MODULE 1

# Tissues, cells and molecular studies

**This module focuses on** the study of …

Unit 1: Microorganisms and microbiology

Unit 2: Health and disease

Unit 3: The body's defence against invaders

# UNIT 1 MICROORGANISMS AND MICROBIOLOGY

**KEY CONCEPTS**

- microbiology
- culture
- sterilisation
- pasteurisation
- inoculation
- aseptic technique
- incubation
- resolving power
- staining
- virus
- bacteriophage
- ultramicroscopic
- anaerobes
- conjugation
- budding
- mycelium
- sporozoan

**After you have completed** this section, you should be able to:

- define a microorganism and its role in microbiology
- understand the need for specialised tools, methods and techniques for studying microorganisms
- understand the need for different types of microscopes
- explain why it is important to stain microorganisms for study
- know the structure, classification and characteristics of some viruses, bacteria, fungi and protozoans.

## What is a microorganism?

The word *microorganism* is derived from the Greek word *micro*, which means 'small'.

**Microorganisms** are very small life forms, which cannot be seen with the naked eye. These microorganisms include viruses, bacteria, fungi, algae and protozoa.

To describe the size of microorganisms, use is made of the metric system measure of length based on a metre. Fractions of a metre are described by using prefixes such as milli- (one thousandth), micro- (one millionth), and nano- (one billionth).

*Note:* Viruses are small particles that exist at a level of complexity somewhere between large molecules and cells. They are much simpler than cells, composed of some hereditary material surrounded by a protein coat. Some scientists regard viruses as parasitic particles while others refer to them as primitive organisms.

The smallest object that can be seen with the naked eye is approximately one tenth of a millimeter (0,1 mm), which is equal to 100 micrometres (100 µm). Most microorganisms are smaller than this. The largest of bacterial cells, for example, are only 5 µm (0,005 mm) in length, and small bacterial cells are about 0,1 µm in length. Hence it is necessary to magnify the images of most bacteria about 1 000 times to be able to see them. Viruses are even smaller. The largest viruses are almost 0,1 µm and the smallest about 0,01 µm. Thus the images of the viruses must be magnified 10 000 to 100 000 times to be seen.

Although microbiology is primarily the study of microorganisms, it inevitably leads to the study of microorganism-human and microorganism-environment interactions. One need only consider the arrival of HIV/AIDS on the world scene, cholera and tuberculosis epidemics and the explosion in biotechnology to understand the extent to which microbiology is woven into our everyday lives.

Microbiology is valuable to most people because of its widely practical and sensible nature. Its application becomes immediately significant to everyday situations, such as those involving food

preparation and preservation, soil fertility, sewage treatment and waste disposal and the prevention and treatment of infectious diseases.

WORK IN GROUPS

## Activity 1

LO2: AS1

Name as many different areas that you can think of where microbiology is applied today.

WORK IN PAIRS

## Activity 2: Case study

LO2: AS2, AS3

### Discovery of microorganisms

Considering their small size, it is no wonder that the existence of microorganisms was not recognised until only a few centuries ago. The advent of the microscope, which is an instrument used to enlarge objects and images, permitted us to see them. Nor is it surprising that we still have a lot to learn about microorganisms. The invention of the microscope occurred at the same time as the introduction of the telescope. As some scientists looked upward and outward toward the stars with telescopes, others began to search inward with microscopes.

The first microscopes were simple ground-glass lenses that magnified images of previously unseen objects. By the late seventeenth century, microscopes permitted magnifications of several hundred times, making it possible to discover the microbial world. Among the first to observe the previously invisible microbial world was Robert Hooke, an English scientist. His detailed drawings of fungi, made in 1667, reflect hours of tedious observations (Figure A).

FIGURE A

Robert Hooke made microscopic observations and provided the earliest descriptions of many fungi. Various species of fungi can clearly be identified in his drawings made from 1635 to 1703 and recorded in his book, *Micrographia*.

Antonie van Leeuwenhoek, an amateur scientist and official winetaster of Delft, Holland, made the first recorded observations of bacteria in 1670 (Figure B). Van Leeuwenhoek's interest in microscopes was probably related to the use of magnifying glasses by drapers to examine fabrics.

His hobby was microscopy and he made over 100 microscopes, each consisting of a simple glass lens (Figure C on the next page). These microscopes were little more than magnifying glasses, each capable of magnifying an image about 300 times, so that bacteria could barely be seen as fuzzy images. Van Leeuwenhoek must have had great patience and persistence to squint through the lens of his handheld microscope at dimly lighted specimens and record drawings of microorganisms as he did. He thought the bacteria he observed were like little animals because they moved, therefore he called them 'animalcules'.

FIGURE B

Antonie van Leeuwenhoek (1632–1723)

Van Leeuwenhoek's observations of bacteria (animalcules) set the stage for the development of the field of microbiology (Figure D).

Although not a professional scientist, van Leeuwenhoek asked questions and performed experiments. For example, after observing animalcules in rainwater from his garden, Van Leeuwenhoek decided to test whether microorganisms came from heaven or whether they came from earthly sources. He washed a porcelain bowl in fresh rainwater, set it out in his garden during a storm, and observed no microorganisms in the freshly collected rainwater sample. After allowing the water to sit for a few days, he observed numerous microorganisms in the sample. He concluded that a few microorganisms in the sample had multiplied and that 'life begets life – even for animalcules'.

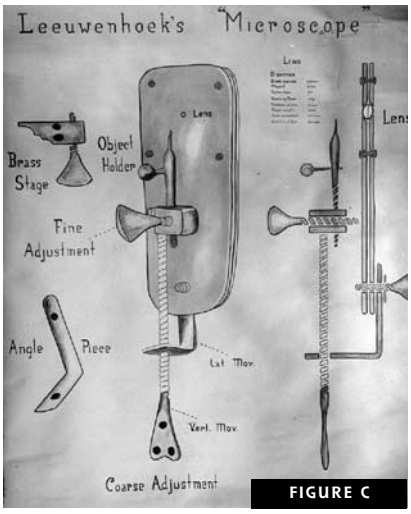


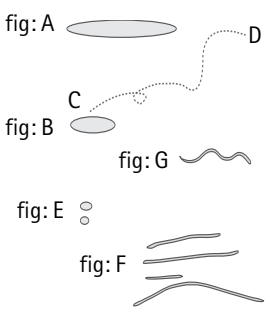

FIGURE C

Van Leeuwenhoek's microscopes were little more than magnifying glasses

Although his observations stimulated much controversy, no one at that time made a serious attempt to repeat or extend them. Van Leeuwenhoek's animalcules remained mere oddities of nature to scientists of the day. It was not until two centuries later that the significance of the observations made by Van Leeuwenhoek became evident, when the science of microbiology began to flourish and to develop into the vibrant field of scientific study it is today.

*From "Microorganisms in our world" by Ronald M Atlas, 1995*

FIGURE D




Reproduction of Van Leeuwenhoek's sketches of bacteria from the human mouth illustrates several common types of bacteria, including rods and cocci.

**A** – motile *Bacillus*
**B** – *Selenomonas sputigena*
**C** and **D** – the path of *S. sputigen*
**E** – micrococci
**F** – *Leptothrix buccalis*
**G** – spirochete

MODULE 1 UNIT 1 DOCUMENT 1

1 Who were the first persons to observe and record the following types of microorganisms
   **a** bacteria?
   **b** fungi?
2 Study Figure C and compare Leeuwenhoek's microscopes with the light microscope of today.
3 Study Figure D and describe in your own words the organisms represented by A, B, C, D, E, F, and G on Figure D.

# Tools, methods and techniques for studying microorganisms

In microbiology two basic methods are used to study microorganisms. These are the pure culture methods and microscopy.

## Pure culture methods

When studying macroscopic organisms such as animals and plants, they can be seen and differentiated from their environment and from one another. However, due to the minute nature of microorganisms and their occurrence throughout the environment, several challenges arise when studying them.

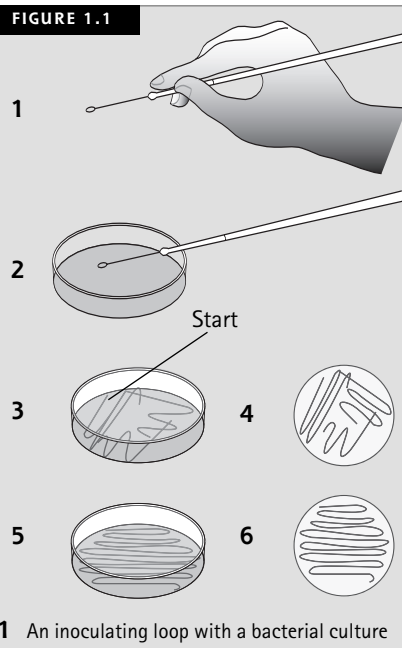
WORK ALONE

### Activity 3

LO1: AS1

Suggest and describe three challenges that are likely to arise when studying microorganisms.

FIGURE 1.1




1 An inoculating loop with a bacterial culture
2 Position flat into agar and streak across the surface.
3 Pattern for a concentrated bacterial culture
4 Reflame loop before changing direction.
5 Pattern for a dilute bacterial culture
6 Streak forward and backward across the plate.

A streak plate

A **pure culture** is a population of identical micro-organisms all derived by asexual reproduction from a single cell. The growth can be seen as colonies. Each colony contains millions of identical cells.

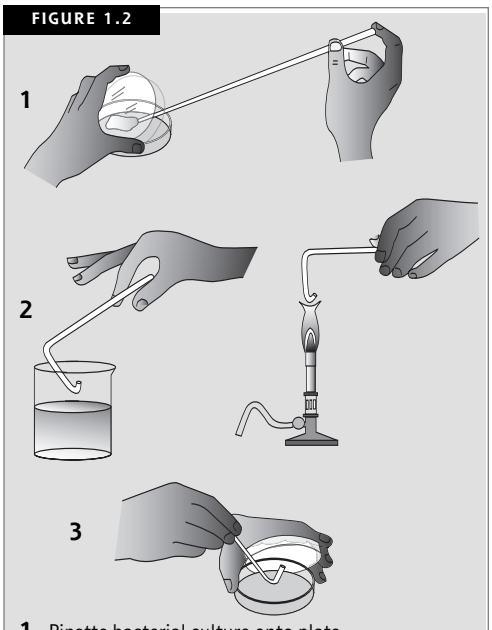
The preparation of a pure culture involves the introduction of a tiny sample of cells into a container of nutrient medium and encouraging the cells to propagate. This process is called **inoculation**.

Pure cultures of microorganisms can be isolated using **aseptic** transfer techniques and sterile media. Isolation involves the separation of the microorganism that is being cultured from a mixture of microorganisms.

The **streak plate** (Figure 1.1), **spread plate** (Figure 1.2 on the next page), and **pour plate** (Figure 1.3 on the next page) methods are used to isolate pure cultures of microorganisms. These isolation techniques are designed so that individual microbial cells are sufficiently separated on the surface of solidified agar so that when they reproduce they form well developed colonies.

Once the microorganisms are transferred onto the plate it must be incubated at the right temperature.

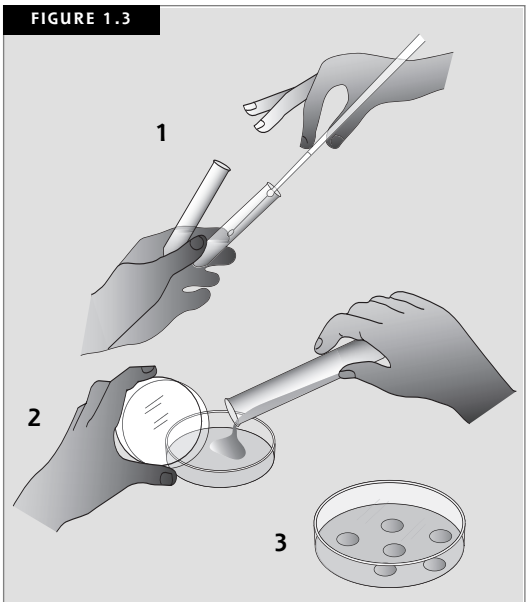
FIGURE 1.2




1 Pipette bacterial culture onto plate.
2 A spreading rod is sterilised by first dipping it into the alcohol and then flaming over a bunsen burner.
3 Allow the spreader to cool before spreading the sample across the agar.

A spread plate

FIGURE 1.3




1 Using an inoculating loop or a pipette, add 0,1 ml bacterial culture to dilution in melted agar.
2 Pour.
3 Colonies develop after incubation.

A pour plate

WORK IN PAIRS

## Activity 4

LO2: AS1, AS2, AS3

1 What do the following terms mean: inoculation, sterilisation and aseptic technique?
2 Explain the importance of sterilisation in the preparation of a pure culture.
3 Differentiate between the following types of cultures: pure, mixed and contaminated.

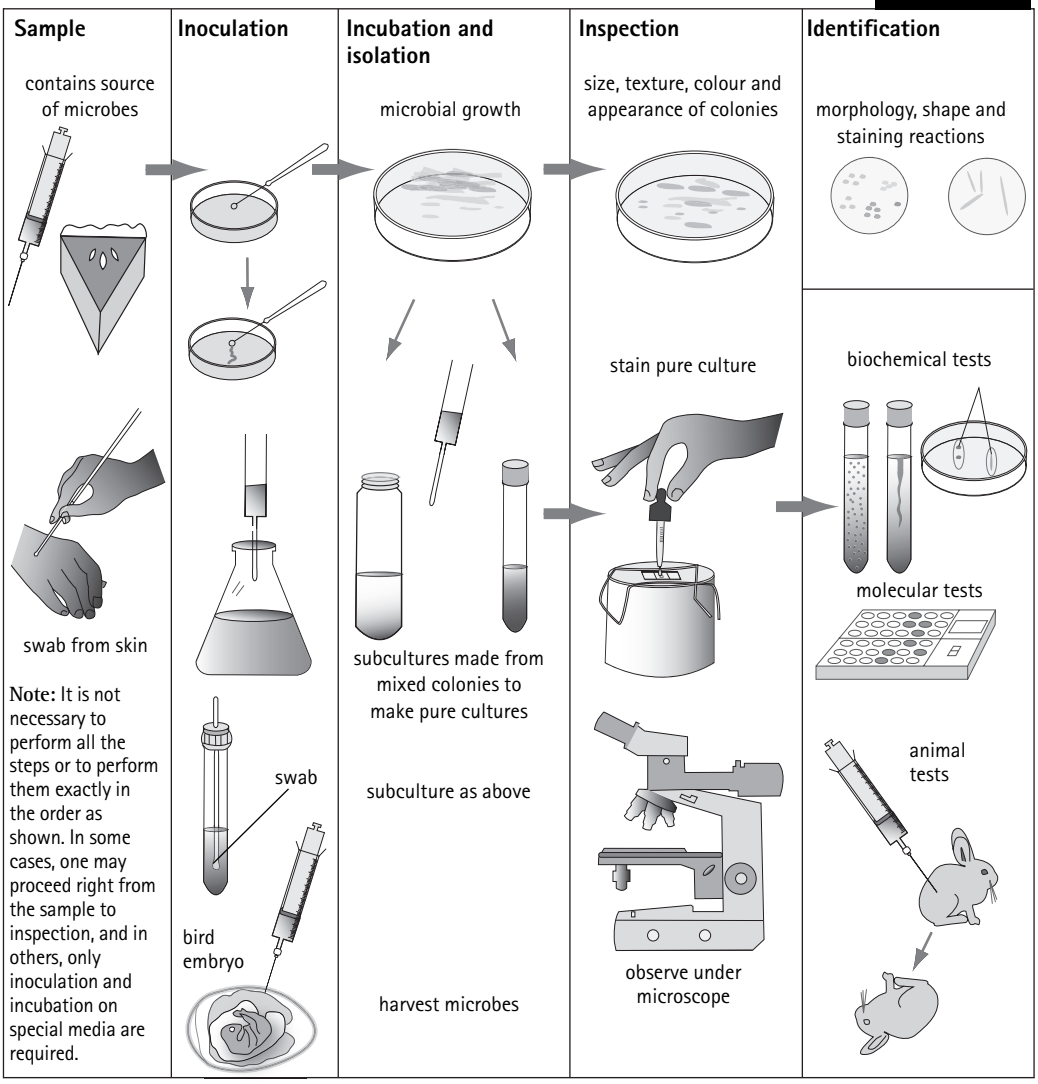
## Microscopy

### Magnifying power versus resolving power

The microscope is an instrument used to magnify (enlarge) images of objects that are too small to be seen with the naked eye. The light microscope can be used to view objects as small as bacteria, while the electron microscope may be used for viewing even smaller objects like viruses and large molecules.

A light microscope allows the formation of an enlarged image of a specimen due to the presence of multiple lenses. Light is refracted

FIGURE 1.4



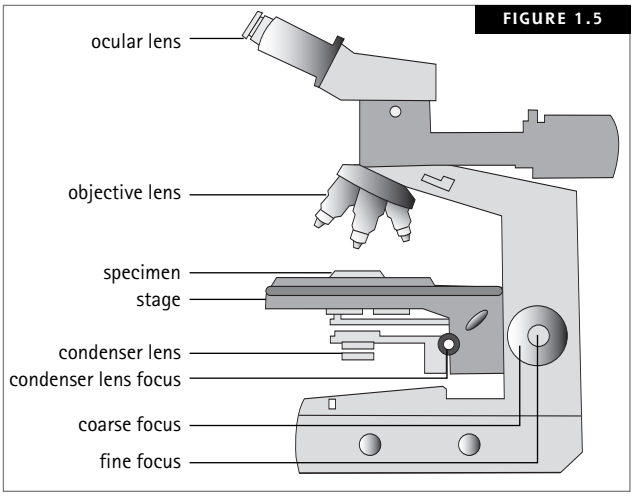

A summary of the general laboratory techniques used in microbiology

(bent) as it moves through these lenses. The *condenser lens* focuses light on a specimen, the light then passes through the *objective* and *ocular lenses* to produce a magnified image.

The **magnifying power** of a compound light microscope is calculated by multiplying the magnifying powers of the objective and ocular lenses.

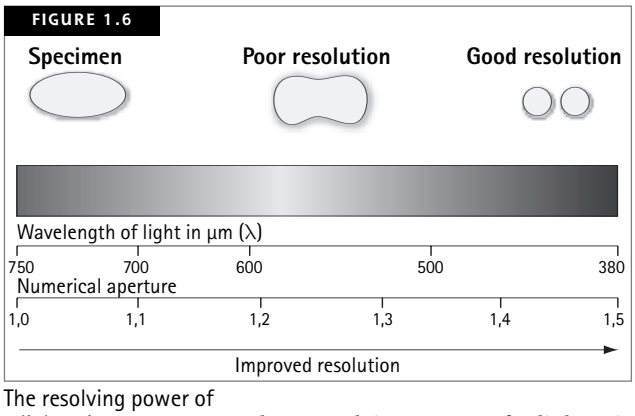
It may be assumed that adding lenses of increased magnifying power can produce more and more powerful microscopes. For example, if a 100× ocular lens is combined with a 100× objective lens, an image would be magnified 10 000×. While this image will be larger, it will not

FIGURE 1.5




A light microscope

FIGURE 1.6




The resolving power of a light microscope

show additional details of the specimen. The ability to distinguish detail in an object is referred to as **resolution**. Therefore, the ability to see detail is determined by the resolving power and not the magnifying power of the microscope. The **resolving power** is a distance that is defined as the closest spacing between two points at which they can still be seen clearly as separate entities.

Objects that are closer than the resolving power of the microscope cannot be seen as separate and distinct, and objects that are smaller than the resolving power cannot be seen at all.

At low resolution, objects blur, while at greater resolution, more details are visible.

Resolution depends on the wavelength of light and the numerical aperture of the lens. Blue light has a short wavelength (380 µm), while red light has a longer wavelength (750 µm). The best resolving power of a light microscope occurs when a short wavelength of light and a high numerical aperture objective lens are used.

To achieve a high numerical aperture, a drop of clear oil is inserted between the specimen and the objective lens. Therefore, for studies in microbiology, microscopes are generally fitted with a special objective lens called an *oil immersion objective lens*.

WORK IN GROUPS

## Activity 5

LO2: AS1, AS2

1 Differentiate between magnifying power and resolving power.
2 Explain how the use of oil can improve the resolution of objects.

### Staining of specimens

Most microorganisms are colourless and cannot easily be seen without staining or dyeing. Stains are therefore used to increase the contrast between the specimen and the background. Various types of staining procedures are used for different purposes in microbiology.

A general staining procedure follows these steps:

1. A suspension of the specimen is transferred onto a clean glass microscope slide.
2. The suspension is spread as a thin film across the slide.
3. It is allowed to dry in the air.
4. The slide is then quickly passed through a flame to fix the cells to the slide.
5. A stain is added onto the specimen and allowed to stand for a period of time.
6. Excess stain is rinsed off from the slide.
7. The slide is now ready for viewing under the microscope.

WORK ALONE

**Activity 6** LO1: AS3

Suggest two reasons for fixing the cells onto the slide.

### Simple staining procedures

Simple staining procedures make use of a single stain.

A **stain** is a salt compound that has positively and negatively charged ions. If the coloured part of the stain is positively charged, it will be attracted to the cells of the microorganism, which have negative charges. Staining with a positive stain is referred to as *positive staining*. Methylene blue is an example of a positive stain that colours the microbial cells blue.

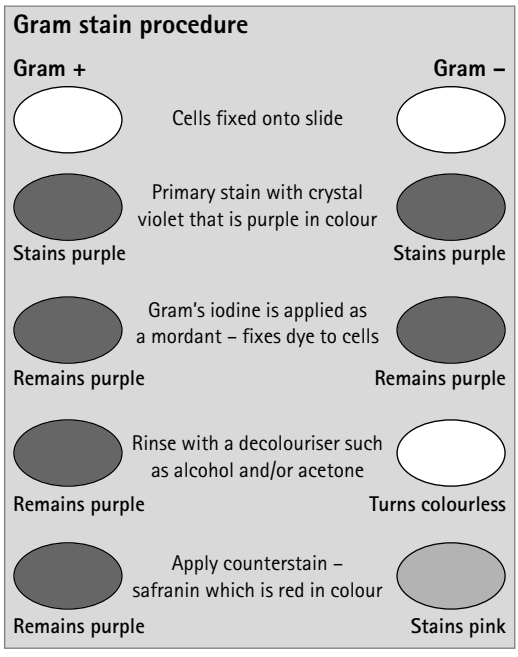
If the coloured portion of the stain is repelled by the negatively charged microbial cells, clear unstained cells will be seen against a dark background. Staining with a negative stain is called *negative staining*. India ink and nigrosin are examples of negative stains.

WORK IN PAIRS

**Activity 7** LO1: AS1

Find out for what purpose(s) positive and negative staining procedures are used.

## Differential staining procedures

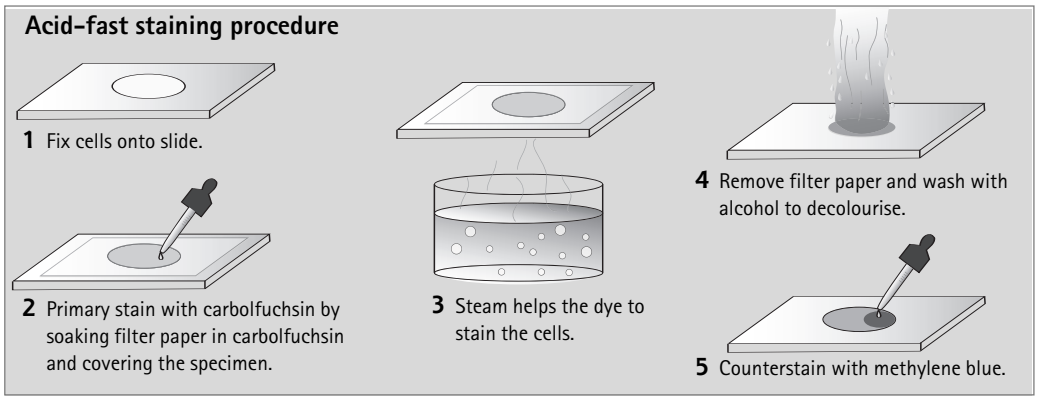



Multiple stains are used in this procedure. Different types of microorganisms and/or parts of the cells exhibit different affinities for certain stains. By making use of this procedure, structural differences between microbial species can be observed.

The Gram stain, acid-fast staining and endospore staining procedures are the most commonly used in microbiological studies.

*Staphylococcus* and *Streptococcus* are examples of Gram-positive bacteria while *Escherichia coli* is an example of a Gram-negative species.

Acid fast staining is used to identify *Mycobacterium* and is important in identifying the causative organism for tuberculosis, namely *Mycobacterium tuberculosis*. These are acid-fast bacteria, which appear red after the staining procedure.




### Endospore staining procedure

The steps involved are the same as that for the acid-fast procedure, except that the primary stain is malachite green, the decolourising agent is water, and the counterstain is safranin.

WORK ALONE

### Activity 8

LO1: AS2; LO2: AS2

1 What is a mordant?
2 Why are the slides steam-heated for the acid-fast and endospore-staining procedures?
3 What is an endospore?