Culturing microorganisms

4.1.1.6 · Cell biology · revision notes

How bacteria multiply by binary fission, how to grow an uncontaminated culture using aseptic technique, and how to calculate bacterial numbers and the areas of colonies or clear zones. It includes the microbiology required practical on antiseptics or antibiotics. This subtopic is in GCSE Biology only, not in Combined Science.

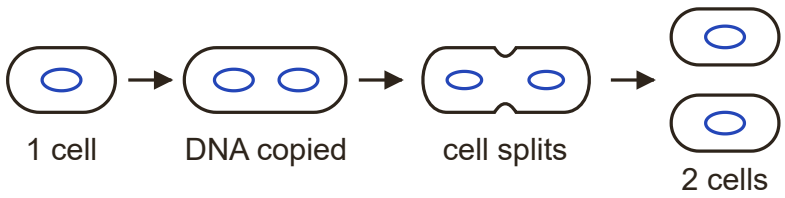
Key facts

- Binary fission: one bacterial cell splits into two; as often as every 20 minutes
- Number of divisions = time ÷ mean division time (same units)
- Sterilise Petri dishes and culture media before use; flame the inoculating loop
- Incubate at 25 °C in school laboratories
- Number of bacteria = starting number × 2^n (n = number of divisions)
- Area of a colony or clear zone = πr^2, where r = diameter ÷ 2
- Tape the lid on; store the dish upside down
- Larger clear zone = more effective antiseptic or antibiotic

Notes

How bacteria multiply

- Bacteria multiply by simple cell division called **binary fission**: one cell splits into two.



Binary fission: one cell becomes two, so the number doubles each time.

- With enough nutrients and a suitable temperature, some bacteria divide as often as once every 20 minutes.
- Each division doubles the number of bacteria: 1 → 2 → 4 → 8 → 16 and so on.
- Number of bacteria = starting number × 2^n, where n is the number of divisions (n = time ÷ mean division time).
- Give a large population in standard form, e.g. 19 200 bacteria = 1.92×10^4. Grade 7+ only

Growing a culture

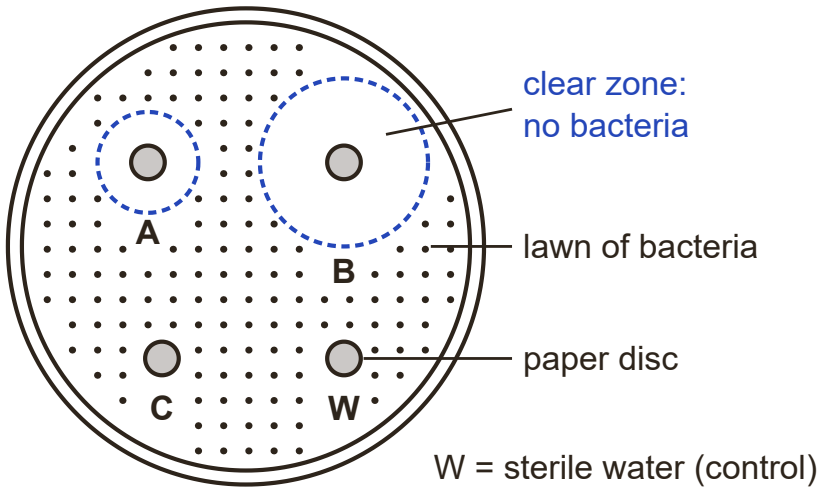
- Bacteria can be grown in a **nutrient broth** solution or as **colonies** on an **agar gel plate**. These contain the nutrients bacteria need, such as carbohydrate for energy and mineral ions.
- Uncontaminated cultures (containing only the microorganism being studied) are needed to investigate the effect of chemicals such as disinfectants and antibiotics.

Aseptic technique, and why

- Sterilise Petri dishes and culture media before use, to kill any microorganisms already present.
- Pour the melted sterile agar into the sterile Petri dish, replace the lid and let the agar set.
- Sterilise the **inoculating loop** by passing it through a Bunsen burner flame (then let it cool), to kill any microorganisms on it.
- Dip the loop in the culture and spread the bacteria over the agar, lifting the lid only slightly.
- Secure the lid with a few strips of adhesive tape, to stop microorganisms from the air getting in. Do not seal it all the way round: oxygen must still get in, so harmful anaerobic bacteria do not grow.
- Store the dish **upside down**, so drops of condensation do not fall onto the agar.
- In school laboratories, incubate cultures at **25 °C**. This is well below body temperature (37 °C), so harmful pathogens are less likely to grow.

Required practical: clear zones

- Aim: compare how well antiseptics or antibiotics stop bacteria growing by measuring the **zone of inhibition** (clear zone) around each one.
- Spread bacteria evenly over an agar plate. Add paper discs soaked in different antiseptics or antibiotics, and a control disc soaked in sterile water. Tape, invert and incubate at 25 °C for about 2 days.
- Change the antiseptic or antibiotic; measure the diameter of each clear zone. Keep the same: type of bacteria, concentration of chemical, disc size, incubation time and temperature.
- After incubation, a **clear zone** forms around a disc where the chemical has killed the bacteria or stopped them growing. The bigger the clear zone, the more effective the chemical against that bacterium.



The bigger the clear zone, the more effective the antibiotic. Here B is the most effective; C has no effect.

- Area of a clear zone or colony = πr^2. Measure the diameter and halve it to find the radius, r.

How to answer each type of question

Explain the steps of aseptic technique

1 to 2 marks each | Grade 5

1. Say what the step prevents or kills: usually 'kills microorganisms' or 'stops contamination'.
2. For 25 °C, compare with body temperature (37 °C) and mention pathogens.
3. For 2 marks, give the reason and its consequence.

Example. A student grows bacteria on an agar plate in a school laboratory.
(a) Why must the agar be sterilised before it is poured into the Petri dish?
(b) Why is the Petri dish stored upside down?
(c) Explain why the plate is incubated at 25 °C.

Model answer.

(a) To kill any microorganisms already in it, so the culture is not contaminated (1)

(b) So that condensation does not drip onto the agar / colonies (1)

(c) Harmful pathogens are less likely to grow (1), because 25 °C is well below body temperature (37 °C), at which pathogens grow best (1)

Analyse the results of the clear zone practical

1 to 3 marks | Grade 5

1. The most effective chemical has the largest clear zone.
2. The water disc is a control: it shows the clear zones are caused by the chemicals.
3. No clear zone means the chemical did not kill or stop the growth of that bacterium.

Example. A student tested three antibiotics, **A**, **B** and **C**, on one type of bacterium. The table shows the results.

Disc	Diameter of clear zone in mm
Antibiotic **A**	12
Antibiotic **B**	20
Antibiotic **C**	0
Sterile water	0

(a) Which antibiotic was the most effective? Give a reason.
(b) Why did the student include the disc soaked in sterile water?
(c) Suggest why there was no clear zone around antibiotic **C**.

Model answer.

(a) B, because it had the largest clear zone (1)

(b) As a control, to show that it is the antibiotic that stops the bacteria growing, not the disc or the liquid (1)

(c) The bacteria are resistant to antibiotic C / antibiotic C does not kill this type of bacterium (1)

Calculate the area of a clear zone

2 to 3 marks | Grade 5

1. Halve the diameter to find the radius.
2. Square the radius, then multiply by π.
3. Round as asked and give the unit (mm^2).

Example. The clear zone around an antibiotic disc has a diameter of 14 mm.
Calculate the area of the clear zone. Give your answer to 3 significant figures.

Model answer.

$r = 14 \div 2 = 7$ mm (1)

area $= \pi \times 7^2 = 153.9\ldots$ (1)

$= 154\ mm^2$ (1)

Calculate the number of bacteria after a given time

2 to 3 marks | Grade 7

1. Put the time and the division time in the same unit.
2. Number of divisions = time ÷ mean division time.
3. Multiply the starting number by $2^{\text{number of divisions}}$.
4. Give the answer in standard form if asked.

Example. A type of bacterium divides once every 25 minutes. A culture starts with 300 bacteria.
Calculate the number of bacteria after 2 hours 30 minutes. Give your answer in standard form.

Model answer.

2 hours 30 minutes = 150 minutes; $150 \div 25 = 6$ divisions (1)

$300 \times 2^6 = 300 \times 64 = 19\ 200$ (1)

1.92×10^4 (1)

Don’t lose marks

- Using the diameter instead of the radius in πr^2, which gives an answer 4 times too big.
- Working out $2 \times n$ instead of 2^n, or multiplying the time by the division time instead of dividing.
- Mixing hours and minutes in the number of divisions.
- Saying the loop is flamed 'to clean it'. Say it is flamed to kill microorganisms (sterilise it).
- Saying 25 °C is used 'so bacteria grow faster'. It is used so harmful pathogens are less likely to grow.

More tips

Memory tricks

- Powers of 2 to know: $2^5 = 32$, $2^{10} = 1024$ (about a thousand), 2^{20} is about a million.
- Aseptic checklist: sterilise the dish and agar, flame the loop, tape the lid, store upside down, incubate at 25 °C.
- Given a diameter? Halve it before you square it.
- Calculator: use the x^y (or ^) key for 2^n.

Exam technique

- Aseptic 'explain' questions need the reason, usually 'to kill microorganisms' or 'to prevent contamination'.
- In population calculations, show the number of divisions as a separate step: it usually earns a mark.
- When comparing antibiotics or antiseptics, name the one with the largest clear zone and quote the numbers.
- In standard form the first number must be between 1 and 10: 1.92×10^4, not 19.2×10^3.

What each grade needs

What you need to be able to do, from the first marks up to the top grade.

Grade 3 — **State how bacteria reproduce**
Bacteria multiply by simple cell division called binary fission.

Grade 4 — **Name two ways of growing bacteria**
In a nutrient broth solution, or as colonies on an agar gel plate.

Grade 5 — **Describe aseptic technique for preparing a culture**
Sterilise the dishes, media and inoculating loop, tape the lid, store the dish upside down and incubate at 25 °C.

Grade 5 — **Explain the reason for each aseptic step**
e.g. flaming the inoculating loop kills microorganisms on it, so the culture is not contaminated.

Grade 5 — **Calculate the area of a clear zone**
Use πr^2, halving the diameter to find the radius.

Grade 7 — **Calculate bacterial numbers after a given time**
Find the number of divisions, then multiply the starting number by 2 to the power of that number.

Grade 8 — **Give population sizes in standard form**
e.g. $300 \times 2^6 = 19\,200 = 1.92 \times 10^4$ bacteria.

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