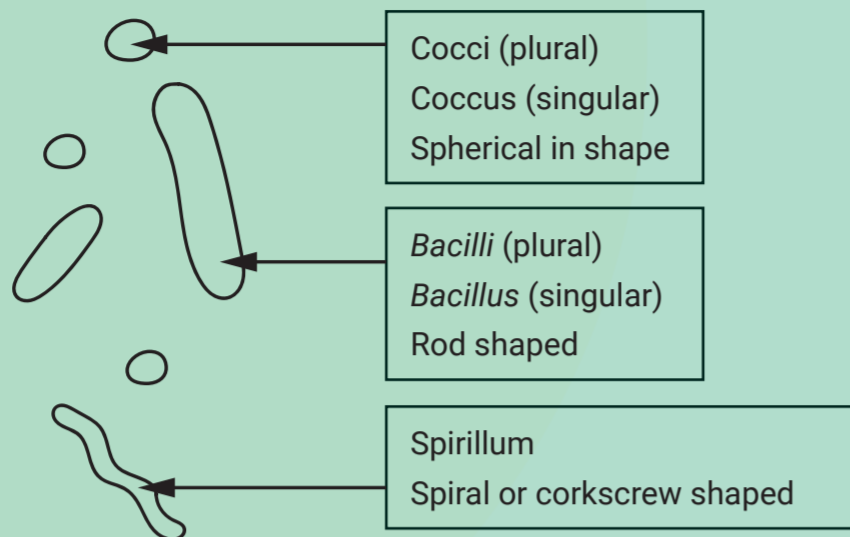


## Microbiology

### Classifying bacteria by shape

Bacteria can be distinguished from each other by their size, shape, staining characteristics, and their metabolic, antigenic and genetic features.

The shapes are shown here:



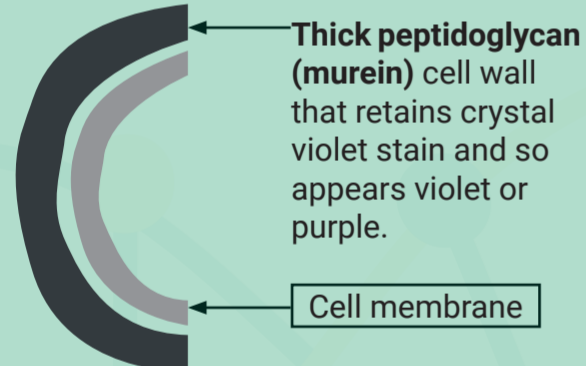
### Classifying bacteria by Gram staining

Bacteria can be classified as: **Gram positive - purple** or **Gram negative - red** according to how they react to the Gram stain.

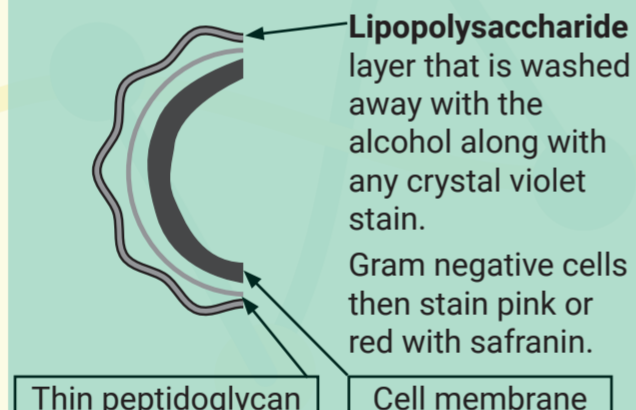
#### Method

1. Smear a glass slide with the bacterial sample and use heat to fix.
2. Stain with crystal violet which binds to peptidoglycan.
3. Treat with mordant Lugol's iodine to fix the stain to the peptidoglycan.
4. Decolourise with alcohol to remove unbound crystal violet stain.
5. Counter stain with safranin.

#### Gram positive



#### Gram negative



The lipopolysaccharide layer of the Gram negative bacteria provides protection from attack by lysozyme and penicillin antibiotics. They are therefore more difficult to control.

### Growing microbes

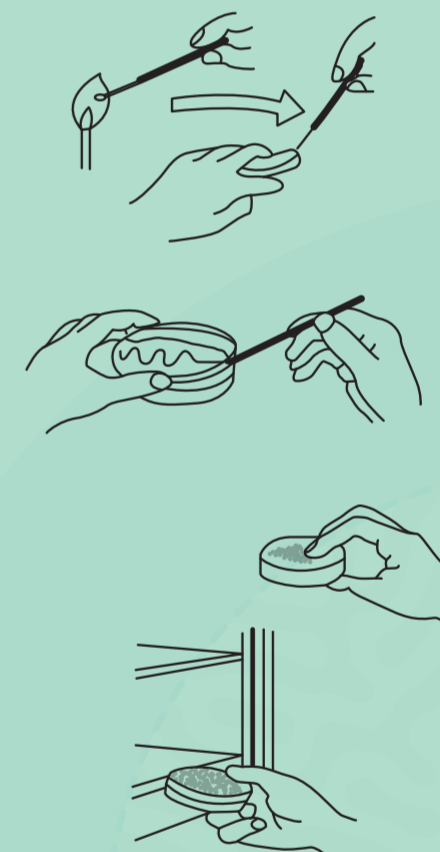
Microbes, such as bacteria, can be cultured in Petri dishes or in larger containers. Generally, they are grown on agar, a jelly like substance, to which the required resources can be added.

<b>Nutrients</b>	<b>Carbon source</b> - Bacteria require a source of carbon for growth, generally an organic compound such as glucose.
	<b>Nitrogen source</b> - Nitrogen needs to be provided for synthesis of nitrogenous compounds. It can be provided as organic or inorganic compounds.
<b>Other</b>	Growth factors, such as water, vitamins and mineral salts, can also be added.
<b>Temperature</b>	The <b>optimum temperature</b> of the bacteria you wish to grow should be taken into account. It is good practice to not grow bacteria at 37°C (body temperature) unless attempting to grow bacteria that live in the human body, e.g. human pathogens.
<b>pH</b>	The <b>optimum pH</b> of the bacteria should be considered when making media. Acid or alkali can be used to change the pH.
<b>Oxygen</b>	<b>Obligate aerobe</b> - Can only grow or metabolise in an oxygen rich environment.
	<b>Facultative anaerobe</b> - Thrive in an oxygen rich environment but are able to grow in an anaerobic environment also.
	<b>Obligate anaerobe</b> - Are unable to grow or metabolise where there is oxygen present.

### Aseptic technique

Aseptic technique must be used to:

- prevent contamination of the environment by the microbes being handled
  - prevent contamination of the cultures by microbes from the environment.
1. Petri dishes and nutrient agar should be sterilised before the agar is poured.
  2. An inoculating loop is used to transfer bacteria and is sterilised before and after use by heating it to red heat in a Bunsen flame.
  3. Only lift the Petri dish lid slightly as this prevents microorganisms from the air contaminating the culture and vice versa.
  4. After inoculation the lid of the Petri dish should be secured in place by strips of adhesive tape labelled and dated.
  5. Inoculated agar plates are incubated at 25°C in school laboratories for 24-48 hrs, which encourages growth of the culture without growing pathogens.
  6. Sterilise plates and equipment after use.



### Counting microbes

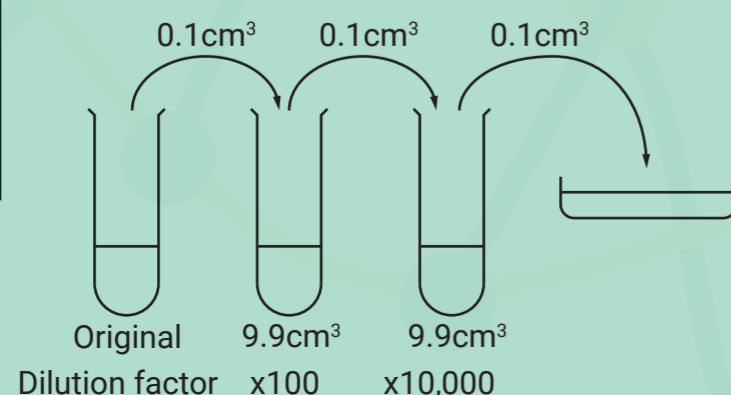
In order to count microbes reliably it may be necessary to dilute the original sample to give between 20-200 colonies which grew from the bacteria in the original sample.

**Total count** - Living and dead cells.

**Viable count** - Living cells only.

Count the colonies on the plate and multiply by the dilution factor to find the number of bacteria in 1cm<sup>3</sup> of the original sample.

#### Serial dilution



Too many colonies to reliably count. They may merge together.



Between 20-200 colonies can be reliably counted. Colonies are not merged so it can be presumed that each colony is derived from 1 bacterium in the original sample.



Too few colonies to give a reliable estimate.