

Units 3 and 6 are written papers about experiments: planning a fair test, handling the numbers, choosing a statistical test and evaluating the result. Questions are set around the eighteen core practicals, so for each one you know what is varied, what is measured, and what must be kept the same. 3 6

Planning a valid investigation

Name the independent variable with its range and at least five values, the dependent variable with how and in what units it is measured, and three controlled variables with HOW each is kept constant.

Control experiment – a run identical except that the factor under test is absent – boiled enzyme, no plant extract, an empty respirometer tube. It shows the effect is caused by that factor. It is not the same as a controlled variable

Biological material varies, so take at least three replicates, from the same source and of the same size, age or mass; use the mean, and a measure of spread.

Control temperature with a thermostatically controlled water bath, pH with a buffer, light with a lamp at a fixed distance in an otherwise dark room.

Risk: name the hazard and the precaution for THIS practical – a scalpel cuts away from the fingers on a tile; cultures are handled aseptically and autoclaved before disposal; ethanol is flammable, so no naked flames.

Data and statistics

Table: independent variable in the first column, quantity and unit in every heading, raw data then the mean, all to a consistent number of decimal places.

Rate = change ÷ time, with its unit. From a curve, the initial rate is the gradient of a tangent drawn at $t = 0$.

Standard deviation – the spread of the replicates about the mean; a larger standard deviation means less precise data. Error bars of ± 1 SD that overlap suggest a difference may not be significant

t-test t – is there a significant difference between two MEANS? Compare the calculated t with the critical value at $p = 0.05$ and the right degrees of freedom ($n_1 + n_2 - 2$)

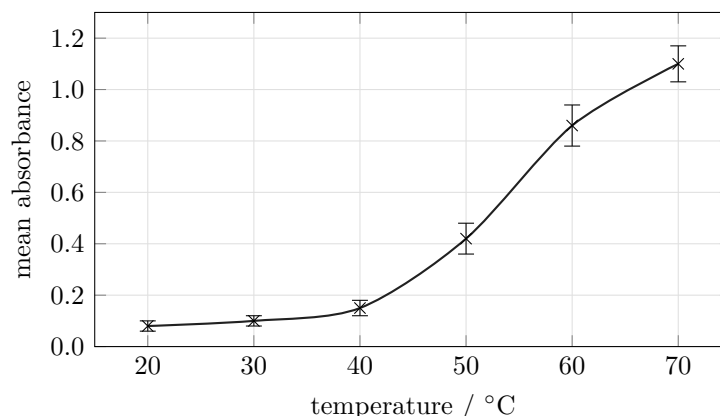
Spearman's rank correlation – is there a significant CORRELATION between two variables? Needs at least five pairs; compare r_s with the critical value for that number of pairs

Chi-squared test – do observed COUNTS differ significantly from those expected? Used with categories – genetic ratios, species found together. Degrees of freedom = number of categories – 1

Write the null hypothesis first ('there is no significant difference / correlation...'), then the decision: calculated value greater than critical value, so reject the null hypothesis; the probability that the result is due to chance is less than 5%.

Worked analysis: temperature and membrane permeability (CP3) :

Beetroot cylinders of the same diameter were cut to 1.0 cm, washed in running water to remove pigment from cut cells, and placed in 10 cm³ of water at each temperature for 30 minutes. The absorbance of the water was read in a colorimeter with a blue-green filter, after zeroing it with distilled water. Three replicates at each temperature.



Mean absorbance with error bars of ± 1 SD. More pigment in the water gives a higher absorbance.

Describe: little pigment leaked between 20 °C and 40 °C (absorbance 0.08 to 0.15), then it rose steeply to 1.10 at 70 °C.

Explain: the red pigment is inside the vacuole, behind the tonoplast and the cell surface membrane. Above about 40 °C the membrane proteins denature and the phospholipids move more, so gaps form in the membranes and the pigment leaks out.

Evaluate: tissue from one beetroot only; cylinders of slightly different surface area; the time each cylinder spends at temperature. Improvements: cut all cylinders with one cork borer and ruler; use five replicates; take the water's temperature at the start and end of the 30 minutes.

Worked calculation: mitotic index (CP6) :

In a stained root-tip squash, 200 cells in randomly chosen fields of view were counted; 34 were in a stage of mitosis (prophase to telophase).

Mitotic index = cells in mitosis $\div$ total cells counted = $34 \div 200 = 0.17$ (17%). The highest mitotic index is in the meristem, just behind the root cap.

Method points: hydrochloric acid breaks down the middle lamellae so the cells separate; a stain such as toluidine blue or acetic orcein colours the chromosomes; pressing the coverslip gently spreads the cells into one layer.

The eighteen core practicals – what each one tests 18

CP1 Food tests with colour standards – Benedict's test on a dilution series of glucose makes colour standards; the unknown is matched against them. It is semi-quantitative – matching by eye; a colorimeter makes it quantitative

CP2 Vitamin C C – the volume of juice needed to decolourise a fixed volume of DCPIP; less juice means more vitamin C. Calibrate against a vitamin C solution of known concentration

CP4 Enzyme initial rate – vary one factor (temperature, pH, enzyme or substrate concentration); measure product formed or substrate used early on, and use the gradient at the start

- CP5 and CP7 Microscopy** – calibrate the eyepiece graticule with a stage micrometer for each objective; tissue plans show tissue outlines only, cell drawings show a few cells in detail
- CP8 Tensile strength of plant fibres** – add masses until the fibre breaks; compare fibres of the same length, and divide by cross-sectional area for a fair comparison. Keep feet clear of falling masses
- CP9 and CP14 Antimicrobial properties** – aseptic technique; discs of plant extract or antibiotic on a bacterial lawn, with a disc of solvent alone as the control; compare the areas of the clear zones. Incubate at no more than 25 °C
- CP10 Photosynthesis** – pondweed in sodium hydrogencarbonate solution; collect the oxygen in a capillary tube (photosynthometer) at each light intensity, wavelength or temperature, with the plant acclimatised first
- CP11 Ecology** – random quadrats to compare two areas, a transect along a gradient; measure an abiotic factor at each quadrat and test for a correlation with Spearman's rank
- CP12 Temperature and development** – e.g. the hatching rate of brine shrimp eggs, or seedling growth, at several temperatures; same number of eggs or seeds, same volume and salinity
- CP13 Microbial growth** – absorbance (turbidity) of a culture at intervals; dead cells also scatter light, so turbidity overestimates the number alive. Plot log of cell number against time for the phases of growth
- CP15 Yeast respiration** – methylene blue (or another redox indicator) turns colourless as it is reduced; time how long it takes at each temperature or substrate. Shake gently and equally, as oxygen from the air re-oxidises it
- CP16 Respirometer and RQ** – with potassium hydroxide to absorb CO₂, the drop moves by the oxygen taken up; without it, by O₂ uptake minus CO₂ given out. $RQ = \text{CO}_2 \text{ produced} \div \text{O}_2 \text{ consumed}$. A control tube with glass beads corrects for temperature and pressure changes
- CP17 Spirometer traces** – tidal volume from the height of a normal breath, breathing rate from breaths per minute; minute ventilation = tidal volume $\times$ breathing rate. Oxygen uptake from the downward slope of the trace as the CO₂ is absorbed by soda lime
- CP18 Germinating grains** – halved grains placed cut face down on starch agar; after incubation, flood with iodine – a clear zone round germinating grains shows amylase made by the aleurone layer. Boiled grains are the control

What examiners keep reporting

Controlled variables named without the method of control. 'Temperature' scores nothing; 'temperature kept at 30 °C with a thermostatically controlled water bath' scores.

The wrong statistical test: a t-test for a correlation, or Spearman's for a difference between two means. Decide first whether the question asks 'is there a difference?' or 'is there a relationship?'

Describing a trend without data. Quote values from the graph, with units, at the points where the pattern changes.

Calling a correlation a cause. A significant correlation shows the variables change together; the experiment, with controlled variables, is what shows cause.