

Culturing and measuring microorganisms

Revision notes · Aseptic technique, culture media, ways of measuring microbial growth, the bacterial growth curve and core practical 13 on growth in liquid culture.

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.

Culturing and measuring microorganisms Spec 6.1-6.4 A Level only

Culturing microorganisms using aseptic technique Spec 6.1

Microorganisms are grown (**cultured**) in a **culture medium** that supplies everything they need: a carbon source (such as glucose), a nitrogen source (such as amino acids or ammonium ions), mineral ions, water and sometimes growth factors such as vitamins. The medium is either a solid **nutrient agar** gel poured into Petri dishes, or a liquid **nutrient broth** in a flask or tube.

The aim of **aseptic technique** is to stop two things: unwanted microorganisms from the air, skin and bench entering the culture (contamination), and the cultured organisms escaping into the environment or infecting the worker.

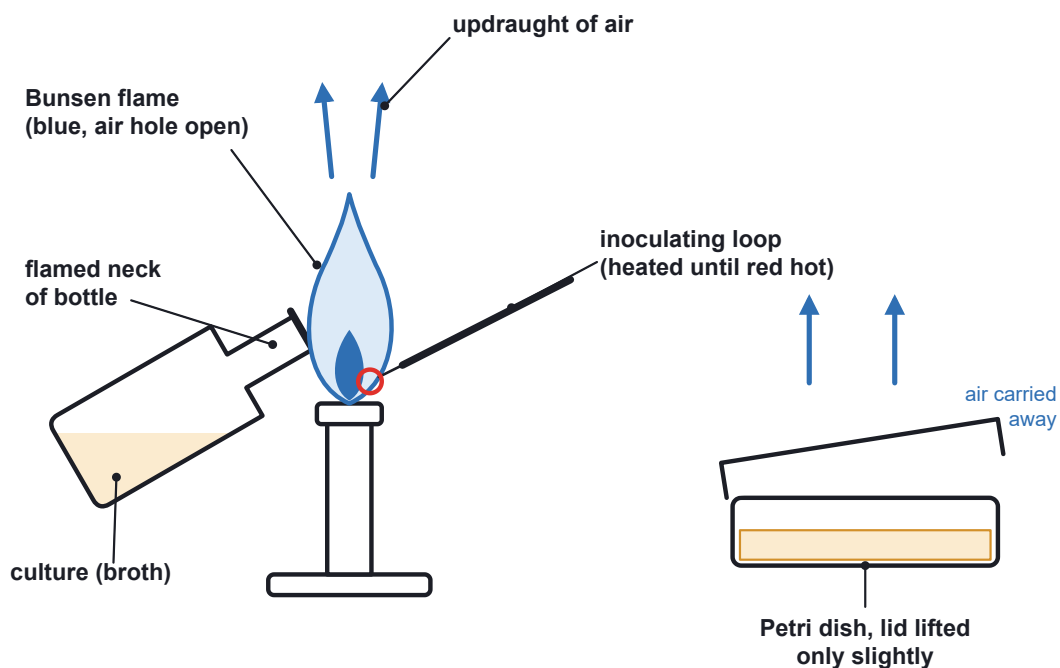
Principles and techniques

Aseptic technique: what is done and why	
Technique	Reason
Sterilise media, glassware and pipette tips in an autoclave (steam at 121 °C under pressure for at least 15 minutes) before use	Kills all microorganisms, including heat-resistant spores, so only the organism being studied grows
Wipe the bench with disinfectant and wash hands before and after	Removes microorganisms that could contaminate the culture or infect the worker
Pass the neck of a bottle or tube and the inoculating loop through a Bunsen flame; heat the loop until red hot and let it cool before use	Kills microorganisms on the surfaces; the flame creates an updraught of air that carries airborne microorganisms away from the opening; a cool loop does not kill the culture being transferred
Keep lids on, lifting the Petri dish lid only slightly and only for as long as needed	Reduces entry of airborne microorganisms
Seal the lid with two or four short strips of adhesive tape, not all the way round	Stops the lid being removed accidentally, but still lets oxygen in, so anaerobic pathogens are not selected for
Incubate plates upside down (inverted)	Condensation collects in the lid and cannot drip on to the agar and spread the colonies
Incubate at 25 °C in schools and colleges (not above 30 °C); commercial and clinical work often uses 37 °C	Slower growth of human pathogens, which grow best at body temperature, is safer
Autoclave or disinfect all used cultures and equipment before disposal	Prevents release of microorganisms

Inoculating a plate

1. Sterilise the inoculating loop in a flame until red hot, then let it cool for a few seconds near the flame.
2. Remove the bottle cap with the little finger of the hand holding the loop, flame the neck of the bottle, and take a loopful of culture.
3. Flame the neck again, replace the cap, then lift the Petri dish lid slightly and streak the loop gently across the agar surface (or spread a measured volume with a sterile spreader to give a lawn).
4. Replace the lid, tape it, label the base with a marker pen (name, date, organism) and incubate inverted.
5. Re-sterilise the loop in the flame.

Exam tip: Always give the reason with the technique. Marks go for 'flame the neck of the bottle to kill microorganisms and create an updraught', not just 'flame the bottle'.



Aseptic technique: flame the loop and the bottle neck, keep the dish lid lifted only slightly, and work in the updraught of the flame.

Measuring the growth of microorganisms Spec 6.2

Growth of a population of microorganisms is an increase in the number of cells (or in their total mass). Four methods are named in the specification.

Cell counts

A sample of the culture is placed on a haemocytometer (a slide with a grid etched on it that holds a known small volume) and the cells in a number of squares are counted under a microscope. The mean count per square is scaled up to give cells per cm^3 . A total cell count includes dead cells as well as living cells. A stain such as methylene blue, which living yeast cells decolorise but dead cells do not, can be used to count only living cells.

Dilution plating (viable count)

A serial dilution of the culture is made, for example by adding 1 cm^3 of culture to 9 cm^3 of sterile water (a 10^{-1} dilution) and repeating this step each time, giving 10^{-2} , 10^{-3} and so on. A measured volume (often 0.1 cm^3) of the more dilute tubes is spread on agar plates, incubated, and the colonies counted. Each colony

is assumed to have grown from one **colony-forming unit** (cfu), a single living cell or a small clump. Only plates with about 30 to 300 colonies are used, because fewer is unreliable and more cannot be counted accurately.

cfu per cm³ of original culture = (number of colonies ÷ volume plated in cm³) × dilution factor

Dilution plating

0.1 cm³ of a 10⁻⁴ dilution of a bacterial culture was spread on an agar plate. After incubation 56 colonies grew. Calculate the number of colony-forming units per cm³ of the original culture.

1. Colonies per cm³ of the diluted sample = 56 ÷ 0.1 = 560 cfu per cm³.
2. The dilution factor is 10⁴ (10⁻⁴ dilution).
3. Original culture = 560 × 10⁴ = 5.6 × 10⁶ cfu per cm³.

Answer: 5.6 × 10⁶ cfu per cm³

Maths skill: Dilution factor and sample volume are the usual traps. Divide by the volume you plated, then multiply by the dilution factor, and write the answer in standard form with units.

Mass

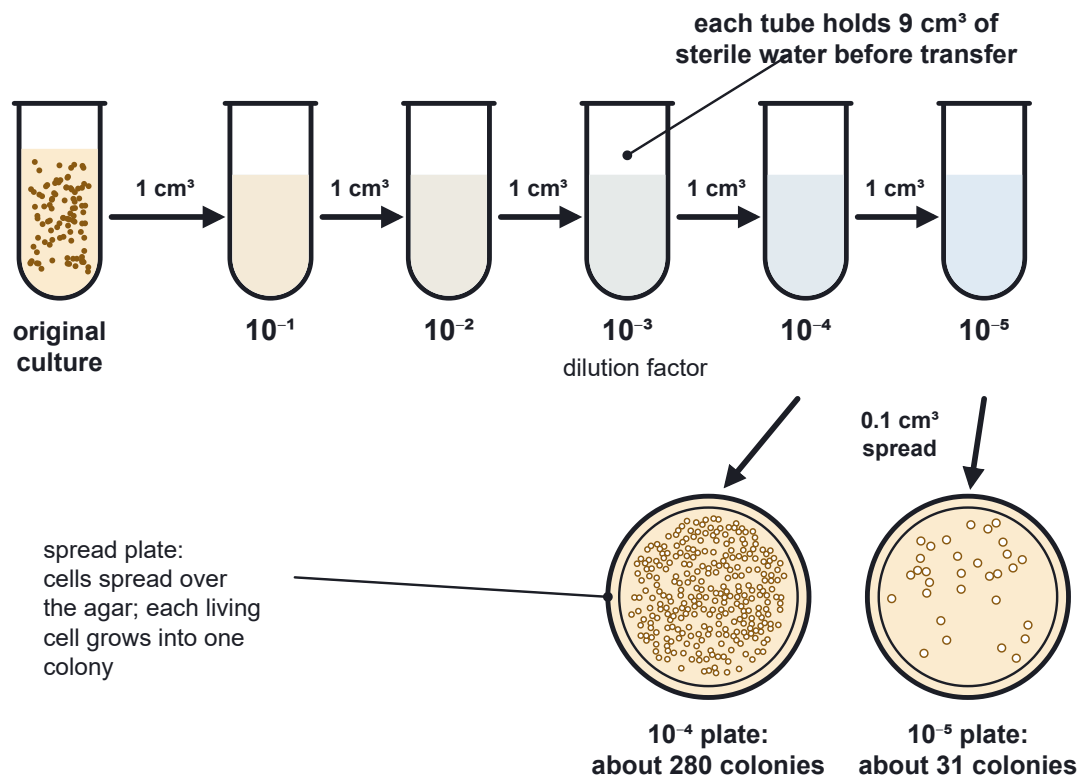
A known volume of culture is centrifuged, the supernatant discarded, and the cells dried in an oven at about 80 to 100 °C until the mass is constant. **Dry mass** is a better measure of growth than wet mass, because wet mass includes a variable amount of water. Mass is used when cells are too small or too many to count, or for filamentous fungi. It cannot tell living from dead cells, and the sample is destroyed.

Optical methods: turbidity

As cells multiply, a liquid culture becomes cloudier (more **turbid**). A **colorimeter** (or spectrophotometer) shines light through the culture and records the absorbance, or the percentage transmission. More cells scatter more light, so absorbance rises as the population grows. It is quick, does not destroy the sample and allows repeated readings from the same culture. A **calibration curve** (absorbance plotted against cell number found by another method) turns readings into cell numbers. Turbidity counts dead cells too, and is unreliable at very low or very high cell densities.

Comparing the methods

Method	Measures	Living and dead cells	Main advantage	Main limitation
Cell count (haemocytometer)	Number of cells per cm ³	Both, unless a viability stain is used	Direct count of cells	Slow; small sample; hard to count at high density
Dilution plating	Number of colony-forming units	Living only	Counts only viable cells	Slow (incubation); clumps counted as one
Dry mass	Mass of cells per cm ³	Both	Works for cells too small or many to count	Destroys sample; time-consuming
Turbidity	Absorbance of the culture	Both	Quick; repeated readings; sample kept	Needs calibration; unreliable at extremes

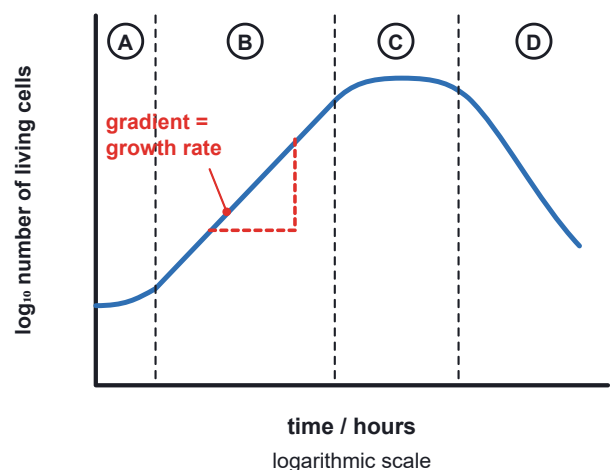
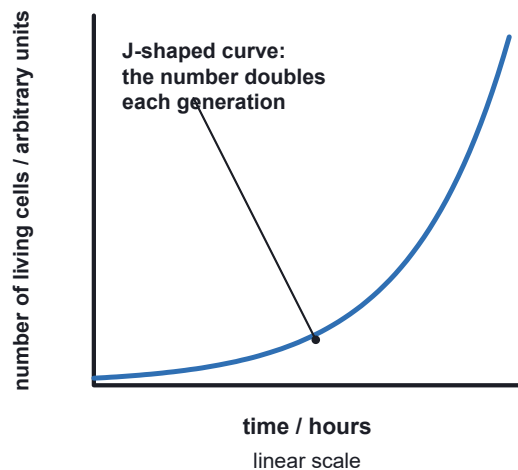


Serial dilution and spread plating: each transfer of 1 cm³ into 9 cm³ of sterile water gives a ten-fold dilution.

The bacterial growth curve and growth rate constants Spec 6.3

When bacteria are inoculated into a fixed volume of broth (a closed culture, with no nutrients added or waste removed) and the number of living cells is plotted against time, the curve has four phases. Cell number is usually plotted as log₁₀ so that the very large range fits on the graph and the exponential phase appears as a straight line.

Phases of the growth curve		
Phase	What happens to cell number	Why
Lag phase	Little or no increase	Cells are adjusting to the new medium: they take up nutrients, synthesise enzymes and ribosomes, and grow in size; few cells divide yet
Exponential (log) phase	Doubles every generation: fastest rate of increase	Nutrients and space are plentiful and there is little waste; cells divide by binary fission at a constant rate, so the number of cells doubles in each generation time
Stationary phase	Constant: births equal deaths	Nutrients become limiting, toxic waste (for example acids that lower the pH) accumulates and oxygen may run short, so division slows until the rate of division equals the rate of death
Death (decline) phase	Falls	Nutrients are exhausted and toxic waste is high, so more cells die than divide



- (A) Lag phase: cells adjust to the medium (make enzymes, grow); few divide yet.
- (B) Exponential phase: cells divide by binary fission, so the number doubles each generation.
- (C) Stationary phase: births = deaths (nutrients limiting, toxic waste building up).
- (D) Death phase: deaths > births (nutrients exhausted).

The **growth curve** of a closed culture: on the log graph the exponential phase is a straight line whose gradient is the growth rate.

Exponential growth and growth rate constants

In the exponential phase each cell divides once per **generation time** (the time for the population to double). After n generations a population starting with N_0 cells has $N_n = N_0 \times 2^n$ cells. Rearranging with logarithms gives the number of generations from the start and end numbers.

$$\text{number of generations, } n = (\log_{10} N_t - \log_{10} N_0) \div 0.301$$

$$\text{exponential growth rate constant, } k = n \div t \text{ (generations per hour) } \text{ generation time} = 1 \div k$$

The constant 0.301 is $\log_{10} 2$. The growth rate constant k is the number of generations per unit time during the exponential phase; it is the gradient of the straight line on a graph of $\log_2$ (cell number) against time, or the gradient of $\log_{10}$ cell number against time divided by 0.301.

Growth rate constant

A broth culture contained 2.0×10^3 cells per cm^3 at the start of the exponential phase and 1.28×10^5 cells per cm^3 6 hours later. Calculate the exponential growth rate constant.

- $\log_{10} (1.28 \times 10^5) = 5.107$ and $\log_{10} (2.0 \times 10^3) = 3.301$, so the difference is 1.806.
- Number of generations, $n = 1.806 \div 0.301 = 6.0$.
- $k = n \div t = 6.0 \div 6 \text{ h} = 1.0$ generation per hour (generation time 1 hour).

Answer: $k = 1.0$ generation per hour

Maths skill: Logarithms on a calculator and reading a log scale are IAL-only skills (A.0.5, A.2.5). If a question does not give the formula, show the doubling idea: $1.28 \times 10^5 \div 2.0 \times 10^3 = 64 = 2^6$, so 6 generations.

Exam tip: Do not say that cells 'stop dividing' in the stationary phase. The correct idea is that the rate of division equals the rate of death, so the number of living cells is constant.

Core practical 13: rate of growth in liquid culture Spec 6.4

Core practical: Core practical 13: Investigate the rate of growth of microorganisms in a liquid culture, taking into account the safe and ethical use of organisms.

A typical investigation uses a harmless organism, such as baker's yeast (*Saccharomyces cerevisiae*) or a non-pathogenic strain of *Escherichia coli*, in nutrient broth, and asks how the rate of growth is affected by an independent variable such as temperature, pH or glucose concentration.

Method

1. Prepare sterile broth and set up identical flasks or tubes, each with the same volume of broth and the same starting cell density. Use aseptic technique throughout.
2. Set the independent variable (for example water baths at 20, 25, 30, 35 and 40 °C for yeast, or broth at different pH).
3. Inoculate each flask with the same volume of culture, mix gently, and take a time-zero reading.
4. At regular intervals (for example every 30 or 60 minutes for several hours) swirl to resuspend and remove a sample with a sterile pipette. Measure absorbance in a colorimeter using sterile broth as the blank, or do a haemocytometer count or dilution plating.
5. Repeat each condition at least three times and calculate mean readings.
6. Plot cell number or absorbance (or $\log_{10}$ of it) against time and compare gradients of the exponential phase, or calculate the growth rate constant k for each condition.

Variables and controls	
Type	Examples
Independent variable	Temperature, pH, glucose concentration or antibiotic concentration
Dependent variable	Cell number or absorbance over time (rate of growth = change in cell number $\div$ time, or k)
Controlled variables	Volume and type of broth; starting cell density and volume of inoculum; strain of organism; aeration or shaking; light; time between readings; same colorimeter wavelength and cuvettes

Processing results

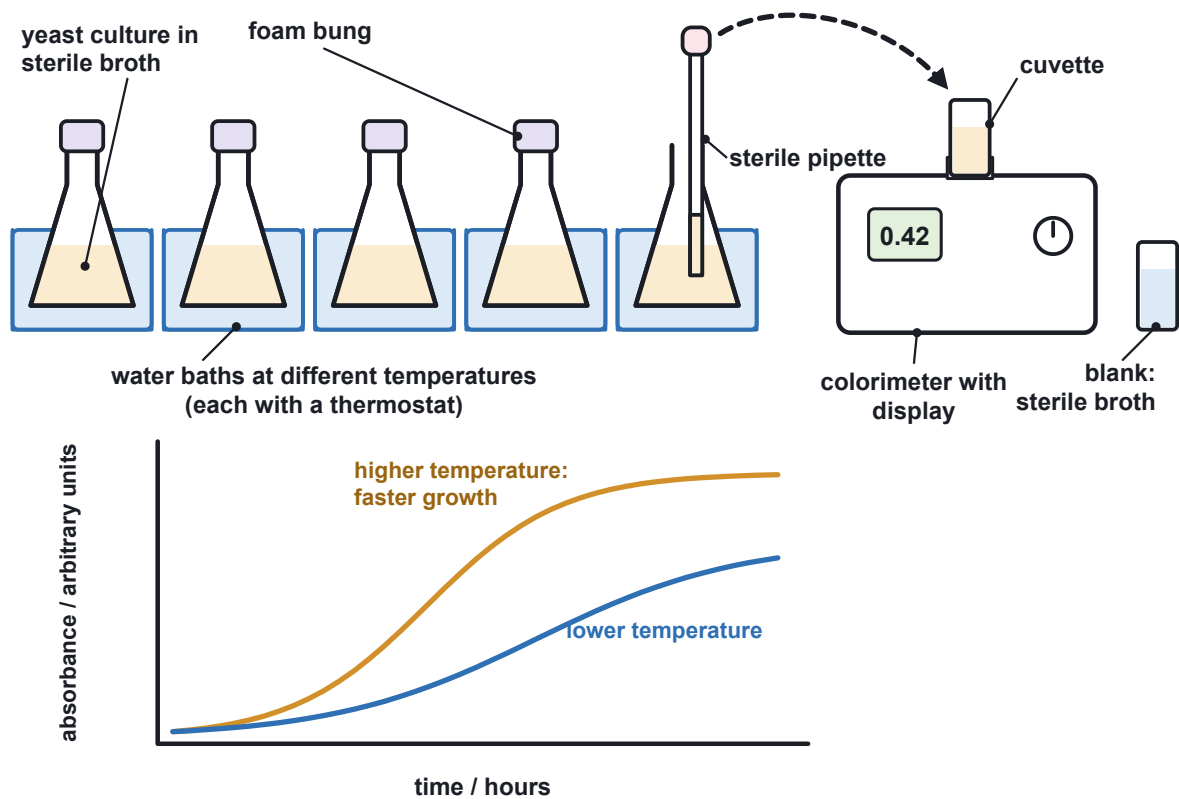
Rate of growth in the exponential phase is the gradient of a line on the graph. For a curved graph, draw a **tangent** to find the gradient at a given time. A growth rate constant can be found from the number of generations per hour. Compare the means for each condition and say whether differences are likely to be real, using error bars or range.

Safe and ethical use of organisms

- Use only non-pathogenic strains, such as laboratory strains of yeast or *E. coli* K12, and never culture samples from the environment or from people, which could contain pathogens.
- Carry out a risk assessment; wear a lab coat and gloves, tie back hair, cover cuts, wash hands, and disinfect the bench before and after.
- Use aseptic technique, incubate at 25 °C or lower for bacteria in school, and tape the lids on any plates.
- Autoclave or disinfect all cultures and equipment before disposal; do not tip live cultures down the sink.

- Ethical use: microorganisms are living organisms, so use only as many as are needed, plan to avoid repeating work unnecessarily, and kill (autoclave) and dispose of cultures responsibly once finished.

Exam tip: In a planning question give a named strain that is safe, a reason for each control, and what you will do with the cultures afterwards. These are easy marks that are often missed.



Core practical 13: growth of a culture at different temperatures measured as absorbance in a colorimeter zeroed with a sterile broth blank.

Quick check

- Why are Petri dishes incubated upside down?
- What does a viable count measure that a total cell count does not?
- Name the four phases of the bacterial growth curve in order.
- What is the formula for the number of generations from the start and end numbers of cells?
- Why is a colorimeter blank of sterile broth used in core practical 13?

Quick check answers

Culturing and measuring microorganisms

1. So condensation collects in the lid and does not drip on to the agar and spread colonies.
2. Only living cells that can divide to form colonies.
3. Lag, exponential (log), stationary, death (decline).
4. $n = (\log_{10} N_t - \log_{10} N_0) \div 0.301$.
5. To set zero absorbance for the broth alone, so readings show only the cloudiness caused by the cells.