Stage 2 Biology

Assessment Type 1: Investigations Folio

Note: This is a report for a Completion Practical Investigation

Practical report

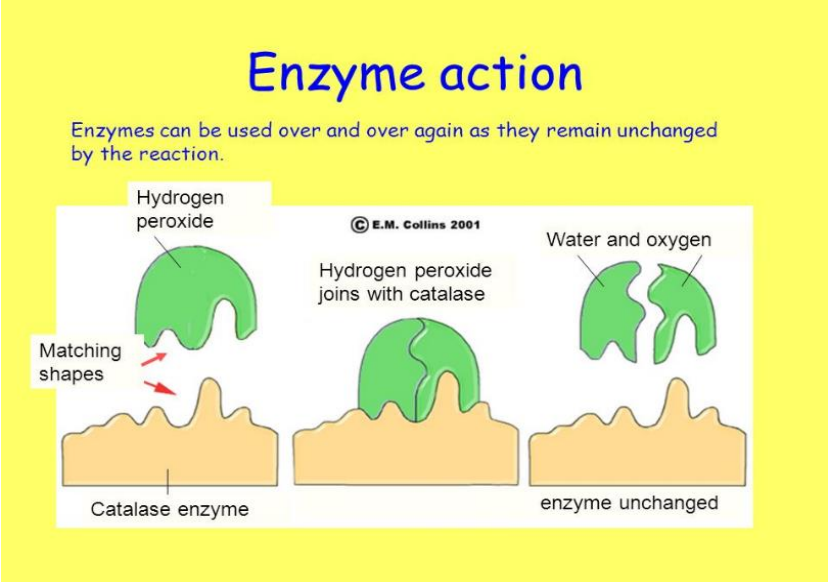
Investigative Question – Does the concentration of Hydrogen Peroxide (H_2O_2) (substrate) effect the rate of reaction when catalysed by the catalase enzyme?

Introduction and Aim -

Catalase is an important enzyme in many organisms as an accumulation of hydrogen peroxide is deadly for cells, so it must be catabolically broken down.

Figure 1 shows how the active site of catalase binds to the substrate (H_2O_2) and then breaks it down into water and oxygen.

Figure 1



Introduction: Generally relevant and some link to the research question.

The equation for this reaction is –

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

There are many factors that can influence the rate of reaction of an enzyme including pH, variations in temperature, presence of inhibitors, concentrations of enzymes or substrates.

Substrate concentration will affect the rate, as increasing the amount of substrate will increase the rate of reaction, with a given number of enzyme molecules. At a particular substrate concentration, the enzymes active site will be saturated, and the rate will not continue to increase (figure 2).

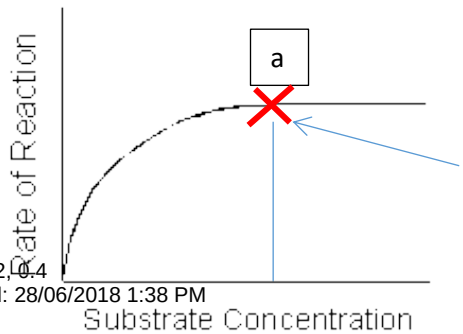


Figure 2: Point of equivalence where set numbers of enzymes are saturated by that substrate concentration, a.

In this practical the catalase enzyme from spinach is used to determine the effect that the concentration of substrate has on the rate of reaction.

Hypothesis –

As the concentration of hydrogen peroxide, the rate of reaction will increase. This will occur until saturation point of the enzyme.

Hypothesis: appropriate format.

Variables –

Independent – concentration (%) of hydrogen peroxide substrate in solution (H_2O_2) : 1, 2, 3, 4, 5, 6 % v/v.

Control: 0% hydrogen peroxide = water. Should have little to no reaction as the breakdown of hydrogen peroxide without the enzyme, catalase is very slow.

Dependent – rate of reaction, calculated using volume of foam produced in measuring cylinder over a time of 30 seconds. The volume of foam produced is measuring the amount of oxygen because the foam is the oxygen production caused by breaking down the hydrogen peroxide.

Appropriate independent and dependent variables.

Formula to calculate the rate:

$$\text{Rate of catalase activity (mL/second)} = \frac{\text{volume of foam produced (mL)}}{\text{30 seconds}}$$

Controlled Variables –

Controlled variables	How is the variable controlled	Why is it controlled?
Temperature of enzyme	The catalase enzyme was in a solution kept at room temperature. 20◦C	Temperature will affect enzyme activity. Can increase or decrease it.
Glassware	The glassware for each concentration of H_2O_2 measurement and the syringe for each catalase extract measurement were kept the same by labelling the apparatus so that the same one could be used for the second replicate.	Could add different volumes if the glassware was not the same throughout the experiment.
Catalase enzyme mixture	The same catalase extract mixture from spinach was used throughout, each time with the same concentration of enzyme.	Ned to have the same amount of enzyme. Enzyme amounts could increase or decrease the rate of the reaction.
Person reading meniscus and timing	The same student read meniscus level each time to minimise variations. While the same student timed each 30 second period.	To keep consistent and increase the accuracy of the readings.
Stopwatch	The same stop watch was used for each trial for control.	Different timers may have different times.
Volume of enzyme	3mL of catalase extract was used each trial	Use a different volume would mean the amount of enzyme will be different.

Variables: generally relevant. Explanation provided is generally appropriate, however, basic or no effect indicated

Factors that cannot be controlled	Explanation
Rate at which catalase enzyme mixture is added to hydrogen peroxide	The syringe being used may squirt out contents at different rates for each trial
Temperature of one trial compared to next	As the trials for each concentration are conducted at different times the temperature of the solutions may be hard to control as there are general fluctuations in air temperature over time
The amount of enzyme in the mixture added to the test tube	The same mixture is used, but there is no way of knowing if the same amount of enzyme is used in each trial.

Appropriate discussion of factors that may not be able to be controlled.

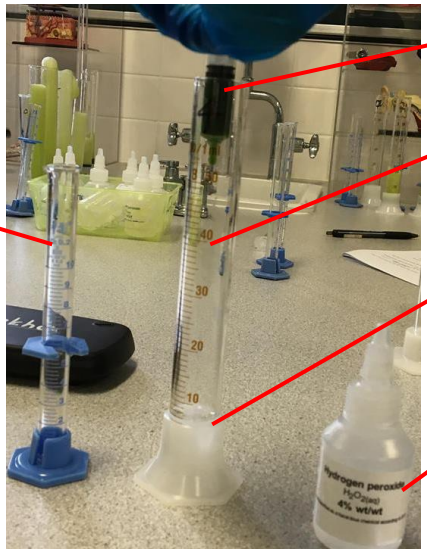
Apparatus and Materials

- 1 x Stopwatch
- 7 x 50mL measuring cylinder
- 7 x 10mL measuring cylinder
- 1 x 5mL syringe
- 1 x 50mL beaker
- 10mL cold hydrogen peroxide (H_2O_2) solution for the concentrations of – 0, 1, 2, 3, 4, 5, 6% wt/wt
- 18mL catalase extract (spinach, purified and filtered)

Note: In a completion practical investigation, the Method/procedure must be included and is assessed using PS KA4 – considering the conventions of a practical report.

Labelled Diagram

Image 1 – experimental set up



Method

1. Decant premixed catalase extract into 50mL beaker
2. Using a 10mL measuring cylinder, measure out 6mL of H_2O (0%), ensuring that the meniscus line sits above the 6mL line when reading from a horizontal eye level position, and pour it into a 50mL measuring cylinder and label accordingly.
3. Extract 3mL of catalase extract from 50mL beaker using 5mL syringe.

4. Add the 3mL of catalase extract to the H_2O in the 50mL measuring cylinder and immediately start timing with the stopwatch.
5. After 30 seconds record the level of foam (mL) in the measuring cylinder and record.
6. Calculate the volume of foam produced: final volume (after 30s) – 9mL = volume of foam produced and record.
7. Calculate a rate of reaction the formula -

$$\text{Rate of catalase activity (mL/second)} = \frac{\text{volume of foam produced}}{\text{30 seconds}}$$

8. Replicate steps 2-7 for 0% concentration 3 times.
9. Repeat steps 2-8 for the concentrations of H_2O_2 (1, 2, 3, 4, 5, 6 % v/v).

Clear discussion of safety.

Safety and ethical concerns

Safety or ethical concern	How managed
Hydrogen peroxide stains and is corrosive	Safety glasses, aprons and gloves are to be worn by experimenters when handling hydrogen peroxide, to ensure that burns and contact with eyes are avoided.
Broken glass	Leather uppers are to be worn to ensure that broken glass does not cause injury or harm. While also the glass is to be disposed of into a designated glass bin.
Disposal of hydrogen peroxide	Hydrogen peroxide is not dangerous enough to worry about rinsing down the sink so marine life will not be detrimentally influenced.

Results

Observations

- For some trials with a high concentration the foam went over the 50 mL limit in the time of 30 seconds
- In 30 seconds the 5 and 6 % concentrations had lower average reading of foam. However the foam bubbled over the top of the measuring cylinder by more than the lower values of 4 and 3 % which had a higher average reading in 30 seconds
- The green catalase extract turned lighter green when reacting with H_2O_2 in bubble form
- Some of the green catalase solution squirted back up the side when entering the substrate solution

Table 1 – Individual Raw data

Concentration of H_2O_2 (%)	Total volume (mL) (±0.5mL)		Total Volume of foam produced (mL) (= Total volume – 9mL)		Rate of Catalase Activity (mL/s)	
	Trial 1	Trial 2	**Trial 1**	Trial 2	**Trial 1**	Trial 2
0	**9**	12	**0**	3	**0**	0.1
1	**12**	12	**3**	3	**0.1**	0.1
2	**28.5**	27	**19.5**	18	**0.65**	0.6
3	**44**	44	**35**	35	**1.17**	1.16
4	**52**	50	**43**	41	**1.43**	1.37
5	**52**	47	**43**	38	**1.43**	1.27
6	**47**	52	**38**	43	**1.27**	1.43

Examples of calculations –
total volume of foam produced trial 1 mL – 6% concentration H_2O_2: 47-9=38
Rate of catalase activity trial 1 mL/s – 6% concentration H_2O_2: 38÷30=1.27

Table 2 – Averages for individual total volume of foam produced data

	Volume of foam produced (mL)		
Concentration of H_2O_2 (%)	**Trial 1**	**Trial 2**	**Average**
0	0	3	1.5
1	3	3	3
2	20	18	19
3	35	35	35
4	43	41	42
5	43	38	40.5
6	38	43	40.5

Example of calculations –
Average volume of foam produced (mL) for concentration of H_2O_2 2 %: (20+18)÷ 2 = 19

Table 3 - class results and averages

Concentration of H_2O_2 (%)	**Individual trials for volume of foam produced (mL)**											**Average**
0	1	1	2	1	0	0	1	1	3	0	1	0.95
1	18	18	2	2	5	4	5	4	3	3	2	5.9
2	17	16	17	19	17	16	17	15	18	20	17	17.1
3	37	37	36	37	34	34	35	31	35	35	36	35.2
4	46	44	41	40	43	48	42	41	41	43	43	42.9
5	41	44	39	43	44	39	42	43	38	43	45	41.9
6	41	44	39	40	42	38	41	36	43	38	42	40.4

Examples of calculations –

Average of volume of foam produced from class for concentration 6%

41+44+39+40+42+38+41+36+43+38+42=40.4

Table 4– manipulated individual data and average rate reaction data from class results

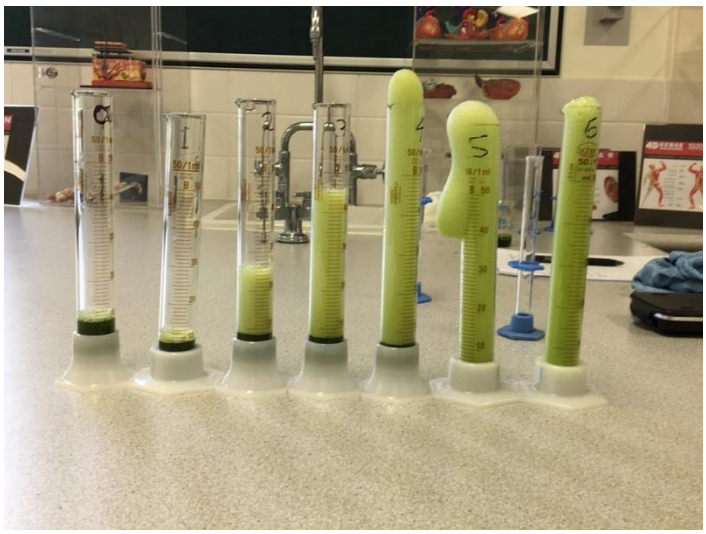
Concentration of H_2O_2 (%)	Rate of catalase activity, (mL/s) individual trial 1	Rate of catalase activity, (mL/s) individual trial 2	Average rate of catalase activity for individual trials (mL/s)	Class average rate of catalase activity (mL/s)
0	0.00	0.10	0.05	0.031
1	0.10	0.10	0.10	0.20
2	0.65	0.60	0.63	0.57
3	1.17	1.16	1.17	1.17
4	1.43	1.37	1.40	1.43
5	1.43	1.27	1.35	1.39
6	1.27	1.43	1.35	1.35

Examples of calculations –
Average rate of catalase activity for individual trials H_2O_2 (3%): (1.17+1.16)÷2 = 1.17

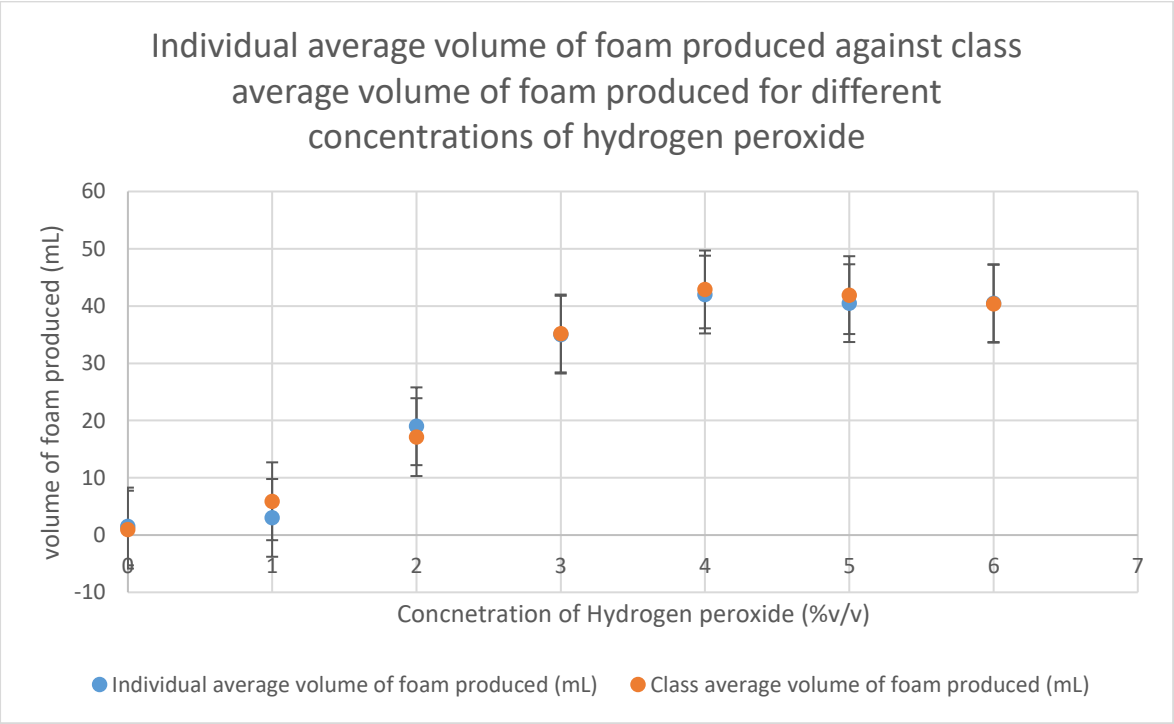
Effective and detailed data presentation.
(Could reduce into 2 tables.)

Class average rate of catalase activity H_2O_2 (4%):
46+44+41+40+43+48+42+41+41+43+43=42.9(Average volume of foam produced)
42.9÷30=1.43mL/s (class average rate of catalase activity)

Image 2 – individual results from trial 2

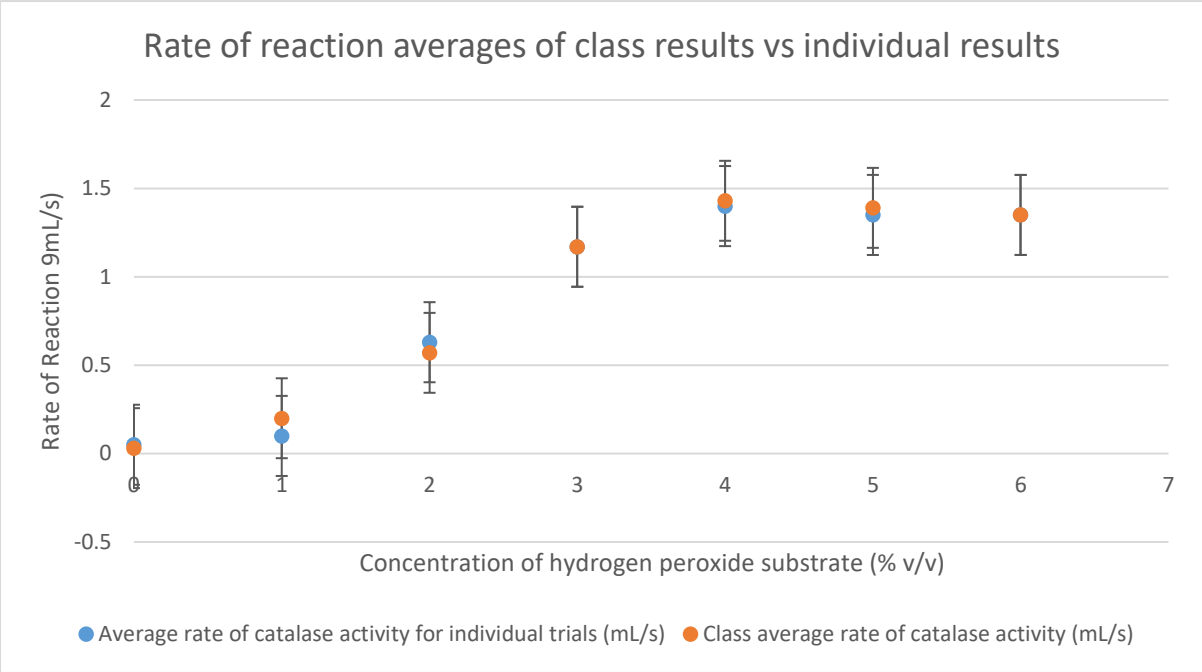


Graph 1 – with standard deviation error bars



Graphs: accurate. No line indicated for best fit or connecting data points.

Graph 2 – with possible max/min error bars



Basic analysis of data. No reference to the data obtained, therefore demonstrates basic interpretation.

Analysis of results

From the results, the individual results compare to the class results e.g. Graph 2 shows how they are almost the same, this allows for the results to be considered valid and reliable.The hypothesis is supported because by relating the results back to the scientific knowledge they show once the substrate concentration increases past 4% the rate of reaction plateaus and even drops a little because the rate at which the catalase enzyme can react is not able to be any quicker, as the solution is saturated with substrate, and therefore the rate stays constant. This is shown in graphs 1 and 2.

Limited connection the relevant theory.

Evaluation

Identifies some potential sources of random error.

Random errors are always present in a practical and are unavoidable. A possible source of random error is that through assuming the foam grows uniformly and that it contains all oxygen produced through the breakdown of hydrogen peroxide the precision of the results are affected. This is because it cannot be certain that the foam bubbles uniformly to make the same sized bubbles everywhere, this means that the reading from the measuring cylinder of the volume of foam produced is compromised and less precise. Also because oxygen may be able to escape the measuring cylinder from the bubbles and therefore each measurement may be more or less than the true value. Another possible source of random error is that hydrogen peroxide is an unstable substance and reacts with itself at room temperature. This means that it must be kept cool in fridge prior to the experiment otherwise the catabolic reaction may take place without the enzyme outside the measuring cylinder. This leads to each trial having a different temperature because the time elapsed from when it exits the fridge to the measuring cylinder is different for each trial because they are not being all done simultaneously. Therefore, the results' precision is being influenced because the results are being skewed in a different direction for each trial. This would consequently increase the reliability of the data because the random error has been minimised. A possible source of random error could be that when squirting the catalase extract out of the

Indicates some effect on the data. No reference to the data

More potential sources of

syringe into the measuring cylinder for the reaction to take place there is splashback up the sides of the cylinder whilst possibly not emptying properly, and it does not all enter at the exact same time. A way to minimise the effect of all random errors and to improve the reliability of the data is to do as many replicates as possible (at least 3) to get an overall average where the results are as close as possible to the true value. In this practical the results were pooled from the class, this would amplify the errors if the experiment was being carried out improperly.

Conclusion relevant? Logical?

A systematic error affects the data in the same way each time, therefore decreasing the accuracy of the results.A possible source of systematic error for this practical could have been that the calibration of equipment may have been incorrect. This includes measuring cylinders, stopwatches and syringes being faulty in their measurement labels contributing to the accuracy of the experiment decreasing. This would have led to the results being either higher or lower than the true value for each trial because systematic errors influence the results the same manner each time. To identify whether systematic errors did influence the practical the practical could have been repeated with different equipment and apparatus whilst using the same method. This then could have identified the different data set and then could be eliminated to improve accuracy. Another possible source of systematic error could have been the concentrations of the different hydrogen peroxide solution could have been incorrect leaving intervals not evenly spaced apart. The results therefore would have been inaccurate as they could have been too high or too low. To improve the practical and to identify this source of systematic error the experiment count have been repeated using different mixtures of hydrogen peroxide solutions to gain insight into whether the results were detrimentally influenced. Parallax error is another possible source of systematic error that could have decreased the validity of the experiment. To identify whether parallax error influenced the results the practical could have been repeated to see if any different results came about. If parallax error does prove to have influenced the results it technically is an error because the experimenters have incorrectly carried out the procedure. The data was pooled for this experiment which means that different apparatus was used for each groups trial meaning that different results could come about, if results are too different they can be eliminated from the data set to improve the accuracy.

Identifies some systematic errors accurately

General link to effect on data

Being repetitive will affect word count and depth of evaluation.

In this case the parallax error is random error.

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Conclusion

Basic conclusion.

From the practical it can be concluded that the hypothesis is supported, and the investigation question was successfully answered. The hypothesis was fully supported by the results showing that the concentration of the substrate increased to a certain concentration (4%) the rate of reaction increased accordingly until it plateaued because the substrate concentration saturated the enzyme concentration. The investigation question can be answered 'yes' the concentration of Hydrogen Peroxide (H_2O_2) (substrate) does affect the rate of reaction when catalysed by the catalase enzyme. The results of the practical are not definitive conclusions however it does support the claims made.

Some justification for the hypothesis being supported. No limitation for the conclusion presented.

Overall grade C

	Investigation, Analysis and Evaluation	Knowledge and Application
A	Designs a logical, coherent, and detailed biological investigation. Obtains, records, and represents data, using **appropriate** conventions and formats **accurately and highly effectively.** **Systematically** analyses and interprets data and evidence to formulate **logical** conclusions with **detailed** justification. **Critically** and **logically** evaluates procedures and their effect on data.	Demonstrates **deep and broad** knowledge and understanding of a **range** of biological concepts. Develops and applies biological concepts highly effectively in new and familiar contexts. Critically explores and understands in depth the interaction between science and society. Communicates knowledge and understanding of biology **coherently,** with **highly effective** use of appropriate terms, conventions, and representations.
B	Designs a **well-considered** and **clear** biological investigation. Obtains, records, and represents data, using **appropriate** conventions and formats **mostly accurately and effectively.** **Logically** analyses and interprets data and evidence to formulate **suitable** conclusions with **reasonable** justification. **Logically** evaluates procedures and their effect on data.	Demonstrates **some depth and breadth** of knowledge and understanding of a **range** of biological concepts. Develops and applies biological concepts mostly effectively in new and familiar contexts. Logically explores and understands in some depth the interaction between science and society. Communicates knowledge and understanding of biology **mostly coherently**, with **effective** use of appropriate terms, conventions, and representations.
C	Designs a considered and generally clear biological investigation. Obtains, records, and represents data, using **generally appropriate** conventions and formats with **some errors** but **generally accurately and effectively.** Undertakes **some** analysis and interpretation of data and evidence to formulate **generally appropriate** conclusions with **some** justification. Evaluates procedures and **some** of their effect on data.	Demonstrates knowledge and understanding of a **general range** of biological concepts. Develops and applies biological concepts generally effectively in new or familiar contexts. Explores and understands aspects of the interaction between science and society. Communicates knowledge and understanding of biology **generally effectively**, using **some** appropriate terms, conventions, and representations.
D	Prepares the outline of a biological investigation. Obtains, records, and represents data, using conventions and formats **inconsistently**, with **occasional** accuracy and effectiveness. **Describes** data and undertakes some **basic** interpretation to formulate a **basic** conclusion. **Attempts** to evaluate procedures or **suggest** an effect on data.	Demonstrates **some basic** knowledge and **partial** understanding of biological concepts. Develops and applies some biological concepts in familiar contexts. Partially explores and recognises aspects of the interaction between science and society. Communicates **basic** biological information, using **some** appropriate terms, conventions, **and/or** representations.
E	Identifies a simple procedure for a biological investigation. **Attempts** to record and represent **some** data, with **limited** accuracy or effectiveness. **Attempts** to **describe** results **and/or** interpret data to formulate a **basic** conclusion. **Acknowledges** that procedures affect data.	Demonstrates **limited** recognition and **awareness** of biological concepts. Attempts to develop and apply biological concepts in familiar contexts. Attempts to explore and identify an aspect of the interaction between science and society. **Attempts** to communicate **information** about biology.