*Preparing Solutions of Different Dilution*

When you dilute a solution you are making it much weaker, or less concentrated. This is the same as when you make squash from concentrated juice.

During your biology practicals you may be asked to prepare solutions of different concentrations and investigate the effects of each on enzyme activity or plasma membranes, for example.

## How do I prepare a dilution?

*Molar dilutions*

If you are given 1M ('M' stands for **molarity**; it is a way of measuring concentration) stock solution of NaCl (sodium chloride) and need to make $20cm^3$ of a 0.2M NaCl solution, then you will have to carry out simple dilutions with distilled water. You do this by combining different proportions of solution with distilled water, as shown in the table below.

| Volume of NaCl solution/cm³ | Volume of distilled water/cm³ | Overall volume/cm³ | NaCl concentration/M |
|---|---|---|---|
| 20 | 0 | 20 | 1 |
| 16 | 4 | 20 | 0.8 |
| 12 | 8 | 20 | 0.6 |
| 8 | 12 | 20 | 0.4 |
| 4 | 16 | 20 | 0.2 |
| 0 | 20 | 20 | 0 |

You can use this method to make other required concentrations or different volumes. Bear in mind that you would need to adjust the above ratios if the starting concentration of your original solution is not 1M.

*Serial dilutions*

If you are given a stock solution, X, that has a concentration of 1,000g/per litre ($dm^3$), this could be too strong and toxic in cells.

You would therefore need to make a diluted solution of X. For example, you may need to make a solution with a concentration of $0.01g/dm^3$, which would be a suitable concentration for cell activity.

To do this accurately, you would need to make a series of dilutions that gradually reduce the concentration of the stock solution X to the required concentration of $0.01g/dm^3$. This process is known as **ten-fold dilution**, which, when carried out carefully and accurately, reduces the errors created when small volumes of liquid are measured.

**How is a serial dilution set up?**

The instructions below show how to prepare a solution of $0.01g/dm^3$ from a stock solution of $1,000g/dm^3$, using ten-fold dilution (these instructions can be adjusted accordingly for different concentrations).

*Preparing Solutions of Different Dilution*

**1** Add 9cm$^3$ of distilled water to each of five test tubes.

**2** Add 1cm$^3$ of the stock solution into the first test tube.

**3** Mix the solution thoroughly using a glass rod.

**4** Now add 1cm$^3$ of this solution to the second test tube containing 9cm$^3$ of distilled water and mix.

**5** Repeat a further three times (or until you have reached your desired concentration).
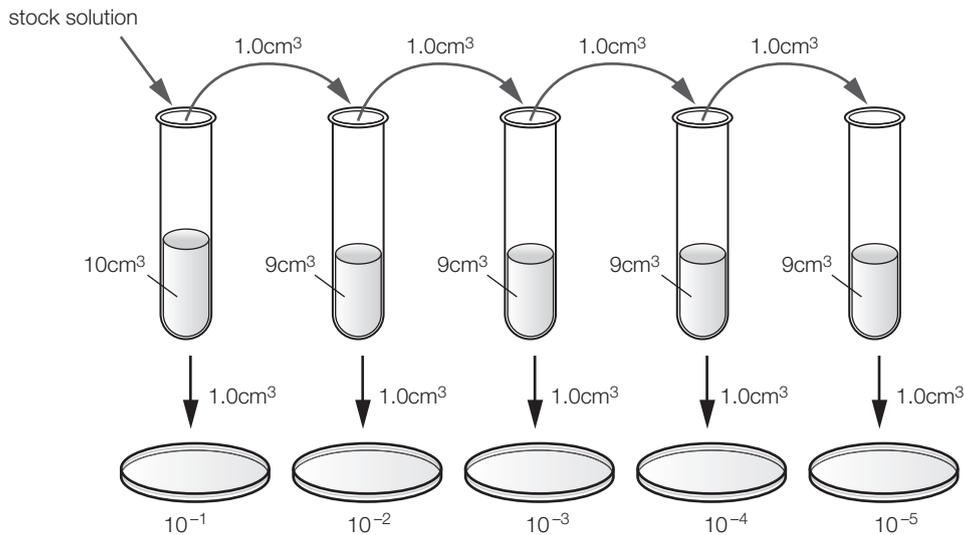


You can usually check your dilutions against the standards already made by your teacher. This allows you to compare the colour of your solutions with those already prepared.

The following table shows how many dilutions are needed to prepare a 0.01g/dm$^3$ solution from a 1,000g/dm$^3$ stock solution, along with the concentrations at each intermediate stage.

| Test tube number | Concentration g/dm³ |
|:---:|:---:|
| 1 | 100 |
| 2 | 10 |
| 3 | 1 |
| 4 | 0.1 |
| 5 | 0.01 |

## Questions

**1** If you were given a stock solution of 100g/dm$^3$, how many ten-fold dilutions would you need to carry out to prepare a 0.1g/dm$^3$ solution?

**2** Draw a table to show how you would dilute a 0.5M stock glucose solution to make a final concentration of 0.1M in 10cm$^3$.

**3** Explain the term *dilution.*

*Biochemical Tests for Molecules*

Biochemical tests are used to detect certain molecules that may be present in nutrients, for example carbohydrates, proteins and lipids.

You will need to know the names of different tests that can be used to do this, how they are carried out and what they show.

## Biochemical tests for carbohydrates

Carbohydrates include sugars and starch, and there are several simple tests that can be carried out to find out which molecule is present.

- **Reducing sugars**, such as glucose, fructose and maltose, are tested using **Benedict's solution**. The procedure is as follows:

    i.  Add equal volumes of the sample and Benedict's solution (blue) into a test tube.
    ii.  Heat the mixture to boiling point for a few minutes.
    iii. Look for colour changes; if sugar is present the **blue** solution will turn **green**, **yellow** or **brick red**, depending on the concentration of sugar. This is due to copper sulphate in the Benedict's solution being reduced to copper oxide, which forms a red precipitate.



blue    green    yellow    orange    brick red

none    low    high
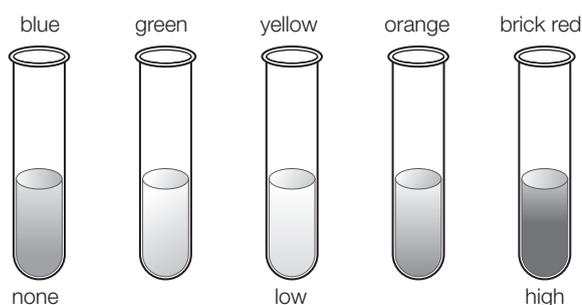
- **Non-reducing sugar**, sucrose, has a few extra steps in the procedure:

    i.  Add an equal volume of **dilute hydrochloric acid** to the sample and boil.
    ii.  Cool, then neutralise with **sodium hydrogen carbonate**.
    iii. Add Benedict's solution and boil.
    iv. Look for a colour change from **blue** to **brick red**, which indicates a positive result.

- **Starch** is tested using **iodine** in a solution of **potassium iodide**.

    i.  Add a few drops of iodine solution to the sample.
    ii.  Colour change should be from **yellow-brown** to **blue-black**; this indicates a positive result.

## Biochemical test for proteins

The simplest and most reliable test for proteins is the **Biuret test.**

    i.  Add a few drops of **Biuret solution** to the sample.
    ii.  Warm gently.
    iii. Look for colour changes from **blue** to **lilac/mauve/purple**; this indicates a positive result.

## Biochemical test for lipids

Lipids are soluble (dissolve) in organic solvents such as ethanol, but are insoluble in water. The test for lipids is called the **emulsion test**.

 i. Place the sample in a clean, dry test tube and add **ethanol**.
 ii. Shake the test tube so that any lipid present dissolves.
 iii. Pour the mixture into a test tube containing (de-ionised) water.
 iv. Look for a white cloudy suspension (like milk); this **white emulsion** indicates a positive test for lipids.

## Task and Questions

### Task

Complete the table below by placing a tick (✔) if the statement is correct for any of the biological molecules.

| Statement | Carbohydrates | Proteins | Lipids |
|---|---|---|---|
| Positive result is a white cloudy suspension | | | |
| Produces a colour change from yellow-brown to blue-black with iodine | | | |
| Benedict's solution is used to test for a molecule in this group | | | |
| There is a colour change in Biuret solution from blue to lilac | | | |
| Only soluble in organic solvents such as ethanol | | | |
| Gentle warming required (no boiling) | | | |

### Questions

 **1** Describe the test for starch.

 **2** What is the test for proteins called?

 **3** What are lipids soluble in?

 **4** Explain how you would carry out a test for glucose.

 **5** Name the non-reducing sugar.

 **6** On which molecules would you carry out an emulsion test?

 **7** When testing for sucrose, what is the purpose of sodium hydrogen carbonate?

 **8** When testing for reducing sugars, what do the different colours indicate?

## *Correlation and Causal Relationships*

**Correlation** and **causal relationships** describe links that may exist between two variables. Data you obtain can be plotted as a graph in order to try to identify these links.
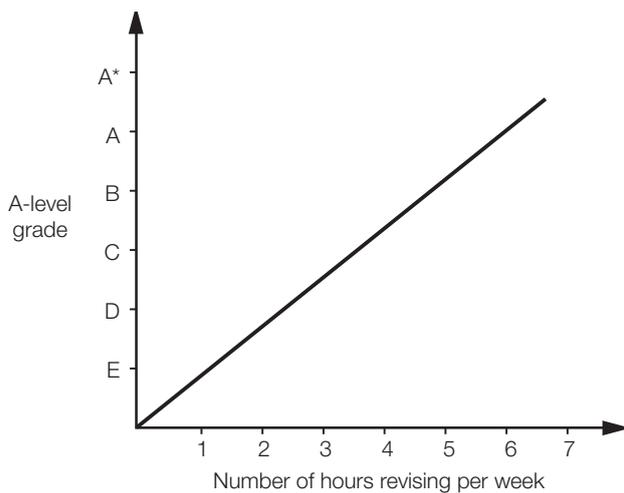
## Correlation

This gives us information about the relationship or the pattern between two variables. It can be the **association** between two variables. If one variable changes, this will result in a change in the other variable; they appear not to be independent of one another.

Data can show:

- positive correlation;

- negative correlation; or

- no correlation at all.

**Positive correlation** is seen when one set of values increases and alongside this another set tends to increase. For example, the correlation between the number of hours spent revising each week and the link with the A-level grade obtained.



**Negative correlation** is seen when one set of values increases and alongside this another set tends to decrease. For example, if you increase the number of hours you exercise, this may be linked with a lower risk of heart attack.
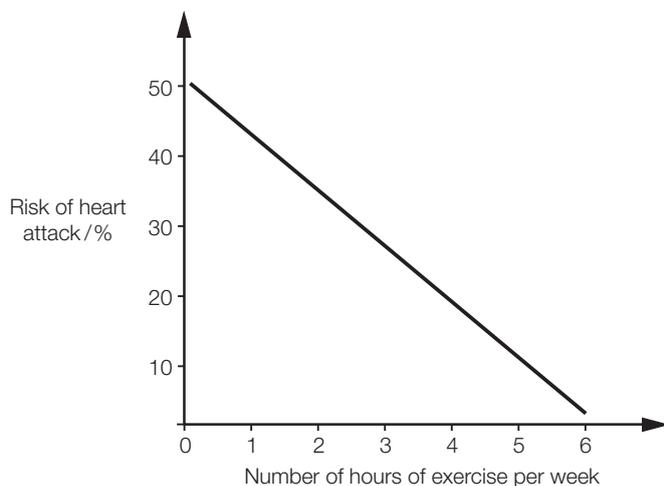
**No correlation** is when the value of one set of data does not appear to affect the other. The results may fluctuate for example for the relationship between the mass of eggs laid and chicks hatched.



Mass of chicks/g

Mass of eggs/g

## Causal relationships

A causal relationship between two variables is possible if the occurrence of the first variable *causes* the other. The first event is sometimes called the **cause** and the second event is called the **effect**.

A correlation between two things does not imply causation. But if there is a causal relationship between two variables, then they *must* be correlated.

### Examples

- A study shows that there is a positive correlation between drinking alcohol and breast cancer. But we cannot conclude that drinking alcohol is the cause of breast cancer. It may be that women who are stressed drink more alcohol and that it could be the stress rather than alcohol that causes breast cancer.

- A study shows that there is a negative correlation between a student's anxiety before a test and their result in the test. However, you cannot conclude that the anxiety causes a lower grade as there may be other reasons for the poor performance, such as students simply not revising enough. So, the correlation here does not imply the causation.

## *Questions*

1  Define the term 'correlation'.

2  Plot a graph of the below data.

| Number of cigarettes smoked per day | Incidence of lung cancer per 100,000 of population |
|---|---|
| 0 | 5 |
| 5 | 12 |
| 10 | 70 |
| 15 | 90 |
| 20 | 150 |
| 25 | 160 |
| 30 | 280 |

**3**    What are the two correlated factors shown by the graph you have plotted in Q2?

**4**    If two factors are correlated, one must cause the other (they are causal) – true or false?

**5**    Would it be correct to say that eating lower amounts of fibre causes bowel cancer?

**6**    What type of correlation is seen when data fluctuates (goes up and down)?

**Practical Skills for the A-level Biologist**

Data interpretation and evaluation are essential skills needed for practical components of your A-level Biology course. You should be able to explain biological information and ideas in a clear and appropriate manner. This can be done using:

- tables;

- line graphs;

- histograms;

- annotated diagrams; and

- concise continuous prose (for example long answers or essays).

You should be able to recognise, and explain, trends and patterns in the data you are presented with. The data should be used, along with supporting points from relevant biological knowledge and understanding, to draw conclusions.

## Data collection

During any investigation, you will be making measurements and observations, which you can record as **raw data**.

You will then need to use this data to produce a **summary table** that shows how you have organised and manipulated the data.

## Data manipulation

Once you have collected results, you may want to find out values such as the **rate of reaction**, **percentage increases** or **percentage decreases** during the investigation. Manipulated data helps you to see if there are any trends or patterns in your results/data.

For example, for an enzyme-catalysed reaction it is important to work out the rate of reaction by using the equation 1/t, where t is time.

| Temperature/°C | Time taken for breakdown of starch by amylase/min | Rate of reaction $1/t$ $min^{-1}$ |
|:---:|:---:|:---:|
| 10 | 28.0 | 0.04 |
| 20 | 20.0 | 0.05 |
| 30 | 12.0 | 0.08 |
| 40 | 6.0 | 0.17 |
| 50 | 10.0 | 0.10 |
| 60 | 18.0 | 0.06 |

Your data manipulation will involve some form of simple maths, such as calculating the mean or a percentage increase (*see Basic Skills for the A-level Biologist – 7: Calculation Skills on page 17*).

## *Data Interpretation and Evaluation*

Refer to the exam board specification you are following to check which data manipulation skills you need to be able to demonstrate.
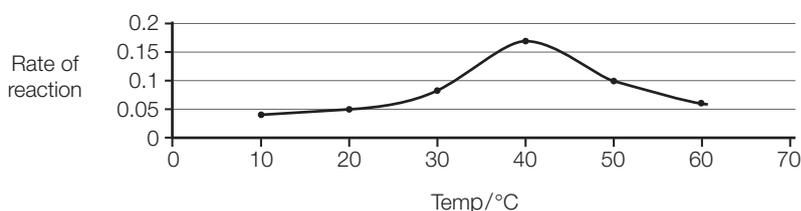


Once you have plotted your graph, look at it very carefully: notice the overall shape of the curve. Ask yourself the following questions to help you analyse your graph correctly:

- Does your graph show an increase or a decrease?

- Is the change very steady or rapid (exponential)?

- Is there a peak in the curve?

- At what precise point does it peak?

- Does the curve level off/plateau after a certain point?

You must be able to describe any trends or patterns shown in the curve concisely. You do not need to mention or explain every data point or measurement, but you do need to highlight significant data points. Explain where there is a large change in the data, or where there is a maximum reading for the independent variable (IV), that is, the biggest change. You could state the **minimum** and **maximum values** within a set of data.

If you have plotted the mean values on a graph, it is possible to show the variability of the data on the graph using **error bars**. These are vertical lines that go above and below the data plotted on the graph. Error bars can be calculated using **standard deviation (SD)** (*see Practical Skills for the A-level Biologist – 14: Statistical Tests on page 39*).

Remember to explain any **anomalous measurements or observations**, and decide on how you are going to deal with them.

- If you have a large set of data with a few outliers that are obviously different from the other values, you could leave them out from your calculations. If they are included, they may have an effect on your mean, leading to an incorrect conclusion.

- If you have one anomalous data point from three repeat values, it may be difficult to ignore and should be included in your calculations.

It can be difficult to decide on how to handle any anomalies; it will all depend on your overall results. You must mention these anomalies when you provide the overall conclusion for your experiment.

You may be required to analyse data presented in a table. Remember, it is very important to look carefully at the data and see where the data increases, if and where it peaks, and if there are any decreases.

*Example*

The table below shows the blood pressure changes in the left ventricle of the heart during one cardiac cycle.

| Time/s (IV) | Blood pressure in left ventricle/kPa (DV) |
|---|---|
| 0.0 | 0.4 |
| 0.1 | 2.1 |
| 0.2 | 11.5 |
| 0.3 | 14.5 |
| 0.4 | 4.0 |
| 0.5 | 1.5 |
| 0.6 | 0.4 |
| 0.7 | 0.4 |

Here, you would look at the time and compare what is happening at that moment to the blood pressure. The trend is an increase in pressure up to 0.3s (seconds) followed by a decrease. The highest blood pressure of 14.5kPa is at 0.3s. The blood pressure drops to 0.4kPa at the end of the cardiac cycle.

## Evaluation skills

Once you have completed your investigation, plotted your results and carried out data interpretation, you are then ready to evaluate your investigation. In your evaluation you should look at the following key features:

- **Reliability of your results** – data is reliable if, after repeating the same investigation, the results are very close together or very similar. Carrying out repeats increases the reliability of data.

- **Precision of your results** – this refers to the accuracy (closeness to the true value) of the data, which could be improved by using equipment that gives you more detailed measurements. For example, to monitor pH changes during an experiment, use a pH meter rather than indicator paper; or to measure $0.5cm^3$ of required solution, use a $1cm^3$ syringe rather than a $5cm^3$ syringe.

- **Limitations of the method** – this involves you looking at your method very carefully and deciding if you could have improved the design in any way to obtain a more accurate set of results. For example, in an investigation designed to monitor the effects of temperature on the rate of an enzyme-controlled reaction, use a wide range of temperatures rather than a narrow range, so that you can determine the optimum temperature with confidence.

## *Tasks and Questions*

For each of the graphs below, give a description of the curve and an interpretation of whether a change in one variable has an effect on the other variable.

### *Task 1*

| Graph | Description and interpretation |
|---|---|
|  | |
|  | |
|  | |
|  | |
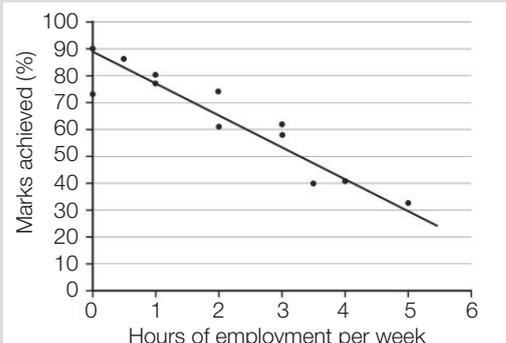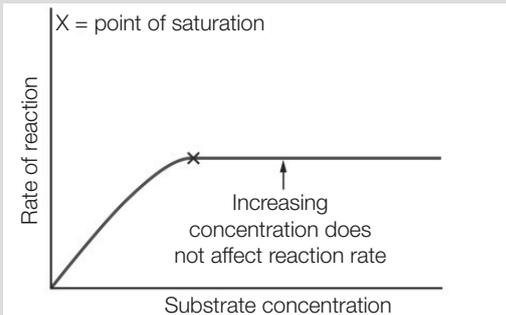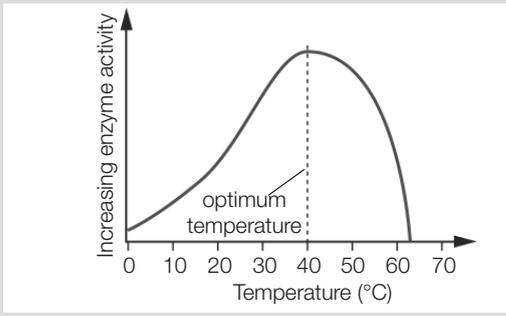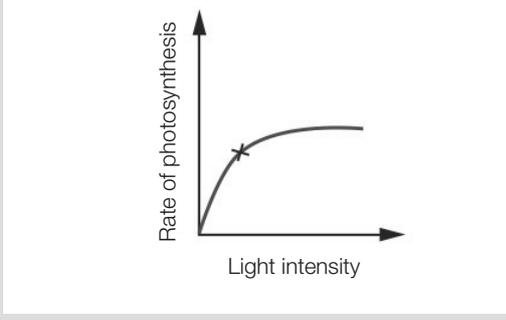
## Task 2

Using the data in the table below, describe and explain the results of an investigation on the effect of different enzymes on apple juice production at 20°C.

| Time/minutes | Vol. of juice with PECTINASE/cm³ | Vol. of juice with CELLULASE/cm³ |
|---|---|---|
| 5 | 5.6 | 0.8 |
| 10 | 8.5 | 1.5 |
| 15 | 12.0 | 2.8 |
| 20 | 14.5 | 3.5 |
| 25 | 17.9 | 4.2 |
| 30 | 18.2 | 4.3 |

## Questions

1   What are the key features that must be included as part of any evaluation?

2   How can the reliability of data be increased?

3   What is the term used to describe data collected during a practical, before it has been manipulated?

Even if biology investigations are repeated a number of times, you are unlikely to get identical results due to **natural variation** and **random errors**.

A histogram can be plotted if you have enough measurements and it may show **normal distribution**, where most of the repeats are more likely to be near the central value – the mean. A typical histogram is shown below.
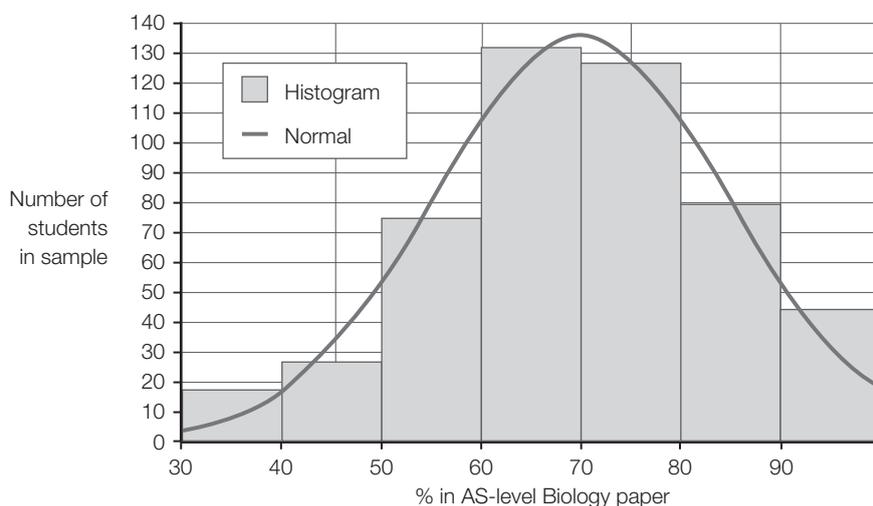


# Descriptive statistics

It can be very useful to summarise data you have collected using descriptive statistics. These include the **mean**, **median**, **mode** and **range**, which all help to highlight trends and patterns in your results.

- **Mean** – this is the average of all the data you have collected and is worked out by adding up all the data and dividing it by total number of repeats or pieces of data (*see Basic Skills for the A-level Biologist – 7: Calculation Skills on page 17*).

- **Median** – this is the middle value when the results are placed in rank order. When you arrange the data in increasing rank order, you will be able to identify the middle value. If you have an even number of results, the median is the mid-point of the two middle values.

- **Mode** – this is the most common data value and can be determined by identifying the category with the highest number of data, often by using a tally chart (that is, the value that appears most often).

- **Range** – this is the difference between the smallest and the largest data value.

# Standard deviation (SD)

The spread of data either side of the mean gives you an indication of how reliable your results are.

- If your results or your data are very close to the mean, then the results obtained can be considered reliable.

- If your data points are scattered widely, then your calculated mean may not be very reliable.
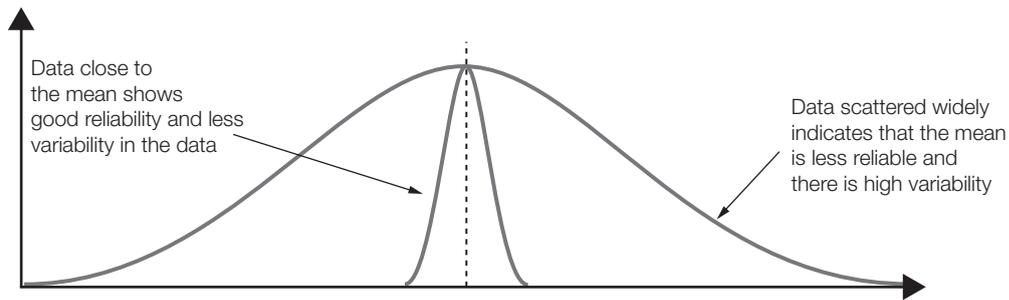
Standard deviation (SD) gives information about how wide the **normal distribution** is for the investigation; a larger SD indicates less reliable data.

When you are comparing different sets of data, a better measure is a **95% confidence limit**; this gives you the confidence to say that 95% of the measurements are within the range of **mean ± 2 SE**. It shows the range of data above and below the mean.

When you calculate the mean, you should also calculate the confidence limit, which will give you a good indication of the quality of your data.

You may be required to calculate the SD for your AS Biology – check your exam board specification.

Calculate the SD using the following equation:

$$\sigma = \sqrt{\frac{\Sigma\,(x - \bar{x})^2}{\eta}}$$

$\sigma$ = standard deviation

$\Sigma$ = sum of

$x$ = each value in the data set

$\bar{x}$ = mean of all values in the data set

$\eta$ = number of values in the data set

(Note: you may be taught an alternative equation.)

## Example

A student carried out an investigation into the effect of an inhibitor on the growth of pea plants by measuring the root length after five days.

| Results without inhibitor | | | |
|---|---|---|---|
| **Pea plant** | **Length of root/cm** | **Difference $x - \bar{x}$** | **$(x - \bar{x})^2$** |
| 1 | 6.0 | −2.7 (6.0 − 8.7) | 7.29 |
| 2 | 8.9 | 0.2 | 0.04 |

(*Continued*)

## Statistical Tests

| Pea plant | Length of root/cm | Difference x – x̄ | (x – x̄)² |
|---|---|---|---|
| 3 | 11.9 | 3.2 | 10.2 |
| 4 | 5.8 | −2.9 | 8.41 |
| 5 | 10.2 | 1.5 | 2.25 |
| 6 | 9.4 | 0.7 | 0.49 |
| Σx | 52.2 | | |
| Mean x̄ | 8.7  (52.2/6) | | |
| Σ(x–x̄)² | | | 28.68 |
| Σ(x–x̄)²/η | | | 4.78 (28.68/6) |
| √Σ(x–x̄)²/η | | | 2.19 |

| Results with inhibitor | | | |
|---|---|---|---|
| **Pea plant** | **Length of root/cm** | **Difference x – x̄** | **(x – x̄)²** |
| 1 | 3.2 | −120 | 1.44 |
| 2 | 4.1 | −0.30 | 0.09 |
| 3 | 3.5 | −0.09 | 0.81 |
| 4 | 5.6 | 1.20 | 1.44 |
| 5 | 6.1 | 1.70 | 2.89 |
| 6 | 3.9 | −0.50 | 0.25 |
| Σx | 26.4 | | |
| Mean x̄ | 4.4 | | |
| Σ(x–x̄)² | | | 6.92 |
| Σ(x–x̄)²/η | | | 1.15 |
| √Σ(x–x̄)²/η | | | 1.07 |

Once you have calculated the SD, you will need to use this value to work out the **standard error (SE)**, using the equation below:

$$SE = \frac{SD}{\sqrt{n}}$$

(n = number of samples)

| | Without inhibitor | With inhibitor |
|---|---|---|
| Mean | 8.7 | 4.4 |
| Standard deviation (SD) | 2.19 | 1.07 |
| Standard error (SE) | 0.89 | 0.44 |

You can now plot a graph of the mean results for the two conditions and add the error bars, SE ± 2.
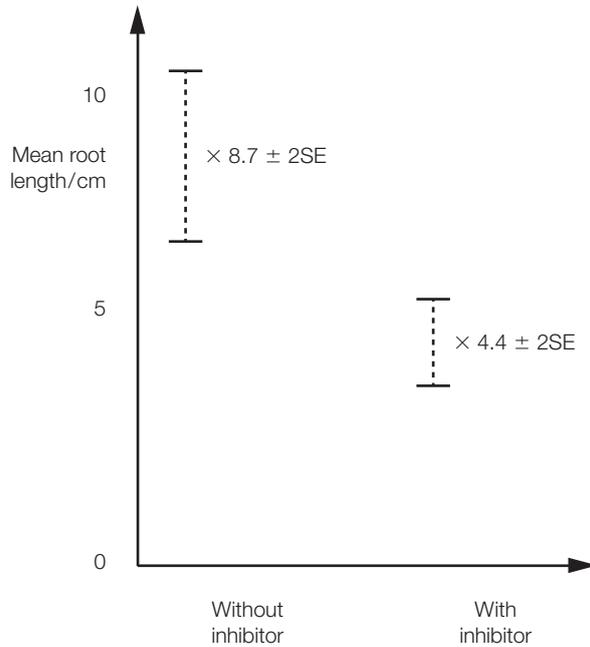


The greater the standard error (SE), the greater the variation of the mean.

## Tasks and questions

### Task 1

Complete the equation for standard error.

$$SE = \frac{?}{\sqrt{?}}$$

## Statistical Tests

### Task 2

Draw a line to connect the following terms with their correct definition.

| Term | Definition |
|---|---|
| **Mean** | The difference between the smallest and the largest data value |
| **Mode** | The average of all the data you have collected. This is worked out by adding up all the data and dividing it by the total number of repeats or pieces of data |
| **Range** | The middle value when the results are placed in rank order. If you have an even number of results, it is the mid-point of the two middle values |
| **Median** | The most common data value. Usually identified using a tally chart (it is the value that appears most often) |

### Questions

**1**   Why is repeated biological data unlikely to be identical?

**2**   What is the function of descriptive statistics?

**3**   What information does standard deviation provide?

**4**   Calculate the standard deviation for the data presented in the table below.

| Cress seedling | Stem length/mm | Difference $x - \bar{x}$ | $(x - \bar{x})^2$ |
|---|---|---|---|
| 1 | 10.0 | | |
| 2 | 9.0 | | |
| 3 | 11.0 | | |
| 4 | 15.0 | | |
| 5 | 8.0 | | |
| 6 | 12.0 | | |
| $\Sigma x$ | | | |
| Mean $\bar{x}$ | | | |
| $\Sigma(x-\bar{x})^2$ | | | |
| $\Sigma(x-\bar{x})^2/\eta$ | | | |
| $\sqrt{\Sigma(x-\bar{x})^2/\eta}$ | | | |