

Bacterial growth, plant medicines and drug testing

Revision notes · Conditions for bacterial growth, antimicrobial and therapeutic plant substances, aseptic technique, and how drug testing developed.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

Bacterial growth, plant medicines and drug testing Spec 4.10–4.13

Conditions required for bacterial growth Spec 4.10

Bacteria are prokaryotes that reproduce asexually by **binary fission**. In ideal conditions some species can divide every 20 to 30 minutes. For growth to happen, several conditions must be met.

Conditions required for bacterial growth		
Condition	Why it is needed	In the laboratory
Nutrients	A carbon source (for example glucose) for respiration and making organic molecules; a nitrogen source for amino acids and nucleotides; mineral ions and sometimes vitamins	Nutrient agar or nutrient broth
Temperature	Enzymes have an optimum temperature. Too cold: slow growth. Too hot: enzymes denature	Incubate at a suitable temperature; in school laboratories usually 25 °C and never above 30 °C, to reduce the risk of growing human pathogens (which grow best near 37 °C)
Oxygen	Aerobic bacteria need oxygen for aerobic respiration. Anaerobic bacteria are inhibited or killed by oxygen	Plates are incubated with lids on, taped in only a few places, so that oxygen can enter
pH	Enzymes work best in a narrow pH range; most bacteria grow best near neutral pH	Buffers or media at a suitable pH
Water	Metabolic reactions take place in solution	Agar gel or broth is mostly water

Providing the conditions safely

- Culture media and equipment are sterilised before use (for example in an autoclave, at 121 °C), so that unwanted microorganisms do not compete with the bacteria being grown.
- Cultures are grown at temperatures that make human pathogens unlikely to grow, and plates are not opened after incubation.
- At the end, cultures are sterilised before disposal.

Exam tip: Do not just list "warmth". Say the bacteria need a suitable temperature because enzymes have an optimum, and name nutrients (carbon and nitrogen sources).

Antimicrobial and therapeutic substances from plants Spec 4.11

Plants cannot move away from pests and pathogens, so many make chemicals that kill or deter microorganisms, fungi and herbivores. Some of these chemicals have been used in traditional medicine,

and some have been developed into modern drugs.

Examples of therapeutic substances from plants		
Plant	Substance	Use
Garlic	Allicin	Antimicrobial properties (antibacterial and antifungal)
Willow bark	Salicin, from which aspirin was developed	Pain relief and reducing fever and inflammation
Foxglove (<i>Digitalis purpurea</i>)	Digitalis (digoxin)	Treating some heart conditions
Cinchona bark	Quinine	Treating malaria
Sweet wormwood (<i>Artemisia annua</i>)	Artemisinin	Treating malaria
Madagascan periwinkle	Vincristine	Treating some cancers

Using plant substances

- Extracts may contain many compounds, so the active compound must be identified and tested.
- The dose in a plant varies with species, age, growing conditions and part of the plant, so a purified, standardised dose is safer.
- Many plants have not been tested, so loss of plant species may lose possible medicines.
- Plant substances can be toxic, as with digitalis, and must be tested before use.

Exam tip: Name at least one plant, the substance and its use. "Antimicrobial" means kills or inhibits the growth of microorganisms such as bacteria.

Core practical 9: antimicrobial properties of plants and aseptic technique Spec 4.12

Core practical: Core practical 9: investigate the antimicrobial properties of plants, including aseptic techniques for the safe handling of bacteria.

Aseptic technique

Aseptic technique prevents unwanted microorganisms contaminating the culture and prevents the bacteria escaping into the environment or infecting the worker.

- Disinfect the bench before and after work, and wash hands.
- Work near a lit Bunsen burner, where rising hot air carries microorganisms away from the work area.
- Sterilise inoculating loops by heating in a flame until red hot, then let them cool before touching the culture.
- Flame the neck of bottles after opening and before closing, hold lids rather than put them down, and lift the lid of a Petri dish only as far as needed.
- Use sterile Petri dishes, agar and spreaders, and keep dishes closed when not in use.
- Seal the lid with only two or four small pieces of tape, never fully around, so oxygen can enter and anaerobic pathogens are not encouraged. Incubate upside down, so condensation does not drip on the agar.
- Do not open dishes after incubation. Sterilise all equipment and cultures in an autoclave afterwards.

Method

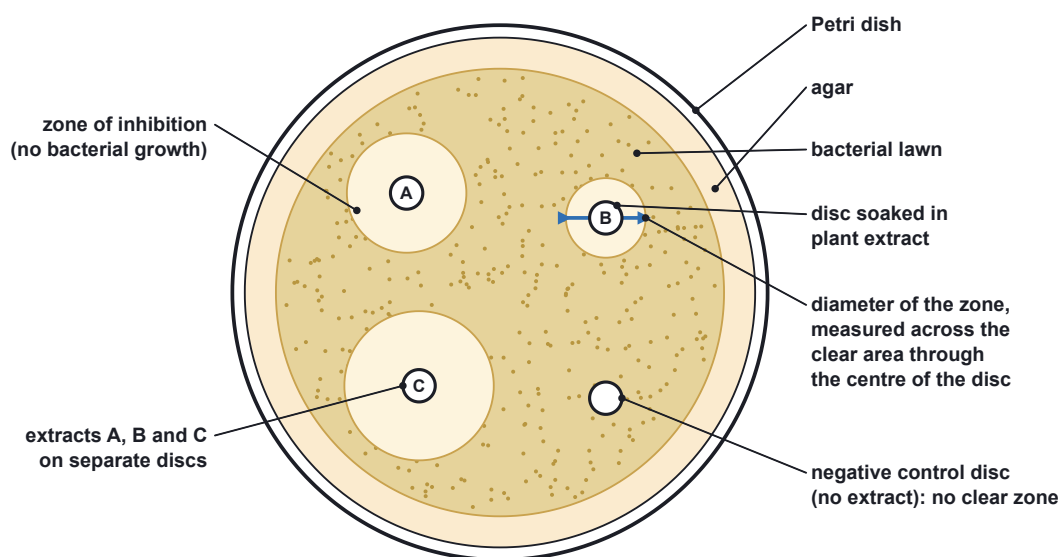
1. Prepare plant extracts, for example by crushing fresh leaves or cloves in a mortar with a measured volume of solvent and filtering. Keep the same mass of plant material and volume of solvent for each

plant, and make a dilution series if concentration is being tested.

2. Using aseptic technique, spread a standard volume of a non-pathogenic bacterial culture evenly over the surface of nutrient agar in a Petri dish to make a bacterial **lawn**.
3. Place sterile filter paper discs, soaked for the same time in each plant extract, onto the lawn with sterile forceps. Include a negative control disc soaked only in the solvent and, if available, a positive control disc with a known antibiotic.
4. Tape, invert and incubate the dishes at 25 °C for 24 to 48 hours.
5. Measure the diameter of the clear zone around each disc (zone of inhibition) in two directions at right angles and calculate a mean diameter. Calculate the area of the zone (area = πr^2).

Variables and controls

Type	Detail
Independent	Type of plant extract (or concentration of the extract)
Dependent	Diameter or area of the clear zone of inhibition
Control (kept constant)	Volume of bacteria spread; type of bacteria; agar depth and type; size of disc; volume and concentration of extract; incubation temperature and time
Control experiment	Disc soaked in solvent only (negative control) shows that the solvent does not prevent growth; a disc with antibiotic (positive control) shows that bacteria can be inhibited



Plan view of an agar plate. The **zone of inhibition** is the clear area where bacteria have not grown; measure its diameter through the centre of the disc.

Comparing zones of inhibition

A clear zone of diameter 14 mm formed around a disc soaked in garlic extract. Calculate the area of the zone of inhibition.

1. radius = $14 \div 2 = 7$ mm

2. area = $\pi r^2 = \pi \times 7^2$

Answer: 154 mm² (to 3 significant figures)

Maths skill: The area of the clear zone (πr^2) is often used because it describes the whole region where growth was inhibited; ranking extracts by diameter gives the same order. If asked for the area of inhibition only, subtract the area of the disc.

Common mistake: A zone of inhibition does not show that bacteria are killed, only that growth is stopped. A negative control is needed to show that the effect is due to the extract and not the solvent.

The development of drug testing Spec 4.13

Historic testing: William Withering and digitalis soup

In the 18th century, William Withering investigated a traditional herbal mixture ('digitalis soup') used to treat dropsy (swelling due to fluid build-up, often caused by heart failure). He found that foxglove was the active ingredient. He recorded the effects of different doses on his patients, and found the dose that worked without causing toxic effects. His work showed that plants contain active chemicals, but there was no control group, no placebo, patients and doctor knew the treatment and the results were open to bias.

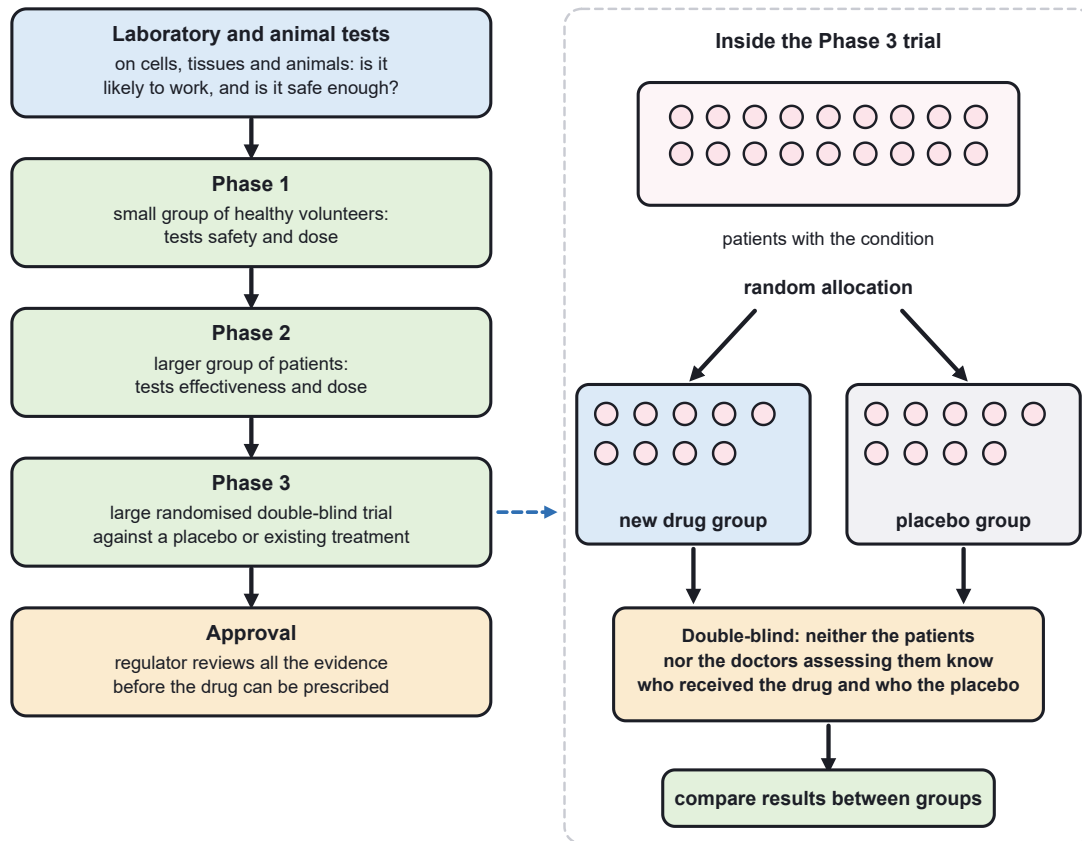
Modern drug testing

1. Laboratory tests: computer models, then tests on cells and tissues, and on animals, to check effects, toxicity and dose.
2. **Phase 1**: a small number of healthy volunteers receive the drug to test safety, side effects and how the body handles it.
3. **Phase 2**: a larger group of patients with the condition is given the drug to find out whether it works and what dose is best.
4. **Phase 3**: a very large group of patients, in a randomised, controlled trial, compares the new drug with a placebo or the best existing treatment to confirm effectiveness and side effects.

Placebo	An inactive substance that looks identical to the drug, given to the control group so the effect of the drug can be separated from the effect of believing one is being treated.
Double-blind trial	A trial in which neither the patients nor the doctors and researchers assessing them know who is receiving the drug and who is receiving the placebo, to avoid bias.

Why these features matter

- A control group and placebo allow for the placebo effect, where patients improve because they expect to.
- Random allocation makes the groups similar in age, health and other factors.
- Double-blind design stops patients and doctors influencing the results, consciously or not.
- Large samples reduce the effect of chance and allow rare side effects to be detected.
- Patients must give informed consent, and trials can be stopped if the drug causes harm or is clearly beneficial.



Stages of drug development, and how a randomised **double-blind** trial is organised.

Exam tip: If asked why a trial is double-blind, say who is not told (patients and the researchers assessing them) and what this prevents (bias). If asked why a placebo is used, mention the placebo effect.

Quick check

1. Name two nutrients that bacteria need to grow.
2. Why is a Bunsen burner lit during aseptic work?
3. What does a zone of inhibition show?
4. What is a placebo?
5. What is the purpose of Phase 1 of a clinical trial?

Quick check answers

Bacterial growth, plant medicines and drug testing

1. A carbon source (for example glucose) and a nitrogen source (for amino acids), plus mineral ions.
2. Rising hot air carries microorganisms away from the work area, reducing contamination.
3. That the substance inhibits bacterial growth.
4. An inactive substance that looks the same as the drug, given to the control group.
5. To test the safety, side effects and dose of a drug on a small number of healthy volunteers.