluids can transmit HIV. The infected fluid must come into contact with a mucous membrane or with blood for infection to be transmitted. Mucous membranes are found inside the rectum, vagina, penis and mouth. The risk of transmitting HIV is reduced by using a condom during sexual intercourse. HIV is not spread through saliva.

Some infectious diseases can be spread from animals to people, we have learnt that these types of disease are called zoonotic diseases. Some diseases such as rabies can be transmitted directly from an animal if the infected animal bites or scratches. Rabies virus can be carried by dogs and cats, but in the UK this is highly unlikely. Rabies is extremely rare in the UK but is found in Asia, Africa and Central and South America. Rabies can be transmitted to humans through a bite from an infected animal. The symptoms of rabies are very similar to those of flu but progress to confusion, anxiety, delirium, and hallucinations. Rabies is almost always fatal if treatment has not begun before symptoms appear. There are vaccinations to prevent rabies infection. Some infectious diseases can also be spread through contact with infected animal faeces. The parasite *Toxoplasma gondii c*an be found in the faeces of cats and can be present in cat litter. This parasite can be dangerous to pregnant women and those with a weakened immune system. There is a small risk that the parasite can cause miscarriage or still birth in pregnant women. If infection occurs in early pregnancy then there is only a small chance that it can spread to the baby but if it does, the problems that develop are more likely to be serious. If infection occurs later in the pregnancy, there is more chance of it spreading to the baby, but any consequences are less likely to be severe.

Indirect transmission

Indirect transmission is when the pathogen is transferred to an individual by an intermediate agent such as the air or an insect.

Some zoonotic infections can be spread when a vector carries the infectious agent. A vector is an animal that carries the disease-causing agent from an infected individual to an uninfected individual. A protozoan parasite called *Trypanosoma* causes Chagas disease and sleeping sickness and can be transmitted by fleas, ticks and tsetse flies. Lyme disease is the most common tick-borne disease in the Northern Hemisphere. It is caused by infection with *Borrelia burgdorferi* bacteria and has the symptoms of fever and chills, headache, joint pain, muscle pain and extreme **fatigue**. Lyme disease can usually be treated effectively with antibiotics but in some people, the symptoms can last for years and disrupt normal life. The bacteria are transmitted by ticks. Ticks are **arachnid** parasites that live by feeding on the blood of mammals. A tick can pick up the bacteria when feeding on infected blood and transmit it to a new host when feeding on an uninfected individual. One of the tell-tale signs of Lyme disease is a circular red rash surrounding a tick bite on the skin, this is often described as looking like a bullseye. Malaria is another disease that is transmitted by a vector. Malaria is serious and sometimes fatal. The symptoms of malaria are high temperatures (about 38°C), vomiting, diarrhoea and headaches. The most serious type of malaria is caused by *Plasmodium falciparum*, infection with this parasite can lead to the development of breathing difficulties and organ failure, which can be life-threatening. Malaria can be transmitted by the female *Anopheles* mosquito from person to person. The mosquito feeds on blood it obtains by piercing the skin. When the mosquito takes a blood meal from a person with the parasite in their blood stream, it ingests a form of the parasite called the **gametocyte**. These gametocytes reproduce in the gut of the mosquito and another form called the **sporozoite** travels to the mosquito's salivary gland. The *Anopheles* mosquito will then take another blood meal from another human and when biting will inject both the parasite and an **anticoagulant** into the person's blood stream. The parasite travels to the person's liver to grow and multiply before releasing gametocytes into the bloodstream to infect and destroy red blood cells.

Key points

Fatigue – extreme tiredness.

Arachnid – a class of invertebrate animals having eight legs.

Gametocyte – a cell produced by protozoa that is used for the purposes of reproduction.

Sporozoite – a cell produced in the lifecycle of some parasites that can move.

Anticoagulant – a substance that prevents blood from clotting.

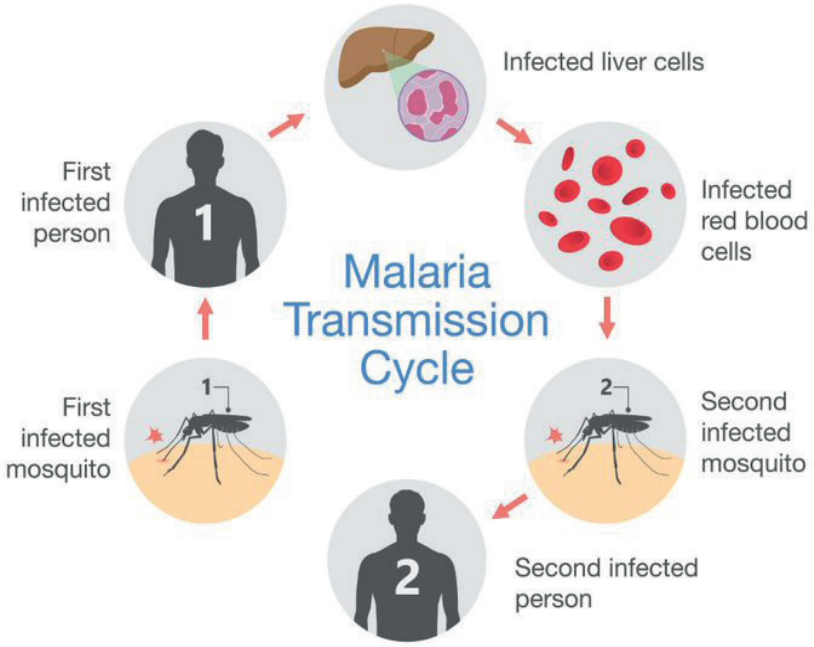


Figure 2.15: The transmission cycle of malaria

Airborne transmission

Some infectious agents can also be transmitted through the air. This type of transmission is referred to as airborne transmission. The common cold (caused by rhinovirus) and flu (caused by the influenza virus) are commonly transmitted from person to person by droplets of mucus suspended in the air. Mucus droplets are expelled into the air when an infected person coughs or sneezes. The mucus droplets can remain suspended for a short period of time and can be breathed in by an uninfected person in close proximity to the sneeze or cough. The mucus will contain thousands of viral particles which may then cause infection in the new host. In 2018, the UK government used a campaign to prevent the spread of flu using the slogan "Catch it, Bin it, Kill it", which encouraged people to sneeze or cough into tissues to prevent mucus becoming airborne. Measles is another infection that can be spread by airborne droplets and aerosol particles. When an infected person coughs or sneezes, the virus in mucus droplets or aerosol is released into the air. Measles is highly infectious as a person expels viral particles in their coughs and sneezes before they develop any symptoms. They could therefore be transmitting the virus to others while they are not aware they are doing so. Measles symptoms include a cough, fever, runny nose, sore throat and white spots (Koplik spots) inside the mouth. Most people who have measles infection will recover fully although there is a higher risk of complications in young children, pregnant women, people with a weakened immune system and people who are malnourished. About 30% of people with measles will develop complications such as ear infections and bronchitis, but measles can also cause more serious complications.

Vehicle borne transmission

Infectious agents may also be transmitted non-directly through vehicles. A vehicle is a non-living entity containing pathogens that can then be picked up by an individual having contact with that vehicle. An example of a vehicle is a kitchen surface. Salmonella bacteria can be found in uncooked chicken meat. If this is left on a kitchen surface and the surface is not cleaned effectively, a person could touch the surface and then ingest the bacteria by hand to mouth action such as eating. The person may then develop food poisoning, symptoms of which include diarrhoea, vomiting, nausea and stomach cramps. Door handles, computer keyboards, telephones and handles on shopping trolleys are other common places where bacteria and viruses can be found. Regular cleaning of surfaces and hands can help to prevent the spread of infection in this way.

Disease can also be food and waterborne. This means that the infectious agent can be transmitted through the consumption of infected food or water. There are many different disease-causing pathogens that can contaminate food. Usually cooking food thoroughly and ensuring it is consumed before it is spoilt can prevent enough of the pathogen being present to cause illness. In the earlier example of salmonella bacteria being present in chicken meat, cooking the chicken fully would ensure that these bacteria are killed and not transmitted to the person when they consume it. *Escherichia coli* is another bacterium that causes "food poisoning", the symptoms of *E. coli* infection are severe stomach cramps, watery diarrhoea and the presence of blood in diarrhoea. There are different strains of *E. coli* that cause varying severities of infection. Most people will fully recover from infection but those who are young, elderly, or vulnerable may develop severe dehydration from diarrhoea, which can be fatal. *E. coli* bacteria can be transmitted by inadequately cooked meat, unwashed fruit and vegetables and unpasteurised milk. Waterborne diseases are caused by pathogens that are transmitted when contaminated water is consumed. This can be through drinking the water or washing fruit and vegetables in contaminated water and then eating them. The bacterium *Vibrio cholerae* can contaminate drinking water and cause the disease cholera. Cholera affects around five million people worldwide and is the cause of tens of thousands of deaths each year in countries in the Global South.

The chain of infection

The chain of infection is the events that must take place to allow pathogens to cause infection in a person. The chain begins with an infectious agent, the bacteria, virus, fungi, protozoan or prion, that has the potential to cause infection. The second stage in the chain is the reservoir, a place where the pathogen can live and reproduce. You have already seen different types of reservoir earlier in the chapter. The next step in the chain is portal of exit. This refers to how the microorganism leaves the reservoir. Once the pathogen has left the reservoir using the portal of exit, it must be transmitted. The mode of transmission, as you have seen, can be direct or indirect. The next step in the chain is the portal of entry, the route that the pathogen uses to enter the host. The host is the next step in the chain. Once in the host the pathogen multiply and cause damage to the host cells and tissues. The chain of infection includes all the necessary features required for infection to happen. It is often useful to think of each feature as a link in the chain. To stop an infection from happening we try to break at least one of the links in the chain.

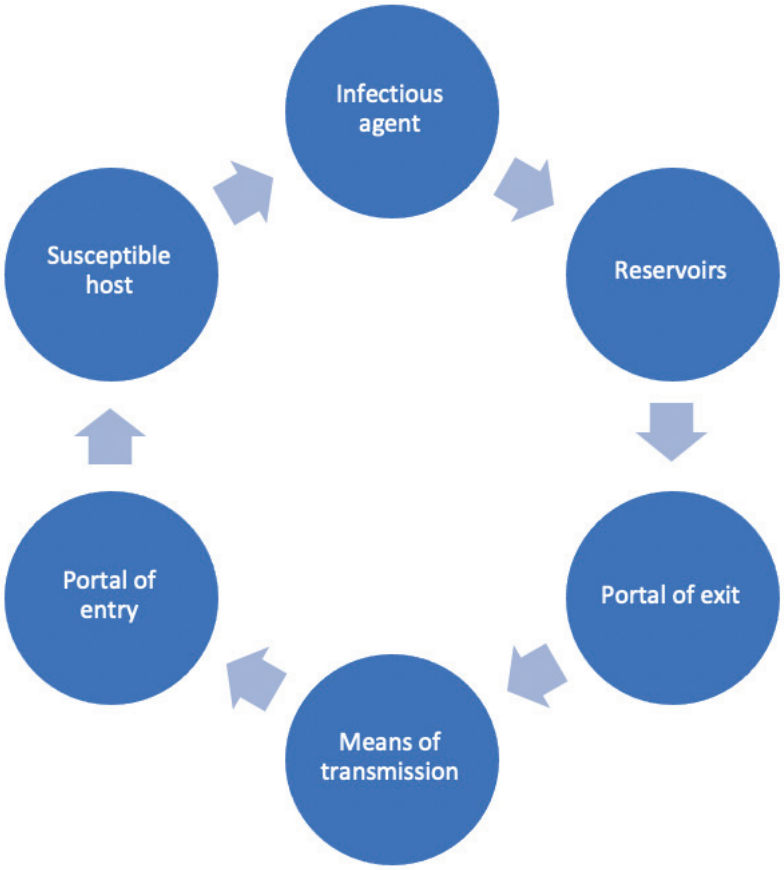


Figure 2.16: The chain of infection

B3 Infectious diseases

This section will cover the causes, signs and symptoms, and progression of a range of infectious diseases. The signs of an infection disease are objective and refer to the physical effects that can be seen and monitored in different patients with the same infectious disease. The symptoms of the disease are subjective and relate to how the patient feels, based on their own interpretation of feeling unwell, examples include headache, nausea and shakiness. The progression of a disease is the change in the way the infection affects the patient over time.

Bacterial diseases

Meningitis

Meningitis is the term used to describe severe inflammation of the membranes (the meninges) that protect and cover the brain and spinal cord.

Causes

Meningitis can be caused by a viral or bacterial infection. Bacterial meningitis is rarer but can be very serious if not found and treated quickly. Different bacteria lead to different forms of meningitis in people of different ages. In premature and newborn babies, *group B Streptococcus* bacteria can cause meningitis. These bacteria, if present in the mother's vagina, can be passed to the baby during childbirth. Older children can develop meningitis after infection with *Neisseria meningitis* or *Streptococcus pneumoniae* bacteria. Another bacterium called *Listeria monocytogenes* can also cause meningitis in adults.

Signs and symptoms

The symptoms of meningitis include headaches, photophobia (aversion to lights), phonophobia (aversion to sounds), vomiting, muscle and joint pain. If meningitis occurs in babies or young children, they may not be able to communicate their symptoms and so doctors must look for key signs of infection. These signs include a high-pitched cry, refusal to feed, becoming stiff, floppy or unresponsive and a bulging soft spot on the top of the head. Not all of these signs may be present. In older children and adults signs of meningitis include high temperature, stiff neck (doctors can test this), seizures and spots or a rash.

If a person is suspected of having meningitis they will be admitted to hospital. A sample of cerebrospinal fluid is usually taken in a procedure called a lumbar puncture. The fluid is checked for bacteria and viruses. The patient is usually started on antibiotic treatment, often given directly into a vein, before the test results are returned, this can then be stopped if needed. The patient may also be given steroid medication to reduce any swelling on the brain.

Progression of disease

The bacteria that cause meningitis usually enter the body in aerosol droplets. These aerosol droplets are created when an infected person coughs or sneezes, resulting in a non-infected person inhaling them. The bacteria then bind to receptors on cells in the upper part of the throat before being transferred to the bloodstream. The bacteria multiply in the bloodstream and the person may start to experience symptoms. The bacteria eventually end up in cerebrospinal fluid found in the brain and spinal cord. Most people with meningitis infection will fully recover but it is possible that long-term problems can arise. Complications range from hearing loss, seizures, memory problems, learning difficulties, vision loss, kidney problems to loss of limbs. In around 10% of patients, bacterial meningitis is fatal, the chances of this rapidly increase if treatment does not begin within 24 hours. It is important that if a person is suspected of having meningitis, that they receive medical attention immediately.

The chances of developing meningitis can be reduced by ensuring you are fully vaccinated against the common causes. Children receive vaccinations against meningitis as part of the normal vaccination schedule in the UK. Young teenagers in England are offered a further meningitis vaccine (MenACWY vaccine) as part of the "teenage booster" vaccine programme in school Years 9 or 10 (UK).

Chlamydia

Cause

Chlamydia is caused by a bacterial infection. It is a sexually transmitted disease that is particularly common in sexually active teenagers and young adults. The bacteria, *Chlamydia trachomatis,* can be spread through sex or contact with infected semen or vaginal fluids.

Signs and symptoms

Many people who are infected with chlamydia do not get any symptoms. In some cases, it might take several months for symptoms to develop and in other cases, symptoms may disappear even though there is still an active infection. Symptoms in females include pain when urinating, unusual discharge from the vagina, pain during sex and bleeding after sex or in-between periods. Males can experience pain when urinating, a white discharge from the penis and pain in the testicles. As a chlamydia infection does not usually cause symptoms, it is recommended that those under 25 and sexually active are tested for chlamydia once a year and when they change sexual partners. Chlamydia infection does not usually have signs that can be observed by a doctor but there are simple and painless tests for infection. A swab of the vagina or a sample of urine from a male or female can be sent to be tested for the presence of the bacteria. The test works by detecting the genetic DNA of *Chlamydia trachomatis* and takes around two days to give results.

Progression of disease

Treatment of chlamydia infection is a short course of the antibiotic azithromycin or doxycycline. In the majority of people, this antibiotic treatment will cure the bacterial infection provided that the antibiotics are taken correctly and completely. It is important that chlamydia is detected and treated as it can be spread to other areas of the body and can lead to serious complications. In males, untreated chlamydia can spread to the testicles and to the tubes that carry sperm from the testicles to the penis. These tubes, the epididymis, can become painful and inflamed. Inflammation can lead to infertility. Males can also develop sexually acquired reactive arthritis (SARA) which is a painful inflammatory condition affecting the joints in the body. In females, chlamydia infection can spread to the womb, ovaries and fallopian tubes. This can cause a serious and painful condition called pelvic inflammatory disease (PID). PID can lead to further serious complications such as infertility, persistent pain and an increased risk of ectopic pregnancy.

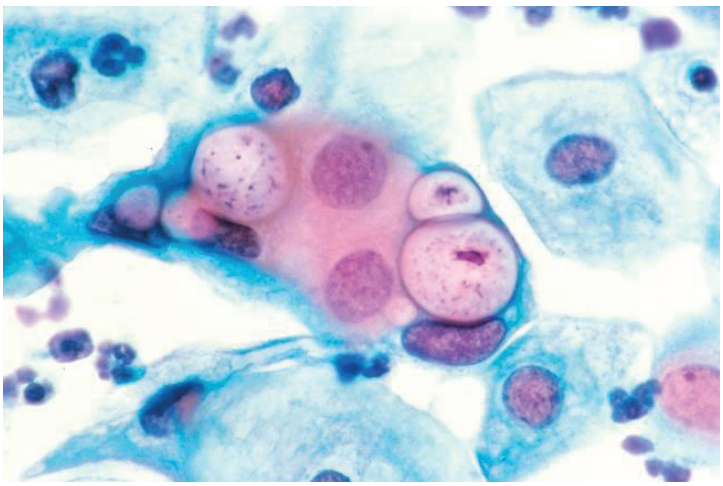


Figure 2.17: A human Pap smear showing chlamydia infection

Cholera

Cause

Cholera is a bacterial infection caused by *Vibrio cholerae*. Cholera is not present in the UK but there is a risk of catching it when travelling abroad, particularly to areas where cholera is **epidemic** and **endemic**, typically where access to clean drinking water, treatment and appropriate medical care is limited. The bacteria are spread by faeces contaminating drinking water and reinfection can occur where waste contaminates drinking water. This combined with lack of medical care can lead to a fatal cycle of infection, dehydration and reinfection.

Signs and symptoms

Symptoms of cholera can start from two hours to five days after exposure. In some cases, infections with certain strains of the bacteria can be asymptomatic (no symptoms) while some strains may cause minor infections. 5% of infections can be severe and may become life- threatening. The symptoms of severe cholera are diarrhoea, which is so acute that dehydration can occur within a few hours. The diarrhoea produced is clear and has a distinctive fishy smell. The infected person may also experience vomiting and muscle cramps. Dehydration can lead to sunken eyes, wrinkling of the hands and feet and cold skin. Patients may have deep and difficult breathing, which can be observed by a doctor. Blood tests can also measure an imbalance of electrolytes (salts) present in the blood. A person with cholera may also have a rapid pulse and experience drops in their blood pressure.

Progression of disease

If untreated, fluid loss can lead to dehydration. Dehydration can cause damage to the nephrons of the kidneys, which can develop into kidney failure. Severe fluid loss can also cause the blood volume to decrease causing a drop in blood pressure, collapse of blood vessels and shock. Fluid loss can lead to imbalances in electrolytes (salts) in the body, which can cause the body pH to become acidic which can lead to coma and death. Treatment with oral rehydration therapy (ORT) is usually effective at replacing the fluids lost if enough is given to the patient.

Key points

Epidemic – a rapid increase in the number of new cases of disease in one particular location.

Endemic – cases of the disease are always present in the population of a particular location.

Pause point

Explain why early detection and treatment of bacterial diseases improve the chances of the patient making a full recovery.

Hint

Use an example of a bacterial disease to describe how the disease progresses over time.

Extend

Explain why there is a higher fatality rate from bacterial diseases in areas with limited access to clean drinking water and appropriate medical care compared to areas where clean drinking water, health facilities and appropriate treatment are readily available.

Viral diseases

HIV

Cause
HIV stands for Human Immunodeficiency Virus; it is a virus that infects and damages cells of the immune system. HIV is transmitted through direct contact with body fluids during sexual intercourse with an infected person or through the sharing of infected needles. People who have unprotected sex with casual partners or who are intravenous drug users are encouraged to have regular tests for HIV.

Signs and symptoms
Most people who have HIV develop symptoms similar to flu around two to six weeks after infection. Symptoms include increased temperature (fever), sore throat, tiredness, muscle pain and a rash. Symptoms usually last for two weeks but then disappear. An infected person may not experience any further symptoms for up to 10 years despite having an active viral infection.

Progression of disease
Inside the body, the virus binds to receptors on CD4 (T helper) cells and inserts its RNA into the cell. Once inside the cell, a DNA copy of the RNA is made, which becomes part of the CD4 cellular DNA. When the cell replicates as part of the normal cell cycle, copies of the viral genome are made. This can happen immediately or after a period of dormancy. New virus particles are assembled that leave the infected cell, destroying it in the process. The new viral particles can attach to and infect other CD4 cells. Over time more and more CD4 cells are destroyed and the person's ability to fight off other infections decreases. A person with HIV will have regular blood tests to measure their CD4 counts. When the CD4 count falls below 400 per microlitre of blood, the person is diagnosed with AIDS. AIDS stands for Acquired Immunodeficiency Syndrome and is used to describe the point in HIV infection where the immune system is no longer able to defend against usually minor infections. Once the immune system has become damaged, a patient may experience weight loss, night sweats, skin problems and recurrent infections. Patients may begin to develop opportunistic infections such as tuberculosis, thrush, chronic diarrhoea and meningitis. The only measurable sign of HIV is found during a HIV test. Early detection of HIV can allow doctors to start patients on antiretroviral treatment. This treatment can delay the destruction of CD4 cells and allow infected people to live relatively normal lives for many years.

Ebola

Cause
Ebola is a viral disease that is caused by viruses of the *Ebolavirus* genus. One of these viral species, *Zaire ebolavirus,* is responsible for the largest number of outbreaks in humans. The virus spreads through direct contact with the blood or bodily fluids of an infected person who has developed symptoms of the disease. Infection usually occurs through contact with blood, faeces or vomit. Healthcare workers who care for and treat infected patients are most at risk of infection and must protect themselves by wearing masks, gowns, gloves and eye protection.

Signs and symptoms
After infection it takes from 2 to 21 days for symptoms appear. These symptoms are influenza- like symptoms that come on suddenly. Patients experience tiredness, decreased appetite, muscle and joint pain, headaches and sore throats. Increased body temperature above 38.3°C is a typical sign of Ebola infection. Symptoms progress to vomiting, diarrhoea, stomach pain and severe dehydration. Five to seven days after first symptoms, all infected people show some decrease in blood clotting. This can cause bleeding from mucous membranes, vomiting blood, coughing up blood or blood in faeces. If bleeding occurs, it indicates that the infection is likely to be fatal. Death, if it occurs, typically happens between 6 to 16 days after symptoms begin and can be caused by low blood pressure resulting from loss of fluid.

Progression of disease
If an infected person survives, they usually make a full recovery. Sometimes long-term complications such as muscular pain, hair loss and inflammation of the eyes leading to light sensitivity and vision loss can occur. The sooner a person receives treatment for Ebola infection, the greater the chance of complete recovery.

Norovirus

Cause
Norovirus is also known as the "winter vomiting bug" and is the most common cause of gastroenteritis. It is caused by the *Norwalk virus,* which can be transmitted by indirect contact with infected faeces via contaminated surfaces, food, water or air. Norovirus is extremely contagious with only a small number of viral particles required to cause infection.

Signs and symptoms
Between 12 to 48 hours after exposure to the virus, the person experiences diarrhoea, projectile vomiting, stomach pain and headache. The only other clinical sign in addition to the symptoms experienced by the infected person may be a slightly elevated body temperature. Transmission often occurs when infected vomit or diarrhoea becomes an aerosol due to flushing the toilet, for example. Infection can also happen if a person eats or breathes air near to where vomiting or diarrhoea has recently occurred. It is possible for traces of the virus to be detected a few weeks after a person has recovered from infection.

Progression of disease
Norovirus does not usually require any treatment and clears up within two to three days with the person making a full recovery.

Fungal diseases

Ringworm

Cause
Ringworm is a highly infectious skin infection that despite its name, is actually caused by fungus. There are different fungi that can cause infection of different parts of the body. Ringworm is caused by a type of fungi called a dermatophyte that lives on the protein keratin found in the skin. Some examples of the species of fungi that can cause ringworm include *Epidermophyton floccosum*, *Tinea manuum* and *Tinea unguium*. Fungal infections can occur on the face (tinea faciei), scalp (tinea capitis), groin (tinea cruris), foot (tinea pedis) and body (tinea corporis).

Signs and symptoms
Symptoms of ringworm are red rings of scaly, outward growing skin on the affected body parts. Figure 2.17 shows the characteristic ring produced in ringworm infection. The rash rings are itchy and can spread by scratching the affected area. The most common form of ringworm is an infection of the foot known as athlete's foot. Athlete's foot can be difficult to treat, often requiring long- term use of antifungal creams and sprays. It is called athlete's foot because those who walk barefoot in public areas, such as changing rooms at gyms or swimming pools, are at a higher risk of catching the fungal spores that may have been left behind by an infected person. Athletes are more prone to the infection, as the fungi grows best in damp areas with poor ventilation such as the footwear of a runner. Symptoms of athlete's foot includes dry scaly patches on the soles of feet, clusters of blisters on the sides of feet, peeling and moisture between the toes and dry patches on the top of the feet. The signs of ringworm that a doctor will observe are typically the presence of the characteristic red ring rash.

Progression of disease
Ringworm can be treated with antifungal creams applied to the affected skin.

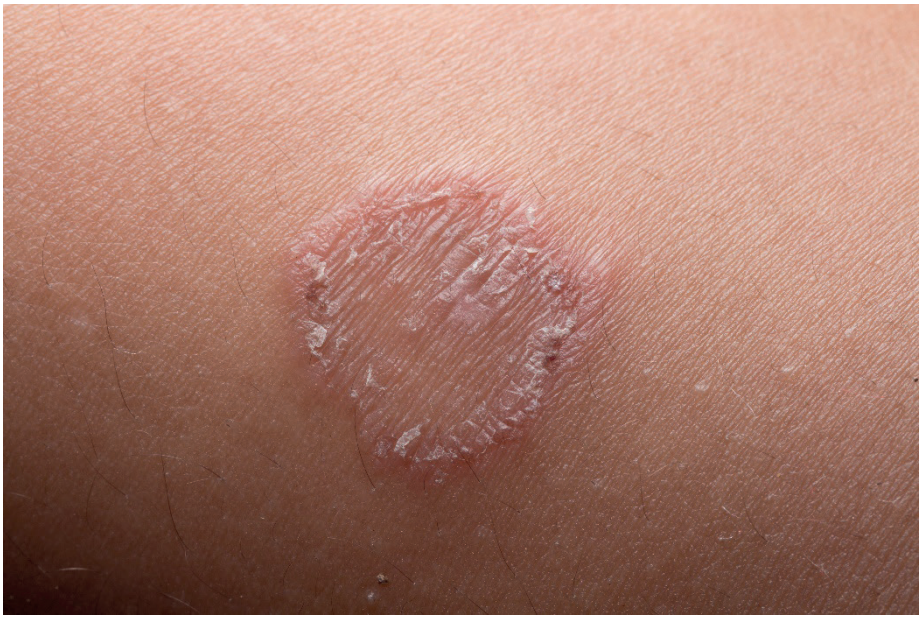


Figure 2.18: The common red circular rash typical in ringworm infection

Candidiasis

Cause

Candidiasis is another infection caused by fungus. The name refers to an infection caused by any type of a yeast called *Candida*. This type of infection is most common in the mouth (oral thrush) or vagina (yeast infection or vaginal thrush), though it can affect other parts of the body such as skin and fingernails. In people who are immunocompromised and those who have weakened immune systems, far more serious infections can occur, potentially leading to severe morbidity and mortality.

Signs and symptoms

Symptoms of candidiasis vary depending on the body part affected but typically the symptoms are redness, itching and discomfort. In oral thrush a white or cream coloured coating of the tongue may be observed. Oral thrush is most common in babies less than one month old. This type of infection is not considered to be a concern unless it persists for more than a few weeks. It can however cause discomfort when feeding and the infant will be given a mouth gel to apply to the insides of the mouth and tongue. Candidiasis infection of the vagina can cause symptoms such as severe itching, burning and soreness. There may also be an unpleasant discharge. The most causative agent of vaginal thrush is the organism *Candida albicans*. It is estimated that 2/3 of females will experience vaginal thrush in their lifetime. HIV/AIDS, diabetes and repeated treatments with antibiotics increase the chances of vaginal thrush occurring. In people who are immunocompromised, candidiasis of the oesophagus can occur.

Progression of disease

This type of infection has a higher potential for becoming systemic, producing a very serious infection called candidemia. This can produce mild to extreme flu-like symptoms, pain, chronic tiredness and other infections. Systemic infection occurs when the yeast enters the bloodstream and spreads to other organs. Systemic candidiasis is more serious and can lead to sepsis which can be fatal.

Prionic diseases

CJD

Cause

CJD stands for Creutzfeldt-Jakob Disease. It is an extremely rare condition that affects the brain and is always fatal. CJD is caused by a prion (abnormally folded protein) that causes other proteins to become misfolded. This affects the processes the brain uses to send signals and results in damage to the neurones (nerve cells). This gives the brain a porous appearance, which is described as spongiform. Over time the number of misfolded proteins increases causing cell death and loss of function.

Signs and symptoms

The condition is progressive, which means that the symptoms worsen over time. This condition is always fatal as there is no treatment. Patients are given medication such as steroids, painkillers and antidepressants to alleviate symptoms. Most people with CJD will die within six months of first symptoms although they can live for up to 2.5 years after initial onset. There are different forms of CJD.

- Sporadic CJD (sCJD) is caused by spontaneous misfolding of proteins in a person. This is the most common form of CJD but still in the UK there are only one or two cases per million people each year.
- Familial CJD (fCJD) is caused when a mutation is passed from parent to child. This type affects around one in every nine million people in the UK.
- Acquired CJD (aCJD) or iatrogenic CJD (iCJD) is caused when infection is accidently transmitted during a medical or surgical procedure. It can also occur if instruments used during brain surgery are not effectively cleaned before use during a subsequent operation. This type of transmission is extremely rare as increased awareness has improved sterilisation techniques in surgery.
- Variant CJD (vCJD) is caused mostly by consuming meat from a cow that is infected with the bovine form of the disease. The form of the disease in cows is called bovine spongiform encephalopathy (BSE) or “mad-cow” disease for short. Between 1995 and 2000 there was an increase in the number of cases of vCJD in the UK. This was caused by the practice of using animal remains to feed other livestock such as cattle. This practice was banned in 1996. There have been no reported cases of vCJD in anyone born after 1982 and cases of vCJD in people born before this date are extremely rare. There are now strict rules in place to stop any meat from cows infected or suspected of being infected from being consumed.

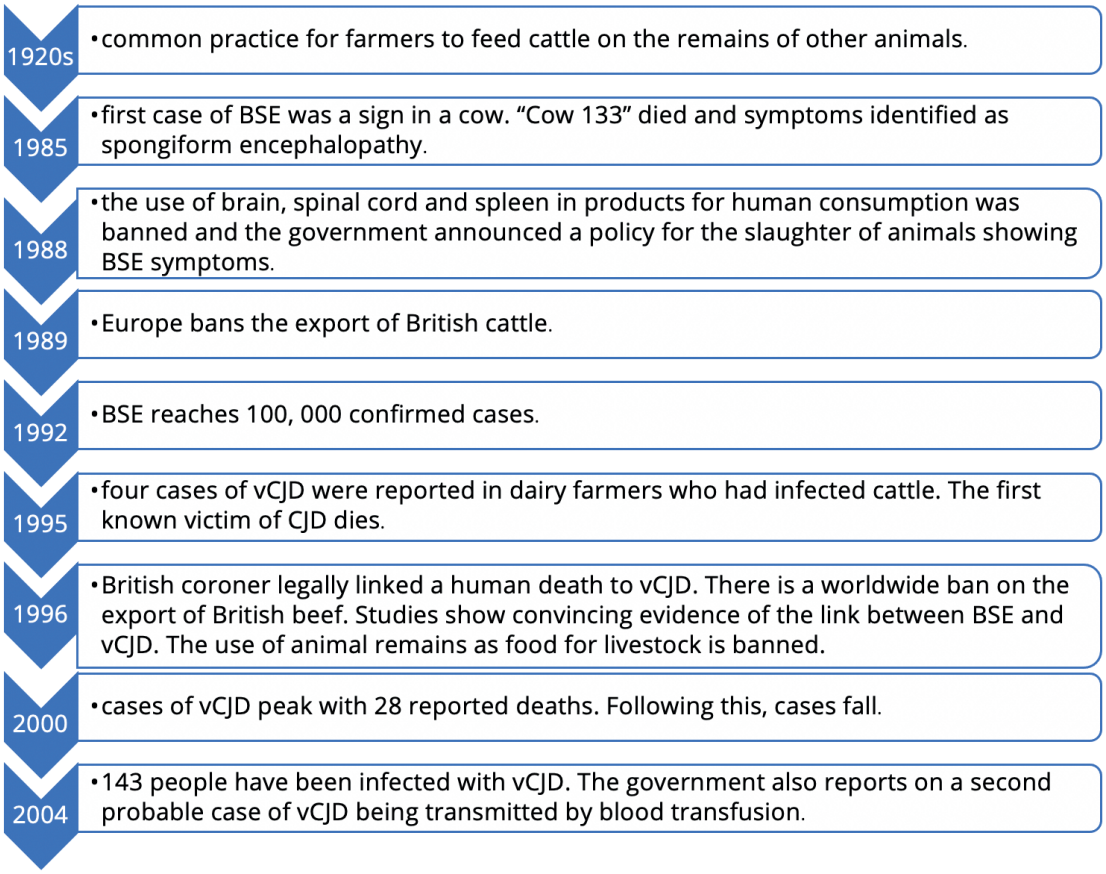


Figure 2.19: Timeline of the vCJD outbreak in the UK

Progression of disease

CJD can have a long incubation period with no symptoms for up to 40 years after initial infection. Symptoms usually begin with memory loss, impaired judgement and blurred vision. Some people may also experience an inability to sleep. As the disease progresses symptoms worsen, with people developing problems with coordination, jerky body movements and blindness. This will eventually develop into the patient not being able to move or speak and they will fall into a coma. There is no test to confirm diagnosis of CJD as this would require a brain biopsy, which is too dangerous to perform while a patient is alive. Doctors will look for specific signs that indicate CJD. A physical examination can observe problems with reflexes and muscle spasms and an eye test can detect partial blindness. A lumbar puncture can be performed where fluid is taken from around the spine. This fluid is tested for a protein, which, if present, indicates that the person has a high chance of having CJD. A doctor will also observe a patient for changes similar to dementia that progress quickly.

Pause point

There have been no new vCJD cases in anyone born after 1982. Explain why.

Hint

Consider how vCJS is transmitted and how the disease progresses.

Extend

Discuss why diseases caused by prions are extremely rare.

Parasitic protozoan diseases

Malaria

Cause

Malaria is a tropical disease that is caused by a plasmodium, a single- celled eukaryotic organism. The most common causative organisms are *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*. The female *Anopheles* mosquito can transfer infective stages of these organisms called sporozoite in their saliva. The sporozoite is transferred to a person's bloodstream when the mosquito bites to take a blood meal. The sporozoite travels to the liver where it reproduces to produce another stage in the plasmodium lifecycle called the merozoite. Merozoites can then infect red blood cells and reproduce to produce more merozoites. These turn into the male and female gametes of the organism. When another mosquito comes along and feeds on an infected person, they take up some of these gametes. The gametes move to the mosquito gut and merge to form immature sporozoites. Sporozoites then travel to the mosquito salivary glands to develop and can then be passed onto another person when the mosquito takes another blood meal.

Signs and symptoms

Symptoms of malaria begin 8 to 25 days after infection. Commonly people experience flu-like symptoms such as headaches, fever and vomiting. Infected people may also experience jaundice, blood in the urine, damage to the eyes and convulsions. There is a typical cycle of sudden coldness followed by shivering and then fever and sweating. The timing of the cycle varies with the organism causing infection. For example, *P. vivax* produces a three-day cycle and P. falciparum produces a 36-48-hour cycle. Severe malaria is caused by P. falciparum. Severe malaria has the additional symptoms of drowsiness and weakness. Red blood cells are destroyed, which leads to a decrease in the oxygen transported around the body. This causes tissues to carry out a lower rate of aerobic respiration producing less ATP.

Progression of disease

If left untreated malaria can progress into a complication known as cerebral malaria. This complication results in blockages in the blood vessels carrying blood to the brain. This can cause seizures, brain damage and coma. Other complications can arise from severe malaria infections including liver failure, shock, a build-up of fluid in the lungs, kidney failure and rupturing of the spleen.

Giardia

Cause

Giardia is an infection caused by a parasitic protozoan organism called *Giardia lamblia*, the infection is commonly known as beaver fever. Giardia causes gastrointestinal infection but 10% of infected people do not experience symptoms.

Signs and symptoms

When symptoms occur, they include diarrhoea, stomach cramps and weight loss. Some people may also experience vomiting and fever. Infection is characterised by chronic, greasy and foul-smelling diarrhoea that can last for several weeks. The long period of diarrhoea can result in weight loss, malnutrition and dehydration. The protozoa are transmitted by contaminated water or food that contain faeces from an infected person. The faeces will contain *Giardia lamblia* cysts. A cyst is a dormant stage in the organism's life cycle that allows it to exist in hostile environments. An individual may start to see symptoms between one to three weeks following transmission of the parasite as the ingested cysts dissolve and release the organism. The symptoms can last for several weeks but usually clears up on its own. A patient may be given treatment to prevent dehydration, but people may experience intestinal discomfort for some time after infection.

Progression of disease

In countries where access to healthcare and treatment is readily available, giardia infection is rarely fatal. However, in countries where there is a lack of access to clean water supplies, poor healthcare and a lack of education the infection can have more severe consequences. The main complication that can arise from giardia infection is dehydration as a result of severe diarrhoea. Children who have giardia infection can develop malnutrition, which can affect their development and lead to a failure to thrive. Some people develop lactose intolerance following a giardia infection, which can persist for a long time after the infection has cleared up.

Helminthic and ectoparasitic diseases

Some infections can also be caused by parasitic worms (helminthic) or by parasites that live on the outside of the body (ectoparasites). The table below shows the cause, signs and symptoms and progression of some of these types of infection.

Table 2.6: The cause, signs and symptoms and progression of common parasitic disease of humans

Name of disease	Causative organism	Signs and symptoms	Progression of disease
Roundworm	Caused by nematode worm. Most common cause of roundworm infection is *Ascaris lumbricoides.*	Does not usually cause any noticeable symptoms. Signs of infection include presence of worms in faeces. Some people develop a high temperature and dry cough during the initial larvae stages of infection.	Roundworm eggs are ingested and move into the duodenum. After 1 to 2 weeks the eggs hatch into larvae. These move into the bloodstream and move to the lungs. The larvae pass from the lungs to the throat and are swallowed. The larvae then mature into adult worms in the intestines and produce more eggs. Adult worms can live for 2 years and produce thousands off eggs per day. These eggs are released in faeces and can be passed onto others through contact with infected faeces. The more worms present, the more severe the infection. Severe infections can lead to blockages in the bowel.
Tapeworm	The most common tapeworms that affect humans are: *Taenia solium* (pork tapeworm) *Taenia saginata* (beef tapeworm) *Hymenolepsis nana* *Diphyllobothrium latum* (fish tapeworm) *Dipylidium caninum* (dog tapeworm)	Does not usually cause any noticeable symptoms. Signs of infection include the presence of flat, rectangular, pale yellow worms in faeces. Infection with tapeworm is rare in the UK but common in other countries. Symptoms can include abdominal pain, diarrhoea, nausea and vomiting and weight loss.	Tapeworm eggs are ingested through consumption of raw or undercooked pork, beef or fish. They can also be ingested through drinking contaminated water or by close contact with an infected person. Worms hatch in the intestine and produce new eggs, which are released in faeces. Complications can arise if worms migrate to other organs such as the brain or liver. This only happens when worms are caught from ingesting raw pork and can lead to headaches, seizures, jaundice and coughing up blood.

Name of disease	Causative organism	Signs and symptoms	Progression of disease
Pediculosis	Infestation of lice. Lice are blood feeding insects that belong to the Phthiraptera order. Human pediculosis can be divided into the following: Pediculosis capitis (head lice) Pediculosis corporis (body lice) Pediculosis pubis (public lice)	The characteristic symptom of lice infection is itching (pruritus) which intensifies 3 to 4 weeks after initial infection. Lice and eggs can often be seen in the hair of the infected part of the body	After infestation, lice feed by taking a blood meal from their host and must stay close to the skin in order to maintain their temperature. Eggs (nits) are laid by adult females and take 6 to 9 days to hatch. When eggs hatch, a nymph is released. Nymphs mature into adult lice around 7 days after hatching. Adult lice can live up to 30 days as long as they are able to feed on the blood several times a day. Transmission occurs when direct contact allows adult lice to jump to an uninfected person or where eggs are able to be transferred between individuals. Body lice can be transmitted by the presence of eggs on clothing.

B4 Prevention and treatment of infectious diseases

To reduce the chances of catching an infectious disease, there are various preventative measures that can be taken.

Prevention

Vaccinations

There are vaccinations for some of the more serious infectious diseases and these can either be given as part of the normal childhood vaccination programme in the UK, to high-risk groups such as the elderly or immunocompromised or to those who are travelling to places where there is an increased risk of disease. The routine childhood vaccination programme in the UK can be seen in Figure 2.20.

The routine immunisation schedule — **from June 2020**

Age due	Diseases protected against	Vaccine given and trade name		Usual site
Eight weeks old	Diphtheria, tetanus, pertussis (whooping cough), polio, *Haemophilus influenzae* type b (Hib) and hepatitis B	DTaP/IPV/Hib/HepB	Infanrix hexa	Thigh
	Meningococcal group B (MenB)	MenB	Bexsero	Left thigh
	Rotavirus gastroenteritis	Rotavirus	Rotarix	By mouth
Twelve weeks old	Diphtheria, tetanus, pertussis, polio, Hib and hepatitis B	DTaP/IPV/Hib/HepB	Infanrix hexa	Thigh
	Pneumococcal (13 serotypes)	Pneumococcal conjugate vaccine (PCV)	Prevenar 13	Thigh
	Rotavirus	Rotavirus	Rotarix	By mouth
Sixteen weeks old	Diphtheria, tetanus, pertussis, polio, Hib and hepatitis B	DTaP/IPV/Hib/HepB	Infanrix hexa	Thigh
	MenB	MenB	Bexsero	Left thigh
One year old (on or after the child's first birthday)	Hib and MenC	Hib/MenC	Menitorix	Upper arm/thigh
	Pneumococcal	PCV booster	Prevenar 13	Upper arm/thigh
	Measles, mumps and rubella (German measles)	MMR	MMR VaxPRO[2] or Priorix	Upper arm/thigh
	MenB	MenB booster	Bexsero	Left thigh
Eligible paediatric age groups[1]	Influenza (each year from September)	Live attenuated influenza vaccine LAIV[2, 3]	Fluenz Tetra[2, 3]	Both nostrils
Three years four months old or soon after	Diphtheria, tetanus, pertussis and polio	dTaP/IPV	Repevax or Boostrix-IPV	Upper arm
	Measles, mumps and rubella	MMR (check first dose given)	MMR VaxPRO[2] or Priorix	Upper arm
Boys and girls aged twelve to thirteen years	Cancers caused by human papillomavirus (HPV) types 16 and 18 (and genital warts caused by types 6 and 11)	HPV (two doses 6-24 months apart)	Gardasil	Upper arm
Fourteen years old (school year 9)	Tetanus, diphtheria and polio	Td/IPV (check MMR status)	Revaxis	Upper arm
	Meningococcal groups A, C, W and Y disease	MenACWY	Nimenrix or Menveo	Upper arm
65 years old	Pneumococcal (23 serotypes)	Pneumococcal Polysaccharide Vaccine (PPV)	Pneumococcal Polysaccharide Vaccine	Upper arm
65 years of age and older	Influenza (each year from September)	Inactivated influenza vaccine	Multiple	Upper arm
70 years old	Shingles	Shingles	Zostavax[2]	Upper arm

Figure 2.20: The routine vaccination programme in the UK as of Autumn 2020

Vaccinations provide immunity to specific diseases through the presence of antibodies. In response to an infection, a type of white blood cell called B plasma cells produce proteins called antibodies. This happens naturally when the body detects a non-self-antigen. Antibodies neutralise pathogens or toxins produced by the pathogen or work by clumping the non-self-cells together, so they cannot attach to body cells. Vaccinations work either by triggering the body to produce antibodies against a specific antigen or by providing the person with the necessary antibodies. The antibodies produced are specific to the antigen that has entered the body. This is why a vaccination against measles would not offer protection against any other diseases.

Mode of action of vaccines

Active immunity

Vaccinations can work by providing the person with active immunity against a disease. A killed or weakened (attenuated) form of the pathogen may be used to produce this type of vaccination. Vaccines that contain killed or attenuated pathogen include MMR (measles, mumps and rubella), polio and hepatitis A. Some vaccines may contain specific pieces of the pathogen that would trigger the immune response such as a particular protein or capsid. Examples of these vaccinations are the whooping cough, HPV and pneumonia vaccines. A third type of vaccine that also works by providing active immunity are vaccines that contain the toxin produced by the microorganism. This toxin will also trigger an immune response in the person receiving the vaccine. Examples of toxoid vaccines include diphtheria and tetanus. All of these vaccine types work by activating the specific immune response that results in the production of antibodies and memory cells. These can remain in the body for a long time (in some cases a lifetime) and provide protection against infection by the actual pathogen. Should the vaccinated person be infected by the pathogen, the body will be able to produce a faster and greater immune response, which will allow the infection to be dealt with before it progresses.

Passive immunity

Vaccinations can also work by providing the person with passive immunity against a disease. Antibodies against the pathogen are used to produce the vaccine. These types of vaccine are less common but include the vaccination against rabies. These types of vaccine produce short- lived immunity and so are usually given as a preventative measure when travelling to countries where risk of infection is high. In the case of rabies, the vaccination could also be given following an incident such as a dog bite where there is a chance that a person has become infected with the virus.

Table 2.7: Comparison of the modes of action of vaccines

Mode of action	Vaccine contains	Immune response	Protection
Active immunity	Dead or weakened pathogen, antigenic material or toxin	Yes – antibodies and memory cells produced	Long-lived (often lifelong)
Passive immunity	Antibody serum	No	Short-lived

Antibiotics

Antibiotics are sometimes prescribed to people to prevent infection. They can act as a **prophylaxis** against infection. Antibiotics can be used against bacterial infections but do not work against viruses, fungi or protozoa. Prophylactic antibiotics can be given as a precaution when a patient is having an operation or has a bite or wound that could get infected. A person with a health condition that means they have a higher risk of infection may be prescribed antibiotics. A patient who has had spleen removal surgery or is undergoing **chemotherapy** may prescribed antibiotics to prevent them catching bacterial infections.

Disinfectants

Disinfectants are antimicrobial agents that can be used on non-living objects such as surfaces and medical instruments. Disinfectants kill microorganisms on the surfaces they are used. Washing with warm water and detergent may only remove visible dirt but leave microorganisms on the surface. Cleaning with disinfectants such as phenol, bleach, alcohol, hydrogen peroxide or iodine solution can kill these microorganisms and prevent them passing to individuals and causing infection. In this way, disinfectant can prevent the spread of infection. It is important that disinfectants are used in hospitals and food preparation areas as the risk of pathogens being present on non-living objects is increased.

Key points

Prophylaxis – a measure taken to prevent infection. This could be an action or treatment.

Chemotherapy – type of treatment used in cancer.

Pause point

Distinguish between antibiotics and disinfectants.

Hint

Explain differences and similarities, referring to specific examples.

Extend

Discuss the advantages and disadvantages of using antibiotics and disinfectants to prevent disease.

Preventative behaviours

Increasing the use of disinfectant when there has been an outbreak of an infection is one behaviour that can prevent the spread of infection. Other strategies used by hospitals also act to prevent the spread of infection. Hospitals encourage patients, visitors and staff to use antibacterial hand gel regularly by placing hand gel on patient beds and at the entrance and exit to hospital wards and departments. Staff working in hospitals have training on how to prevent the spread of infection and must be "bare below the elbows". This means they cannot wear any clothing or jewellery that is on the lower part of the arms or hands. Hospitals have strict infection prevention strategies that must be followed before and after medical procedures and surgery.

Outside of hospitals, individuals can also behave in ways to prevent infections from spreading. An example of a preventative behaviour is regular hand washing especially after going to the toilet and before preparing or eating food. People should also use tissues when sneezing or coughing and stay off work or school when they have an infectious illness. Washing raw fruit and vegetables and ensuring that meat is thoroughly cooked before eating can also prevent a person from catching food-borne infections. Safe sex is a preventative behaviour that can stop the spread of sexually transmitted infections such as HIV and chlamydia. Safe sex involves using contraception such as a condom for vaginal penetrative sex, anal penetrative sex and oral sex. Ensuring people have all the vaccines that are part of the routine vaccination programme as well as having any necessary boosters and travel vaccinations, can help to prevent the spread of infection.

Environmental measures

Precautions can be taken to reduce the spread of infection from the environment. In the UK the mains water supply is screened and treated to ensure it is safe to drink and use in cooking. This ensures that any potential water borne pathogens are killed before the water reaches people's taps. At first the water is passed through a screen that captures and removes any branches or leaves. Following this, a solution is added to the water to make impurities such as dirt solidify and float. This is called flocculation. After this the water is filtered twice to remove large and small particles. Sand filters are used to do this. Some water treatment plants also use carbon and ion exchange processes to remove further microscopic and dissolved particles from the water. Once the water has been screened and filtered a small amount of chlorine is added. Less than one milligram of chlorine is added per litre of water. Chlorine acts as a disinfectant and kills any bacteria or fungi present in the water making it safe to drink. Wastewater from toilets, baths, sinks, washing machines etc. in all homes and industrial settings in the UK is treated before it enters natural water sources. Many infectious diseases can be spread by the contamination of water with faeces. Effective treatment of water means that the water can be returned to the environment with no risk to wildlife or humans. Mosquito nets can be used to prevent the spread of infections such as malaria that can be transmitted by mosquitoes. A mosquito net is made of a fine mesh and stops insects from getting through. They are used to cover beds or tents. They can also be installed on windows or doors. The most effective mosquito nets are those soaked in an insecticide which can reduce malaria cases by up to 70%.

Treatment of infectious disease

If an infection occurs there may be treatments available that can kill the infectious agent or prevent it from multiplying further. These treatments may eradicate the infection and allow the patient to fully recover. In the cases of other infections such as CJD, there may be no available treatment to eradicate the cause and treating the symptoms is the only option.

Antibiotics

Antibiotics can be used to treat bacterial infections. They do not work on infections caused by other infectious agents. Antibiotics can either be bactericidal, which kill the bacteria or bacteriostatic, which stop the growth of bacteria. Antibiotics called beta-lactam antibiotics work by disrupting the cell wall formation in bacteria. Without a cell wall, the bacterial cell bursts. Beta-lactam antibiotics include penicillin and cephalosporin. Macrolide antibiotics work by affecting bacterial ribosomes, stopping them from carrying out protein synthesis. Blocking this cellular process kills the bacteria as it cannot survive without protein synthesis. Erythromycin is an example of a macrolide antibiotic. Other antibiotics such as ciprofloxacin are called quinolones. These antibiotics work by inhibiting the replication of DNA. This prevents the bacteria from reproducing. Antibiotics can also be classed as broad or narrow spectrum antibiotics. Broad spectrum antibiotics are effective against a wide range of bacteria, they work against Gram-positive and Gram-negative bacteria and are often prescribed when the infection has an unknown cause or is caused by multiple bacteria. Narrow spectrum antibiotics are prescribed less frequently as they only work on certain strains of bacteria.

Antifungals

Antifungal agents can be used to treat fungal infections. Most of these treatments are topical and are applied to the affected area but some can also be taken orally. Echinocandins are a type of antifungal that work by disrupting the production of cell walls as the fungi reproduce. An example of this type of antifungal agent is the treatment for *Candida* yeast infections. Other antifungal medication works by preventing the production of new cell membranes when the fungi reproduces. They do this by targeting the pathway used to produce a molecule called ergosterol. Ergosterol is a steroid found in the cell membranes of fungi. Without ergosterol the cell membrane does not work correctly, and molecules can freely leave the cell, this causes the cell to die.

Antivirals

Antiviral medication can be used to treat viral infections. There are only a small number of antiviral drugs available, which are specific to certain viral infections. Extensive research has to be carried out to produce a safe and effective antiviral drug. This is because they replicate inside host cells, so any medication must minimise damage to host cells. The most commonly used antiviral drugs are against HIV, herpes virus, hepatitis B and C and influenza A and B. Typically viruses follow the same general lifecycle in order to replicate inside host cells. This cycle can be seen in Figure 2.20. Antiviral medication works by disrupting one of the stages in the cycle.

Antiviral medication can work by preventing the virus from entering host cells. One way to do this is to inhibit the production of the antigens on the virus surface so that the viral particles cannot attach to receptors on the host cells. If a virus cannot enter the host cell, it cannot replicate. Other antiviral medication works by preventing the replication of the virus inside the host cell or by stopping the successful assembly of new viral particles. The synthesis of viral DNA can be inhibited, which prevents the virus from reproduction. Another mechanism is to stop the assembly of amino acids into the proteins needed to form the new virus particles.

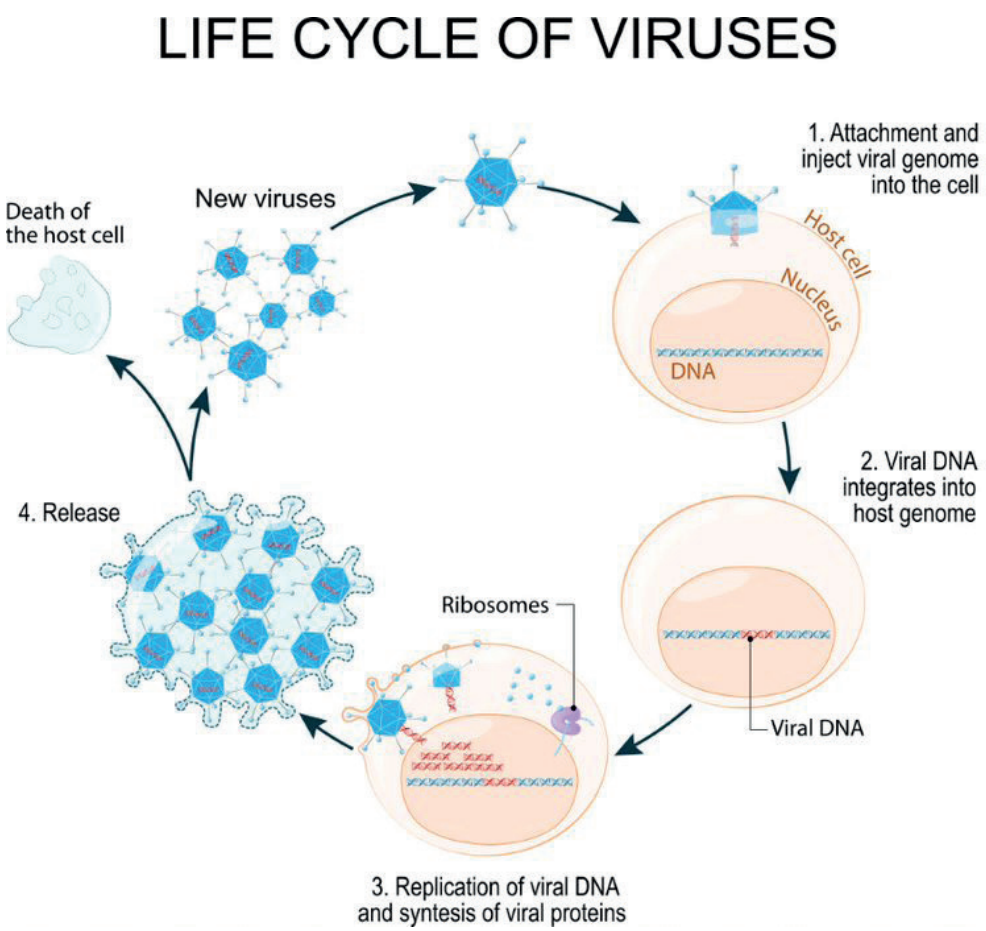


Figure 2.21: The stages in the lifecycle of a typical virus

Antiprotozoal drugs

Antiprotozoal drugs are those that can be given to treat an infection caused by a protozoan. The mechanism of action of these drugs varies widely from drug to drug. Malaria is a tropical disease that is caused by a plasmodium, a single-celled eukaryotic protozoan organism. The most common causative organisms are *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*. Different drugs used to treat malaria work by targeting different stages of the plasmodium lifecycle and different possible causative agents. Chloroquine works on the blood stage of the infection whereas primaquine works by killing the merozoites during the part of the lifecycle that happens in the liver. Doxycycline is another drug that can be given. This drug targets the stage of the plasmodium lifecycle that occurs in red blood cells of the infected person. This type of malaria treatment is only effective in *Plasmodium falciparum* infection. A combination of drugs is often used to treat malaria infections to ensure that different possible causative species and lifecycles are targeted.

Assessment activity 2.2 B.P3 B.P4 B.M2 AB.D1

1. Choosing one infectious disease that is caused by bacteria, produce a patient information leaflet that describes how the disease develops. Explain how the patient can protect themselves against the disease and the possible treatments available.
2. Repeat activity 1 for one viral disease, one fungal disease, one disease caused by a protist and one prionic disease.
3. Choose one infectious disease that is caused by bacteria and evaluate the treatment available. Look at the advantages and disadvantages of the treatment and what the benefits and risks are of having and not having the available treatment.
4. Evaluate the development of new treatments for HIV using links to current issues facing scientist trying to develop these antiviral drugs.

C Explore the application of techniques to culture and identify microorganisms

In the laboratory microbiologists will use techniques to grow (culture) various microorganisms and identify them. This section looks at some of the techniques that may be used and things that scientists need to consider when working with microorganisms.

C1 Health and safety

When working in an industry including microbiology laboratories, or schools or colleges working with microorganisms, scientists must ensure they follow the legislation in place to keep workers safe. This is required by law. The most important piece of legislation that workers have to comply with is the Control of Substances Hazardous to Health Regulations of 2002. This piece of legislation is often referred to as COSHH. COSHH includes many substances that are hazardous to health including chemicals but also biological agents such as microorganisms. To comply with COSHH, workplaces must have procedures in place for workers to complete risk assessments to prevent or control the potential risk that could arise. This could include making the correct personal protective equipment (PPE) available and the safe storage, labelling and disposal of materials and equipment. COSHH also includes the Approved List of Biological Agents, which classifies microorganisms into one of four hazard groups. Hazard group 1 is the least hazardous and hazard group 4 is the most hazardous. This list gives workers an indication of what kind of measures they must put in place for each group of microorganisms. This helps to protect workers from infection with the microorganism being used in that workplace. It is important that any workers in the workplace are considered, not just those in direct contact with the microorganism. This means that safety measures must be put in place for scientists, cleaners, office staff and visitors.

Employers also have a duty under The Health and Safety at Work Act (1974) to inform their employees about potential hazards and risks in the workplace. Employers also need to consult their employees on health and safety matters and consider their points of view before making decisions on health and safety. The Health and Safety at Work Act states that all workplaces must have a risk assessment that reduces the chances of harm happening and describes procedures that must take place in the unlikely event that a risk occurs.

Classification of biosafety (levels 1-4)

Biosafety refers to reducing the risk of infection that could arise from working in areas where microorganisms are used. A biosafety level (BSL) is a set of containment precautions used to reduce the risk of infection in laboratories. A laboratory will decide which level precautions to take based on the organisms they work with and where the microorganism is on the Approved List of Biological Agents. The lowest level is BSL-1 and the highest is BSL-4. In Table 2.8 you can see the different safety procedures at the four biosafety levels and the types of microorganism that might be used.

Table 2.8: Safety protocols of the biosafety levels and examples of their use

BSL	Risk levels	Example microorganisms	Typical lab safety precedures to follow
1	Low risk to individuals and communities	B. subtilis, Staphylacoccus aibus, Saccharomyces cerevisiae and non-pathogenic E.coli (Any organisms that do not cause disease in healthy humans)	This is the type of lab found in schools and colleges. Hand washing on entry to lab and before exit; attention to personal hygiene; no eating or drinking or mouth pipetting; no requirement for containment cabinets,; work can be carried out on open benches; lab has a lockable, but this is not usually locked; people carrying out investigations observe aseptic techniques and wear some protective clothing. such as disposable aprons; all potentially infectious material has to be decontaminated before disposal.
2	Moderate risk to individuals and low risk to communities	Salmonella spp, Staphylacoccus aurea, hepititis B and C virus, adenoviruses, HIV, pathogenic strains of E coli, Plasmodium falciparum, Toxoplasma gondii	Pathology and research labs. Procedures of BSLq plus: personnel need more training for handling pathogens, given by a senior qualified and competent scientist; limited access to the lab while work is in progress; extreme precautions with contaminated sharp items; safety cabinets used if aerosols will be generated.
3	High risk to individuals and moderate risk to communities	Mycobacteriuem tuberculosis, yellow fever virus, Yersinia pestis (cause of bubonic plague), SARS, Chlamydia, rabies virus	Pathology and research labs dealing with various bacteria, parasites and viruses that can cause serious or potentially lethal diseases, but for which there are treatments. Procedures of BSL 1 and 2 plus: specific training for lab personnel for handling pathogens; all procedures for handling microorganisms are carried out in containment cabinets; personnel wear protective clothing and may be required to remove make up or jewellery.
4	High risk to individuals and communities	Ebola virus, smallpox virus, Herpes B virus, Marburg virus, Lassa virus, Clostridium botulinum	Public health labs and some medical research labs. Procedures of BSL 1–3 plus: high levels of security for access – via director; double-door entry with an airlock; airflow systems and negative pressure in the lab, so air always enters the lab and does not flow outwards; air is filtered; multiple containment rooms; lab separate from other buildings; pressurised personnel suits; operators manipulate cultures of pathogens by putting their hands inside special gloves inside sealed cabinets; established protocols for all procedures; extensive training for personnel; personnel may be required to shower before entry and before leaving; own clothes not worn in lab; protective clothing decontaminated.

Biosafety cabinets

When working with potentially dangerous microorganisms in a laboratory, work is carried out in a biosafety cabinet. The purpose of these cabinets is to protect the worker against infection and to prevent the accidental release of pathogens into the environment. Biosafety cabinets (BSCs also called laminar flow cabinets) are enclosed spaces made of stainless steel with no gaps or joints where the microorganism could collect. BSCs are connected to an air supply, which creates a negative air pressure inside

the cabinet. This means that air is always flowing into the cabinet from the outside. The air from the cabinet is filtered so that no microorganism leaves the cabinet, protecting those in the outside environment from infection. BSCs may also have an ultraviolet lamp, which can be switched on to kill any microorganisms within the cabinet.

Classes of BSCs

There are three classes of BSCs offering increasing levels of protection to the worker and the environment and increasing levels of protection to the work being carried out.

- Class I: These BSCs provide worker and environmental protection but do not offer any protection to the work being done. This means that the microorganism being worked with could still become contaminated by other microorganisms present in the environment, but the worker is unlikely to become infected if they use the cabinet correctly. Class I BSCs are often used to hold pieces of equipment such as centrifuges or for procedures that could generate potentially harmful aerosols.
- Class II: These BSCs protect the worker, the environment and the microorganism being worked with. To protect the work, a fan on top of the cabinet draws sterile air over the work being handled. This air is then filtered before it leaves the cabinet to protect the worker and environment.
- Class III: These BSCs are sometimes called glove boxes. They are used where maximum biocontainment is used as they give the maximin protection. The enclosure is airtight and all material leaves through a double-door autoclave. Workers use gloves attached to the front of the cabinet to have any contact with the microorganism. This type of cabinet will also have a fan to draw sterile air over the work and a filter to ensure any air leaving is safe.

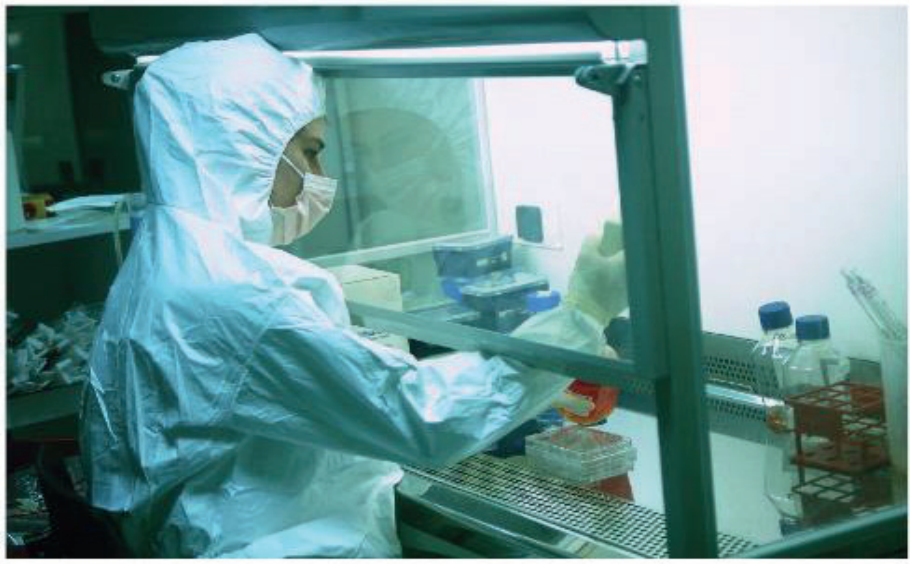


Figure 2.22: A biosafety cabinet

Personal protective equipment (PPE)

Personal protective equipment (PPE) refers to the safety equipment that a worker can wear to minimise the risk of exposure to the microorganisms they work with. Different PPE would be worn according to the biosafety category being followed. Workers may wear a laboratory coat that fastens to the neck and has cuffed sleeves. These may either be laundered regularly or may be sterilised after each use. Scientists may also wear googles or a face shield. They may wear gloves and overshoes if required. There are some pieces of PPE that are not specific to working with microorganisms but may need to be worn by the scientist. In the highest level BSL-4, further PPE would be required including full suits or individual sterile air supply masks. This is because infection with this category of infection could be serious or even lethal. In a school or college laboratory, which would be BSL-1, it is appropriate for teachers and pupils to wear basic protective clothing. This includes laboratory coats or aprons and goggles. Gloves may also be worn but are not necessarily required.

Pause point

Explain the differences between different levels of biocontainment.

Hint
Include the types of organism in each type of laboratory, specialist equipment they may use and personal protective equipment that will be worn.

Extend
Discuss the level of training that staff would need in each type of laboratory and the security they may have.

Methods of sterilisation and disinfection

When working with microorganisms it is important that all equipment used and surfaces worked on are kept as free as possible from microorganisms. This serves two purposes.

- Protection of the scientists and other personnel in the laboratory from the microorganism they are working with. This stops people getting infections from the microorganisms they work with.
- Protection of the work from contamination with other microorganisms that may be present in the environment or on the equipment used. This ensures that any results from the experiment are **valid.**

Sterilisation is the process of killing and removing all the microorganisms from a particular location. This includes the killing/removal of both potentially harmful and harmless microorganisms. Sterilisation is important, for example, when instruments are used in surgery or in producing canned food. In the school or college microbiology laboratory equipment has usually been sterilised before it is required for use. It may be possible to observe sterilisation techniques in a college or school laboratory used to dispose of contaminated waste or bacterial **cultures**. Plastic equipment such as Petri dishes will typically arrive at a school or college in sealed plastic bags that have been sterilised by the manufacturer using irradiation. This is where x-rays or gamma rays are used to kill any microorganisms present. This makes the Petri dishes sterile and free of microorganisms until the packet is opened. Other methods including the use of heat, steam and filters to carry out sterilisation can be seen in Table 2.9.

Table 2.9: Examples of sterilisation methods

Sterilisation method	Description
Irradiation	Electron beams, x-rays or gamma rays are used to kill and remove microorganisms on disposal equipment.
Heat	Flaming –using a flame to heat metal equipment until it glows red. Incinerating– destroying contaminated waste using high temperatures in an incinerator or furnace. Tindilisation – boiling in water for 20 minutes in three heating and cooling cycles.
Steam	An autoclave is a machine that uses steam heated to 120°C - 135°C. Contaminated equipment that can withstand this temperature can be sterilised by placing in an autoclave for at least 15 minutes. Cultures of microorganisms used in school or college and other BSL-1 laboratories can be placed in an autoclave to be destroyed before being disposed of in the normal waste.
Filtration	Liquids that would be damaged by other sterilisation methods can be sterilised using mechanical filtration. The liquid would be slowly passed through a filter. The filter would have pores that allow liquid through but trap microorganisms. Note that in this method, the microorganism would still need to be killed. Filtering only separates the microorganism from the liquid.

Disinfection is the use of chemicals on non-living objects such as laboratory benches and glass test tubes to kill or remove the majority of microorganisms' present. Disinfection is used when sterilisation is not possible and is the next best thing to prevent contamination or infection. Different disinfectants target different microorganisms, so it is important that the correct one is used in different laboratories. It is also important that the disinfectant is used in accordance with the manufacturer's guidelines for diluting with water. This ensures that it works effectively in killing the microorganisms present. Examples of disinfectants include bleach, hydrogen peroxide and alcohols. Bleach usually contains the active ingredient sodium hypochlorite, which is effective at killing a wide range of bacteria. Hydrogen peroxide works by oxidising compounds that the microorganism needs to survive and reproduce. Alcohols work by denaturing the proteins in microorganisms causing them to die. Alcohols are widely used as one method of preventing the spread of microorganisms on hands in hospitals and microbiology laboratories, with hand gel made available for use by staff and visitors. Alcohol wipes may also be used to disinfect hard surfaces such as benches and chairs in laboratories.

Key points

Sterilisation – the killing or removal of all microorganisms present at a particular location.

Disinfection – the killing or removal of most microorganisms on a physical surface.

Valid – information or a result that can be trusted.

Culture – a method of multiplying microorganisms by providing resources for them to reproduce in a medium under controlled laboratory conditions.

Aseptic technique

Aseptic technique refers to the practices used in a microbiology laboratory when working with microorganisms. Aseptic techniques are carried out for two purposes.

1. To minimise the risk of infection from the microorganism. This protects scientists working with the microorganism and any other person who may come in to contact with the microorganism, room or contaminated waste.
2. To minimise the risk of contamination of the experimental work from microorganisms that may be present on the scientists or in the laboratory.

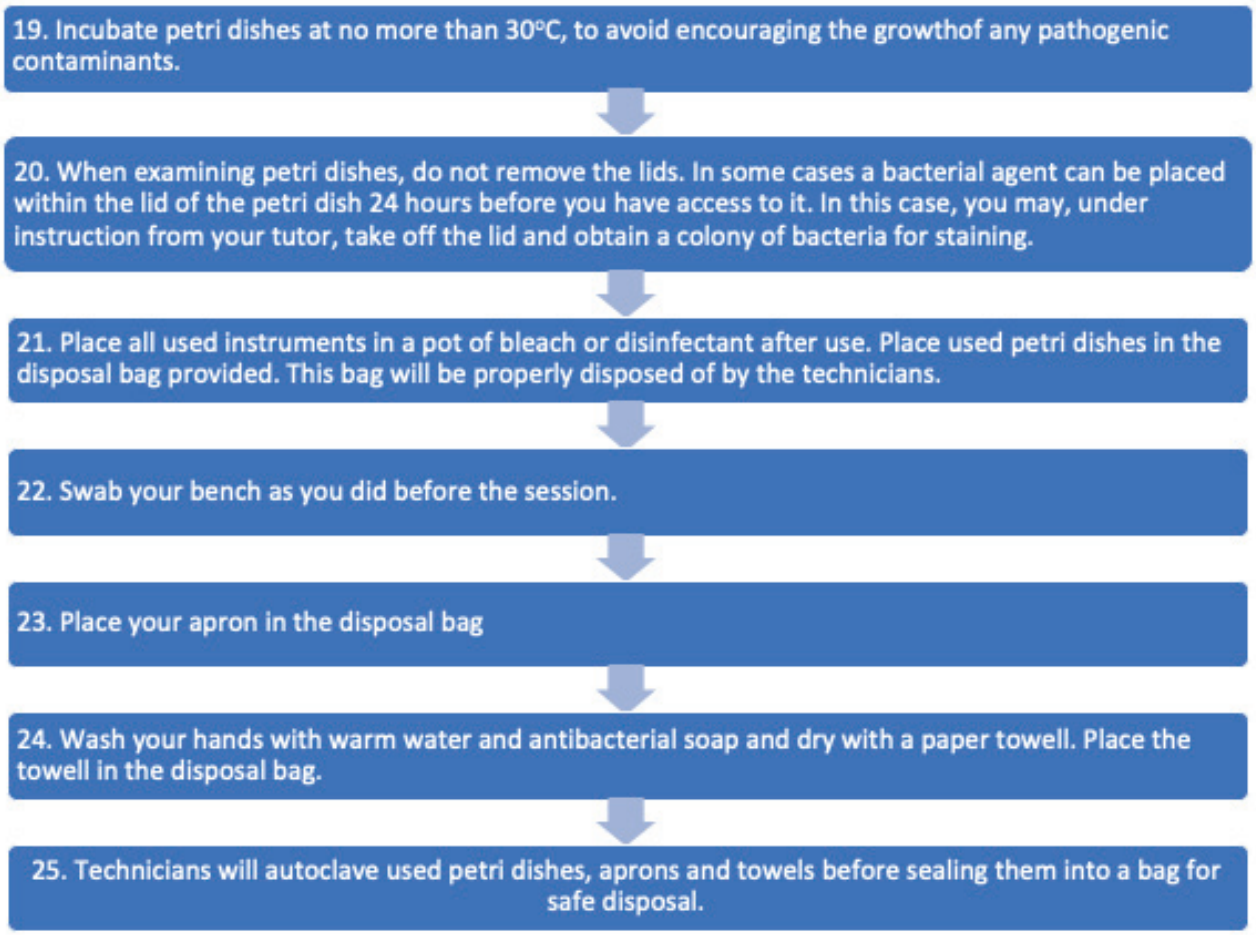
When working with microorganisms in a laboratory, other activities being carried out in the immediate vicinity should be reduced and where possible relocated. For example, if you were working in the classroom laboratory to produce a streak plate of a bacterial culture, other people should not work on your bench to complete work such as working on a computer.

When working with microorganisms, you must always thoroughly wash and dry your hands using antibacterial soap and warm water. You must also wear a laboratory coat or apron. You must disinfect the work surfaces before and after use. When carrying out experiments with microorganisms, you will always use sterile equipment. Sometimes you may sterilise the equipment yourself using a Bunsen burner, in other cases the equipment will arrive to you sterile and only be used once before it is sent for re-sterilisation. It is common aseptic technique to always carry out microbiology experiments around a Bunsen burner flame. A Bunsen burner flame is used to draw air currents upwards, which reduces the chances that any microorganisms fall onto your work and therefore reduces the risk of contamination.

After you have completed microbiological experiments, any contaminated equipment must be made safe before it can be disposed of. Metal equipment such as wire loops can be sterilised by holding them in a hot Bunsen burner flame until the metal glows red. This is called flaming. Other equipment may be placed in disinfectant. Contaminated Petri dishes or microbiology cultures will be autoclaved before they are disposed of in normal waste in a sealed bag.

Table 2.10: Step-by-step aseptic technique

1. If you have a heavy cold or a stomach upset check with your tutor before you begin a microbiology practical.
2. Cover any cuts and grazes with a clean waterproof dressing.
3. Do not eat or drink anything in the lab; do not do any hand to mouth operations, such as chewing pen tops; no mouth pipetting, always use mechanical pipettes and micropipettes.
4. Always wear a clean dispoable plastic apron; this protects your clothing and reduces the risk of contamination of the microorganisms you are growing.
5. If possible, close windows and doors to prevent airborne contamination.
6. Wash your hands with warm water and antiseptic soap before you start. Dry them with a paper towel and place this towel in the disposal bag provided.
7. Make sure benches have smooth surfaces, so that bacteria cannot collect in crevices (you should cover any benches with cracked surfaces using a clean plastic sheet).
8. Spray your bench area with disinfectant, leave it to work for 10-15 minutes and then wipe with a paper towel. Place the paper towel in the disposal bag.
9. Work near a lit Bunsen burner (hot flame). You will need this to flame loops, but the updraft will also prevent airborne bacteria from falling onto the plates you want to inoculate. Place the Bunsen flame on yellow when not in use, as this is cooler and more visable.
10. Sterilise your equipment before use. Hold wire loops in the hottest part of a blue Bunsen flame, until they gow red hot. Allow them to cool in the air, dip them in methanol and then quickly flame the loops again to burn off the methanol.
11. Sterilise glass instruments, such as spreaders, by dipping them in 70% methanol and then flaming them lightly.
12. Always keep the methanol in a small beaker, well away from the Bunsen flame.
13. Never place bungs or caps from flasks of bacterial cultures on the bench; hold them in the crook of your little finger.
14. *Before* and *after you use a (sterilised) loop or pipette to withdraw a sample from a culture tube, pass the neck of the culture through the Bunsen flame.*
15. Before you inoculate plates or tubes label them with your name, date and culture. Label petri dishes on the bottom. Use a permanent marker pen. Do not lick labels.
16. Always tape a petri dish lid to the petri dish in two places, with sticky tape. Do not seal the dish all the way round, as this excludes air and could encourage the growth of anaerobic contaminants.
17. Incubate the plates upside down, so that any condensation runs onto the lids and not onto the microorganisms.
18. Report any spillages immediately.



Safe culturing of microorganisms

When growing microorganisms in laboratories steps must be in place to ensure that these cultures remain safe. In school and college laboratories, you will work with non-pathogenic microorganisms. Although these are safe, culturing could cause them to become pathogenic and so all microorganisms have to be treated as potentially pathogenic. When growing microorganisms, they must be incubated (grown at warm temperatures) to ensure they have the energy for adequate growth. Bacterial cultures should be incubated at temperatures that do not encourage the growth of potential pathogens. This means that they should be incubated below body temperature. To achieve this, a maximum incubation temperature of 30°C is used. Aseptic technique should be followed to avoid contamination of cultures. Contamination could lead to the incubation of human pathogens that have come from the scientist carrying out the work. Petri dishes should also be sealed with two pieces of tape, one on each side, to stop the lid from coming off. Petri dishes that have been incubated should never be opened.

C2 Microscopy and staining techniques

Microscopy

There are different types of microscope that can be used to observe microorganisms. Some microorganisms such as fungi, bacteria and protozoa can be seen with a light microscope. Others including viruses and prions are too small and can only be observed with an electron microscope. In school or college, you will prepare slides of microorganisms and use a compound light microscope to view them.

Light microscopes are also called optical microscopes, they use visible light to view specimens. Compound light microscopes have an objective lens that magnifies the specimen before a second lens, the eyepiece lens, causes further **magnification** to produce an image of the specimen. The person using the microscope can see the image by looking down the eyepiece lens(es). A light microscope may have more than one objective lens, which can be rotated into place. These lenses will have different magnifications such as x4, x10 and x40. When each objective lens is used, the total magnification used to produce the image can be calculated by:

Total magnification = magnification of objective lens x magnification of eyepiece lens

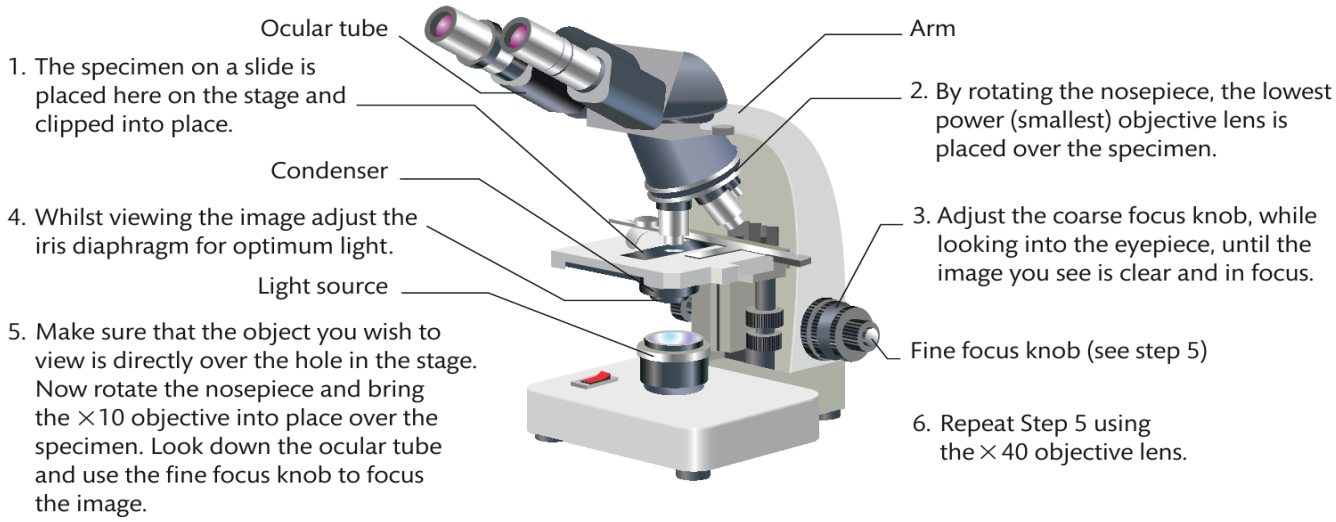


Figure 2.23: Annotated diagram describing how to use a light microscope

The maximum magnification that can be achieved by a light microscope is x1500. As light is used as the energy source, the wavelength of light limits the maximum **resolution** that can be achieved. The maximum resolution of a light microscope is 200nm. This means that if any objects are closer together than 200nm, they will be viewed as one object. Light microscopes can be used to view cells, but intracellular detail cannot be seen.

Some light microscopes have a special objective lens called an oil immersion lens. This lens allows for greater magnification than can be achieved by a normal objective lens. In normal objective lenses, there is air between the objective lens and the specimen. To use this lens, a drop of immersion oil is placed between the oil immersion lens and the specimen being viewed. The oil has a **refractive index** similar to the glass of the lens, so a higher magnification can be achieved.

Key points

Magnification – how many times an image appears compared to the actual specimen.

Resolution – the smallest distance that two separate objects can be apart in a specimen and still be seen as two separate objects in the image produced. It determines the detail of the image produced.

Refractive index – the speed of light changes as it passes from one medium to another, for example from air to glass. This causes the light to change direction, this is known as refraction. The refractive index of a material is the ratio of the speed light travels in a vacuum to the speed it travels in that material.

Electron microscopes

Electron microscopes use a beam of electrons to produce very clear, highly magnified images. Electrons have a much shorter wavelength than light and so electron microscopes have a much higher resolution than light microscopes, typically 400nm. There are two main types of electron microscope each of which produces different types of image.

Transmission electron microscopes work by passing a beam of electrons through the specimen. They have a magnification of up to x 50,000, 000 and produce 2D images.

Scanning electron microscopes work by passing a beam of electrons over the surface of the specimen. They have a magnification of up to x 1, 000,000 – 2, 000, 000 and produce 3D images.

Both types of electron microscope:

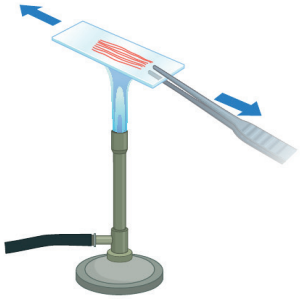
- are very large and expensive
- need a lot of skill and training to use
- can only observe very thin specimens
- involves complex and expensive slide production
- require the provision of a vacuum for the electrons to travel through.

Producing slides for light microscopy

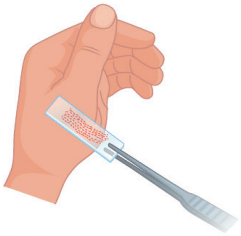
In schools and colleges, it is not possible to carry out electron microscopy, but it might be possible to prepare slides for observation with a light microscope. To view a microorganism, place a small amount of the culture on a clear, colourless glass slide. This is called mounting. Once mounted on a slide, a cover slip is placed over the top of the specimen to protect it. There are different types of slide that could be produced.

Flat slides

For most specimens you will use a flat microscope slide. When producing a microbiological slide, it is important that you follow aseptic technique and use a sterile microscope slide. To observe bacteria, a heat-fixed smear is produced on a flat microscope slide. A wire loop is used to transfer a loopful of bacteria from a bacterial colony into a drop of sterile water on a slide. If a liquid culture is being observed, then a loopful of the liquid bacterial culture can be added directly onto the slide surface. The slide is left to dry in air before being heat fixed. Heat fixing is achieved by swiftly passing the slide through the flame of a Bunsen burner. Heat fixing ensures that the bacteria are not washed off the slide during staining. All specimens produced for observation by light microscope must be stained in order to be seen.



a. Passing the slide through a Bunsen flame



b. Testing to ensure that the slide is not overheated

Figure 2.24: Use of a Bunsen burner to heat fix a slide

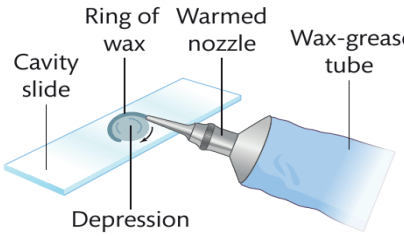
Concave slides

Some species of microorganisms are motile. This means that they move around, this movement can be observed using a suspension of the living microorganism. This is often used for bacteria, yeast and protists. This method of slide preparation is known as the hanging drop method.

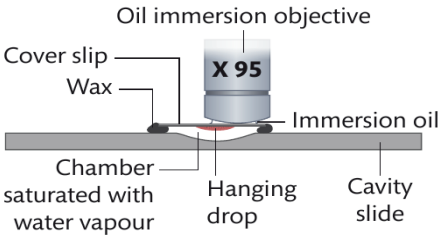
Step-by-step: Hanging drop method

1. Place a drop of microorganism suspension onto a cover slip using aseptic technique.
2. Place a ring of petroleum jelly around the depression of a concave slide.
3. Position the concave slide over the drop of microorganism suspension
4. Turn the slide and cover slip over so that the suspension is hanging from the cover slip into the depression of the slide.
5. View the preparation using the oil immersion lens.

a. Warming the wax-grease tube



b. A circular wall of wax is made around the depression



c. The slide is shown correctly set up for oil immersion viewing

Figure 2.25: The hanging drop method, with an oil immersion objective lens, used to view, live motile specimens

Staining techniques

Slide preparations must be stained in order to be seen. If a specimen is colourless you would not be able to see anything when you observed it with a light microscope. Different stains work by targeting different structures within microorganisms. The most common types of staining you will use in schools and colleges are staining with methylene blue, Grams staining of bacteria and India ink staining.

Simple staining with methylene blue

Once a slide has been heat-fixed, a drop of methylene blue can be added. Excess stain is then washed off before the slide is viewed with or without oil immersion. Methylene blue binds to and stains the cell components that have a negative charge. Methylene blue stains the nuclei of cells and can be used to stain fungi and protozoa. Methylene blue can be used to observe the shape of bacterial cells.

Gram staining

Gram staining is the most widely used stain to stain and differentiate between bacteria. Gram staining divides bacterial cells into two major groups based on their cell wall structure. These groups are Gram positive and Gram negative as we have seen previously in the chapter. Gram stain is an example of differential staining as it allows you to distinguish between two groups of bacteria. Gram staining also allows you to see the shapes of the bacteria and how they are arranged.

Gram staining involves two different stains. Crystal violet is a purple stain that binds to the thick layer of peptidoglycan in Gram-positive cell walls. This means that when observed with a light microscope, Gram-positive bacteria appear purple. The cell walls of Gram-negative bacteria do not retain crystal violet and so they do not appear purple. In order to visualise Gram-negative bacteria, a second stain must be used. This second stain is called a counter stain. Typically, safranin or fuchsin is used. These stains cause Gram-negative bacteria to appear red or pink when observed with a light microscope.

Step-by-step: Gram staining

1. Wash your hands with warm water and antimicrobial hand wash.
2. Clean the laboratory bench with antibacterial disinfectants or wipes.
3. Produce a heat-fixed slide of a bacterial culture.
4. Apply the first stain, crystal violet and leave for 30 to 60 seconds.
5. Rinse off the crystal violet by tilting the slide. Use a wash bottle to squirt a gentle stream of distilled water over the smear.
6. Apply iodine to the smear. Iodine acts as a mordant. It reacts with the crystal violet to form a complex that traps the stain in Gram-positive cell walls during the next step.
7. Rinse the smear with a 1:1 mixture of acetone and ethanol. Hold the slide at an angle and rinse for less than 10 seconds.
8. Apply a counterstain such as safranin to the smear. Leave on for 30 to 60 second.
9. Rinse the smear with distilled water by tilting the slide.
10. Dry the slide by allowing it to air dry or by blotting with blotting paper.
11. Observe the slide using a light microscope without and with oil immersion.

Limitations of Grams staining

Grams staining is a good technique because it gives quick results using a relatively simple and inexpensive method. It can be used to determine the best treatment for a bacterial infection. Some antibiotics specifically target Gram-positive bacteria while some specifically target Gram-negative bacteria. Grams staining can help narrow down which antibiotics would be effective against a patient's infection. However, there are many limitations of the technique. The technique requires the scientist to decide on the colour that the sample has stained. This can lead to subjectivity as it can be difficult to distinguish between purple and pink/red. The technique is good because it allows you to identify features that can help narrow down the identification of the species present. The technique does not, however, allow identification of the specific bacterial species present. Some bacteria cannot be classified as either Gram-positive or Gram-negative and so this staining method may not always work.

India ink staining

India ink staining is an example of a negative staining technique. Negative staining is used to study the morphology (shape), size and arrangement of bacterial cells that are difficult to stain. India ink staining can be used to stain cells that would be damaged by heat fixing. India ink is an acidic stain that can donate hydrogen ions and itself become negatively charged in the process. Most bacterial cells are also negatively charged and will repel the stain. The glass of the slide, however, will not repel the stain and will appear dark. The bacteria will appear as clear spots against the dark background. When the slide is observed you can see the shape and arrangement of the cells. Measurements can be made to give an indication of the size of the cells being viewed.

Step-by-step: India ink staining

1. Wash your hands with warm water and antimicrobial hand wash.
2. Clean the laboratory bench with antibacterial disinfectants or wipes.
3. Using 95% alcohol clean the surface of a glass slide.
4. One the alcohol has completely evaporated, pass the slide through a hot Bunsen burner flame to sterilise.
5. Place a very small drop of India ink near the end of the slide.
6. Hold an inoculating loop in the centre of a hot Bunsen burner flame and remove a small amount of the bacterial culture or yeast suspension.
7. Disperse the culture in the stain drop without spreading the stain.
8. Use another sterile slide to spread the drop of stain. Do this by resting the end of the other slide on the centre of the slide containing the stain drop. Tilt the slide forward towards the drop forming an angle of less than 90°. Pull the slide towards the drop causing it to spread along the edge of the spreader slide. Push the spreader slide in the opposite direction to produce a thin smear of stain.
9. Allow the smear to air dry.
10. Observe the slide using a light microscope without and with oil immersion.

Pause point

Explain the difference between Gram staining and India ink staining.

Hint

Include the types of organism in each type of laboratory, specialist equipment they may use and personal protective equipment that will be worn.

Extend

Discuss the level of training that staff would need in each type of laboratory and the security they may have.

C3 Culture of microorganisms

Types of media

When working with microorganisms a substance is needed for them to grow on or in. This is called the growth media. The growth media used contains all the nutrients that the microorganism needs to grow. Some types of media are specialised to promote the growth of specific types of microorganism. Scientists select the growth media depending on the aims of their investigation.

In schools and colleges, people mostly work with bacteria using liquid growth media called nutrient broth or nutrient agar poured into Petri dishes to produce nutrient agar plates.

Nutrient broths

Nutrient broth is made from beef extract that contains peptones (digested proteins). To make nutrient broth you would dissolve 8g of beef extract and peptone in sterile water. You would then transfer to a larger vessel and top up the volume of sterile water to 1L. You would then divide the broth amongst test tubes stoppered with cotton wool and foil caps or in universal bottles with screw-top lids. You would then sterilise the broth in an autoclave. Once it has cooled down you can transfer some of the bacterial culture to the broth using aseptic technique. This is called inoculating. This is done by using a sterilised wire loop to transfer a drop of liquid solution to the broth or by touching a bacterial colony with the loop and transferring this to the broth.

When bacteria are grown in broths, they may show characteristic growth patterns. The bacteria could form a sediment at the bottom of the tube, you would see this as formation at the bottom of the broth. They could also produce turbid growth throughout the broth solution. This occurs when the bacteria growing are insoluble. Another growth characteristic in broths is the formation of a pellicle. A pellicle describes thick growth at the top of the broth tube.

Nutrient agar

Nutrient agar is made by mixing 20g of Bovril (beef extract), 5g of sodium chloride and 15g of agar powder with 100mL of sterile water. It is important that the agar powder is weighed out in a fume cupboard to avoid inhalation. Once the ingredients have been mixed together, they form a paste. Further sterile water is added to make the volume up to 1L. This growth media can also be sterilised in an autoclave and allowed to cool and solidify. This can then be melted when required or kept in a thermostatically controlled water bath set to 50°C until you are ready to pour into Petri dishes.

Pouring nutrient agar plates

Petri dishes are supplied in packs of 10. The required number of plates are removed from the package and laid out on the laboratory bench after it has been disinfected. Care must be taken not to remove the lid of the Petri dishes until you are ready to pour agar into them. This keeps the inside sterile. When ready to pour, the lid is lifted off one of the plates, ensuring that the opening is large enough to pour the nutrient agar in but covering as much of the plate as possible. The lid of the bottle containing the nutrient agar (if required) is removed, and the neck of the bottle is passed through a hot Bunsen burner flame. The nutrient agar contents are then immediately and smoothly poured into the Petri dish to cover a depth of about 5mm. The neck of the bottle is re-flamed after pouring and, finally, the lid is replaced on the Petri dish and the plate is swirled to distribute the liquid agar. This process is repeated with all the plates required. Once the plates have set, they should be stacked and stored, upside down in a fridge until you are ready to inoculate them with bacteria or fungi.

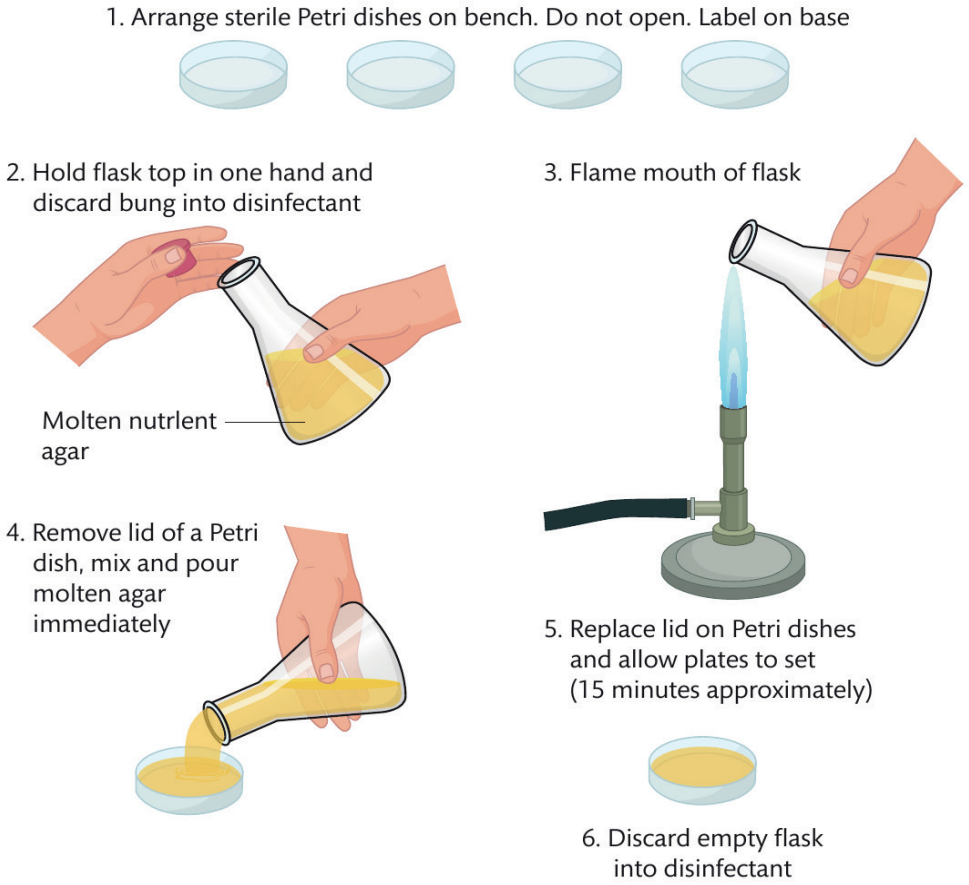


Figure 2.26: How to pour an agar plate

When bacteria are grown on solid media, they form colonies. A colony is the result of a single bacterium landing on the solid media and dividing by binary fission. After 24-48 hours, where there was one bacterium, there will be a visible colony containing several million bacteria that are all genetically identical to each other and to the bacterium that landed there before incubation. Colonies show typical growth characteristics. Colony morphology describes the characteristics of an individual colony of bacteria growing on the agar. It can be used to help identify the bacteria present. Specific terminology is used to describe types of colony. This terminology includes:

- Form – the shape of the colony e.g., circular, filamentous.
- Size – the diameter of the colony.
- Elevation – what the colony looks like side on.
- Surface – how does the colony appear e.g., smooth, rough, wrinkled.
- Opacity – is the colony transparent, opaque etc.
- Colour – what colour is the colony e.g., white, cream, red.

Selective media

Microorganisms can also be grown on what is known as selective media. Selective media refers to any substance that will only allow certain microorganisms to grow. This is different from the ones we have seen previously (nutrient broths and agars), which are known as general growth media as they allow a wide range of microorganisms to grow. Selective media supports the growth of one group of microorganisms while reducing the growth of others. For example, if the only type of sugar in a growth medium is lactose, then only the bacteria that can utilise lactose (lactose fermenters) will be able to grow. Selective media is used when you want to isolate specific groups of microorganisms. Another example is the use of nutrient agar with added antibiotics. If you add penicillin to nutrient agar, this will promote the growth of Gram-negative bacteria while preventing the growth of Gram-positive bacteria. Only Gram-positive bacteria are susceptible to penicillin. On the other hand, if you want to culture only Gram-positive bacteria, you could add potassium tellurite or sodium azide to nutrient agar. These inhibit the growth of Gram-negative bacteria and therefore will allow you to selectively grow only Gram-positive bacteria. Sometimes growth media is enriched with highly nutritious minerals such as blood, serum or yeast extract, to encourage the growth of organism that will not grow on other types of media. Table 2.11 shows some types of specialist agar and their uses.

Table 2.11: Types of specialist agar and their uses

Medium	**MacConkey agar (pH 7.1)**	**Mannitol salt agar (pH 7.4)**	**Blood agar (pH 7.3)**	**Potato dextrose agar (pH 5.6)**
Supplementary ingredients	Lactose (sugar) Bile salts Crystal violet Neutral pH red indicator Peptone	7.5% NaCl Mannitol (carbohydrate) Phenol red (pH indicator)	5% sheep or horse blood	Potato extract Dextrose (sugar)
Used for:	Culturing Gram-negative bacteria (Gram-positive growth inhibited by bile salts) Organisms that ferment lactose appear pink Organisms that do not ferment lactose, appear colourless or cream coloured	Culturing bacteria of the *Staphylococcus* genus. These can withstand high salt concentrations. Can differentiate between pathogenic bacteria such as *S. aureus* which will turn agar yellow and non-pathogenic (agar stays red)	Culturing fastidious microorganisms that require specific nutrients to grow. E.g. *Neisseria gonorrhoeae* Allows scientists to detect when a bacteria may have haemolytic activity (causes the destruction of red blood cells)	Culturing fungi including yeasts and moulds

Differential media

Growth media can also be used to differentiate between different microorganisms that could be present in a culture or specimen. Table 2.11 shows that MacConkey agar allows you to differentiate between bacteria that do and do not ferment lactose. Bacteria that can use the sugar lactose as their energy source, will appear as pink colonies when grown on MacConkey agar. Bacteria that cannot utilise lactose as their energy source will not appear pink.

Starch agar (nutrient agar with added starch) can also be used to differentiate between organisms. Some bacteria produce and secrete an enzyme called α-amylase which hydrolyses (breaks down) starch. To carry out the starch hydrolysis test, the bacteria are grown on starch agar and then incubated. After incubation, iodine solution is added to the agar. Iodine is an indicator that turns blue-black in the presence of starch indicating that the starch has not been hydrolysed. However, if the bacteria grown produces α-amylase, the starch will have been hydrolysed. In this case, the iodine will not turn blue-black.

Methods of cell culture

You will be expected to carry out inoculation of liquid and solid growth media, using aseptic techniques. There are lots of different methods of producing cultures and you may not use all of the ones described in this section.

Step-by-step: Inoculating liquid media to produce broth cultures

1. Labl your tubes of liquid media (broth) with your name, the date and the microorganism to be added.
2. Wash your hands with warm water and an antimicrobial hand wash.
3. Ensure the work surface has been disinfected with a suitable disinfectant.
4. Flame a wire loop by holding in the centre of a hot Bunsen burner flame and allow it to cool (if you are using a disposable, sterile plastic loop, do not flame it).
5. Take the culture tube and loosen the cap. Remove the cap from the culture tube using your little finger and keep hold of the lid. Do not set it down.
6. Flame the neck of the culture tube.
7. Insert the sterile loop into the culture tube. If it is a liquid culture, obtain a loop-full of culture. If it is a slant, touch a colony with the edge of the loop. Hold this loop while re-flaming the neck of the culture bottle and replacing the cap.
8. Pick up the liquid broth tube to be inoculated. Remove the cap (hold onto it) and flame the neck of the bottle.
9. Insert the loop containing the bacteria into this tube and then withdraw it.
10. Flame the neck of the newly inoculated tube again and replace the cap.
11. Sterilise your wire loop by flaming it or dispose of the loop in a container of disinfect if it is plastic.
12. Incubate the broth culture produced. It can then be used for further culturing or analysis of the bacteria.

Stab cultures

Stab cultures are produced by pouring growth media such as nutrient agar into test tubes while it is molten and then allowing it to solidify. One it is solidified bacteria are introduced into the agar by stabbing an inoculating needle containing the bacteria into the centre of the agar. The bacteria grow in the area where the agar has been pierced. Stab cultures are mostly used for short- term transport of cultures. The bacteria will not grow well so this method is not used for culturing or analysis of the bacteria.

Slant tubes

Slant tubes, also known as agar slopes, are used for the long-term storage of microorganisms. A slant tube is produced by pouring molten agar into a tube while it is tilted. As the agar solidifies it forms a slanted surface on which bacteria and yeast can be made. If the purpose of the slant is to store the culture until it is needed at a later date, then an agar plug can be created in the top of the tube to prevent drying out. Slant tubes should be re-cultured once every six to nine months.

Streak plate

Streak plates involve the spreading of bacteria on the surface of solid agar that has been poured into a Petri dish. This method spreads one loopful of bacterial culture, so that the bacteria on the last streak are spread out. The aim of a streak plate is to dilute the bacteria culture so that by the end of the process, a single bacterium lies on the surface of the agar. When the plate is incubated these individual bacteria will form a colony that is distinct and easy to see. Figure 2.26 shows the procedure for carrying out a streak plate.

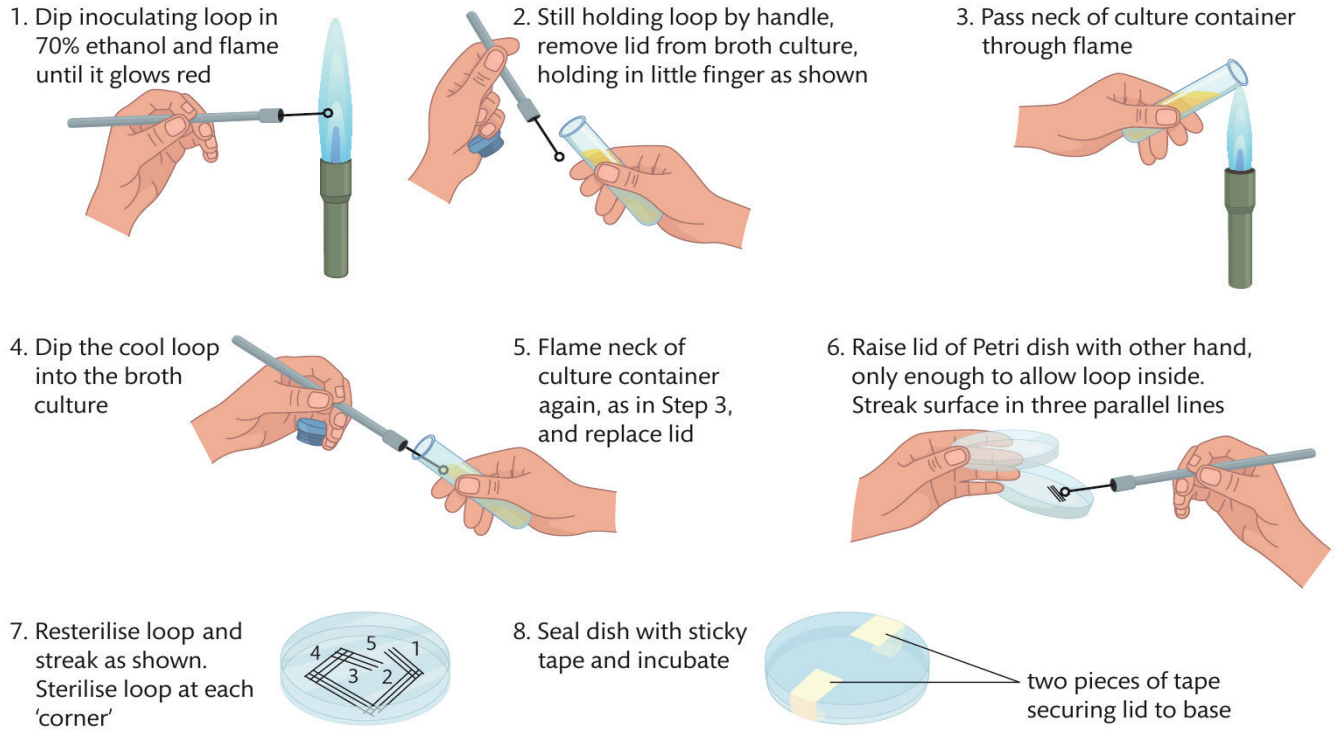


Figure 2.27: How to prepare a streak plate

Pour plates

Another method of culturing bacterium is to use a pour plate. This method also allows analysis of the effect of substances on the growth of bacteria. To produce a pour plate, 1mL of bacterial culture is added directly to molten nutrient agar. Before adding the bacteria to the molten agar, it is important that it has cooled enough not to kill the organism but not so much that the agar solidifies in the tube. Usually when the agar is at a temperature that is comfortable to touch, it is safe to add bacteria. It is important to work safely to ensure the agar does not burn you. Once the bacterial culture is added to the molten agar, the bottle is rolled between the hands to mix it thoroughly. The agar is then poured into a sterile Petri dish and allowed to solidify. Aseptic technique is observed throughout. Alternatively, the bacterial culture is first pipetted onto the centre of a sterile Petri dish before cooled molten agar is poured over the top. When incubated, the bacteria will grow both on the surface and within the agar.

Lawn spreads

Lawn spreads can also be used to culture bacteria. To prepare a lawn spread:

1. Pipette 1mL of bacterial culture into the middle of the solid nutrient agar that has set in a petri dish.
2. Use a sterilised bent glass rod to spread this evenly over the surface of the agar. One effective way to sterilise the bent glass rod before and after use is buy dipping in 95% ethanol and setting the ethanol alight by passing through a hot Bunsen burner flame.

When incubated, lawn spreads produce a lawn of bacteria rather than individual colonies. This type of plate is useful for investigating the effectiveness of antibiotics or other antibacterial substances.

C4 Quantitative analysis of microbes

There are various ways that microbial growth can be measured quantitatively. One of the factors you may investigate is a count of the cell population present in a sample. You may carry **out total population counts** in which the total number of cells, living and dead, are counted in a certain volume of a sample. You can also carry out a **viable cell count** in which only the number of living cells is counted. This type of count is useful in giving an indication of the severity of an active infection as it is only living cells that would cause disease or be transmitted to others.

Yeast cells are large enough to be seen with a light microscope, so a **haemocytometer** can be used to count them. A haemocytometer is a special slide that has a grid etched into its middle section, below the surface of the slide. Figure 2.27 shows a haemocytometer. When a cover slip is placed firmly on the slide, it forms a chamber of depth 0.1mm so you can calculate the volume of liquid over each etched square and make a total cell count using the steps below.

- Shake the tube of liquid medium in which the yeast cells have been growing.
- Transfer 1mL of this liquid and add it to 9mL of sterile water in another test tube. This dilutes the sample by a factor of 10.
- Mix the diluted yeast suspension well and, using a pipette, allow some of the solution to trickle into the grooves under the cover slip of the haemocytometer.
- Observe the haemocytometer grid under the microscope, using low power first.
  Focus on the central grid area where there are 25 squares, each sub-divided into 16 smaller squares.
- Count the yeast cells in five of the 25 squares (80 small squares): count the central square and each corner square. The volume of liquid over 80 small squares is 0.02μL.
- If any cells are on the boundaries, only count those on the left and top boundaries and not those on the bottom and right boundaries.
- Now you know how many yeast cells (n) are in 0.02μL of sample and you can calculate how many are in 1mL of the undiluted liquid medium.

Number of cells in 1 **µL** = n / 0.02

So, number of cells in 1 mL of diluted culture = (n / 0.02) x 1000

And the number of cells in 1mL of undiluted culture = (n / 0.02) x 1000 x 10

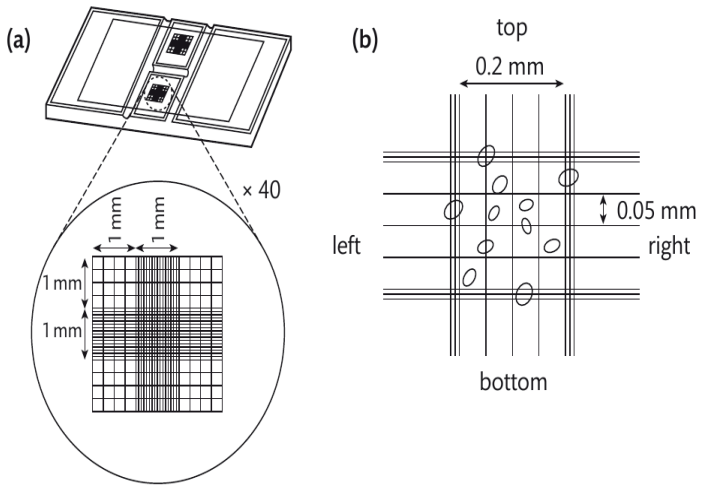


Figure 2.28: (a) A haemocytometer slide and (b) using a haemocytometer slide to make a total population count

Key points

Total population count – a quantitative method used to count the number of living and dead cells in culture.

Viable cell count – a quantitative method used to count the number of living cells in a culture that are growing and reproducing (viable).

Haemocytometer – a slide with a grid etched into its middle section, used to count cells large enough to be viewed with a light microscope.

Turbidimetric methods

The amount of growth in a liquid culture can also be measured using a colorimeter, which measures turbidity. A colorimeter shines a beam of light through a sample that is placed in a special plastic container, called a cuvette. A photoelectric cell picks up the light that has passed through the sample and tells you how much light has been absorbed.

Step-by-step: Measuring turbidity using a colorimeter

1. Fill a cuvette with liquid medium that does not contain any microorganism culture.
2. Set the colorimeter with a blue or green filter.
3. Insert the cuvette into the colorimeter and calibrate the colorimeter by setting the reading to zero.
4. Fill another cuvette with a sample of the microorganism culture that has yeast or bacterial cells growing in it.
5. Place the cuvette in the colorimeter using the same filter as before.
6. Measure the absorbance. The greater the absorbance, the greater the growth.

The colorimeter cannot distinguish between particles in the culture medium and cells, which is why you need to calibrate the colorimeter first so that it "ignores" the particles of the growth medium. You can also use this method to give a measurement of growth rate by taking samples from the culture at timed intervals and measuring the absorbance.

Viable counts

Serial dilutions

Serial dilution and subsequence colony counts can be used to give a viable cell count of bacteria. This gives an estimation of the number of living cells per mL of sample. During a serial dilution the liquid culture of bacteria is diluted over and over again into a volume of sterile water. Each dilution is then diluted further by taking a sample of the diluted culture and transferring it to a new volume of sterile water. This is repeated so that each time the sample is diluted by a factor of 10. Figure 2.28 shows how the bacteria culture can be diluted using serial dilution.

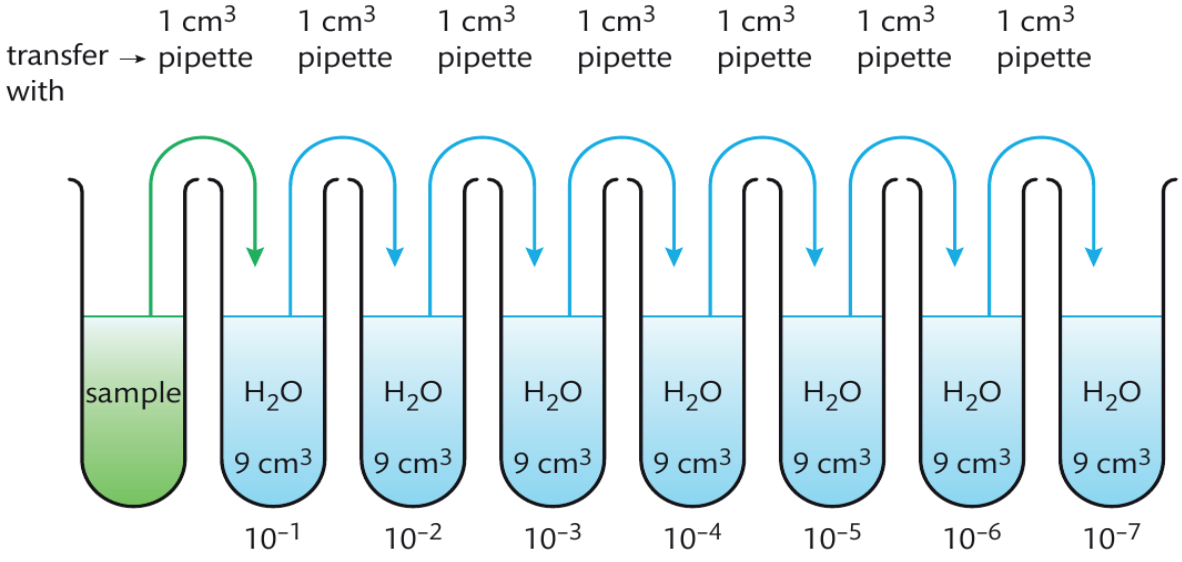


Figure 2.29: Carrying out a serial dilution

Once the serial dilution has been performed, a small volume of each culture is taken and transferred to individual nutrient agar plates. Aseptic technique is used to produce a lawn spread of each dilution. After incubation, a plate with a countable number of colonies is selected. The colonies can be counted and each one represents a single bacterium. A calculation can then be performed to calculate the number of viable cells per mL of sample. It is important that any sample volumes and the dilution factor is taken into consideration.

Step-by-step: Serial dilution and colony counts

1. Label your tubes for serial dilutions and agar plates with your name, the date, the microorganism to be added and dilution to be produced.
2. Wash your hands with warm water and an antimicrobial hand wash.
3. Ensure the work surface has been disinfected with a suitable disinfectant.
4. Mix the contents of the sample and, using a sterile pipette, withdraw 1mL of the solution. Hold the lid of the culture bottle after removing and flame the neck of the sample bottle after removing the lid and before replacing it.
5. Add the sample to a tube containing 9 mL of sterile water. This gives a x 10 dilution factor (dilution factor of 10^{-1}).
6. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 100 dilution factor (dilution factor of 10^{-2}).
7. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 1000 dilution factor (dilution factor of 10^{-3}).
8. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 10 000 dilution factor (dilution factor of 10^{-4}).
9. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 100 000 dilution factor (dilution factor of 10^{-5}).
10. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 1000 000 dilution factor (dilution factor of 10^{-6}).
11. Roll the tube between your hand to mix the contents. Transfer 1mL from this tube using a new sterile pipette and add to another tube containing 9mL of sterile water. This gives a x 10 000 000 dilution factor (dilution factor of 10^{-7}).
12. Using a sterile pipette each time, transfer 1 mL from the 10^{-3}, 10^{-4}. 10^{-5}. 10^{-6} and 10^{-7} dilutions onto five separate nutrient agar plates and evenly spread the bacteria using a sterile glass spreader.
13. Secure the plates using two pieces of tape, one on each side, and incubate upside down at 30°C for 24 hours.
14. Examine the plates (do not open them) and select the plate that has between 20 and 200 colonies. Count the colonies. You can divide the plate into sections and use a colony counter and felt tip pen to spot each colony as you count it. Using this count, you can calculate how many bacteria were present in 1mL of undiluted broth.

Pause point

Discuss the advantages and disadvantages of colorimetry, hemocytometry and serial dilutions for measuring the growth of bacteria.

Hint
Think of the pros and cons of each method.

Extend
How can the growth of a multicellular fungus be assessed?

Assessment activity 2.3 C.P5 C.P6 C.M3 C.M4 C.D2

1. Evaluate the development of new treatment for HIV using links to current issues facing scientists trying to develop these antiviral drugs Carry out Gram staining of a culture of bacteria and use light microscopy to observe the slide produced. Produce biological drawings of what you observe and identify as many features and characteristics of the bacteria as you can.
2. Once you have narrowed down the possible identification of the bacteria, use aseptic technique to produce the following cell cultures:
   a) agar slant
   b) streak plate
   c) pour plate
   d) lawn spread.
3. Compare and contrast the techniques used to identify and cultivate the bacteria. Link your comparison to the aims of the technique used and the quality of the results obtained.
4. Carry out a total and viable cell count to measure the growth of the microorganism you have used in activity 2.

D: Investigate the effect of antimicrobial agents on the growth of microorganisms

You may carry out investigations into the effect of different antimicrobial agents on the growth of microorganisms. Antibiotics and disinfectants are the two most likely antimicrobials you will investigate When designing an investigation, it is important to plan your method carefully so that the results you obtain can be trusted. In your experimental design you will need to identify which variable(s) you change (**independent variable**), the measurements you will make (**dependant variable**) and therefore the results you will record and the variables you will control (**control variables**) to ensure results are valid.

Key points

Antimicrobial – a substance that can destroy or inhibit the growth of microorganisms.

Independent variable – the factor that you change in an experiment.

Dependent variable – the factor that changes as a result of changing the independent variable. It is the factor that you measure.

Control variables – the factors that you set as constant or aim to keep constant to ensure results from an experiment are valid and conclusions can be trusted.

D1 Investigating the substances that inhibit the growth of microorganisms

Designing investigations

You have learnt about different cell culture methods in the previous section. Two of these methods, lawn spread plates and pour plates, can be used to investigate the effect of antimicrobial agents. Pour plates are suitable, as they produce an even spread of bacteria within and on the surface of the agar. If performed correctly, a confluent growth of bacteria occurs. This is where there is a continuous growth of bacteria covering all parts of the agar. As a result of confluent growth, the number of available bacteria for the antimicrobial agent to act on, is controlled. Pour plates can be used when the antimicrobial being tested is able to diffuse into the agar. A lawn spread also produces confluent growth but only on the surface of the agar. This method is suitable if the substance being tested does not diffuse into agar well or if the ability to do so is not known.

A common way to carry out an investigation is to first produce a lawn spread or pour plate of the bacterial culture being investigated. These plates are then incubated with the antimicrobial agent. The antimicrobial agent can be added to filter paper discs that are transferred to the surface of the agar using aseptic technique. Alternatively, wells can be cut into the agar using a sterile cork borer. Controlled volumes of the antimicrobial agent are then added to the wells. In both cases, you would obtain your results by measuring the zone of inhibition around the antimicrobial agent. When the bacteria grow confluent growth will be seen across the plate surface. However, where the antimicrobial agent is effective at inhibiting growth, clear zones will be produced on the agar. These clear zones are called zones of inhibition. The diameter of the zone of inhibition can be measured using a ruler or digital calliper. It is also possible to measure the area of the zones by placing 1mm^2 graph paper over the top and counting the squares.

When investigating the effect of antimicrobials, an important factor to control is the incubation temperature being used. It is important that all bacterial plates being used are incubated at the same temperature as different temperatures may lead to different growth rates and different results.

Case study

Coryn works in a microbiology laboratory and wants to test the effectiveness of different antibiotics on a bacterial culture. In order to produce valid results, she must design her investigation carefully. She decides to produce a lawn spread of the bacterial culture, which will then be incubated with a disc impregnated with the antibiotic. Coryn will measure the diameter of the zone of inhibition (no growth) around the antibiotic disc. She starts planning her investigation by listing her variables. She decides that her independent variable is the antibiotic used and her dependent variable is the diameter of the zone of inhibition produced. She identifies two variables to control; these are volume of bacterial culture added to produce the lawn plate (1 mL) and incubation temperature (30°C).

Check your knowledge

1 What equipment might she use to measure the zones of inhibition produced?

2 How might the two control variables identified be controlled?

3 Identify other variables that should also be controlled in this investigation.

Antimicrobial susceptibility tests

Disinfectants are antimicrobial agents that can be applied to non-living objects such as surfaces and instruments, to kill microorganisms on those surfaces. Examples are phenol, bleach, alcohol, hydrogen peroxide and iodine solution. Antiseptics are antimicrobial agents that can be applied to skin to reduce the risk of infection and sepsis. Examples are TCP, hydrogen peroxide, boric acid and salt water. Some natural compounds such as garlic and essential oils also have antimicrobial properties. Disinfectant, antiseptics and natural compounds can be tested using antimicrobial susceptibility tests.

Step-by-step: Investigating the antimicrobial effect of disinfectants using a lawn spread

1. Wash your hands with warm water and an antimicrobial hand wash.
2. Ensure the work surface has been disinfected with a suitable disinfectant.
3. Using aseptic technique pour molten agar into a petri dish and allow it to set.
4. Remove the lid of the bacterial culture tube using your little finger and do not put it down. Flame the neck of the bottle.
5. Using a sterile pipette, extract 1mL of the culture. Do not put the pipette down. Re-flame the neck of the bottle before replacing the lid.
6. Remove the lid of the Petri dish slightly, keeping it close to the agar. Transfer the bacterial culture from the pipette to the centre of the agar. Replace the lid. Place the pipette in disinfectant.
7. Dip a glass spreader in 95% ethanol and pass through a Bunsen burner flame to ignite. Allow to cool.
8. Use the glass spreader to spread the bacterial culture over the surface of the agar.
9. Make up the disinfectants to be tested to their recommended dilutions.
10. Prepare a filter paper disc.
11. Using sterile forceps dip the filter paper disc into the disinfectant and allow to soak.
12. Using sterile forceps place the filter paper disc on the centre of the agar.
13. Secure the plates using two pieces of tape, one on each side and, incubate upside down at 30°C for 24 hours.
14. Observe the plate and measure any zone of inhibition using a ruler or digital calliper.

Safety tip – ethanol is extremely flammable. Replace the lid of the ethanol dish before passing the glass spreader through the Bunsen burner flame.

The method above could be modified to investigate the effect of:

- different disinfectants
- different antiseptics
- different natural antimicrobials
- disinfectants vs natural antimicrobials
- different concentrations.

Pause point

Write a method for how you could test the effectiveness of different concentrations of a disinfectant.

Hint

Start by thinking about which method of cell culture you will use.

Extend

Evaluate the equipment you have chosen and consider how accurate your results would be.

Antibiotics

Antibiotics are chemicals produced by microorganisms that can kill or inhibit the growth of other microorganisms, mainly bacteria but also some fungi and protozoans. Examples of antibiotics are penicillin, tetracycline and cephalosporins. You can also carry out investigations into the effect of antibiotics on microbial growth using the lawn spread or pout plate methods.

Antibiotics are generally divided into two groups based on how they work: bactericidal or bacteriostatic. Bactericidal antibiotics work by killing bacteria directly. Some of these work by damaging the membrane of the bacteria so that the cellular contents leak out. Examples of bactericidal antibiotics are vancomycin and penicillin. Bacteriostatic antibiotics do not kill bacteria but instead prevent growth or reproduction of the bacteria. In the longer term this can give the body's immune system a chance to remove the bacteria while the population size is not increasing. Tetracycline is an example of a bacteriostatic antibiotic. It works by inhibiting the ribosomes in the bacteria and preventing protein synthesis from taking place. When carrying out investigations into the effect of antibiotics on microbial growth, whether the antibiotic is bactericidal or bacteriostatic will affect your results. A larger zone of inhibition indicates a greater effectiveness at killing bacteria. Bacteriostatic antibiotics may not produce a zone of inhibition but may cause less of a confluent growth in the area surrounding the antibiotic.

Narrow spectrum antibiotics are designed to target a limited range of bacteria.

You could carry out investigations into the effect of antibiotics on the growth of microorganisms. Your school/college will be able to buy rings that are impregnated with different antibiotics or individual discs containing different antibiotics. These can be applied using aseptic technique, onto the surface of pour plates or lawn spreads. The effectiveness of the antibiotic can be measured by measuring any zone of inhibition produced. The bigger the zone, the more effective the antibiotic.

Investigations can be carried out to test the effect of:

- different antibiotics
- different types of antibiotic (bacteriostatic, bactericidal, narrow and broad spectrum)
- the age of antibiotics on their effectiveness.

D2 Interpretation, analysis and evaluation of results

To ensure that any results you obtain are as accurate as possible and to allow you to draw valid conclusions it is also important to consider:

- the data collection method used
- the number of repeats you will perform
- any sources of error in the data
- possible limitations in the investigation.

Data collection methods

When measuring the diameter of the zone of clearance you may use a ruler or a digital calliper. A ruler is typically used to measure in centimetres. When measuring small diameters these units may not be small enough to show you a clear difference between two zones of inhibition. To avoid this, you may choose to measure in millimetres instead. Figure 2.29 shows how a ruler can be used to measure the diameter of a zone of inhibition. Using a ruler, it is only possible to measure to the nearest millimetre. A measurement made by the ruler has an error of ±0.5mm. This is the maximum uncertainty of the ruler.

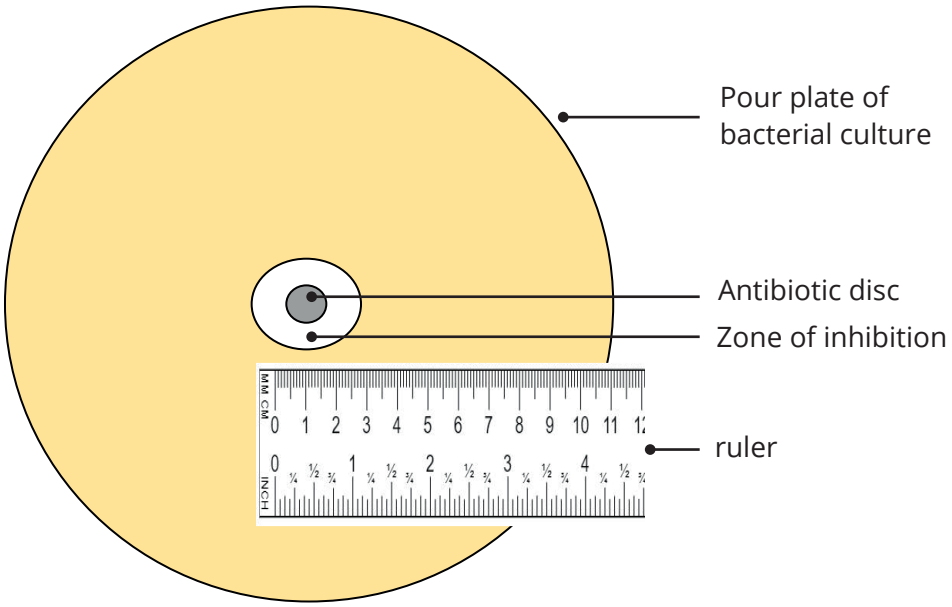


Figure 2.30: Using a ruler to measure the zone of inhibition produced by an antibiotic disc on a pour plate of a bacterial culture. Measured through the lid of the Petri dish

To improve the accuracy of the measurements made, a digital calliper could be used. Some digital callipers are able to make measurements to the nearest 0.01mm. A digital calliper, as shown in Figure 2.30, is placed over the lid of the Petri dish. The wheel is then turned to open the prongs of the calliper so that they represent the size of the diameter of the zone of inhibition. This then gives a digital reading on the screen to the nearest 0.01mm. Measurements made by a digital calliper have less errors due to the increased sensitivity of the measuring equipment. The maximum uncertainty of a digital calliper would be 0.005mm.

Figure 2.31: A digital calliper

Repeats and anomalous data

To ensure the data you are collecting is repeatable and can be trusted, you normally take repeat readings. In an investigation into the effect of antimicrobials on the growth of microorganisms, you might carry out more repeats on different agar plates or you might divide a plate in half and apply two discs of antimicrobial. If repeat readings are in good agreement with each other, you can usually assume that the data is reliable. When you take repeat readings, you can also identify any anomalous results. An anomalous result is a measurement that does not fit the general trend or pattern. If you think a result is anomalous, you can disregard it and take a further repeat reading.

Recording and presenting data

When collecting data, it is important that it is presented in a clear and logical way. Usually you will design a table to collect and display your results. The table should:

- be drawn with ruled pencil lines
- have a complete border around the outside
- have the independent variable in the first column
- have the dependent variable (repeats and mean) in subsequent columns
- have informative headings with units in the columns
- contain measurements recorded to an appropriate level of precision for the measuring equipment you used.

Example

An investigation was carried out into the effect of three disinfectants on the growth of *E.coli* bacteria using the lawn spread method. Measurements of any zones of inhibition produced after 24 hours incubation at 30°C were made using a ruler in millimetres. The results obtained were recorded in a correctly drawn table as shown in Table 2.12.

Table 2.12: Effect of different disinfectants on the growth of *E. coli* bacteria

Disinfectant	Diameter of zone of inhibition /mm			
	Repeat 1	Repeat 2	Repeat 3	Mean
Bleach	65	63	60	62.7
Hydrogen Peroxide	56	54	57	55.7
Iodine solution	10	60	15	12.5

Table 2.12 has correct headings with units. The table shows that three repeats have been carried out and all measurements have been recorded to an appropriate level of precision given that a ruler has been used. Mean values have been calculated. For iodine solution, repeat 2 has not been included in the mean calculation as it is anomalous. The person carrying out the investigation may choose to repeat this test.

To present results from an investigation, a graph may be used. Bar charts may be used to display discrete or categoric data. For the data in Table 2.12, it would be appropriate to draw a bar chart to display the results.

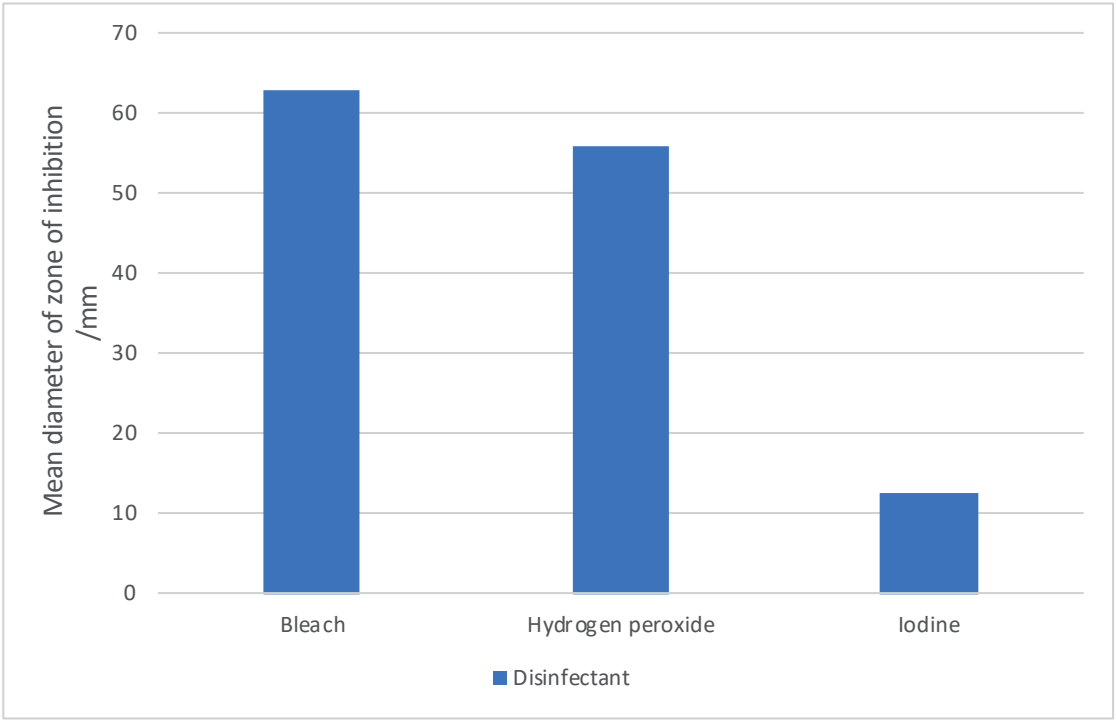


Figure 2.32: Bar chart showing the effect of different disinfectants on the growth of *E. coli* bacteria

Line graphs

Line graph are used when plotting results from continuous data. A line graph produced can result in a straight line or a smooth curve. As with any graph, the axis must be labelled with informative titles with units. The scales used should be ascending and have equal intervals. The scale should be chosen so that the graph area covers as much of the available paper as possible.

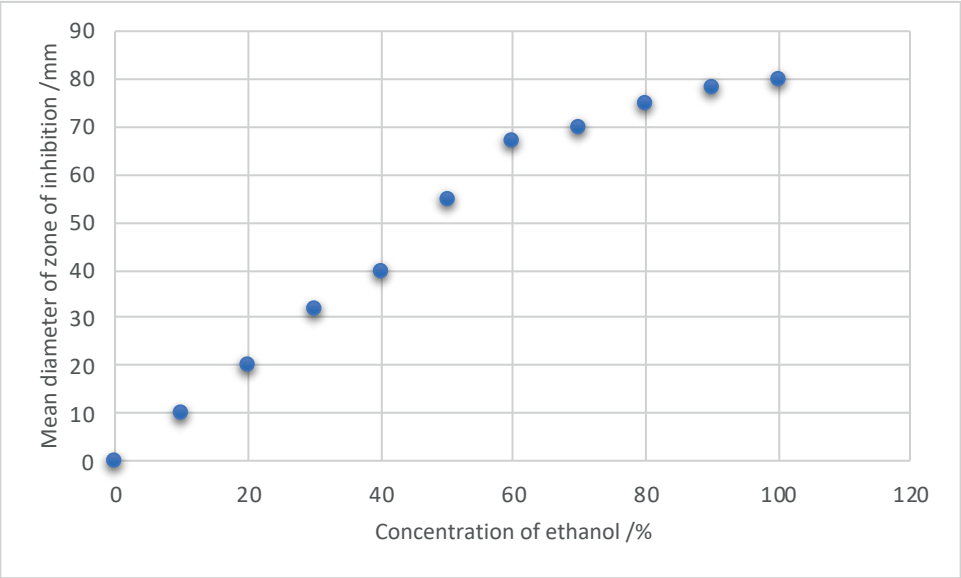


Figure 2.33: Line graph showing the effect of different concentrations of ethanol on the growth of *M. luteus* bacteria

The graph in Figure 2.33 includes a line of best fit. The experimental data displayed does not exactly fit the curve. A line (or curve) of best fit should be drawn so that there are an equal number of points either side of the line.

Sources of error

Different measuring equipment may measure to different degrees of precision. You have already seen this when comparing a ruler to a digital calliper.

Percentage error is used in science to give an indication of how error affects the accuracy of the measurements made. To calculate percentage error, you use the following equation:

$$\text{Percentage error} = \frac{\text{uncertainty of the measuring cylinder x 100}}{\text{measurement made}}$$

Worked example

Bobby is investigating the effect of different concentrations of TCP on the growth of *S.epidermis* bacteria. He decides to use the lawn spread method and cut wells into the agar. He will then fill these wells with TCP and incubate the plates for 48 hours at 25°C. He plans to measure the zones of inhibition produced. Bobby cannot decide whether to use a ruler or a digital calliper to measure his results. He carries out a preliminary experiment using the recommended dilution of TCP and makes the following measurements of the zone of inhibition produced:

Ruler/mm = 55

Digital calliper /mm = 54.58

1 What is the maximum percentage error of each piece of equipment?
2 Which piece of equipment would you choose to make the most accurate measurements?
3 What else could affect the accuracy of the measurements made?

Drawing conclusions

Having processed your data using tables and graphs, you should be able to look for trends or patterns in your results.

You would need to consider:

- Is there a relationship between the variables?
- Do the results support your hypothesis?
- Are your results supported by secondary data?

Before drawing conclusions, you may wish to carry out further statistical analysis of your results. You may choose to calculate standard deviation. Depending on the type of data obtained you may carry out statistical tests such as T-tests, correlation analysis or chi-squared tests. You would link your results and conclusions back to the aims and purpose of the investigation and consider areas where your results could be improved.

Discussion and evaluation

In science, it is typical to evaluate the investigation carried out by producing a discussion of the results obtained. in your evaluation, you would explain any possible reasons for anomalous data and suggest limitations that could reduce the accuracy of any data obtained.

Common limitations you may wish to avoid or include in an evaluation

When carrying out investigations into the effect of antimicrobials, there are some common limitations that could reduce the accuracy of the results obtained. When planning investigations, you may wish to tweak your method to avoid some of these. Alternatively, they could offer possible explanations for any anomalous results.

You may get what looks like zones of inhibition on the agar plate and might assume that this lack of growth is caused by the antibiotic placed on the agar. It could, however, result from non-standard confluent growth. This is where the bacteria do not grow evenly across the agar when incubated. Often this is due to poor technique. You will also need to be aware of any potential contaminating microorganisms that could be competing with the bacteria you are trying to measure. Other bacteria or fungi that end up on the agar due to poor aseptic technique could outcompete the bacteria being investigated leading to inaccurate results.

Other possible limitations include:

- use of antibiotic discs that are old or past their recommended use-by date
- antibiotic discs being used that have not been stored at the correct temperature of 4 °C
- poor pouring of agar plates resulting in inconsistent depth of agar in the Petri dish. This would affect the diffusion of the antibiotic into the agar
- use of incorrect growth conditions for the bacteria.

Assessment activity 2.4 C.P5 C.P6 C.M3 C.M4 C.D2

1. Produce a plan for an investigation into the effects of an antimicrobial on the growth of a microorganism. Speak to the technicians or teachers in your school or college to find out what equipment and microorganisms are available.
2. Consider the method you will use to produce the most accurate results and select appropriate equipment to use.
3. Carry out your investigation using aseptic technique to collect your data.
4. Produce an analysis of your results using the data collected and make valid conclusions.
5. Produce an evaluation of your method, techniques and data collected. Link your conclusions to the wider impact on prevention and treatment and disease.

Answer to Worked example

Ruler: Percentage error = $\frac{0.5 \times 100}{55}$ = 0.91%

Digital calliper: Percentage error = $\frac{0.005 \times 100}{54.58}$ = 0.0092%

Think Future Skills

Joanne Metcalfe - Clinical Microbiologist
I work for a company that analyses samples sent from various places including the NHS. I have a degree in microbiology and have completed masters qualifications in medical microbiology. I have also completed courses that allow me to practice clinical microbiology and to become a registered clinical microbiologist. The company I work for monitors and analyses microbial cultures and samples using computer software and a range of practical methods. Typical tasks I might complete include:

Identifying infections in patient samples using a variety of microbiological, biochemical and molecular methods

Carrying out tests to assess the virulence of different microorganisms

Carrying out research to prevent the spread of infectious microorganisms in hospitals and other clinical settings

Recording, analysing and interpreting scientific data

Writing reports

Communicating with healthcare professionals or clinical colleagues

Focusing your skills

I need the following skills to do my job competently:

- enthusiasm about microbiology
- multi-tasking
- quick learner
- problem solving
- good communication skills
- sound technical judgement
- attention to detail
- ability to be organised and meet deadlines
- analytical skills
- high emotional intelligence
- desire to commit to professional development and improvement.

Getting ready for assessment

Hannah is studying for a BTEC Extended Certificate in Applied Human Biology. She was given an assignment as part of her practical portfolio. She was asked to investigate the effect of garlic, as an antimicrobial, on the growth of bacteria.

How I got started

I gathered all of my notes on antimicrobial agents and techniques used to investigate the effect of antimicrobials on the growth of bacteria. I used relevant and appropriate sources to find out more about the antimicrobial properties of garlic. I found a reference to an article in the magazine Scientific American and the technicians at my college were able to help me find the article. I also found some useful, peer reviewed websites via the internet.

How I brought it all together

I decided to take an extract of garlic and then use the well method on a lawn spread. I first used a bacterial slant that my college had brought in and inoculated nutrient broth to make a liquid culture. After incubating this culture for 48 hours at 25°C, I used it to produce a lawn spread of bacteria on nutrient agar plates. Using a cork borer, I made a well in the centre of each plate. Then, I added a known volume of a known concentration of garlic extract and incubated the plates for 24 hours at 30°C. I repeated this for all of my different concentrations of garlic on separate lawn spreads. I also carried out three repeats of each concentration on different lawn spreads. This would allow me to see if my data was reliable and carry out a statistical test. I got my results by using a digital calliper to measure any zones of inhibition that were produced around the wells where I added the garlic. I recorded my results in a scientific table.

What I learnt from the experience

I learnt that garlic has antimicrobial properties. I found that some concentrations of garlic produced larger zones of inhibition and therefore are more effective at reducing bacterial growth.

I could have extended the investigation by trying other extracts, for example tea tree oil.

Think about it

1. Have you made a plan with timings so you can complete your assignment by the agreed sub mission date?
2. Have you arranged to meet with the science technicians or your tutor to discuss what re sources are available for your work?
3. Have you researched all the background information about the bacteria and the chemicals you will use?
4. Have you made notes of the sources of your information so you can reference all sources properly?
5. Have you considered all the safety aspects of your investigation and practised using aseptic techniques?