I B Biology IA Handbook

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(For use with the IB Diploma programme)

(Third edition)

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Published by IBID Press, Victoria, Australia

Third Edition 2014

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Library Catalogue

1. Biology
2. International Baccalaureate Biology Diploma Programme.
3. Internal Assessment Handbook
4. Talbot C

ISBN: 978-0-9925224-0-7

Published by IBID Press, 36 Quail Crescent, Melton 3337, Victoria, Australia.

Samples of other materials to support the teaching of IBO syllabuses can be found at: <*www.ibid.com.au*>

Foreword

This 'Student Handbook for Internal Assessment - Biology' has been written specifically to support the teaching of the current International Baccalaureate Biology course (© International Baccalaureate Organisation 2014). It has been written by an experienced and practising Senior Biology Teacher with close reference to the Group 4 Internal Assessment criteria.

The material was written with close reference to the IBO Diploma Programme Biology Guide published in March 2014. Some material is reproduced from the Guide and other material is adapted from the Guide. The assessment criteria have been divided into aspects and sub-aspects, but this division has been implemented by the author and is not mandated by the IBO.

This material has been designed to accompany and complement Biology for use with the International Baccalaureate (4th edition) written by Minka Peeters Weem, Antony Mayrhofer and Christopher Talbot published by IBID Press, and the Volumes of Investigations for IB Biology written by Paul Billiet and published by IBID Press.

It will, however, be useful to IB Biology students following any practical scheme of work and using any published Textbook.

The aim of this Student Handbook is to help you, as an IB Biology student, to plan, conduct and write a report for an Individual Investigation for Assessment and conduct and write a report for other practicals or investigations including your Group 4 Project as required by your teacher. It contains a wide variety of information and advice that will help you at various stages throughout your IB Biology practical programme including the completion of your Individual Investigation. A separate chapter is included for students who are studying biology for their Extended Essay.

The Guiding Questions (inspired by the IB MYP approach) have been written by the Author and the Editor and are designed to help you consider the important concepts and understandings inherent in the group 4 assessment criteria. The guiding questions are generative, meaning they generate multiple avenues of inquiry and investigation as you plan, perform and write-up your Individual Investigation. Active questioning will keep your brain engaged: an inquiring mind is absorbing information and constructing meaning. Deliberate and thoughtful questioning involves higher order thinking skills and depth of knowledge.

Both Standard and Higher Level IB Biology students should find this Student Guide useful. It includes material that will be relevant to students of all linguistic and academic abilities. Although the author is European, he has consulted a North American colleague to ensure that the conventions are also recognisable and relevant to American and Canadian students.

Author Profile

Chris taught IB Chemistry, IB Biology and Theory of Knowledge (ToK) in the High School of the Overseas Family School, Republic of Singapore. He now teaches at a leading IB World School in Singapore. He graduated with Honours in Biochemistry from the School of Biological Sciences, University of Sussex in the United Kingdom. He also holds an Advanced Certificate in Molecular Biology from Birbeck College, University of London in the United Kingdom. He has a Masters degree in Life Sciences (Chemistry) from the National Technological University (NTU) in Singapore. He is the co-author of the MYP Student Guide and MYP Practical Portfolio and the author of the Practice Examinations for IB Chemistry and IB Biology. He is a contributing author to the fourth edition of the IB Biology text book published by IBID Press. He has had many Chemistry and Biology articles published in School Science Review and is serving a five year term as an Editorial Associate.

Internal Assessment

The individual assessment allows students to develop and demonstrate their skills in scientific research.

The initial planning is vital to the success of the individual investigation. Students will be guided by the teacher as to the appropriateness of the research question – level of complexity and compatibility with the assessment criteria.

The individual investigation is personal research and each student will undertake a unique Individual Investigation of a Research Question that is of interest.

The formulation of the research question is the responsibility of the individual student.

Each student should decide whether the investigation is a hands on investigation or uses secondary sources such as databases. A mix of various types of investigation is allowed.

There is no expectation to go beyond content of the syllabus. Work can be based on concepts within the course specifications.

One investigation is required and it must be individual work - no partners or sharing of data.

The assessment model uses five criteria to assess the final report of the investigation with raw marks and weightings assigned (*refer to the IBO Syllabus Guide for details*).

Levels of performance are described using multiple indicators per level. Also, not all indicators are always present. It very much depends upon the type of investigation.

The investigation should take about 10 hours of work.

There will be a single investigation by the student and the report can be 6 to 12 pages long, have an academic and scholarly presentation, and demonstrate scientific rigour commensurate with the course.

The IA combines research and experimental work.

Acknowledgements

It is a considerable pleasure to acknowledge the extensive advice and numerous contributions of the following during the production of the fourth edition: Cesar Reyes (Overseas Family School) and Dr John Green (formerly of Li Po Chun United World College).

The author and publisher also grateful acknowledge the permission given by IBO to use and adapt material from the Syllabus Guide (*© IBO 2014)*.

The Individual Investigation

In the second year of the IB Diploma programme you will be required to research, design, perform (carry out) and write-up your own Individual Investigation. The internal assessment (IA) accounts for 20% of your final IB Diploma grade for IB Biology and requires you to spend 10 hours performing laboratory or field work, during which time you will be in communication with your Biology teacher who will act as your supervisor. The time required for you to write the report for your own Individual Investigation cannot be included in the 10 hours and this should be done outside of the laboratory period.

A range of possible types of investigations can be carried out for the Individual Investigation.

You will probably be encouraged by your Biology Teacher to carry out traditional hands-on experimental work in the laboratory or field. This could involve extending some of the investigative approaches that you were exposed to during the IB Biology course, or you might investigate in a practical way an experiment relevant to some of the biological or biochemical concepts you learnt during the IB Biology course.

For example, you may observe a pioneer plant of estuarine salt marshes that colonizes bare mud, eventually helping to increase the height of the mud flat. You may want to investigate whether the cell sap osmotic potential was under any form of control. You could collect samples of the plants from different locations, dry them, grind them up and stir into distilled water and leave for a fixed length of time. The conductivity of the solutions and the water samples could be measured and useful conclusions made.

You may also go on-line and retrieve data from a reliable and reputable Biological or Biochemical web site and process, analyse and present the data for your own analysis and investigation. You may want to retrieve protein sequences from the SWISS-PROT database or study human genetics (OMIM – *Online Mendelian Inheritance in Man*). Many web sites record biodiversity or ecological data, for example, the RSPB (*Royal Society for the Protection of Birds*).

You may use a spreadsheet, such as Excel, to set up a model to simulate some Biological phenomenon, for example, predator-prey relationships, modelling the spread of an infectious disease, some aspect of evolution or population genetics, or axon excitability. You can compare the theoretical data against experimental data and evaluate the experimental data and any assumptions in your model.

In actual practice some combinations of some or all of these approaches may be suitable, depending on the topic of the Individual Investigation.

The subject matter or content may be inside or outside the current of the IB Biology syllabus for example, chemical communication between plants or programmed cell death. The subject matter of your Individual Investigation is your decision, as the student, but you must ensure you are familiar with any new biological mathematical or biochemical facts, principles and concepts. However, your knowledge of IB Biology (SL or HL) will enable you to get the maximum mark when the write-up of your Individual Report is assessed by the IBO.

The Individual Investigation {your Internal Assessment (IA)} will consist of a report 6 to 12 pages long. The report should resemble a scientific paper from the Biological literature. It should be an academic piece of work and show the scientific rigour expected from the an SL or HL Biology student. You are expected to show a high degree of personal involvement and a good scientific understanding of the Biology that lies behind your Individual Investigation. It is important that you summarize the current Biological thinking and knowledge of your chosen topic.

Health and Safety Symbols

Laboratories can be hazardous places. Often scientists, Science teachers and students handle equipment and materials which can be dangerous to their health and safety. Throughout these Volumes you will see a number of symbols and warnings which will represent particular hazards. For each of these we will briefly describe the hazard and indicate what precautions you should take to avoid damage and/or what responses are appropriate. In all cases, of course, you should seek advice and assistance from the teacher or laboratory technician.

A biohazard is any organism or body fluid which could possibly cause illness or disease in your body. This particularly includes micro-organisms.

A flammable substance is one which will readily burn in air. It may be a solid, liquid or gas. If you are using such a substance it is vital that there are no sparks or naked flames which could ignite it. It is vital that you know what to do in the event of fire. This may include the use of fire extinguishers and evacuation procedures.

A radioactive substance is one which emits particles or 'radiation'. This radiation is known to cause damage to cells and may also be cancer causing. If you are using radioactive substances it is vital that you wear protective clothing, use metal tongs and listen carefully to instructions given by your teacher or laboratory technician.

Sharp instruments are often used in Science and particularly in Biology, to cut sections through plant or animal tissue. These instruments, which include scalpels and razor blades are very sharp and obviously will also cut through your tissues. When using these instruments it is essential that you always cut away from your body and preferably onto a cutting board. It is also important to be very careful when carrying these instruments and also ensure they are placed on the workbench in a safe place.

When certain chemicals are mixed together they can become explosive. An explosion is caused by rapid expansion of gas in a confined space and can be very dangerous. Sometimes it is important to ensure that the space is not confined and sometimes it is important to conduct these reactions behind a protective screen.

It is often necessary to protect your hands from heat, chemicals or other hazards and gloves will be made available for these situations. The type of glove needed will depend on the particular hazard and your teacher will provide further advice. In some cases you will be advised to dispose of the gloves after use and in other cases to wash and dry them carefully.

Your eyes are the most vulnerable and easily damaged external part of your body. This is why they must be protected if you are using solids and liquids which could get into them. Whenever you are heating things or using corrosive liquids, and in other cases as instructed by a teacher, you should wear safety goggles. You should also do this if possible even if you wear spectacles to correct your vision. In the event that something gets in your eye you should immediately make use of the eyewash facility in the laboratory as instructed and then notify your teacher.

Some chemicals, which are used in a laboratory, are *corrosive*. This means that they can react with and 'eat away' materials like the bench, your books, clothing and skin. It is essential that you handle these materials, which are usually liquids, with care. Always tip from the container with the label uppermost, never add water to concentrated acid and never have your face anywhere near the container. It is usually advisable to wear both safety goggles and gloves. If protective aprons are available you should also wear one.

As a general rule, 12 or 24 volt *electrical* appliances are unlikely to cause serious injury. However, 'mains' voltage (110V or 240V or higher) can cause serious injury or death. The appliances you use should be regularly tested and certified safe. If you notice sparks or smell insulation burning, turn the power off immediately and notify staff. Be particularly careful not to allow water to get into any appliance as it may cause a short circuit.

Some chemicals are *poisonous* and should not be inhaled or ingested. It will be necessary to use a fume cupboard when using poisonous gases or volatile liquids. They could make you very ill and you may require medical assistance. It is vital that you listen to instructions, follow them carefully and notify your teacher immediately if there is accidental exposure to poisonous or toxic substances.

Lasers are very intense beams of light. They are capable of causing burns to the skin and permanent damage to the eyes. It is essential that these are only ever used under the supervision of a teacher and in a situation where people can not see the beam directly or when it is reflected from a shiny surface. Sunglasses or welding masks do not provide sufficient protection and special 'laser glasses' must be used where there is a risk.

UV light is harmful to skin and especially eyes. Do not expose these areas directly to a UV light source. If it is not avoidable, sunscreen can be applied to the skin and special goggles should be worn.

There are other *dangers* or hazards as well, for example carrying heavy or hot objects. This may also include chemicals which are not poisonous but which may smell unpleasant or irritate the skin. Whenever you see this icon more information will be provided in the adjacent text about the specific danger.

In Science and particularly in Biology, there are situations when ethics and ethical issues need to be considered in experimental work. This is particularly the case when human volunteers are being used, not just for experimental work but also when they are being surveyed to collect personal information. In these cases a consent form should be used to explain the nature of their involvement and to get their approval. Ethics will also be an issue whenever animals are used in experimentation or when they are collected in the field. They should not be exposed to conditions that are outside their natural range of tolerance and wild animals must be released back where they were sampled with the minimum of disturbance.

The environment and environmental issues become important when hazardous substances are used or produced during an experiment. Their disposal must result in minimal impact on the environment. In field work the protocol that is used must reflect practices that minimise the impact of the investigation on the site.

IMPORTANT NOTE

Although every care has been taken in preparing and trialling these investigations, absolutely no responsibility or liability whatsoever can be accepted for any damage or accident which may occur for whatever reason during the conduct of any of these activities. The Safety Warnings and Icons are advisory only and are not intended to be exhaustive or exclusive. It is a strict condition of sale that safety in the laboratory is the responsibility of the staff and students doing the laboratory work and not the author, editor or publisher of this work.

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This criterion assesses the extent to which the student engages with the exploration and makes it their own. Personal engagement may be recognized in different attributes and skills. These could include addressing personal interests or showing evidence of independent thinking, creativity or initiative in the designing, implementation or presentation of the Investigation.

The descriptors in the following table will be used by your teacher to allocate a mark for your performance in this criterion:

MARK	DESCRIPTOR
0	The student's report does not reach a standard described by the descriptors below.
1	The evidence of personal engagement with the exploration is limited with little independent thinking, initiative or insight. The justification given for choosing the research question and/or the topic under investigation does not demonstrate personal significance, interest or curiosity. There is little evidence of personal input and initiative in the designing, implementation or presentation of the investigation.
2	The evidence of personal engagement with the exploration is clear with significant independent thinking, initiative or insight. The justification given for choosing the research question and/or the topic under investigation demonstrates personal significance, interest or curiosity. There is evidence of personal input and initiative in the designing, implementation or presentation of the investigation.

Guiding Questions

- ***How well has the student justified their choice of research question and approach to the investigation?***
- ***To what extent does the student engage with the investigation and make it their own?***
- ***How much has the student developed and modified the method through their own personal initiative?***
- ***To what extent has the student reflected on the significance of their exploration from a personal, and also a scientific, view?***

Your mark for personal engagement will be judged from the written evidence and is based on the your individual work on the Individual Investigation. Your teacher will take into account your self motivation and perseverance that is evidenced in the report for the Individual Investigation.

To score maximum marks under the personal engagement criterion you must provide clear written evidence and evidence to your teacher that you have contributed significant scientific thinking, initiative, or insight into your Individual Investigation. You should take 'ownership' of the Individual Investigation and show all the traits and characteristics of a research student.

Your research question could be based upon some theory covered in class, for example, optimal-foraging but applied to an unfamiliar context, such as bee-flower interaction, or an extension of your own personal interest, for example, biochemistry, which you may be intending to study for a degree at chemistry. You may have extracted chlorophyll but you are interested in extracting and studying the oxidation and antiradical activity of the purple pigment *betalain* from beetroot.

Your teacher may have shown an interesting demonstration that captured your interest, such as the tenderizing of meat with pineapple juice. An enzyme, *bromelain*, digest the protein softening the tissues in the meat. You may want to investigate the effect of temperature on the activity of bromelain.

If you have strong mathematical skills you may want to construct a spreadsheet simulation of population growth (exponential and logistic models), natural selection of a single allele under selection or enzyme kinetics (including inhibition).

You may have a closer personal involvement, for example, you may be colour blind and interested in the evolution and genetics of colour blindness. You may have lived in a country and observed the effects of global warming, or of a particular form of pollution, such as acid rain or heavy metal pollution.

If you are from a country where agriculture is important to the economy you may be personally interested in the crop yields and genetically modified (GM) crops

You can demonstrate personal engagement via personal input and initiative in the design (planning), implementation (carrying out), or presentation (write-up or report) of the Individual Investigation. Perhaps you have improved the accuracy of a method to monitor the rate of an enzyme-controlled reaction or built novel apparatus that allows you to investigate a reaction or biological phenomenon in a new way.

Personal engagement is intended to be a way of crediting your originality in application and design. Superficial investigations or unmodified and unjustified standard methods from biology text books would score poorly in this criterion. Your method should not simply be a 'recipe' where you follow a standard technique with no comment, no justification and no modification.

Your self motivation towards the Individual Investigation will be formally assessed by your Biology Teacher. This means that written work involved must always be handed in on time and complete. You may also be assessed on the issues of plagiarism. This could involve copying someone else's investigation design or conclusion and evaluation. It could also involve using someone else's processed data from their Individual Investigation or copying their calculations.

You will also be assessed on whether you approach your Individual Investigation with integrity. This could involve making up results to fit a preconceived relationship or hypothesis, ignoring results which are unexpected (anomalous data) or not acknowledging if you obtained some of your biological raw data in a book or from the Internet.

Listed in Figure 101 is a summary of what you need to do to score well in the Personal Engagement criterion.

Assessment criteria	Evidence required	What you must do
The evidence of personal engagement with the exploration is clear with significant independent thinking, initiative or creativity.	A justified research question	You must justify the choice of research question and the chemical topic under investigation and demonstrate personal significance, interest or intellectual curiosity.
	Personal engagement during the exploration	You show significant independent thinking, initiative or creativity in the report (write-up) of your individual investigation, especially in the introduction. Your work must be original.
	Personal engagement during, before and after the exploration	You show personal input and initiative in the design, implementation or presentation of the investigation. Any reflective modifications to the method should be outlined and justified.

Figure 101 Summary of the Personal Engagement criterion

This criterion assesses the extent to which the student establishes the scientific context for the work, states a clear and focused research question and uses concepts and techniques appropriate to the Diploma Programme level. Where appropriate, this criterion also assesses awareness of safety, environmental, and ethical considerations.

The descriptors in the following table will be used by your teacher to allocate a mark for your performance in this criterion:

MARK	DESCRIPTOR
0	The student's report does not reach a standard described by the descriptors below.
1–2	The topic of the investigation is identified and a research question of some relevance is stated but it is not focused. The background information provided for the investigation is superficial or of limited relevance and does not aid the understanding of the context of the investigation. The methodology of the investigation is only appropriate to address the research question to a very limited extent since it takes into consideration few of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. The report shows evidence of limited awareness of the significant safety, ethical or environmental issues that are relevant to the methodology of the investigation*.
3–4	The topic of the investigation is identified and a relevant but not fully focused research question is described. The background information provided for the investigation is mainly appropriate and relevant and aids the understanding of the context of the investigation. The methodology of the investigation is mainly appropriate to address the research question but has limitations since it takes into consideration only some of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. The report shows evidence of some awareness of the significant safety, ethical or environmental issues that are relevant to the methodology of the investigation*.
5–6	The topic of the investigation is identified and a relevant and fully focused research question is clearly described. The background information provided for the investigation is entirely appropriate and relevant and enhances the understanding of the context of the investigation. The methodology of the investigation is highly appropriate to address the research question because it takes into consideration all, or nearly all, of the significant factors that may influence the relevance, reliability and sufficiency of the collected data. The report shows evidence of full awareness of the significant safety, ethical or environmental issues that are relevant to the methodology of the investigation*.

Guiding Questions

- ***To what extent has the student stated a clear and focused research question?***
- ***To what extent has the student established the scientific context for the work through a discussion of its significance?***
- ***To what extent has the student devised a methodology that shows awareness of the factors that may influence the collection of data relevant to the research question?***
- ***To what extent does the methodology allow for the collection of sufficient relevant data that could enable a reasoned conclusion to be drawn?***
- ***To what extent has the student shown how their method has been developed and modified?***
- ***When appropriate, to what extent does the investigation indicate an awareness of safety, environmental, and ethical considerations?***

2.1 Defining the Research Question

It is essential that you are given an open-ended problem to investigate where there are several independent variables, from which you could choose one that provides a suitable basis for the practice investigation. This should ensure that a range of plans can be formulated and that there is sufficient scope to identify both independent and controlled variables. Although the general aim of the investigation may be given by your IB Biology Teacher, you must formulate a specific Research Question. Commonly, you will do this by modifying the general aim provided and indicating the variable(s) chosen for investigation.

Your IB Biology Teacher may suggest the general research question only. Asking students to investigate some property of a specific strain of brewer's yeast, where no variables are given, would be an acceptable teacher prompt. This could be focused by the student as follows: '*Do different carbon sources (glucose, fructose, galactose, sucrose, lactose and maltose) affect the rate of respiration (as measured by acid production)*'?

Alternatively, your IB Biology Teacher may suggest the general Research Question and specify the dependent variable. An example of such a teacher prompt would be to ask the student to investigate the effect of a factor that influences enzyme activity. This could then be focused by you as follows: '*Does ethanol concentration affect the activity of immobolised lactase?*' It is not sufficient for you merely to restate the Research Question provided by your IB Biology Teacher.

2.2 Variables

A variable is a factor present in an experiment that can be changed or controlled to see what effect it has on the results of the experiment. Each biological test should change only one variable at a time. In other words, each test should be a '**fair test**'. If more than one variable is changed during a test, it will not be possible to conclude which variable was responsible for the change in results.

For example, in a typical enzyme-controlled reaction the following continuous variables may affect the rate of reaction:

- concentration of hydrogen ions, H^+ (aq), or pH
- concentration of any known inhibitors
- concentration of any known co-enzymes or co-factors
- concentration of the substrate (provided the enzyme is present in large excess)
- temperature
- concentration of the enzyme (provided the substrate is present in large excess)

2.2.1 Selecting variables

Variables are factors that can be measured and/or controlled. **Independent variables** are those that are manipulated, and the result of this manipulation leads to the measurement of the dependent variable.

A **controlled variable** is one that should be held constant so as not to obscure or hide the effects of the independent variable on the dependent variable.

The variables need to be explicitly identified by you as the dependent (measured), independent (manipulated) and controlled variables (constants). Relevant variables are those that can reasonably be expected to affect the outcome of the experiment.

For example, in the investigation 'How does pigmentation in cyanobacteria vary with light intensity?', you must state clearly that the independent variable is the light intensity and the dependent variable is the amount of pigmentation. Relevant controlled variables would include temperature of solution, preparation of cyanobacteria cells (of a specific species), sample size and light wavelength (or frequency).

A continuous variable is a variable that can take can take any numeric value between some practical limits, for example, the concentration of pH could take on any value (theoretically) between 0 and 14, for example, 7.2, 6.1, 5.3 etc.

Experiments will thus consist of manipulating one of these variables (the independent variable) but keeping all the other variables fixed or controlled. The identification and control of variables is a very important part of IB Biology coursework if the 'Exploration' criterion is going to be assessed by your IB Biology Teacher.

An investigation commonly performed as an assessed IB Biology investigation involves establishing the relationship between light intensity and the rate of photosynthesis (as measured by the rate of bubble production) in distilled water. See *Figure 201*.

The following variables may effect the rate of bubble release (the dependent variable):

- Light intensity (continuous)
- Water temperature (continuous)
- Concentration of sodium hydrogencarbonate (sodium bicarbonate) (continuous)
- Wavelength or frequency of light (continuous)
- Species of plant (categoric)
- Number of leaves on plant (discrete)

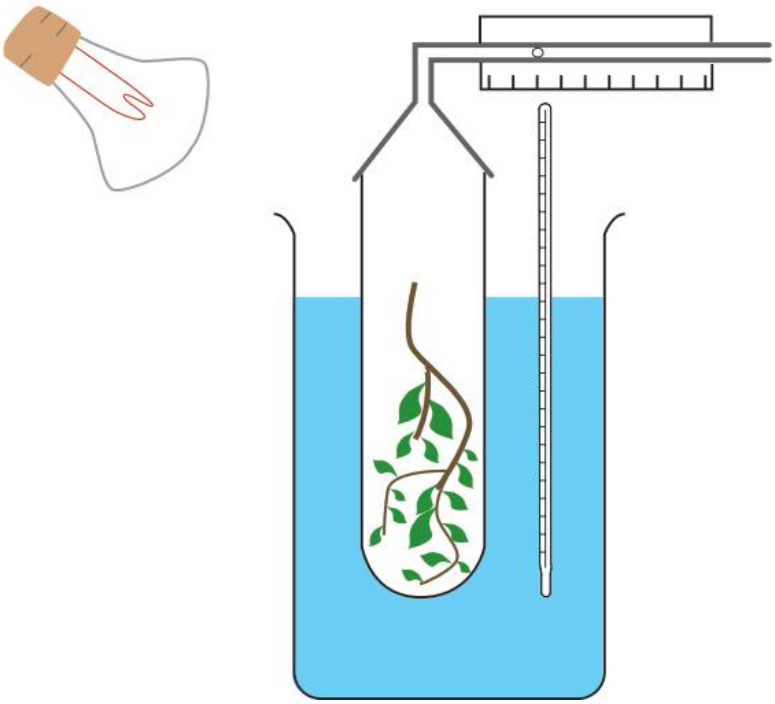


Figure 201 Investigating the rate of photosynthesis in Elodea

A discrete or discontinuous variable is a variable that can take only a small number of whole number or integer values. For example, the number of leaves could be 2, 5, 8, etc. A categeoric variable is a variable that can only be described by words (e.g. *Elodea Canadensis* and *Elodea nuttallii.*)

Again, experiments will involve selecting one of the variables as the independent variable and controlling the others so that they are constant.

Another common investigation involves examining the factors (variables) that affect heart rate. In the human population the variables include: level of 'fitness', age, respiratory efficiency (for example, smokers versus non-smokers), basal metabolic rate, body mass, and circulatory efficiency (for example, presence of atherosclerosis in the arteries).

In a single individual the variables include: the time of the last meal, consumption of any stimulants (for example, coffee), body position (standing, sitting, or lying down), exercise, breathing rate and state of tension.

Figure 202 overpage summarises the variables that affect common biological processes. These processes would make very suitable investigations where the Exploration criterion is assessed.

BIOLOGICAL PROCESS	MAJOR VARIABLES
Enzyme-controlled reactions	Temperature, pH (hydrogen ion concentration), substrate concentration (unless saturation has occurred), enzyme concentration
Photosynthesis	Light intensity, wavelength or frequency of light, temperature, concentration of carbon dioxide gas (or hydrogencarbonate ion concentration in hydrophytes), amount of chlorophyll.
Respiration	Temperature, concentration of respiratory substrate, (e.g. pyruvate, glucose or sucrose) amount of respiring tissue or cells, (e.g. yeast suspension or germinating seeds, pH of medium.)
Transpiration	Relative humidity and pressure of the atmosphere, velocity of air currents, temperature, number and surface area of leaves, thickness of waxy cuticle.
Osmosis	Solute concentration of cell contents relative to surrounding external medium, temperature and membrane permeability.
Diffusion	Solute concentration, temperature, particle size.
Ecological succession	Location, weather, type of habitat, time, external factors, (e.g. mowing and grazing, that is, competition and selection.)

Figure 202 Variables affecting common biological processes

2.2.2 Controlling variables

'Control of variables' refers to your manipulation of the independent variable and your attempt to maintain the controlled variables at a constant value. Your method should include explicit reference to how the control of variables is achieved. If the control of variables is not practically possible, some effort should be made by you to monitor the variable(s).

You may use a standard measurement technique as part of a wider investigation, but it should not be the focus of your investigation. You should be assessed on the individual design of the wider investigation. If a standard measurement technique is used, it should be referenced.

For example, while planning an investigation to study the effect of light wavelength on the rate of photosynthesis in *Elodea* (Canadian pondweed), you may have adapted a method to measure the rate of photosynthesis taken from a textbook. A standard reference would then be expected as a footnote, for example, "Green, Christopher David (1995) 'Investigations in IB Biology', *Garden Press*". Or you may adapt a general protocol provided by a teacher in a previous investigation. The reference may appear as: Smith, David John (2006) "Investigating quorum sensing in bacteria worksheet, *Garden Press*" (fictitious examples).

2.2.3 Controls and Comparisons

A control allows confirmation that no unknown or unidentified variable is responsible for any of the observed changes in the dependent variable. The use of a control ensures the experiment is a 'fair test'.

For example, consider an investigation of meat digestion by the action of the enzyme trypsin. The control for this experiment could consist of a 1.00 gram cube of raw chicken meat in distilled water maintained at a temperature of 20 °C for 15 minutes. The result of this control is expected to be zero change in mass since the enzyme is absent and is assumed to be solely responsible for the digestion of the meat. We need to ensure that the meat does not undergo spontaneous decomposition on exposure to light and/or air.

Consider the hypothesis that '*Plants need light to conduct photosynthesis and produce oxygen*'; the prediction is that if light is not present, a plant will not produce oxygen. Hence, a plant placed in the dark (so light is excluded) is the control.

The comparison consists of a test (whose numeric results are either zero or have been averaged) where results are used as a comparison for the results of other tests. For example, consider, again, an investigation of meat digestion by the action of the enzyme trypsin; the reaction between a 1.00 gram cube of raw chicken meat and a neutral 5% trypsin solution maintained at room temperature for 15 minutes could be used as a comparison in an enzyme investigation.

The results of other tests, involving changes in trypsin solution concentration, trypsin solution temperature, surface area of the chicken meat, pH, etc. could all be compared to the mean results of the control and/or the comparison to see how much faster or slower the new reactions are.

2.3 Hypotheses

A hypothesis is a prediction and explanation of the type of biological behaviour, relationship or result expected during a biological investigation.

The generation of a hypothesis appears not to be a requirement of the Exploration Criterion, but it is often an essential part of the Scientific Method and you are encouraged to include a hypothesis where appropriate in your Exploration Investigations even if it is not formally assessed.

Hypotheses enable the planning of experiments so that the predictions based upon a hypothesis may be tested and either supported or disproved. According to Karl Popper's concept of falsification *(see Theory of Knowledge Program)* a hypothesis is never proved, simply supported). Many IB Biology investigations, in particular, your Group 4 Project, will start with a hypothesis or a pair of competing hypotheses.

In practice many of your hypotheses will be derived from your biological knowledge, Biology text book or notes. However, they should be underpinned by a justification or explanation using relevant biological or biochemical concepts.

Hypotheses may be **qualitative**, for example: '*an increase in sucrose concentration will increase the rate of water loss from potato strips*', assuming that the cell membranes of the potato cells act as selectively permeable membranes.

This behaviour is accounted for by the increase in the rate of osmosis due to an increasing difference between the concentrations of free water in the cytoplasm of the potato cells and the concentration of free water in the surrounding sucrose solution. The difference between the concentrations of free water inside and outside of the cells is known as the concentration gradient. The rate of water loss from the potato cells increases exponentially with an increase in the concentration gradient.

Where possible, the hypothesis should be **quantitative**, and describe the relationship between a dependent and independent variable, for example: '*Every rise of 10 °C will double the rate of reaction between human trypsin and a meat substrate until the internal human body temperature of 37 °C is reached (and perhaps beyond)*'. As a consequence an exponential graph of rate of reaction against temperature is expected until denaturation occurs. This behaviour is accounted for by the doubling in the number of enzyme and substrate molecules possessing combined kinetic energies equal to or in excess of the activation energy, the minimum amount of combined kinetic energy two colliding molecules require for a successful reaction.

On occasions, your hypothesis may prove to be wrong and will be falsified by your experimental data. This is not to be taken as a sign of failure, the result is just as meaningful as proving the hypothesis and you will not be penalised by your IB Biology teacher or moderator appointed by the IBO. However, where possible you need to try and account for the mismatch between your hypothesis and data and then reformulate a new hypothesis to fit the data and then test it.

For example, you might predict a directly proportional or linear relationship between heart rate and work. You would therefore expect the heart rate to double if the amount of work (via exercise) is doubled. Although this hypothesis is easily disproved, it is useful and valid since it is easily testable. The relationship between heart rate and work is not a simple one as follows.

Working muscles require increased levels of oxygen. One might therefore predict that heart rate would increase proportionally. However, at rest a significant amount of oxygen remains in the blood, so that the blood in the veins is never fully deoxygenated. In addition, blood flow through resting muscle is relatively small. Flow increases dramatically in working muscle, partly due to the effect of muscle contractions on the intrinsic vessels. Working muscles also respond by removing some of the available oxygen from the blood. Thus, while the rate of oxygen consumption during exercise increases to around 15 × resting rate, cardiac output may be no more than 5 × (depending on the level of fitness).

Furthermore, since cardiac output is related to both stroke frequency and stroke volume, the rate of increase in heart rate will depend on how much blood is pumped out at each beat - and stroke volume increases during exercise. Peripheral blood vessel resistance also falls, making it easier for blood to flow around the body. These changes can be related to fitness. Blood shunting also plays a role: increasing blood flow to active muscle and decreasing it to inactive muscle.

2.3.1 Predictions

Predictions are based upon your hypothesis. They are descriptions of what you expect to observe and why. Below are two examples.

Hypothesis: Leaves release oxygen gas via their stomata during photosynthesis. Prediction: If the hypothesis is true, then leaf discs, from which we have gently removed gas by aspiration (and therefore sink in solution), will re-float as the leaf produces oxygen gas (and hence loses mass) by photosynthesis in light, but will not re-float so quickly as in the dark since photosynthesis will not be occurring.

Hypothesis: Minerals are actively absorbed and then transported passively in dead xylem by pulling forces generated in leaves, and accumulate in leaves by means of evaporation (transpiration). Prediction: If the hypothesis is true, then shoots should transport an inert coloured dye when a severed stem is placed in the dye. (This only occurs if a bubble of air is not introduced.) The dye should be observed when a cross section of the stem is viewed under a microscope at low power.

Any assumptions inherent in your prediction need to be stated.

Consider the use of a potometer to investigate transpiration to measure the uptake of water by a leafy shoot. It is assumed that a small percentage of water retained by the plant in processes such as photosynthesis, hydrolysis reactions and the maintenance of cell turgour is negligible.

Consider a simple photosynthesis investigation involving testing for starch in leaves using aqueous iodine. It is assumed that boiling will not remove starch from the leaves.

Consider a dihybrid cross involving individual organisms who are heterozygous at the two alleles under investigation. A 9:3:3:1 ratio is predicted for the four phenotypes. However, the ratio above is only expected in the absence of recombination, crossing-over, linkage or mutations for a relatively large population.

You might be counting bubbles from a piece of submerged *Elodea* as a measure of the rate of photosynthesis. One assumption in this simple approach is that the bubbles of oxygen-enriched air are all of the same volume.

If you are performing capture-recapture to estimate the size of a population, the following assumptions are made:

- The marking method employed does not affect the animal.
- The mark will remain throughout the duration of the investigation.
- The marked individuals mix thoroughly with total population before being recaptured.
- The likelihood of an animal being recaptured must not change with age.
- The population is a closed one – no immigration or emigration occurs.
- There are no births or deaths during the period of investigation.
- The organisms are not 'trap happy', this may occur if the traps are baited.

If you are finding the enthalpy, energy or heat content of a dried food by burning it under a metal can with water (of known temperature) then the assumption in your calculation is that all of the heat energy released by the food entered the water, and none was absorbed by the can, the thermometer or surrounding atmosphere. The latter is a significant source of heat absorption and leads to a calculated value that is typically half of the true or literature value. A large systematic error is thus generated.

2.4 Relationships between variables

Many IB Biology investigations involve verifying or establishing the relationships that exist between different variables.

2.4.1 Independence and Dependence

In many IB Biology investigations, readings of two variables are recorded and the relationship between them established.

For example, investigating the effect of temperature on the activity of a particular enzyme and enzyme rate (as measured by rate of disappearance of reactant or rate of appearance of products).

The temperature variable can be easily manipulated or changed by the person performing the investigation and is known as the independent variable. In the enzyme investigation the enzyme activity, the measured variable, is the dependent variable, since enzyme activity is dependent on the temperature. There is an assumption that the change in the temperature causes a change, either directly or indirectly, in the enzyme rate.

However, the converse is not true, that is, a change in enzyme rate (the dependent variable) will not change the temperature (the independent variable). The independent variable is often a physical or environmental variable e.g. time, temperature, pH, concentration of a chemical, surface area, etc.

2.4.2 Interdependence

Frequently during an IB Biology Investigation, we attempt to establish the relationship between two or more variables, where changes in a variable are connected with changes in another variable, but where there is no dependent or independent relationship.

Such a relationship between variables is known as an interdependent relationship and implies that a change in one variable or characteristic is paralleled by a change in another unrelated variable. For example, longer seeds are wider; larger animals have thicker legs and heavier hearts

2.5 Ethics

If you are designing an IB Biology Investigation, it is vital that you consider whether it is necessary to utilise living organisms. If you do have to use them, then you must ensure that the treatment they receive is ethically or morally acceptable. If you are carrying out an ecological investigation, then you must consider the effect your investigation could have on the environment. Does it disturb the animals? Will you have to trample on fragile or delicate plants in order to take your measurements? Are there any long term effects of taking samples repeatedly from an area? It is also very important to consider the laws of the country your school is located in. For example, the Republic of Singapore is very strict about any interference with the local flora and fauna on the island. You must familiarise yourself with the local laws and acts or parliament in the country your school is located.

2.5.1 Plants

Whole plant specimens should not be picked or uprooted from their natural environment. If your proposed biological work requires the use of leafy stems and flowers, you should ensure that they are obtained from gardens or from commercial sources or bred for the purpose. Great care must be taken during investigations involving Option C: Ecology and Conservation that plants are not trampled or damaged during the sampling process.

2.5.2 Invertebrate animals

No experiments should be carried out which may cause harm or suffering to animals. In no circumstances should the animals be subjected to such extreme conditions that they are killed. For example experiments, which investigate the effect of alcohol (ethanol) on *Daphnia*, should use concentrations that do not kill the organisms. This also applies to the effect of alcohol or caffeine on *planaria* (flat worms). It is not acceptable to carry out experiments that measure the amount of force or heat needed to dislodge limpets or other molluscs from rocks.

Any animal removed from its environment for the purpose of counting or measurement should be returned as soon as possible. Particular care should be taken when sampling invertebrates found in rivers, ponds and on rocky foreshores. Some investigations may involve the use of 'mark/recapture' sampling. Before this is carried out, consideration should be given to the effects upon individuals and populations of the organism.

Some ecological investigations may involve the use of pitfall traps. These traps should be checked several times daily to ensure animals are not trapped for long periods of time. The animals should be given protection from the prevailing weather and also a source of suitable food and water.

2.5.3 Vertebrate animals

Experiments should not be carried out using live vertebrates. The only possible exception this is maze learning in rodents (rats and mice) which might be performed during investigations involving Option A: Neurobiology and Behaviour. These behaviour experiments should be carried out without any harm or suffering to the animals and in no circumstances should they be subjected to such extreme conditions that they are killed. No electric shock treatments should be administered. They should not be exposed to any unnecessary stresses such as deprivation of food or water or rest. For example, in experiments using a food reward during maze learning in rodents, the animals must not be starved prior to the tests. Some vertebrates, for example, selected species of bats and amphibians, may be specially protected by the law.

2.5.4 Humans

Many biological experiments are carried out where the subject is one of the students in the school. It is not acceptable to carry out investigations where students are given cigarettes or alcohol. All subjects should give their consent to any activities before they are undertaken. It is also prudent for parental permission to be sought after your IB Biology Teacher has approved your investigation.

When carrying out investigations into fitness and heart rate, it would be essential to determine that the subject was fit and healthy and did not have any medical problems such as asthma, which could put them at risk. In addition, careful consideration should be given before any investigations are conducted where the fellow students may expose the subjects to judgmental comments. This includes exercise experiments, reaction times, memory tests and testing for various genetic phenotypes.

Some investigations compare different age groups. Again, great care must be taken to ensure that none of the subjects are upset by the results of the investigation, for example, slow reaction times or reduced memory in elderly people. Any investigation that requires subjects to fill in a questionnaire must ensure that none of the questions are of a personal nature, there is always an option not to answer any or all of the questions if the person so desires and confidentiality is protected.

2.6 Developing a method for collection of data

The definition of 'sufficient relevant data' depends on the context of your investigation. Your planned investigation should anticipate the collection of sufficient data so that the Research Question can be suitably addressed and an evaluation of the reliability of the data can be made. If error analysis involving the calculation of standard deviation is to be carried out, then a sample size of at least five is needed. The data range and amount of data in that range are also important.

For example, when trying to determine the optimum temperature of a typical mammalian enzyme, using a range of temperature values between 20 and 60 °C would be insufficient. Using a range of values between 10 and 70 °C would be better, but would also be insufficient if only three different temperature values were tested in that range.

2.6.1 Tests

Biological experiments are usually made of a number of tests. For example, you might design an experiment that measures the rate of water loss via osmosis from 50 mm strips of potato. Identical strips are placed in sucrose solutions with the following molarities: 1.00 mol dm^{-3}, 2.00 mol dm^{-3}, 3.00 mol dm^{-3}, 4.00 mol dm^{-3} and 5.00 mol dm^{-3}. This experiment thus consists of five tests.

2.6.2 Replication

The results of a single test are not meaningful in a Biological or Scientific sense since the result may have been anomalous or highly unusual. Biological tests, are usually replicated at least three, preferably five times, under identical experimental conditions.

This can be carried out by one IB Biology student repeating the experiment several times, or by several IB Biology students repeating the same experiment. The results can be compared to establish whether any systematic errors are present and then processed statistically to take into account random errors.

2.7 Guidelines for the Design of IB Biology Individual Investigations

Below are some generic or general questions that will be useful in guiding you in the design of IB Biology experimental investigations, especially the Group 4 Project.

- What is the specific problem or aim of the investigation?
- Is there a relevant biological hypothesis (qualitative or quantitative), with an associated explanation, that can be tested)?
- What method can be used for investigating this problem or testing this hypothesis? For example, if it is an ecological investigation, what classification keys do I need to use?
- If appropriate, what statistical approach or sampling technique are you going to employ?
- Should a 'pilot' study be performed to assess the proposed method?
- What biological principle(s) and assumption(s) , if any, are applicable to the chosen method?
- Identify the independent and dependent variables.
- How will the independent variable be manipulated and how will the other variables be controlled to ensure a 'fair test'?
- What are the controls, if any, in the investigation?
- Outline the method, apparatus (including precision), materials and organisms, where appropriate, you plan to use in the investigation.
- What type of observations and data will be collected and how will measurements be made and recorded?

The following section briefly outlines a possible response to an IB Biology Teacher requesting students to design an investigation into an enzyme of their choice. This may form the basis for a practice Individual Investigation.

Aim

To investigate the effect of temperature on the activity of potato catalase.

Research Question

What is the relationship between temperature and the rate of reaction between hydrogen peroxide and potato catalase?

Introduction

Hydrogen peroxide is an oxidising agent (oxidant) formed in cells as a secondary product of several metabolic reactions. The enzyme catalase, found in all cells, hydrolyses hydrogen peroxide to water and oxygen:

hydrogen peroxide → water + oxygen

$2H_2O_2$ (aq) → $2H_2O$ (l) + O_2 (g)

Catalase thus plays an important role in the detoxifying, radical scavenging process that occurs in cells with high metabolic activities, for example, liver cells.

Hypothesis (and variables)

It is predicted that at low temperatures the catalytic decomposition of hydrogen peroxide by catalase will be slower and that at high temperatures the enzyme will be denatured and there will be no activity.

Denaturation occurs when the high temperature modifies or disrupts hydrogen bonds and other intermolecular forces or non-covalent interactions thereby altering the shape of the active site of the enzyme and prevents enzyme-substrate interaction.

A decrease in temperature is accompanied by a reduction in the mean kinetic energies, and hence diffusion rates, of substrate and enzyme molecules. More importantly, there is a decrease in the number of enzyme-substrate pairs that have a combined kinetic energy equal to or in excess of the activation energy.

An exponential relationship between temperature and rate (up to the denaturation point) is predicted. Specifically every 10 °C rise should result in a doubling of the rate (up to the denaturation point) since the number of enzyme-substrate pairs that have a combined kinetic energy equal to or in excess of the activation energy is doubled. *(Reference to the lock and key hypothesis of enzyme action in your textbook would be most helpful.)*

Method

The method should ideally be written very briefly in the past tense and in the passive voice, and should clearly allow another IB Biology student to replicate your experiment(s) and obtain the same results (within experimental error). Suitable annotated diagrams should be included where appropriate. The nature of any chemicals should be clearly specified, for example, volume and concentration (if a liquid or solution); apparatus should be clearly described including any associated uncertainty and the specification of any instruments identified, for example, a colorimeter. Any safety or environmental precautions should be included. Any dilutions you have performed should be clearly described. You should not give details for common Biological procedures, for example, calibration of a pH probe with a buffer.

Example 1

0.1 gram of the potato (King Edward variety) macerate were transferred to a test tube (diameter 1.00 cm) containing 10.0 cm^3 of 10% by volume aqueous hydrogen peroxide solution. The maximum height of foam in the test tube was measured using a plastic ruler with a scale in cm. The uncertainty in the ruler was ± 0.2 cm.

Any deviations from the original method should be documented and accounted for. The number of readings or measurements should be specified together with time factors, such as the duration of the experiment and the interval between readings (if applicable). The number of repeats or replicates for each measurement should also be stated.

Example 2

The experiments involving the potato macerate were repeated but the reaction performed inside small 25 cm^3 plastic graduated syringes. This improved the accuracy of the results since loss of oxygen to the atmosphere was prevented.

Listed in Figure 203 is a summary of what you need to do to score well in the Personal Engagement criterion.

Assessment criteria	Evidence required	What you must do
Identifies a topic, a relevant and fully focused Research Question is clearly described. Relevant and appropriate background information is provided to increase the understanding of the context of the investigation	A topic, a relevant and fully focused Research Question are described	Identify the topic and state the Research Question, for example: The investigation will determine how the 'independent variable' affects the 'dependent variable'. The following will be kept constant: controlled variable 1, controlled variable 2,… and the method for measuring the dependent variable.
	Relevant and appropriate biological background is included	Give relevant background information including a summary of the literature and a testable hypothesis and perhaps reference to a relevant biological model or theory and identifies the main processes involved and the likely causes at the biochemical level. Makes quantitative predictions in words and in the form of graph.
	State the relevant variables explicitly	Classifies and tabulates key variables. Independent variable (one only) Dependent variable (one only) Processed variables (one or more) Controlled variables (typically more than one) Identify variables over which little control can be exerted
Designs a methodology that allows relevant, reliable and sufficient data to be collected. Shows full awareness of safety, ethical and environmental issues	Appropriate choice of chemicals, materials and apparatus	List of all biological materials and specimens (where appropriate) and apparatus and instrumentation (state manufacturer (where appropriate)) with specifications including precision/random uncertainty. A labelled and cross-sectional diagram of any set-up of apparatus with a justification and explanation for the methodology including any statistics..
	Effective control and manipulation of variables	**A clear description of the method which** • describes how the independent variable is to be varied and measured accurately • describes how the dependent variable is measured • includes a logical sequence of steps to be taken and their rationale • includes details of any modification or adaptations of standard methods and justification for their use • includes a clear account of how and why the controlled variables are kept constant. • includes a statement of how the plan will produce relevant, reliable and sufficient results. • includes a statement of how the plan will produce accurate and precise results. • draws up bank results tables and graph axes and describes how the raw data will be processed • includes where appropriate, an explanation of the biological principles behind your plan. • includes control experiments should be described (if relevant) • includes a detailed statement of how the plan ensures an ethical and safe investigation (risk assessment) that minimises the impact on the organisms and the environment. • describes safety precautions taken to keep risks to a minimum • describes how chemicals are to be disposed of and stored
	Appropriate number and range of readings to be taken	State you will take measurements for at least five values of the independent variable. Also consider what the reading at zero will be. State the range of values for the independent variable i.e. the lowest and highest values and the size of the increment. State the number of repetitions for each value of the independent variable.

Figure 203 Summary of the Exploration criterion

This criterion assesses the extent to which the student's report provides evidence that the student has selected, recorded, processed and interpreted the data in ways that are relevant to the research question and can support a conclusion.

The descriptors in the following table will be used by your teacher to allocate a mark for your performance in this criterion:

MARK	DESCRIPTOR
0	The student's report does not reach a standard described by the descriptors below.
1–2	The report includes insufficient relevant raw data to support a valid conclusion to the research question. Some basic data processing is carried out but is either too inaccurate or too insufficient to lead to a valid conclusion. The report shows evidence of little consideration of the impact of measurement uncertainty on the analysis. The processed data is incorrectly or insufficiently interpreted so that the conclusion is invalid or very incomplete.
3–4	The report includes relevant but incomplete quantitative and qualitative raw data that could support a simple or partially valid conclusion to the research question. Appropriate and sufficient data processing is carried out that could lead to a broadly valid conclusion but there are significant inaccuracies and inconsistencies in the processing. The report shows evidence of some consideration of the impact of measurement uncertainty on the analysis. The processed data is interpreted so that a broadly valid but incomplete or limited conclusion to the research question can be deduced.
5–6	The report includes sufficient relevant quantitative and qualitative raw data that could support a detailed and valid conclusion to the research question. Appropriate and sufficient data processing is carried out with the accuracy required to enable a conclusion to the research question to be drawn that is fully consistent with the experimental data. The report shows evidence of full and appropriate consideration of the impact of measurement uncertainty on the analysis. The processed data is correctly interpreted so that a completely valid and detailed conclusion to the research question can be deduced.

Guiding Questions

- ***Has the student selected and recorded raw data, including uncertainties and qualitative observations where relevant, that allow for coherent analysis?***
- ***Has the student selected (an) appropriate method(s) for analysing the data?***
- ***How successfully has the student analysed the data?***
- ***Is the analysis of data accompanied by evidence of an appropriate consideration of uncertainties?***
- ***How successfully has the student interpreted their analysis to form a conclusion?***
- ***Has the student drawn a conclusion that is relevant to the purpose of the investigation?***
- ***Has the student compared their conclusion to accepted scientific theory?***

Ideally, you should work on your own when collecting raw data. When data collection is carried out in groups, the actual recording and processing of data should be independently undertaken if this criterion is to be assessed. Recording class or group data is only appropriate if the data-sharing method does not suggest a presentation format for you and the other students. Pooling data from a class is permitted where you and the students have independently organised and presented the data. For example, you may have placed it on a real or virtual bulletin board.

3.1 Recording raw data

Raw data is the actual data you measured and may include associated qualitative data (observations). It is acceptable for you to convert handwritten raw data into word-processed form. The term 'quantitative data' refers to numerical measurements of the variables associated with the investigation. Associated qualitative data are considered to be those observations that would enhance the interpretation of your results.

Uncertainties are associated with all raw data and an attempt should always be made to quantify uncertainties. For example, when you say there is an uncertainty in a stopwatch measurement because of reaction time, you must estimate the magnitude of the uncertainty. Within your tables of quantitative data, columns should be clearly annotated with a heading, units and an indication of the uncertainty of measurement. Your uncertainty need not be the same as the manufacturer's stated precision of the measuring device used. Significant digits in your data and the uncertainty in it must be consistent. This applies to all measuring devices, for example, digital meters, stopwatches, and so on. The number of significant digits should reflect the precision of your measurement.

There should be no variation in the precision of your raw data. For example, the same number of decimal places should be used. For data derived from processing raw data (for example, means), the level of precision should be consistent with that of your raw data.

Your recording of the level of precision would be expected from the point where you take over the manipulation. For example, you would not be expected to state the level of precision in a solution prepared for you a technician. You should not be told how to record the raw data. For example, you should not be given a pre-formatted table with columns, headings, units or uncertainties.

3.1.1 Recording observations

The accurate and careful recording of observations, qualitative data or descriptive data is an important part of the assessment of IB Biology coursework. Be aware that simple observations may be the starting point for more detailed investigations involving the collection of quantitative data. Quantitative observations refer to measurements: numbers and associated units.

Description of chemical changes

Some IB Biology investigations may involve visible chemical changes during the experiment, for example, in the colour or clarity of solutions in food tests or enzyme investigations and in the colour of hydrogencarbonate (bicarbonate) indicator in photosynthesis investigations.

Other changes that may need to be described or recorded, for example, by drawing or photography, are the difference between a de-starched leaf and a normal leaf when tested with aqueous iodine solution and changes in anhydrous cobalt(II) chloride paper during transpiration investigations.

However, when using solutions ensure they are thoroughly mixed before recording your observations. If solutions are not mixed then the reaction will only occur at the boundaries giving a banded appearance. This can be achieved by gently tapping the tube against a finger, gently shaking the tube from side to side or stirring the mixture with a clean glass rod.

Inexperienced IB Biology students often use samples of Biological reagents that are far too large. Solids and pure liquids contain considerably greater amounts of substances than solutions. Therefore, use them sparingly. You will see a small amount dissolve more easily and quickly than a larger amount. A large amount may be in excess and thus hide the underlying reaction by forming a suspension.

When recording colour changes ensure that the colour is recorded before, during and after the reaction has occurred. In addition, make sure the correct direction of the colour change is indicated. If a solution loses its colour then state

that the solution was decolourised, rather than it went clear. Never describe a solution as white, it will be either a precipitate (the substance is insoluble) or suspension (the substance is slightly soluble). Be careful when assessing the colour of a precipitate: a white solid at the bottom of a test tube or boiling tube containing highly coloured solution often looks coloured.

If a gas is produced then its colour and odour (if any) should be recorded. Comments should be recorded about the rate of evolution of the gas, for example, as bubbles or thickness of froth layer. Any heat changes should also be recorded. A cooling effect is observed if the reaction is endothermic while an increase in temperature indicates an exothermic reaction.

There are circumstances where you may not be sure if a chemical change has occurred. In these instances use a control where a chemical reagent or reactant is replaced with distilled water for comparison. If no observable reaction occurs then the chemical is responsible for that change.

Avoid contaminating your reaction mixture. If, for example, you want to test the pH of a Biological solution then use a glass rod to remove a small drop and place it on indicator paper or into indicator solution. Do not add the indicator solution or insert the indicator paper into your reaction mixture. Never place anything into a reagent bottle containing a liquid or solution. Pour from it into a second container and insert equipment or apparatus into the secondary container.

The IB Group 4 assessment criteria stress appropriate raw data should be collected. It may not be apparent, particularly if the piece of assessed coursework is an open-ended investigation, what observations are relevant and therefore appropriate to record. However, as an IB Biology student you are expected to develop a capacity for discrimination, choosing which observations to include, and which to ignore.

Although observations should only be based on what is clearly seen, it helps to be aware of what changes are or might be expected during an investigation. This is especially useful when changes are not immediately obvious (for example, during growth investigations) or when the changes are rapid (for example, measuring the pulse rate before and after exercise). Where possible, and appropriate, prepare suitable tables. If no changes occur then it is not acceptable to write that 'there were no changes'. Instead you should comments that the contents of the reaction vessel remained the same, for example, the contents of the test tube remained green and clear.

Description of structures

You may be asked to observe an unfamiliar Biological specimen, or part of a Biological specimen, and to describe distinct features. Unfamiliar Biological specimens will share certain features with specimens which you have observed in your IB Biology class. It is, however, important to observe and record features that are actually present, rather than what might be expected. Observable features could include shape, colour, texture and size.

For example, *Cepaea nemoralis*, the banded wood snail (see *Figure 301*), is found in many parts of Europe and was introduced to the United States. The dimensions of the snail's shell should be measured and its colour bands described. The shell colours vary from yellow, red, pink to olive and the number of bands varies from one to five. The number of whorls should also be recorded.

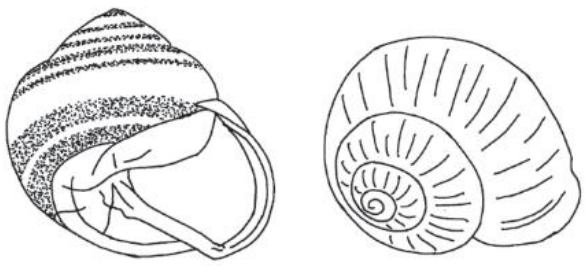


Figure 301 Drawing of Cepaea nemoralis shells

Structures which have a clear biological function are particularly suitable for description. The observation of fine detail might require the use of a hands lens or low power microscope. Photographs and Biological drawings are used to record descriptions of Biological structures.

3.2 Comparison of features

Comparisons in Biology are often based on external features which are usually quite visible to the observer. Both similarities and differences in these functions often reflect corresponding similarities and differences in embryological development, function habitat and other characteristics associated with the organism.

Be aware that organisms having a very similar classification may have obviously different features, due to genetic and environmental variation. In addition, organisms which are not closely related may share several common features due to evolutionary convergence.

The features which are chosen for comparison should be clearly evident, even though such features may only be fully revealed using a hand lens or low power microscope. It is entirely possible that a particular feature might be missing, due to either physical damage to the specimen, poor preparation or simply length of storage. If you suspect this problem, then, where possible, more than one specimen of the same type should be examined.

Comparisons should involve features which are likely to be Biologically significant, that is, features which have some recognisable role in the organism's functioning. For example, in a comparison of the microscopic cross sections of a dicotyledon root and shoot, the relative distributions of vascular (transport) tissues (xylem and phloem) and the presence or absence of root hairs are significant, but any differences in colour of the sections may have resulted from the use of different stains and is probably not Biologically significant.

One common method of presenting systematic comparisons is in the form of a table (see *Figure 302*), with descriptions for each specimen next to a corresponding description for the same features. Comparison can also be made by separate written accounts for each specimen, or by annotated Biological drawings. Regardless of the method chosen, it is important to emphasise equivalent structures for each specimen and to convey clearly whether differences or similarities are being described.

FEATURE	***Lamium album*** **(white dead nettle)** **(insect-pollinated)**	***Poa species*** **(Meadow grass)** **(wind-pollinated)**
Petals	Large, conspicuous, white	Absent (bract present)
Nectary	Present	Absent
Scent	Present	Absent
Anthers	Small, enclosed within flower	Smooth, light and relatively small.
Filament	Short, rigid	Long, flexible.
Stigma	Relatively small, enclosed within flower	Feathery, large surface area

Figure 302 Comparison of an insect-pollinated flower and a wind-pollinated flower.

(*Apart from birds, other pollinators of plants include bats and even slugs and snails; a bract is a leaf from the axil from which a flower or floret develop. An axil is the angle between the leaf and its stem*).

3.2.1 Guidelines for Drawing Biological Specimens

Biological specimens may be living or dead, whole or in part, microscopic or macroscopic, real or photographed. In any case the following general guidelines should apply:

- Use a good quality, sharp pencil of a hardness that suits you – HB is usually the best.
- Draw on good quality plain paper which is capable of withstanding rubbing out.
- Ensure the diagram, along with all labels and annotations, will fit comfortably on the page.
- Your drawing should be relatively large. The more details you need to include, the larger your drawing should be.
- Make large, clear line drawings without the use of ink or coloured pencils.
- Make single pencil lines without the use of ink or shading.
- Keep the drawing simple by providing only an outline of all the basic structures.
- Draw accurately and faithfully what can be seen. Never draw anything you cannot see, even if you expect it be present. Never copy from text books.
- Draw individual parts of a specimen in strict proportion to each other.
- Provide suitable headings which clearly indicate the nature of the drawing. For microscopic drawings, the section (transverse section (T.S.), longitudinal section (L.S.)) should be stated.
- State the magnification, scale or actual size of each specimen. For a small biological specimen your magnification should be greater than three times.
- $$\text{Magnification} = \frac{\text{Length or width of drawing}}{\text{Length or width of specimen}}$$

  Indicate by means of two parallel lines in your drawing where you have taken your measurement.
- Label fully all biological features keeping labels away from the diagram, and never label on the actual drawing.
- Avoid crossing label lines and, if possible, arrange labels vertically, one beneath the other.
- Use annotations (notes added to labels) if at all possible. In particular, try to relate structure to function.

Figure 303 shows a correctly drawn and labeled drawing of palisade cells under high power.

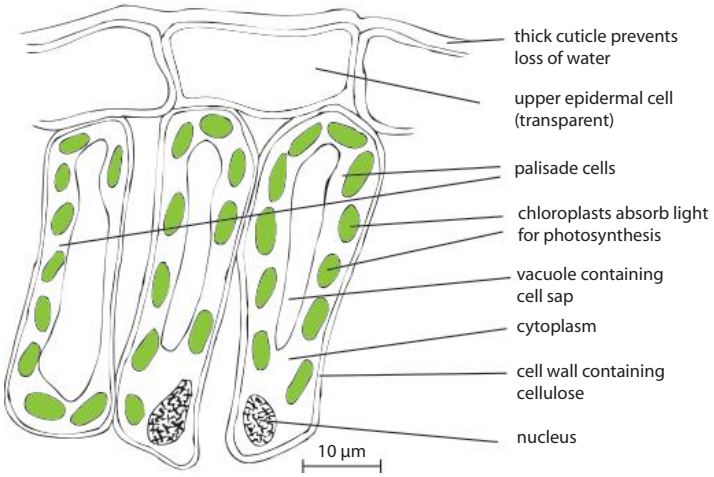


Figure 303 High-power drawing of palisade cells (×1000)

3.2.2 Diagrams

Diagrams are frequently used to illustrate the method or results of an IB Biology investigation. Their main purpose is often to show the relative positions of different pieces of apparatus, chemicals and Biological materials.

- Diagrams should be greatly simplified and should conform to a scale.
- The purpose of diagrams is to avoid writing a lengthy method.
- Pencil should be used for drawing and ink for the labels.
- Colours should not normally be used.
- Diagrams should be drawn in two-dimensional cross section, as shown in *Figure 303*.
- Liquid levels should be drawn horizontal with meniscuses at the sides of the containers. (A meniscus is the curved surface of a liquid).
- Use a ruler.

Note the use of additional comments in *Figure 304* which shows the results of an investigation into photosynthesis.

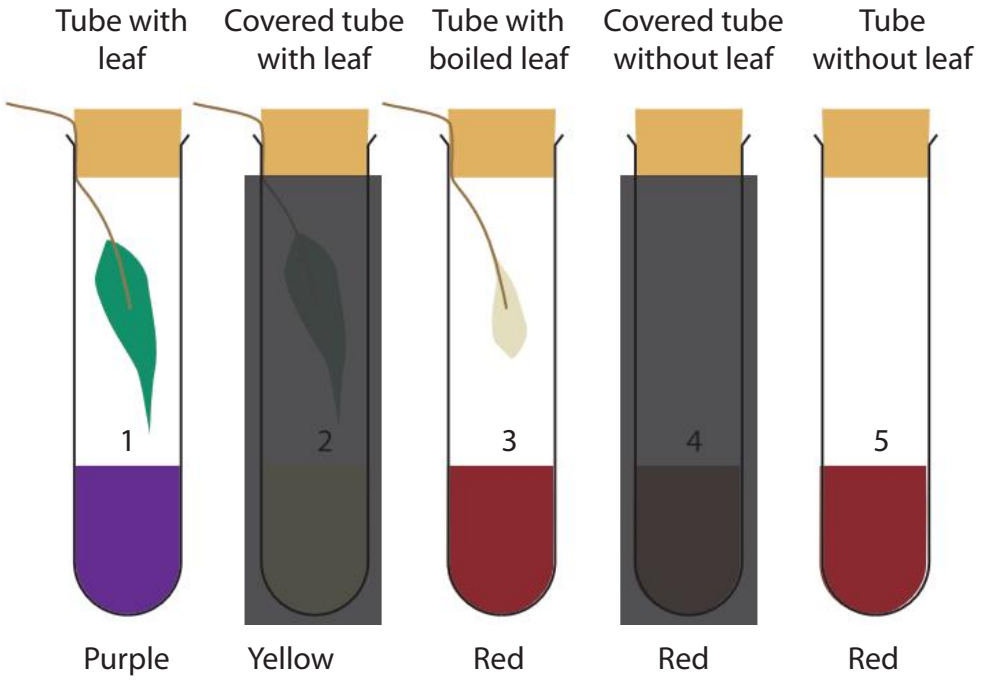


Figure 304(a) The results of an investigation involving carbon dioxide uptake and loss by leaves, the covering on Tubes 2 and 4 is black plastic.

3.3 Data

You will frequently be involved in collecting raw data during an IB Biology Investigation. Raw data is data collected by measuring, observing (e.g. behaviour), drawing, photographing, data-logging or counting in the laboratory or outside the laboratory during field work. This data may be simply be used to describe nature, for example, a population of organisms, or for testing an experimental hypothesis.

3.3.1 Types of Data

Data for IB Biology investigations are produced from two main forms of observation: natural and experimental.

Natural Observations

These are mostly concerned with recording similarities and differences between cells, organs, organisms, samples and populations. They may involve recording measurements or counting.

Experimental Observations

IB Biology Investigations frequently involve recording the values of properties, termed **variables**, that change during an experiment. A single value of a variable is sometimes known as a **variate** and a particular group of variates may be called a variate set.

Ecological investigations include observation *in vivo* (for example, use of quadrats and mark, release and recapture techniques), field manipulation, (for example, the effect of removing two competing species from a field situation), laboratory manipulation, (for example, choice chambers to investigate behavioural responses and mathematical modelling, such as the modelling of exponential or logistic growth using a spreadsheet such as Excel. The term *in vivo* refers to living organisms whereas the term *in vitro* means in the laboratory. Refer to *Figure 304*(b).

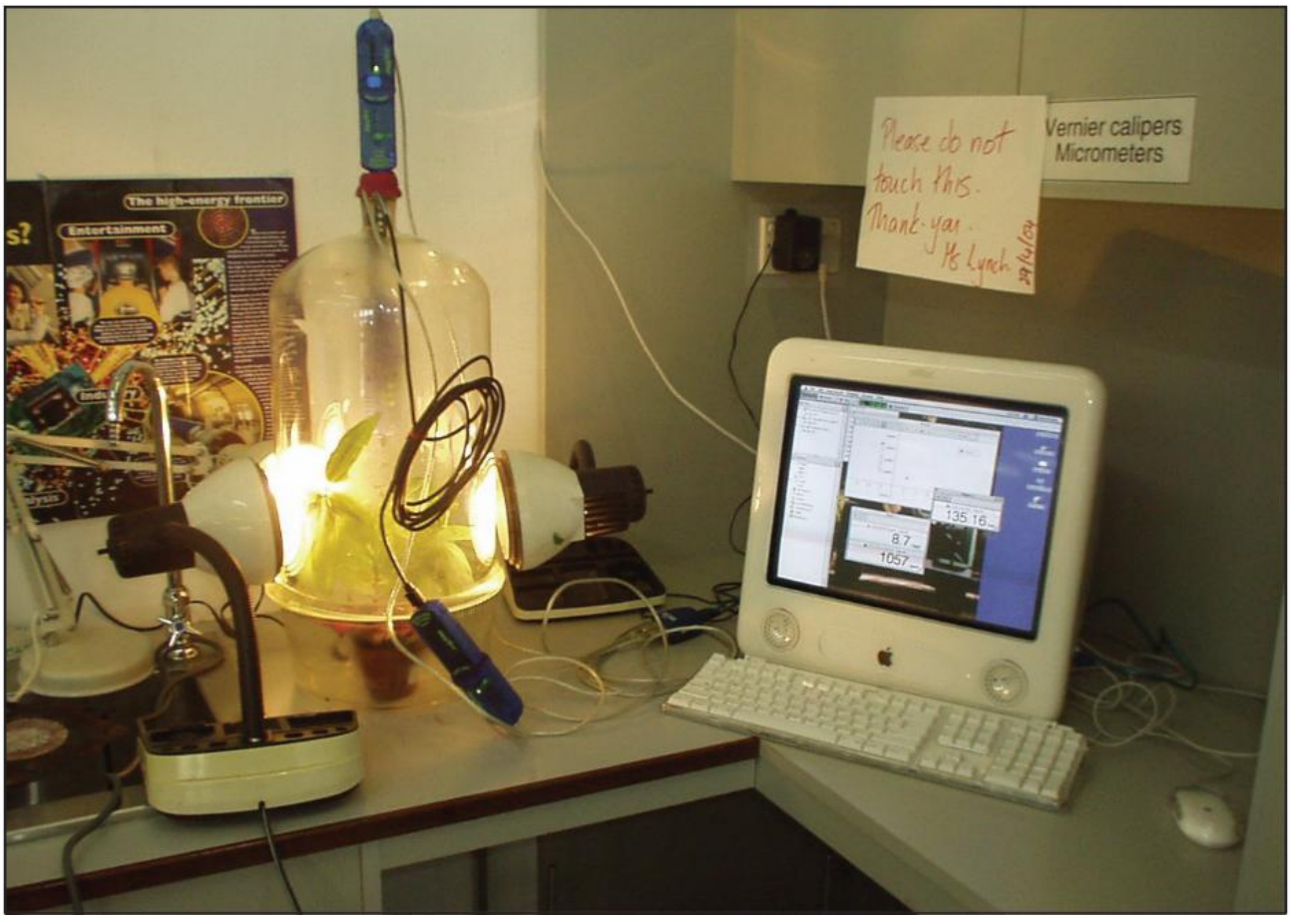


Figure 304(b) Some equipment used to make observations and collect data

3.4 Types of variables

3.4.1 Qualitative

Qualitative variables are non-numerical and since they are descriptive, numbers are not attached to them. Examples are colour and shape. Such variables are often called attributes and such observations are placed in categories. In *Figure 305* (a) the categories are qualitative and the frequencies of the coloured beaks are quantitative.

Green beak	115
Red beak	24
Blue beak	78

Figure 305 (a) Colour of beaks in a sample of parrots

3.4.2 Rankable

Rankable variables are semi-quantitative and there is some meaningful order present so that the data can be ranked. For example, scoring the intensity of a Benedict's test positive result. When describing the distribution of an organism it may be helpful to use an abundance scale and allocate values to your observations. See *Figure 305(b)*.

Rare	*1*
Frequent	*2*
Common	*3*
Very abundant	*4*

Hare	3
Rabbit	4
Fox	1
Badger	1
Grass snake	2

Figure 305 (b) Abundance scale

3.4.3 Quantitative Data

Quantitative or measured data, or observations described by numbers, are of two types: discrete (discontinuous) or continuous.

Discrete (Discontinuous)

When data are obtained by counting, they will have a limited or fixed number of whole numbers. An example would be the number of petals on a flower or the number of plants inside a quadrat. There cannot be any intermediate or decimal values.

Continuous

When the data are obtained by measuring, for example, length, mass (see *Figure 306*), period of time , they can take any value in a continuous interval (within limits set by the accuracy of the measuring scale). Frequently such data will posses decimal points.

MASS OF DRIED SUNFLOWER SEEDS / g						
0.92	1.46	1.60	1.12	1.44	1.30	1.26

Figure 306 Sunflower seed data

The last digit of the measurement should imply or indicate the precision, that is, the limits on the measurement scale where the true measurement lies. For example, a measurement of 0.920 g has implied limits of 0.915 – 0.925, that is, 0.920 g ± 0.005 g.

Frequently Biological data is composed of a number or frequency of observations falling within particular categories or classes, resulting in a grouping of data. The categories or classes may belong to any of the three types of variable, namely, qualitative (non-numeric {see *Figure 307*}), rankable/semi-quantitative (see *Figure 308*) or quantitative (numeric) (see *Figure 309*). The other variable is the number of observations falling within the particular categories.

Qualitative

Tabby cats	44
Siamese cats	2
Burmese cats	5
Tortoise-shell cats	6
White cats	1

Figure 307 The number of pure bred cats in a given area of Singapore

Rankable/semi-quantitative

1990	0.001%
1991	0.009%
1992	0.050%
1993	0.099%
1994	0.101%

Figure 308 The percentage of people infected with HIV in a large city

Quantitative

Frequency distributions are often based on continuous variables, that is, variables which are clearly divided into clearly defined ranges or bands.

LENGTH / mm	NUMBER
3.01 – 6.40	32
6.41 – 9.80	44
9.81 – 12.20	29
12.21 – 14.60	11

Figure 309 A sample from a population of marine organisms

3.5 Organising and presenting biological data in tables

3.5.1 Independent and Dependent Variables

When performing a Biological investigation of an experimental nature all of the variables except one are kept constant and this one is varied to determine its effect. For example, to find the effect of light on the rate of photosynthesis in an aquatic plant, the light intensity could be varied, for example, by varying the light source distance between 10 cm and 30 cm in steps of 5 cm. For each light intensity, the rate of photosynthesis would be measured by counting the number of bubbles of oxygen-enriched air released from the leaves of the plant (see *Figure 310*).

A table is therefore constructed during the planning stage of the investigation. The table will have two columns: the first, by convention, contains the independent variable (which is being varied or manipulated) and the second the dependent variable (which is being measured).

DISTANCE BETWEEN LAMP AND PLANT / cm	RATE OF PHOTOSYNTHESIS (NUMBER OF BUBBLES) / min^{-1}

Figure 310 The headings for a data table for a photosynthesis investigation

The distances between the lamp and plant are entered at the start of the experiment and as the experiment is performed the appropriate values of the rate of photosynthesis can be recorded. (See *Figure 311*). Note that the distances are continuous data and rates are discontinuous or discrete data.

Distance between lamp and plant / cm ± 0.05 cm	Rate of photosynthesis (number of bubbles) / min^{-1}
(Independent variable)	(Dependent variable)
30.00	2
25.00	6
20.00	17
15.00	20
10.00	35

Figure 311 A partially completed data table for a photosynthesis investigation

This set of raw data is totally inadequate and the rate of photosynthesis should be measured at least three times for each light intensity. Such experimental repetitions are known as replicates or trials.

Often class results can be pooled or shared with each group or pair of IB Biology students performing the experiment at one of the light intensities. The random errors inherent in the experiment can be reduced by calculating the mean values. The calculation of means is an example of data processing or data transformation. Processed data by convention is always placed on the right hand side of the data table.

Uncertainties or errors in the distance measurements should be included in the column headings together with the appropriate SI units. Since the rates are exact or counted numbers there is no associated uncertainty or error. Note that the mean values have the same number of decimal points and significant figures as the measurements they were calculated from. Note that only true numbers should be used to record data in a table, for example, write 0.25 rather than .25. Missing values should be recorded as '–' and zero values as '0'. As shown in the *Figure 312* caption every table should have an informative title.

Distance between lamp and plant / cm ± 0.05 cm	Rate of photosynthesis (Number of bubbles of oxygen) / min^{-1}			
	Trial 1	Trial 2	Trial 3	Mean value
30.00	2	8	5	5
25.00	6	10	11	9
20.00	17	13	18	16
15.00	20	28	31	26
10.00	35	34	35	35

Figure 312 The results of an experiment to investigate the number of bubbles produced by an aquatic plant at different light intensities.

Tables should be kept as simple and clear as possible. Ideally, each table of Biological results should show one relationship, in this example, it is the relationship between light intensity and the rate of photosynthesis. Graphs are often drawn from tables of data and conclusions about Biological relationships are often drawn from tables of data. This table clearly shows there is a proportional relationship between light intensity and photosynthetic rate, or an inverse relationship between distance of the lamp from the plant and the rate of photosynthesis.

3.5.2 Interdependent Variables

During an IB Biology Investigation you might want to establish whether there is any connection between two variables, that is, whether the relationship is interdependent. In such examples, you must ensure that the two variables relate to one particular organism (see *Figure 313*). Each of the five shells are therefore numbered and each measured in turn for the two variables. The two variables relate clearly to one particular species.

Shell number	Height / mm	Width / mm
1	23	18
2	24	18
3	24	17
4	15	19
5	22	15

Figure 313 Heights and widths of the shell of a particular species of mollusc

3.5.3 Frequency Distributions

For frequency data distributions, the data must be arranged so that the frequency of each category is known. If a continuous variable is under investigation, it is important to ensure that the classes or groups are mutually exclusive, that is, it must be clear into which class each observation falls (see *Figure 314*).

Length of a particular species of limpet / mm							
5.55	6.60	7.25	8.82	9.92	10.11	5.71	4.02
7.24	9.08	11.21	6.76	5.82	7.01	11.62	7.69
9.12	6.99	4.39	6.93	7.83	6.74	11.01	11.84
7.27	7.12	7.08	6.65	9.85	6.63	4.30	12.00
12.00	9.26	9.24	7.33	7.33	6.30	7.02	8.73

Figure 314 Lengths of a particular species of limpet

The data needs to be grouped into various classes defined by certain length bands. A good 'rule' is that the number of classes should be 5 times the logarithm (to the base 10) of the number of observations. In this example, there are 40 observations, so the number of classes should be $5 \times \log 40 = 5 \times 1.6 = 8$.

Next the class size has to be determined. The smallest value is subtracted from the largest value and the result is divided by the number of classes required minus one. The longest limpet was 12.00 mm and the shortest was 4.02 mm, so (12.00 mm – 4.02 mm) = 7.98 mm.

This value is then divided by (8 –1) = 7, so the class size is 7.98 mm/7 = 1.14 mm. Starting with the shortest limpet, 4.02 + 1.14 (the class interval) = 5.16, so the first class is 4.02 to 5.16 mm. The next class will be 5.17 mm to 6.31 mm etc. (see *Figure 315*). The use of a tally chart with its associated gate scoring is a simple way of establishing the frequency for each class.

Class	Tally chart	Class frequency
4.02 – 5.16	✓ ✓ ✓	3
5.17 – 6.31	✓ ✓ ✓	3
6.32 – 7.46	✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓	18
7.47 – 8.61	✓ ✓	2
8.62 – 9.76	✓ ✓ ✓ ✓ ✓ ✓ ✓	7
9.77 – 10.91	✓ ✓ ✓	3
10.92 – 12.06	✓ ✓ ✓ ✓ ✓	5
12.07 – 13.21		0
TOTALS		41

Figure 315 Tally chart displaying data concerning limpet length

3.5.4 Contingency Tables (Extension Material)

An IB Biology ecological investigation might involve examining an organism for two characteristics and classifying each characteristics into a number of categories. The data can be displayed in the form of an array or matrix known as 2 × 2 contingency table.

(*The use of contingency tables is not mandated by the IB Biology programme and students will not be penalised if they are not used. However, a contingency table does facilitate the application of a chi-squared test of association*).

The 2 × 2 contingency table is the is the simplest type and occurs when two sets of observations have two categories each so that there are four (two multiplied by two) possible combinations. *Figure 316* shows the general format for a 2 × 2 contingency table and *Figure 317* shows a specific Biological example of a 2 × 2 contingency table.

		FIRST CATEGORY		ROW TOTALS
		A	B	
SECOND CATEGORY	A	cell 1	cell 2	
	B	cell 3	cell 4	
COLUMN TOTALS				GRAND TOTAL

Figure 316 General format of a 2 × 2 contingency table

		FIRST CATEGORY		ROW TOTALS
		BANDED snails	UNBANDED snails	
SECOND CATEGORY	Oak wood	40	120	160
	Under bridges	50	20	70
COLUMN TOTALS		90	180	**230**

Figure 317 A specific example of a 2 × 2 contingency table for Biological data

3.6 International System of Units (SI)

3.6.1 SI Base Units

The five basic quantities or base units of the SI system (see *Figure 318*) commonly used in Biology are: the metre for measuring length, the kilogram for measuring mass, the second for measuring time, the mole for measuring the amount of substance and the kelvin for measuring temperature. The two other base units of the SI system are the Ampere or Amp (the unit of electric current) and the candela, (a measure of light intensity). (Do not use the word 'amount' to refer to a mass, a number or a volume).

DIMENSION	SYMBOL	SI UNIT NAME AND SYMBOL
Length	*L*	metre, m
Mass	*m*	kilogram, kg
Time	*t*	second, s
Temperature	*T*	kelvin, K
Amount of substance	*n*	mole, mol

Figure 318 The five commonly encountered physical quantities in Biology

The size of the units given above is not always the most suitable for certain measurements and decimal multiples are frequently used as shown below. A set of common SI prefixes and associated symbols is given in *Figure 319*. (*The asterisk indicates the prefixes that are unlikely to be used in Biological investigations*).

FRACTION	PREFIX	SYMBOL	MULTIPLE	PREFIX	SYMBOL
10^{-1}	deci	d	10^{3}	kilo	k
10^{-3}	milli	m	10^{6}	mega	M
10^{-6}	micro	μ	10^{9}	giga*	G
10^{-9}	nano	n	10^{12}	tera*	T

Figure 319 SI prefixes and associated symbols

3.7 Additional Comments and Guidance

3.7.1 Mass and Weight

It is important to distinguish between mass and weight: mass is a measure of the quantity of matter present, whereas the weight of an object is the force (the 'push') it exerts on a supporting object. This force is generated by the Earth's gravitational field. Mass is measured in kilograms (kg) and weight is measured in newtons (N). Invariably in Biology what you refer to as weight is actually mass.

3.7.2 Volume

One physical quantity that is very important in Biology, is volume, which is derived from length and in the SI system is expressed in cubic metres, m^3. However, the usual unit used in Biology is the cubic decimetre (1 dm^3), which is commonly termed a litre (symbol l). There are 1 000 dm^3 in one cubic metre (1 m^3). Each decimetre can be divided into 1 000 cubic centimetres (cm^3).

(*Note that millilitres (mL) is widely used in many countries and can be regarded (to a good approximation) as interchangeable with cm^3, but it is not an SI unit and cubic centimetres (cm^3) should be used instead.*)

3.7.3 Length

Strictly speaking, all measurements of length should be expressed in metres (m) but often such measurements are expressed in centimetres (cm) and millimetres (mm), where 100 cm = 1 m and 1000 mm = 1 m.

3.7.4 Mass

Strictly speaking, all masses should be expressed in kilograms, but often such measurements are expressed in grams or milligrams. 1 kg = 1000 g; 1g = 1000 mg

3.7.5 Temperature

Strictly speaking, all temperatures recorded during Biological investigations should be expressed as thermodynamic temperatures in kelvin, but temperatures are invariably expressed in Celsius. Temperatures in Biology need only be expressed in kelvin if calculations are being performed with the various 'gas laws'.

However, note that the value of 0 °C is arbitrary and does not represent the absence of heat energy. It is also incorrect to state that a temperature of 50 °C is twice as hot as 25 °C. Such statements are only possible using the kelvin scale.

The numerical value of an thermodynamic temperature expressed in degrees Celsius is given by: $t/°C = T/K - 273.15$ where t is the numerical value of a Celsius temperature and T is the thermodynamic temperature in kelvin. It follows that the degree Celsius is equal in magnitude to the Kelvin, thus, temperature differences or intervals may be expressed in either the degree Celsius or the Kelvin using the same numerical value.

Here are two examples showing the inter-conversion between the two temperature scales:

Convert 78.0 °C to kelvin: $t/°C = T/K - 273.15$; $T/K = 273.15 + t/°C$; $T/K = 273.15 + 78.0 = 351.15$ K

Convert 373.00 kelvin to degrees Celsius: $t/°C = T/K - 273.15$; $t/°C = 373.00/K - 273.15$; $t/°C = 99.85$ °C

3.7.6 Derived SI Units

Many other additional SI units exist and some of the more common ones encountered during Biological investigative work are tabulated in *Figure 320*. All of these derived units can be defined or expressed in terms of the SI base units.

Quantity	S.I. unit	Relationship to other quantities	Basic definition
Molar concentration (formerly, molarity, M)	mole per cubic decimetre (M)		$mol\ dm^{-3}$
Frequency	hertz (Hz)		s^{-1}
Volume	cubic metre		m^3
Area	square metre		m^2
Force	newton (N)	$J\ m^{-1}$	$kg\ m\ s^{-2}$
Energy (Work, heat) (enthalpy)	joule (J)	N m	$kg\ m^2\ s^{-2}$
Pressure	pascal (Pa)	$N\ m^{-2}$ or $J\ m^{-3}$	$kg\ m^{-1}\ s^{-2}$
Density	kilogram per cubic metre	$kg\ m^{-3}$	$kg\ m^{-3}$
Specific heat capacity	joule per kilogram per kelvin	$J\ kg^{-1}\ K^{-1}$	$kg\ m^2\ s^{-2}\ kg^{-1}\ K^{-1}$
Power	watt (W)	$J\ s^{-1}$	$J\ s^{-1}$
Molar mass	grams per mole	$g\ mol^{-1}$	$g\ mol^{-1}$
Potential difference (voltage)	volt (V)	$W\ A^{-1}$	$J\ s^{-1}\ A^{-1}$

Figure 320 SI derived units

Some of these units only appear in a limited number of Biological contexts, for example, water potential, is a measurement of the pressure generated during osmosis. The measurement of frequency in hertz may occur during an investigation into thresholds of hearing. The measurement of force in Newton would occur during the investigation into muscle action. The term specific in front of a physical quantity has the meaning 'per unit mass'.

Use of SI Units

There are various rules regarding the use of the SI system and its associated units:

- A space should be inserted between the number and its associated unit, for example 25 g, not 25g.
- Any S.I. unit may take only one prefix. For example, milli millimetre is incorrect and should be written as micrometre.
- Most prefixes which make a unit larger are written in capital letters (M, G etc.), but when they make a unit smaller then lower case (m, n, p etc.) is used. (One exception to this is that the kilo (k), for example, in the kilometre and kilogram, to avoid any possible confusion with kelvin (K)).
- A unit which is named after a person is written all in lower case e.g. pascal (the S.I. unit of pressure when named in full, but starting with a capital letter (Pa) when abbreviated. Similarly, for the joule, the S.I. unit of energy, 5 joule and 5 J.
- An exception to this rule is the litre (a non SI unit) which, if written as a lower case 'l' could be mistaken for a '1' (one) and so a capital 'L' is allowed as an alternative.
- Units written in abbreviated form are never pluralised. So 'm' will always be either metre or metres.
- An abbreviation (such as 5.00 Pa) is never followed by a full-stop unless it is the end of a sentence.
- To make numbers easier to read they may be divided into groups of three separated by spaces but not commas. For example, 2 000 000.00 and 0.004 34.
- Negative indices are used when symbols are combined, for example, the symbol for moles per cubic decimetre is written as mol dm^{-3}, rather than mol/dm^3. Both are pronounced moles per cubic decimetre, where per means 'divided by' or 'for every'. Similarly, the ecological units of kJ m^{-2} $year^{-1}$ refers to kilojoules (of energy) per square metre per year.

Some examples are show in Figure 321 showing the rules regarding the correct use of SI units.

Rule	Correct use	Incorrect or less correct use
Abbreviations such as sec, cc, or mps should be avoided. Only use standard unit symbols, prefix symbols, unit names, and prefix names.	s or second cm^3 or cubic centimetre m/s or metre per second	sec cc mps
Unit symbols are unaltered in the plural.	51 cm 2.37 m	51 cms
Unit symbols are not followed by a full stop unless at the end of a sentence.	The length of the leaf stalk is 18 cm. The leaf stalk is 18 cm long.	The leaf stalk is 18 cm. long.
A space is used to show the multiplication of units. A solidus (/) or slash is used to show the division of units	The speed of sound is about 346 m s^{-1} (metres per second); the decay rate of caesium-113 is about 22 ms^{-1} (reciprocal milliseconds) m/s m s^{-2}	The speed of sound is about 346 ms^{-1} (metres per second); The decay rate of caesium-113 is about 22 m s^{-1} (reciprocal milliseconds) m ÷ s m/s/s
Information should not be mixed with unit symbols or names.	The biomass of grass was 545 g/m^2.	545 g of grass / m^2.
It must be clear to which unit symbol a numerical number belongs	22 cm × 40 cm 20 °C to 30 °C or (20 to 30) °C 102 g ± 3 g or (102 ± 3) g 74% ± 5% or (74 ± 5)%	22 × 40 cm 20°C – 30 °C or 20 to 30 °C 102 ± 3 g 74 ± 5%
The word 'weight' is often used interchangeably with the term 'mass'. In science, weight is a force and the SI unit for mass is the kilogram.	The mass of grass is 110 g. The weight of the grass is 1.10 N	The weight of grass is 100g.
The term molarity, with the symbol M, is no longer used. Instead the concentration should be expressed as moles per cubic decimetre.	0.4 mol dm^{-3}	0.4 M

Figure 321 Examples of correct and incorrect use of SI units

3.7.7 Non-SI units

SI units of measurement have not gained universal acceptance and a number of non-SI units of measurement are often used in Biology.

Pressure

Blood pressure is expressed in millimetres of mercury (abbreviated to mm Hg). It refers to the height of a column of mercury that can be supported by the pressure being measured.

For example, a systolic pressure of 120 mm Hg means that a column of mercury 120 millimetres high could be supported by blood pressure during the systole of the heart, when the ventricles contract and the blood pressure is at a maximum. Atmospheric pressure at 25 °C = 760 mm Hg = 101 000 Pa = 101 kPa. To convert mm Hg to kPa divided by 7.5; to convert kPa to mmHg multiply by 7.5. The pressure of the atmosphere and partial pressures of gases are also commonly expressed in units of mm Hg and atmospheres (atm) (760 mm Hg=1 atm).

Energy

The energy content of foods in usually expressed in either kilojoules or kilocalories. A calorie (C) is the amount of heat energy required to heat one gram of water through one degree Celsius. However, since this is a relatively small unit of energy it is more common to use a unit known as the kilocalorie or Calorie, which is equivalent to 1 000 calories.

1 Joule = 4.186 Calories; 1 J = 4.186 Cal. To convert calories to joules multiply 4.186; to convert joules to calories divide by 4.186. It is suggested that raw data may be recorded in non-SI units but that such data be converted into SI units before being processed in calculations.

Mixing Ratios

Mixing ratios are frequently used to describe the concentrations of trace or pollutant gases, for example, ozone (trioxygen) in the atmosphere.

For example, nitrogen forms 78.08 % by volume of the atmosphere and its concentration, expressed as a mixing ratio is 780 800 ppmv, where ppmv refers to parts per million by volume. This means that 780 800 out of 1 000 000 particles (atoms or molecules) in the air are nitrogen molecules.

Mixing ratios of gases can also be expressed in ppvb, parts per billion (10^9) by volume.

Mole Fraction

The mole fraction of a gas or liquid expresses the ratio of the number of moles of one gas or liquid to the total number of moles of gas or liquid in the sample.

For example, a mixture of methane and oxygen contains 0.313 moles of oxygen and 0.616 mol of methane.

Mole fraction of oxygen =

$$\frac{\text{amount of oxygen}}{\text{total amount of oxygen and methane}} = \frac{0.313\ \text{mol}}{(0.313\ \text{mol} + 0.616\ \text{mol})} = 0.3369\ \text{(The units cancel)}$$

The mole fraction can be used to calculate the partial pressure of a gas: the partial pressure of a gas is equal to its mole fraction multiplied by the total pressure.

Concentrations

Volume Percent

Concentrations of Biological solutions involving the mixing of two liquids are often expressed as a volume percent.

For example a solution of 70% by volume of ethanol implies that 100 cm^3 of the solution contains 70 cm^3 of ethanol.

$$\text{Volume percent of ethanol} = \frac{\text{volume of ethanol}}{\text{volume of water}} \times 100 = \frac{70\ cm^3\ \text{ethanol}}{100\ cm^3\ \text{water}} \times 100 = 70\%$$

One potentially confusing phenomenon about the concept of volume percent is that the volumes of liquids are not exactly additive: there is frequently a small reduction in volume.

Mass Percent or Weight Percent

A common way of expressing the concentration of a substance dissolved in water is to express it as a weight or mass percent:

For example a 12% by mass of aqueous sodium chloride solution would have 12 grams of sodium chloride for every 100 grams of solution. This solution would be prepared by weighing out 12 grams of sodium chloride and then adding 88 grams of distilled water.

Since mass (unlike volume) is conserved, the masses of the components of the solution, the sodium chloride and water, will add up to the total mass of the solution. To calculate the mass percent of a solution, you divide the mass of the solute (dissolved substance) by the mass of the solution (the combination of the solute and solvent (the dissolving liquid) and of course multiply by 100.

$$\text{12\% sodium chloride solution} = \frac{\text{12 g sodium chloride}}{\text{100 g water}}$$

$$\frac{\text{12 gram sodium chloride}}{\text{(12 gram sodium chloride + 88 gram of water)}} = \text{12\% sodium chloride solution}$$

Mass/ Volume Percent or Weight/Volume Percent

Another method of expressing concentration of a solution is weight/volume percent or mass/volume percent. The solute is measured as a mass in grams and the solution is measured in cubic centimetres.

For example, a 5% mass/volume sodium chloride solution contains 5 gram of sodium chloride for every 100 cm^3 of solution.

$$\text{mass/volume percent} = \frac{\text{mass of solute (g)}}{\text{volume of solution } (cm^3)} \times 100$$

Since there are different units in the numerator and denominator, this expression of concentration is not a true percentage. However, it is relatively easy to prepare solutions since volumes are easier to measure than masses and because the density of a dilute aqueous solution is relatively close to 1 g cm^{-3}. Consequently, the volume of a solution in cm^3 is very nearly numerically equal to the mass of the solution in grams.

Volume strength

Solutions of hydrogen peroxide are often sold according to their 'volume strength'. The volume strength of a solution of hydrogen peroxide is measured by the number of volumes of oxygen released when it is completely decomposed under standard conditions (0 °C, 1 atmosphere pressure).

For example, 10 volume strength hydrogen peroxide solution means 1 cm^3 of the solution will release 10 cm^3 of oxygen gas (when completely decomposed). Volume strengths can be converted to other measures of concentration, for example, molarity (mol dm^{-3}) and percentage by volume.

3.8 Measurement

No measurement is exact; some error or uncertainty is always associated with the measurement since a degree of estimation is involved. The act of measuring gives significance (or meaning) to each digit in the number produced.

Consider the following examples involving measurements made with a graduated scale:

3.8.1 Ruler

The whole numbers on the metric ruler (see *Figure 322*) represent centimetres. The divisions are tenths of a centimetre, or millimetres. The arrow represents the end of an object being measured and zero is on the left.

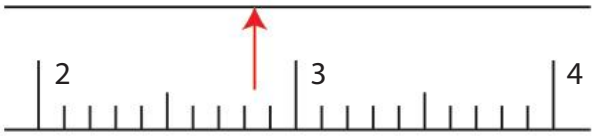


Figure 322 Section of a metre rule

From the markings on the ruler we know that the object is more than 2 cm in length, but less than 3 cm in length and that it is longer than 2.8 cm, but shorter than 2.9 cm. We can then examine the gap between 0.8 and 0.9 cm, where the arrow is located and, mentally, divide that small gap into ten equal divisions. The use of a magnifying glass will assist in this process.

In general, whatever the smallest division in your scale, you can usually estimate to the next decimal place after it. (This very precise approach is only justified if the scale between the scale divisions is relatively large and you are good at estimating between the divisions on the scale). In this example, the smallest division is in the tenth place so we can estimate to the nearest 0.01. You can then report the object's length as 2.82 cm.

(The first two digits are 100% certain, but the last, since it was estimated, has an error associated with it. However, all three digits are significant. Note that only one estimated digit is allowed to be significant in a measurement. It is always the last digit in the measurement).

The implied limits of the measurement 2.820 cm are 2.815 cm – 2.825 cm. This can be written as 2.820 cm ± 0.005 cm, where the ± 0.005 cm is the absolute error or absolute uncertainty.

3.8.2 Liquid-in-glass Thermometers

Liquid-in-glass (red coloured alcohol) thermometers used by IB Biology students typically have a scale marked with only whole numbers. The gap between each whole number does not have any scale marks for tenths of a degree Celsius.

The temperature indicated on the thermometer in *Figure 223* is 28.5 °C. The implied limits of the measurement 28.50 °C are 28.55 °C – 28.45 °C. This can be written or reported as 28.50 °C ± 0.05 °C.

Remember to mentally divide up the gap between 28 and 29 and record your estimate of how many tenths of a degree Celsius are covered by the mercury thread. A magnifying glass is often used to enlarge the scale which greatly improves the accuracy of the estimation process. (*More accurate, but limited range thermometers, calibrated in 0.1 °C if available are preferable for such precise measurements*).

A common student mistake when using this type of thermometer is to record a temperatures to the nearest whole number, for example, 15 °C, as opposed to the correct 15.0 °C (together with the associated error and uncertainty).

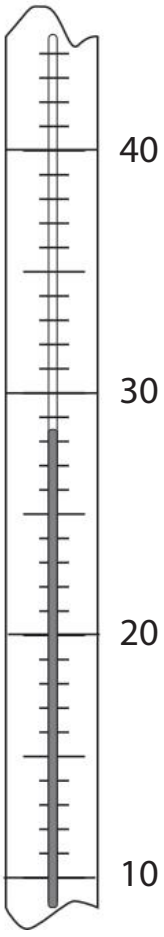


Figure 323
A thermometer scale

3.8.3 Measuring Cylinder

The measuring cylinder (*Figure 324*) indicates a volume of 4.28 cm^3 ± 0.05 cm^3. The smallest division is in the tenth place so we are allowed to estimate to the hundredth place.

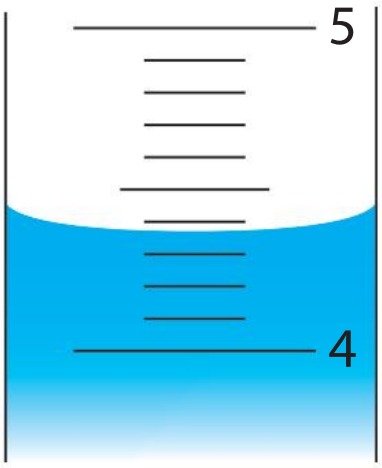


Figure 324 Measuring cylinder

This precision can be obtained by the use a 'burette reader' (see *Figure 325*): a piece of card behind the measuring cylinder, the upper half being white and the lower half black. A magnifying glass can again be used to increase the accuracy of your reading.

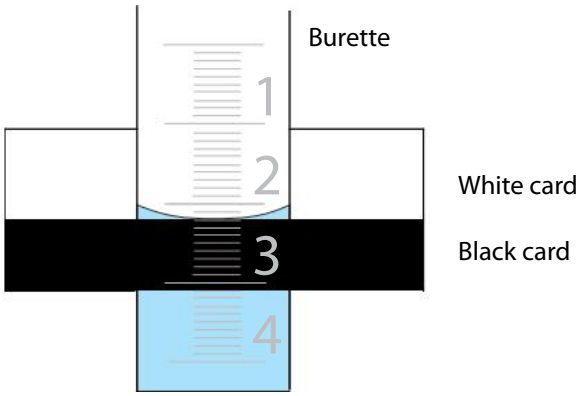


Figure 325 A burette reader

3.8.4 Digital Instruments

Digital instruments commonly used in Biology investigations include pH probes and meters and electronic stop watches. Unless the instrument manufacturer indicates otherwise (on the instrument or instructions), then the precision of measurements made with digital instruments are reported with a precision of ± ½ of the smallest unit of measurement.

For example, during an investigation into the effect of light on photosynthesis a light meter reads 1.493 lux; the precision of the light intensity measurement is ± ½ of 0.001 lux or ± 0.0005 lux. Hence the light meter reading should be reported as 1.493 lux ± 0.0005 lux.

3.9 Significant Figures

The concept of significant figures is extremely important in biology and other sciences since it is concerned with ensuring that numerical results and associated calculations are not given an importance or implied accuracy that is unjustified.

The word 'significant' in the scientific or mathematical sense indicates that the number is 'meaningful'. As shown previously biologists generally report all the digits in a number they know for certain plus one extra digit which is an estimate. All of these numbers, including the estimated number, are regarded as significant.

There is therefore no need for you to describe a measurement as 'about...' or 'approximately…', or 'around …' since the precision of any measurement is incorporated into the number of digits you use to report that measurement. The following rules will help you determine the number of significant figures in a number:

- Non-zero digits are always significant.
- For example, in the number 89.99, there are four significant figures.
- All zeros between other significant digits, or 'internal zeros', are significant.
- For example, in the numbers 303 and 1.01, there are three significant figures.
- Zeros to the left of the decimal place or 'leading zeros' are never significant.
- For example, the number 0.006204 has four significant figures. The '6' is the most significant figure. The left hand '0's are not significant. The zero between the '2' and the '4' is significant.
- Terminal zeros in a number with no decimal places are uncertain; they may or may not be significant.
- For example, the zeros in 100 cm^3 are uncertain. They may be present merely to indicate the size of the number (that is, locate the decimal point). If so, these zeros are not significant and are described as place holders. If the zeros are place holders then the number 100 therefore has only one significant figure, the one. Alternatively, the zeros may be significant and indicate the precision of the volume. The number 100 may therefore have three significant figures.

This ambiguity is avoided if the number is expressed in scientific notation, since the number of digits before the exponent are used to express the number of significant figures. (The exponential term is not added to the number of significant figures). For example, 1×10^2 has only 1 sig. fig. and 1.00×10^2 has 3 sig. figs).

3.9.1 Significant Figures in Calculations

Measured quantities are often used in biological and other scientific calculations. The precision of the calculation is limited by the precision of the measurements on which it is based.

Addition and Subtraction

When measured quantities are added or subtracted, the result should be reported to the same number of decimal places as the number with the least number of decimal places. For example: 2.487 + 330.4 + 22.59 = 355.477 g (uncorrected) should be reported as 335.5 g (corrected) which has one decimal place since the number 330.4 has the least number of decimal places, namely one.

If you are adding numbers expressed in scientific notation then you must, if necessary, adjust the numbers so they have the same exponent. For example: $1.46236 \times 10^8 + 4.293 \times 10^7 = 1.46236 \times 10^8$ (5 decimal places) + 0.4293×10^8 (4 decimal places) = 1.89166×10^8 (uncorrected) = 1.8917×10^8 (corrected to 4 decimal places).

Losing Significant Figures

Sometimes significant figures are 'lost' while performing calculations. For example, if you find the mass of a beaker to be 53.110 g, add water to the beaker and find the mass of the beaker plus water to be 53.987 g, the mass of the water is 53.987-53.110 g = 0.877 g.

The final value only has three significant figures, even though each mass measurement contained five significant figures.

Multiplication and Division

When measured quantities are multiplied or divided, the result should be reported to the same number of significant figures as the number with the least number of significant figures.

For example: $2.34 \times 1.8 = 4.212$ (uncorrected) = 4.2 (corrected to 2 sig. figs.) since 1.8 has the least number of significant figures, namely two.

For example: 17.32/2.4 = 7.2166667 (uncorrected) = 7.2 (corrected to 2 sig. figs.) since 2.4 has least number of significant figures, namely two.

Combination of Multiplication, Division, Addition and Subtraction

Adjust for significant figures at each separate operation, using the two rules given previously.

For example: $\frac{2.423}{1.24} + 0.37 = 2.32403$ (uncorrected) = 2.3 (corrected)

A useful way to remember the order of mathematical operations is BIDMAS:

Brackets Indices Division Multiplication Addition Subtraction

So the first operation is division: $\frac{2.423}{1.24} = 1.95403$ (uncorrected)

This number should be corrected to three significant figures since 1.95, has the least number of significant figures, namely three.

The second operation is addition: 1.95 + 0.37 = 2.32 since 0.37 and 1.95 both have two decimal places.

3.9.2 Rounding Numbers

When correcting your final answer of a calculation to the appropriate number of significant figures, you frequently have to round your final answer. This is because during a calculation with significant figures you may have to reduce the number of digits in the answer to indicate the precision of the answer. Apply the following three general rules for rounding:

Rule One

If the number beyond the last digit to be reported is less than 5, then drop the last digit.

For example, 1.32 corrected to two significant figures (2 sig. figs.) is 1.3. Two digits are to be reported and the number beyond the last reported digit, namely 2, is less than 5, hence it is removed.

Rule Two

If the number beyond the last significant figure is greater than 5, then increase the final digit by 1.

For example, 5.798 corrected to two significant figures (2 sig. figs.) is 5.8. Two digits are to be reported and the number beyond the last reported digit, namely 9, is more than five, hence the final reported digit, namely 9, is increased by 1 (see *Figure 326*).

Number	Number of significant figures used to report number	Number corrected to appropriate number of significant figures
49.5149	five	49.515
49.5149	four	49.51
49.5149	three	49.5
49.5149	two	50
49.5149	one	50

Figure 326 Examples of rounding

Rule Three

If the number beyond the last significant figure is exactly 5, then round the final digit to the closest even number.

For example, 3.55 rounded to two significant figures would be 3.6 (rounding up) and 6.450 rounded to two significant figures would be 6.4 (rounding down).

3.9.3 Exact and Irrational Numbers

Sometimes the numbers used in a calculation are exact rather than approximate. This is true when using defined quantities, including many conversion factors, for example, 10 mm = 1 cm, and when using exact or counted numbers, for example, three (3).

The use of exact numbers does not affect the accuracy of a calculation. You may think of them as having an infinite number of significant figures, for example, with a conversion, such as 1 cm = 10 mm, the precision is absolute and could be written as 10.000. with an infinite number of zeros to the right of the decimal point.

Irrational numbers, for example, ratios, such as 4/3, or pi (π): 3.1415926 … (used to calculate the volume of a sphere or cylinder) have an infinite number of digits. When you have to a number like pi, use sufficient digits so that it has one more digit than the least precise measurement.

For example:

You want to calculate the mean height of three plants and measure the following heights: 30.1 cm, 25.2 cm, 31.3 cm. The mean height is (30.1 + 25.2 + 31.3)/3 = 86.6/3 = 28.87 = 28.9 cm.

There are three significant figures in the heights; even though you are dividing the sum by a single digit with one significant figure, the three significant figures should be retained in the calculation. The three is an exact number and does not affect the accuracy of the calculation.

3.9.4 Uncertainties and Significant Figures

Absolute Uncertainty or Error

The absolute uncertainty or absolute error is the number which, when combined with a measurement or reported value, gives the range of true values.

For example, the length of a small tropical insect as measured by an imprecise ruler may be reported as 1.3 mm ± 0.4 mm (see *Figure 327*). In this example, the reported value is 1.3 mm, the absolute uncertainty or absolute error is 0.4 mm, and the range of true values is 0.9 mm – 1.7 mm. ((1.3 mm – 0.4 mm = 0.9 mm (lower limit of range) and (1.3 mm + 0.4 mm = 1.7 mm (upper limit of range)).

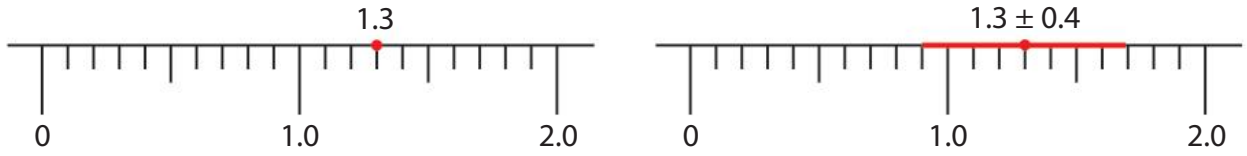


Figure 327 The exact quantity 1.3 mm and the uncertain quantity 1.3 mm ± 0.4 mm

Absolute uncertainties or absolute errors have the same units as the reported value with which they are associated. Whenever you record measurements during an IB Biology investigation you should record the absolute uncertainties or errors.

The uncertainty in a measurement should be significantly smaller (less than 10%) than the measurement itself. For example, 0.2 s ± 0.1 s implies that the uncertainty or error is 50% of the measured time. Such data is useless and should be discarded.

The number of significant figures used should be such that the uncertainty is in the last significant figure. It is illogical to report values with more significant figures than indicated by the uncertainty or error. For example, in 9.63 ± 0.6 the three has no meaning and the number should be reported as 9.6 ± 0.6. When using scientific notation, both the value of the quantity and its uncertainty should have the same exponent, for example $(3.33 \pm 0.01) \times 10^{-4}$.

Absolute uncertainties or errors can be used to decide whether or not your experimental results are in agreement within experimental uncertainty or error with those of another IB Biology student. For example, the two measurements 9.6 ± 0.3 cm and 10.4 ± 0.3 cm do not have overlapping ranges as shown. Hence, even taking into account the experimental uncertainty or error the two measurements do not agree (see *Figure 328*).

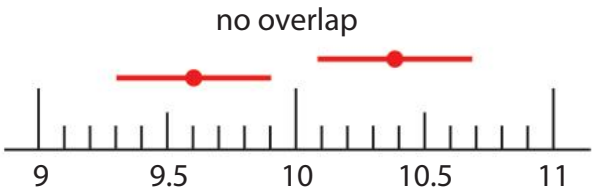


Figure 328 Two measurements that do not agree within experimental error or uncertainty

However, the two measurements 9.6 cm ± 0.6 cm and 10.4 cm ± 0.6 cm do agree when experimental error or uncertainty are taken into account since the uncertainty or error ranges now overlap (see *Figure 329*).

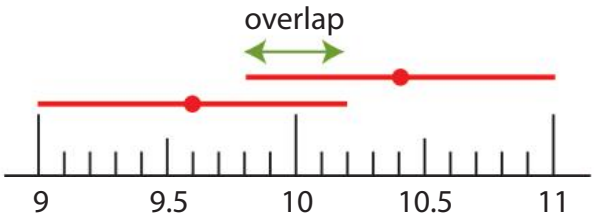


Figure 329 Two measurements that do agree within experimental error or uncertainty

When measurements are combined by adding or subtracting, the absolute uncertainties or errors are added to give the resultant uncertainty.

For example

(720 g ± 1 g) + (640 g ± 1 g) = 1360 g ± 2 g

(172 g ± 1 g) - (64 g ± 1 g) = 108 g ± 2 g

Here is another example involving addition and subtraction.

Let A = 5.23 ± 0.07, B = 4.67 ± 0.04, and C = 7.11 ± 0.09. Calculate F = A + B – C.

F = 5.23 + 4.67 – 7.11 = 2.79

Resultant uncertainty = (0.07 + 0.04 + 0.09) = 0.20

Therefore, F = 2.79 ± 0.20

Only one figure can be kept in the uncertainty or error, hence

F = 2.8 ± 0.2

When measurements are combined by multiplying or dividing by a constant then the absolute uncertainty is multiplied or divided by the same constant.

For example

100 insect wing cases have a total thickness of 9 mm ± 0.1 mm.

The mean thickness of one insect case = 9 mm/100 ± 0.1 mm/100

Therefore the result can be reported as 0.0090 mm ± 0.0001 mm

In order to handle the uncertainties or errors in multiplication and division it is necessary to introduce the related concept of percentage uncertainty or percentage error.

Percentage Uncertainty or Error

Percentage uncertainty or error is also known as relative error or uncertainty.

The percentage uncertainty or percentage error is another method of reporting errors or uncertainties in measurements. The percentage uncertainty or error is the ratio of the absolute uncertainty or error to the reported value.

For example, a length of 100 cm ± 1 cm has a percentage uncertainty or error of 1 cm/100 cm, that is, 1%. The measurement can therefore be reported as 100 cm ± 1%. Note that percentage uncertainties or errors, unlike absolute uncertainties or errors, are dimensionless, that is, they lack units.

When measurements are combined by multiplying or dividing, the percentage uncertainties or errors are added to give the resultant uncertainty or error.

For example:

$(25\ m \pm 1\ m) \times (5.0\ m \pm 0.1\ m) = 125\ m^2 \pm 8\ m^2$

Working:

Resultant uncertainty or error = (1 m/25 m) + (0.1 m/5.0 m) = 0.06 or 3/50

$125\ m^2 \times 3/50 = 7.5\ m^2$ (rounding up)

Hence, the resultant uncertainty or error is $125\ m^2 \pm 8\ m^2$.

When raising a measurement to a power *n*, the resultant uncertainty or error is *n* times the relative uncertainty or error.

For example:

Let $A = 5.23 \pm 0.07$. Calculate $F = A^3$.

$F = (5.23)^3 = 143.056$

Percentage error in $A = \frac{0.07}{5.23} \times 100 = 1.34\%$

Percentage error in answer $F = 3 \times 1.34\% = 4.02\%$

Absolute error in answer $F = 143.056 \times \frac{4.02}{100} = 5.75$

Resultant uncertainty or error in $F = 3 \times (5.23)^3 \times (0.07/5.23) = 5.744$

Therefore $F = 143.056 \pm 5.744$

Only one figure can be kept in the uncertainty or error, hence

$F = 143 \pm 6$

Percentage Error

The percentage error is defined as.

$$\text{percentage error} = \frac{|\text{accepted value} - \text{experimental value}|}{\text{accepted value}} \times 100$$

For example

If your experimental value for a particular experiment is 10.3 and the reported value in your text book or biological literature is 9.80, then your percentage error is 5.1%.

$$\text{percentage error} = \frac{|9.8 - 10.3|}{9.8} \times 100 = 5.1\%$$

3.10 Sources of error in biological data

Human Error

For example, 575 recorded as 757 is an example of a transposition error. Human errors or mistakes can be eliminated by taking great care, repeating readings and data checking.

Instrumentation limitation

Be aware of the uncertainties inherent in the instruments or laboratory glassware you are using in your investigation.

Uncontrolled Factors

Even the best experimental designs or circumstances will leave some variables uncontrolled, for example, experiments may be performed on different days or different times of the same day.

Unrepresentative Samples

This should not occur if the sampling is controlled. Be certain of the limits of the population from which you are sampling.

Random Errors

Random measurement errors or experimental errors are small fluctuations that occur when measurements are recorded. This means that when the measurement is repeated then slightly different results are obtained. A random measurement error is one that is inherent in the type of measurement, for example, recording resting heart rate measurements.

A random experimental error is inherent in the measuring device. They occur when interpolating a reading on a scale, that is, estimating the position of a pointer that lies between two scale markings. The reading will vary slightly with the position of the eye and the student's eye sight. They may also occur during to timing events where the error or uncertainty is due to the random variation of the experimenter's reaction times.

The majority of statistical tests assume random errors exist – errors can be reduced by averaging a series of measurements.

Systematic Errors

Systematic errors occur when equipment is not calibrated accurately. This type of error, unless compensated for, will introduce bias into the results. A simple example of the source of such an error is the use of a pH probe and meter that reads 6.50 when placed into a fresh buffer of pH =7.00. All the readings measured with the pH probe and meter will be in error by a fixed amount of 0.50 pH units.

Rounding Errors

Perform any 'rounding off' at the end of a calculation. If you round off during a calculation you may introduce errors.

3.11 Processing raw data

Data processing involves, for example, combining and manipulating raw data to determine the value of a physical quantity (such as adding, subtracting, squaring, dividing), and taking the average of several measurements and transforming data into a form suitable for graphical representation.

It might be that your data is already in a form suitable for graphical presentation, for example, distance travelled by *Paramecium* against temperature. If your raw data is represented in this way and a best-fit line graph is drawn, the raw data has been processed. Plotting raw data (without a graph line) does not constitute processing data.

3.11.1 Processing Data in Tables

The raw data recorded in your table is frequently going to need processing or transforming. This makes it easier to establish trends and relationships between variables. The processing of data involves carrying out mathematical operations or transformations. Examples are shown below of various tables of Biological data being subjected to some relatively simple data processing operations.

Finding the Total

This operation involves finding the total value of all the data or data entries in a particular column: first data entry + second data entry + … etc =. Example (14 + 22 + 36) = 72. (See *Figure 330*).

Time / minutes	Number of bubbles produced
1	14
2	22
3	36
Total	72

Figure 330 A data table showing a total

Maintaining a Running Total

This operation involves adding each new data value or data entry to the earlier ones in a column. (See *Figure 331*). Total of earlier data + next data entry = running total. Example: (36 + 36) = 72

Time /minutes	Number of bubbles produced	Total number of bubbles
1	14	14
2	22	36
3	36	72

Figure 331 A data table showing a running total

Calculating the Mean

This operation involves finding the central value around which your data is spread (see *Figure 332*). Total ÷ number of data entries. Example: $\frac{69}{3} = 23$. Note that a mean cannot be more accurate than the original data.

Time/minutes	Number of bubbles produced
1	14
2	20
3	35
Total	69

Figure 332 A data table showing the total (prior to calculating the mean).

Sorting into Order

This operation involves sorting the data in a column into ascending or descending order, see *Figures 333 (a) and (b)*. This type of operation can sometimes be avoided by careful planning before commencing the Investigation.

Species	Number of individuals
A	24
B	3
C	52
D	98

Table 333 (a) An unsorted data table

Species	Number of individuals
D	98
C	52
A	23
B	23

Table 333 (b) A sorted data table

Calculating Percentages

This operation involves calculating what fraction a data value or data entry is of the total value of a column. This fraction is then expressed as a percentage (see *Figure 334*).

(data entry ÷ column total) × 100. Example: (150 ÷ 400) × 100 = 37.5

Gender	Number	Percentage of population
Female	150	37.5
Male	250	62.5
Total	400	100

Figure 334 Data table showing percentages

Calculating Reciprocals

This operation involves finding the inverse of a data value or data entry (see *Figure 335*). 1 ÷ data value = or data value^{-1}. Example: 1 ÷ 4 = 0.25

pH	Time for solution to decolourise / s	Reciprocal of time or rate / s^{-1}
1	8.0	0.12
2	6.0	0.17
3	4.0	0.25

Figure 335 Data table showing reciprocals of time (rate).

Calculating Differences

This operation involves calculating how much a variable has changed between consecutive readings (see *Figure 336*) i.e. new data entry – previous data entry.

Time (minutes)	Total distance moved by bubble / mm	Distance moved each minute / mm
1	9	9
2	22	13
3	37	15

Figure 336 Data table showing differences between consecutive readings

Calculating Rates

This operation involves calculating how rapidly a particular variable changes with time. (See *Figure 337*).

i.e. data entry ÷ time interval. Example: (84 ÷ 60) = 1.4.

Time/minutes	Number of bubbles produced	Number of bubbles produced per second
1	30	0.5
2	72	1.2
3	84	1.4

Figure 337 Data table showing average rate

Note that some data processing or transformations, for example, running sum, produce a new column of data or even a completely new table, for example, sort into order. Other data processing or transformations, for example, calculate a mean, produce a summary figure which is usually entered at the bottom of the appropriate column. Other more complex data transformation may involve calculating ratios, multiplying by constants, for example pi, calculating logarithms or anti-logarithms.

NATURE OF IB BIOLOGY INVESTIGATION	RAW DATA	PROCESSED DATA
Enzyme-controlled reactions	• Mass or volume of substrate formed or product formed measured in mass (m) or volume (v)	• Rate of reaction - mass or volume of substrate consumed or product formed per unit time. $\left(\frac{m}{t} or \frac{v}{t}\right)$
Enzyme-controlled reactions	• Time taken for completion of reaction (t)	• Rate of reaction $\left(\frac{1}{t}\right)$
Distribution of specimens in a location	• Number of individual specimens in each count (x) • Total number of counts (n) • Area of location (a)	• Average number of individual specimens per unit area $\left(\frac{\Sigma x}{(n)\times(a)}\right)$
Osmosis in (plant cells)	• Number of plant cells exhibiting plasmolysis (x) • Total number of plant cells in sample (n) • Initial length or mass of plant tissue sample (i) •Final length or mass of plant tissue sample (f)	• Percentage of plasmolysis $\left(\frac{x}{n}\times 100\right)$ • Percentage change in the length or mass of plant issue $\left[\left(\frac{f-i}{i}\right)\times 100\right]$
Transpiration	• Potometer reading - distance travelled by air bubble within a calibrated capillary glass tube (d) • Time taken (t) • Radius of the bore of the capillary tube (r). • Time taken (t) • Initial mass (m_1); final mass (m_2)	• Volume of air evolved per unit time. • Mass change ($m_1 - m_2$)
Respiration	• Respirometer reading - distance travelled by an ink droplet in a calibrated capillary tube (δ) • Time taken (t) • Radius of the bore of the capillary tube (r)	• Volume of oxygen absorbed per unit time.
Estimation of population size.	• Number of marked individuals (n_1) • Sample size (n_2) • Number of marked individuals in sample (n_3)	• Population size. $\frac{n_1 \times n_2}{n_3}$
Hemocytometer (improved Neubauer)	• Number of cells counted in 80 small squares (n)	• Number of cells per mm^3 $\left(\frac{N}{80}\times 4000\right)$
Chromatography	• Distance moved by solute (x) • Distance moved by the solvent (y)	• Retention factor. $R_f = \frac{x}{y}$

Figure 338 Common examples of processing raw data during IB Biology investigations

3.12 Descriptive Statistics using Excel

Excel has three formulae for the measurement of the central tendency of a sample.

The arithmetical mean or mean is given by the formula:

=AVERAGE (range)

The median is given by the formula:

=MEDIAN (range)

And the mode is given by the formula:

=MODE (range)

The use of these formulae are illustrated in *Figure 338*. In many cases the quantities measured during an IB Biology Investigation will show a normal distribution, and so the arithmetical mean is the most appropriate descriptive statistic to employ. If the mean of a sample is calculated then a measure of the variation, dispersion or spread of the data should also be calculated.

The range is given by the Excel formula:

=MAX (range) – MIN (range)

where MAX returns the largest value in a range and MIN the smallest.

This is the simplest, but least useful function since it is a relatively crude measure that does not take the other (intermediate) values into account.

The variance (of a population sample) is given by the Excel formula:

=VAR (Range)

This has little use as a descriptive statistic since it is not in the same units as the measurements.

The standard deviation (of a population sample) is given by the Excel formula:

=STDEV (range)

(*If the numbers you are analysing represent an entire population, rather than a sample, then use the VARP and STDEVP functions to calculate variance and standard deviation*).

SD gives a good indication of the variability or 'spread' of a set of data around the mean. However, it is not the best statistic to use when comparing different sets of data, especially if the data sets are of different sizes.

The standard error of the mean is given by the formula

=STDEV (range)/SQRT (COUNT (range))

This gives an indication of the confidence of the mean.

The 95% confidence interval is given by the formula:

=CONFIDENCE (0.05, STDEV(range), COUNT (range))

The value of 0.05 is used to give the 95% (0.95) confidence interval which means there is a 95% probability that the real or true mean lies within ± confidence interval (CI) from the measured mean (see *Figure 339*). In other words, it gives the percentage number of times that the calculated interval actually contains the true population mean when the process of taking n samples is repeated a large number of times. However this means that there is still a 1 in 20 chance that the calculated confidence interval will not contain the true population mean. The upper and lower limits of this range are called the confidence limits and can be shown as error bars on line graphs or bar charts.

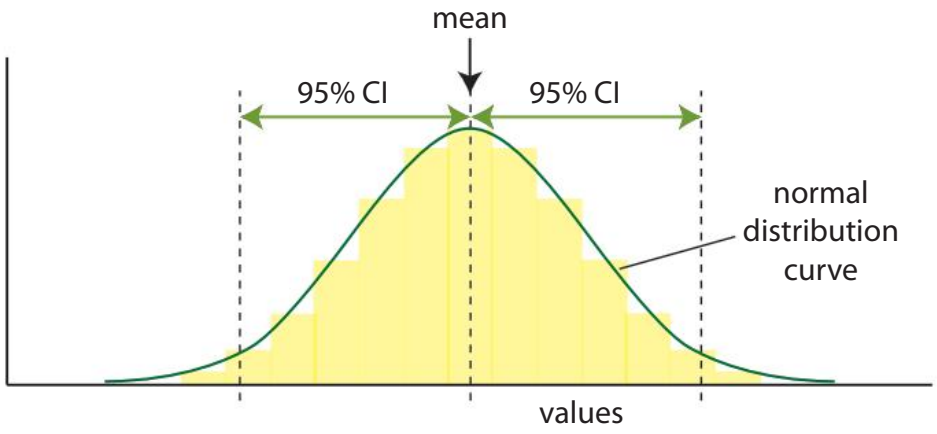


Figure 339 The normal distribution curve and 95% confidence intervals

A confidence interval should always be calculated whenever a mean is calculated. If the confidence interval is small compared to the mean then the mean is reliable, but if the confidence interval is large compared to the mean then the mean is unreliable (see *Figure 340*).

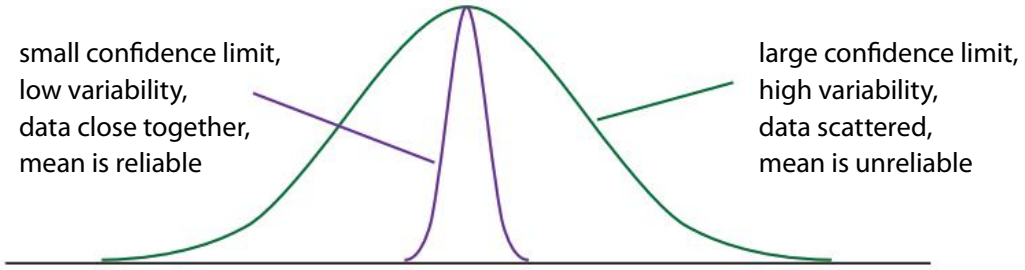


Figure 340 The relationship between the confidence limit and the mean

Excel BioIA.xls

	A	B	C	D
1		Group A	Group B	
2		14.20	10.30	
3		15.70	18.20	
4		11.50	26.40	
5		13.00	6.10	
6		15.60	22.20	
7		15.30	2.10	
8		15.30	2.10	
9				
10	Mean	14.37	12.49	
11	Median	15.30	10.30	
12	Mode	15.30	2.10	
13	Standard Deviation	1.59	9.85	
14	Sample Variance	2.53	96.98	
15	Range	4.20	24.30	
16	Standard Error	0.60	3.72	
17	95% confidence interval	1.18	7.30	
18				
19				
20				
21				
22				

Sheet1 Sheet2 Sheet3
Ready

Figure 341 Eight descriptive statistics for two sets of sampled data: Groups A and B

Refer to *Figure 341*. In group A the confidence interval is small compared to the mean, so the sampled data can be regarded as reliable and the real or true mean is relatively close to the calculated sample mean.

However, in group B the confidence interval is relatively large compared to the mean, so the data is regarded as unreliable since the real or true mean is relatively far from the calculated sample mean.

The formulae in cells C9 to C16, respectively, are:

=AVERAGE (C2:C8)

=MEDIAN (C2:C8)

=MODE (C2:C8)

=MAX (C2:C8) – MIN (C2:C8)

=VAR (C2:C8)

=STDEV (C2:C8)

=STDEV (C2:C8)/SQRT (COUNT (C2:C8))

=CONFIDENCE(0.05, STDEV(C2:C8), COUNT (C2:C8))

Note that the MODE formula will return #N/A if no values are duplicated. In addition, note that Excel will always return the results of calculations to about eight decimal places. This precision cannot be justified when processing Biological data so the calculated results should be formatted to a more sensible precision (Format menu > Cells > Number tab > Number).

(Excel outputs the confidence interval based on a normal distribution, rather than the Student t distribution. However, the calculation of confidence intervals should be based on the Student t distribution if the number of replicate measurements, as in the example above, is relatively small. If a Student t distribution is used then the confidence intervals for Groups A and B should be 1.47 and 9.11, respectively).

3.13 Presenting processed data

You are expected to decide upon a suitable presentation format for yourself (for example, spreadsheet, table, graph, chart, flow diagram etc.). There should be clear, unambiguous headings for calculations, tables or graphs. Your graphs need to have appropriate scales, labelled axes with units, and accurately plotted data points with a suitable best-fit line or curve (not a scatter graph with data-point to data-point connecting lines).

You should present the data so that all the stages to the final result can be followed. Inclusion of metric/SI units is expected for your final derived quantities, which should be expressed to the correct number of significant figures. The uncertainties associated with your raw data must be taken into account. The treatment of uncertainties in graphical analysis requires the construction of appropriate best-fit lines.

The complete fulfillment of Aspect 3 does not require you to draw lines of minimum and maximum fit to the data points, to include error bars or to combine errors through root mean squared calculations. Although error bars on data points (for example, standard error) are not expected, they are a perfectly acceptable way of expressing the degree of uncertainty in the data. In order to fulfill Aspect 3 completely, you should include a treatment of uncertainties and errors with their processed data, where relevant.

3.13.1 Displaying Data

Drawing Graphs

During your IB Biology coursework programme you will be frequently required to draw graphs and interpret them. A graph is a visual presentation of data and helps to make the relationship between two variables more obvious. A line graph is drawn when the data relates to an independent and a dependent variable. The following guidelines should be followed when plotting line graphs of two variables:

- There should be a title located at the top of the graph. Frequently, this will take the form of Effect of (independent variable) on (dependent variable). The date should be included next to the title.
- Each of the axes should be labelled with the name of the variable and appropriate units with a solidus located in between them, for example, Molarity of sucrose/mol dm^{-3}. This is to convert the units into pure numbers.
- The independent variable is plotted on the X axis or abscissa and the dependent variable is plotted on the Y axis or ordinate. The two axes meet at the origin which is denoted by O.
- Time is usually plotted along the horizontal axis since it is frequently an independent variable. However, time can be a dependent variable. For example, when finding the time taken for an enzyme-controlled reaction to occur at various temperatures.
- Draw the graph to ensure that the data fills as much of the space on the graph as possible (unless extrapolation is involved). This ensures the graph paper is not wasted and, more importantly, accuracy is maximised. Therefore, choose scales for the x and y axes that cover the range of the experimental data. If both scales start from zero, then the origin should be shown on both axes, but your scales do not necessarily need to commence with zero at the origin.
- When choosing the scale, always choose values for the major divisions that make the smaller sub-divisions easy to interpret. If an axis must be broken to make best use of the scale, the break should be shown by means of displaced origins.
- If the graph is used for extrapolation then ensure that the range of scales covers the range of extrapolation.
- Draw a small dot or small vertical cross, +, for each data point at the appropriate place on the graph and draw a circle round it for easy detection.
- If you plot two or more different sets of data on the same set of axes (for easy comparison), then label each line at the end, for example, with A, B, C and D and then identify these in a suitable key. Alternatively you could encircle the points with different colours for each line or even use different symbols for the points.
- The data points on a line graph can be 'joined up' in one of three different ways.

  The most common approach in Biology is to use short straight lines to connect each data point to the next (the 'zig-zag method').

  Straight lines between data points indicate that the accurately plotted recorded points are fixed, but intermediate values are unknown and cannot be predicted.

  A smooth rounded curve or line of best fit can only be justified if have a good reason to think that the intermediate values lie on the interpolated line and that there is a definite and continuous relationship between the two variables.

3.13.2 Logarithmic Scales

Sometimes during an IB Biology investigation, the data collected are not easy to plot on a graph because of the very wide range of numbers involved. An example arises when measuring the growth rate of micro-organisms, such as yeast in culture by the use of a hemocytometer. Idealised results are plotted in *Figure 342* and take the form of an exponential curve.

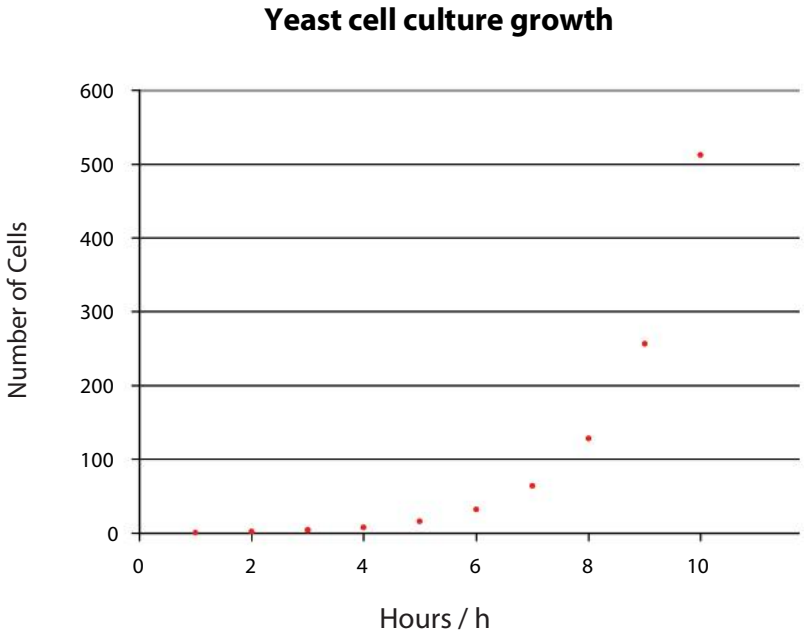


Figure 342 A graph of yeast cell culture growth

Rather than plotting the arithmetical value, that is, the raw data, it is preferable to calculate the logarithm to the base ten of the numbers. This can be done with a calculator or Excel: the formula is =LOG10(cell reference).

The logarithm of the number only increases relatively slowly and a straight line plot (*Figure 343*) is generated.

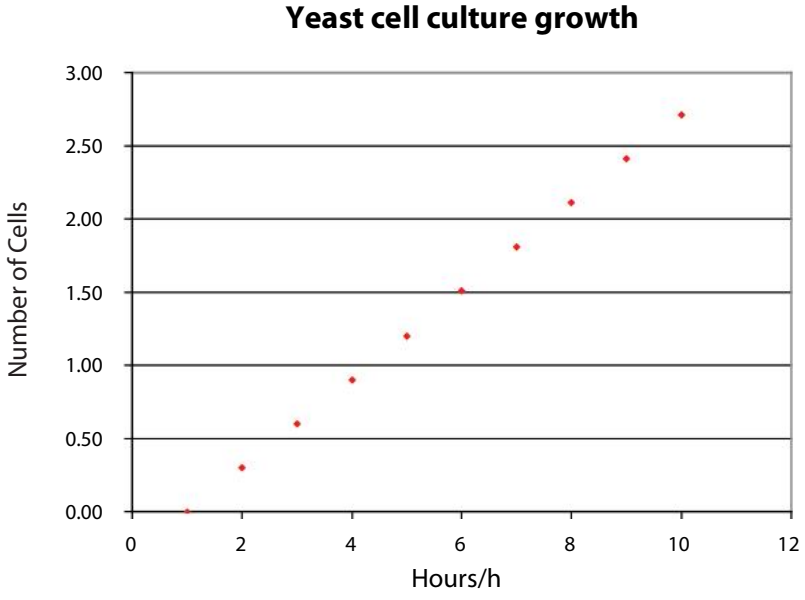


Figure 343 A logarithmic plot of yeast cell culture growth

3.13.3 Scattergrams

A scattergram (see *Figure 244*) is drawn when during an IB Biology investigation it is not possible to alter or manipulate either of the two variables.

For example, if you wanted to establish the relationship between heart beat rate and breathing rate in humans. In such investigations you simply record the data available and there is no independent (manipulated) and dependent variables.

Figure 344 shows a scattergram which displays the relationship between heart rate and breathing rate in humans

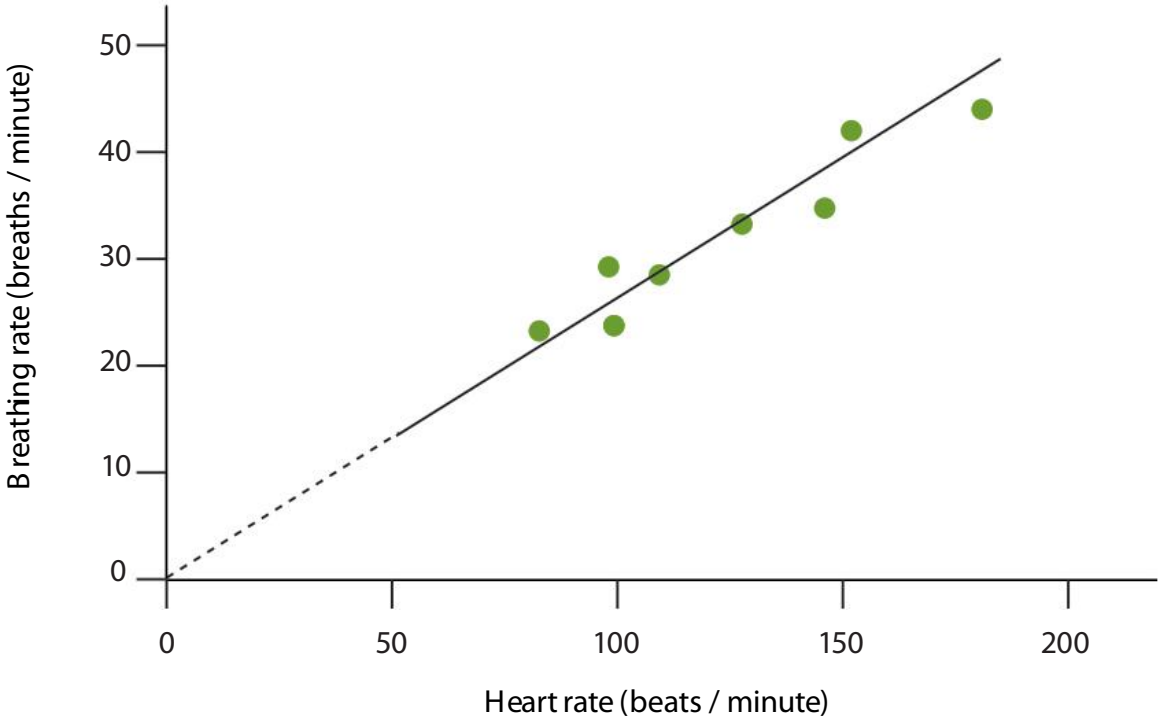


Figure 344 A scattergram

A line of best fit may be drawn through the data of a scattergram, but its presence implies a definite relationship between the variables.

3.13.4 Histograms and Bar Charts

Histograms and bar charts are used to display discontinuous, grouped data. Each group of data is related to the other groups, but there is no progressive change as with data plotted in a line or scattergram. For example, the distribution of leaf number within a plant population.

In histograms, the groups are shown as intervals along the x axis and there is no 'overlap' between groups so any value can only belong to one group. The number of values in each group, or frequency, is shown on the y axis. This type of graph is often known as a frequency histogram. *Figure 345* shows the distribution of number of flowers within a plant distribution

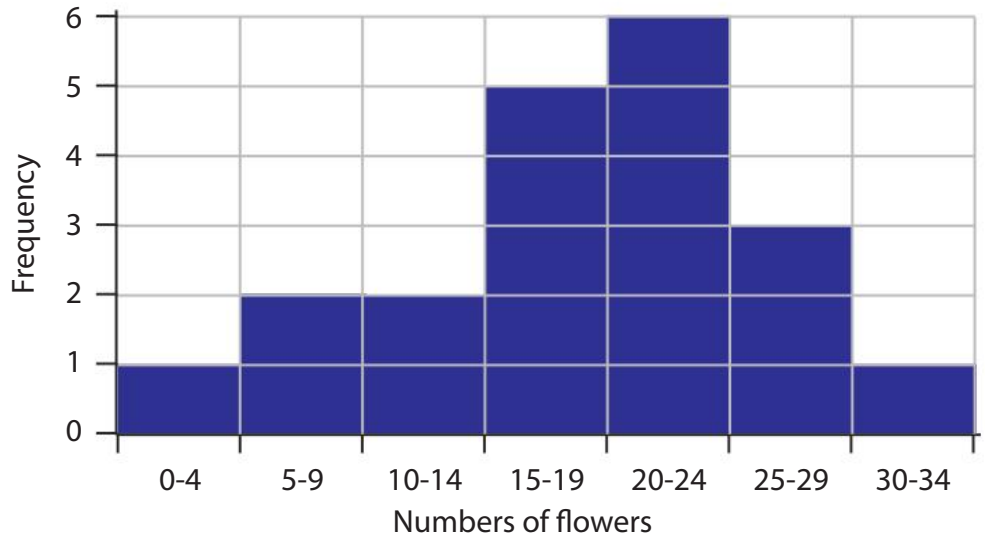


Figure 345 Example of a histogram

3.13.5 Pie Charts

Pie charts are useful when the data are discrete or discontinuous (categories) and when there are six or fewer categories. A circle is divided according to the proportion of counts in each category (see *Figure 346*).

The number of degrees of a circle to represent each category is then calculated (see *Figure 347*).

Blood group	Percentage of United Kingdom Population
A	47
B	7
AB	2
O	44

Figure 346 Tabulated blood group data

47/100 × 360° = 169°	8/100 × 360° = 29°
2/100 × 360° = 7°	44/100 × 360° = 158°

Figure 347 Pie chart angle calculation

The pie chart can then be drawn with a pair of compasses and a protractor to measure the angles. Alternatively, Excel can be used to plot the pie chart. The segments should then be labeled and a key provided, as shown in *Figure 348*. *(An alternative method of presenting this type of data is a bar chart.)*

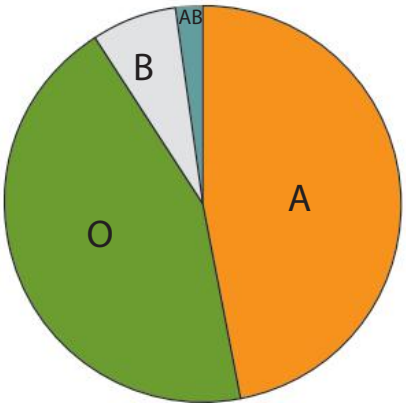


Figure 348 Pie chart showing blood group data from the United Kingdom

3.13.6 Nomograms

A nomogram or nomograph is a graphical method of presenting data regarding two or three variables that can be related to each other. It is a form of a conversion chart which allows values of one or more variables to be converted into values of another variable.

The nomogram shown in *Figure 349* can be used to determine the energy needs of males aged 10 to 15. For example to calculate the energy need for an active person (physical arbitrary level =1.4) with a body mass of 50 kg we draw a straight line through these coordinates on the first and third lines. A direct reading of the energy requirement of the person, (approximately 9.2 MJ), can be obtained from where the line crosses the middle or second line.

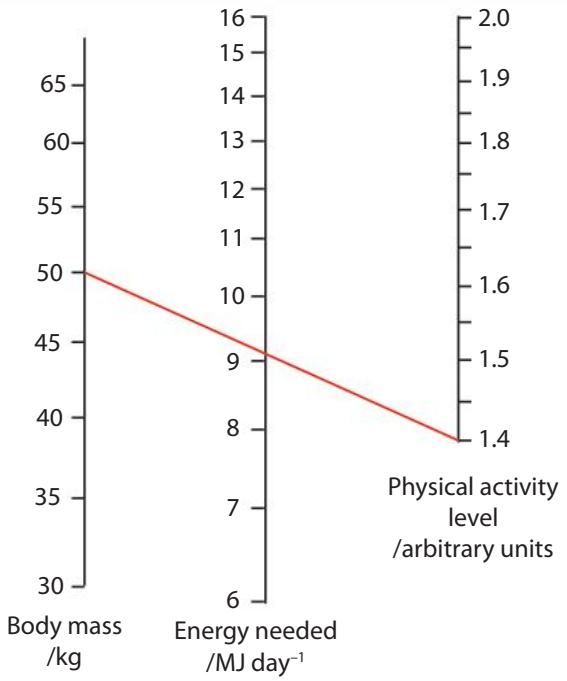


Figure 349 A nomogram for physical activity (for males aged 10 –15)

3.13.7 Kite Diagrams

If an area is being studied to determine the changes in the distribution of certain species along a transect, the data collected can be plotted as a kite diagram (see *Figure 350*).

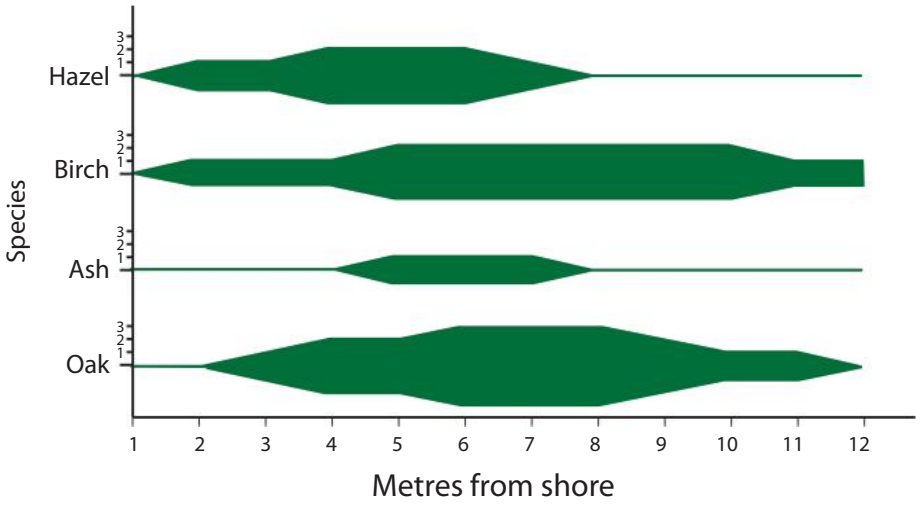
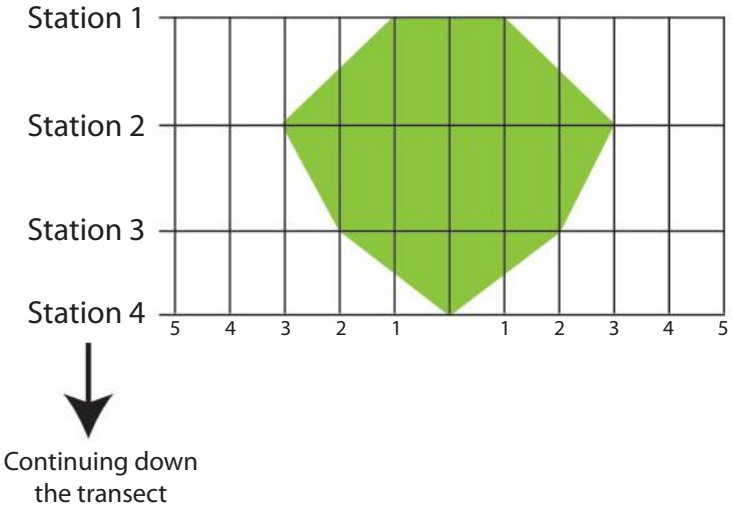


Figure 350 A kite diagram

A kite diagram consists of one or more kites drawn along a series of baselines. The kites along a single baseline represent the abundances of a species, usually a plant, across the transect. The wider the kite, the more frequent or abundant the species is. If the kite converges into a line then the species is absent at that point on the transect. The actual or relative abundance of a particular species at a particular sample point can be established by reading the width of the kite from the kite diagram. Kite diagram abundances are sometimes presented using a relative abundance scale known as the ACFOR scale (see *Figure 351*). The qualitative letter scale is then converted to a numeric scale.



A	Abundant	5
C	Common	4
F	Frequent	3
O	Occasional	2
R	Rare	1
N	Not found	0

Figure 351 Constructing a kite diagram, using the ACFOR scale

3.14 Graphing Data

The production of graphs is an important aspect of the presentation and processing of data. Excel can plot scattergrams, line graphs, bar charts and pie charts. If you are investigating an association between two variables, then you should plot a scattergram ; if you are comparing different sets of data, you should plot a bar chart; and if you are collecting frequency data, then you plot a bar chart or a pie chart. To produce a graph using MS Excel, enter your data into columns or rows, and select them. Then click on the Chart Wizard from the Toolbar (or Insert menu > Chart). This Wizard has four steps:

1. **Graph Type:** select the type you want and press 'Next'. Choose 'Pie' for pie charts, 'Column' for bar charts or 'XY (Scatter)' for line and scattergrams. Do not choose 'Line', which plots the data against row number.

2. **Source Data:** if your sample graph looks as expected, then simply press 'Next'.

If it looks wrong, you can correct it by clicking on the series tab, and then the red arrow at the end of the data range Box. Then highlight the cells containing the X data in the spreadsheet and press the red arrow again. Repeat this procedure for the Y values box.

3. **Chart Options:** the most important tasks are to enter appropriate titles for the graph and the two axes: labels and associated units. The gridlines and legend can be turned off which improves the appearance of the graph.

4. **Chart Location:** Enter 'Return' when you are finished. This places the chart adjacent to the data. It can then be copied and pasted into a Word document. The graph can also be saved as a template: new data can be entered into the spreadsheet cells and the graph will automatically be updated.

You are advised not to use three dimensional or shadow effects, which may obscure the trend in the graph.

Excel graphs are easily edited; simply double-click (or sometimes right-click (on a PC mouse)) on the part you want to alter.

For example, you can:

- move and re-size the graph.
- change the background colour (white is usually preferable).
- change the shape and size of the data markers (dots).
- change the axes scales and shape and tick marks.
- add a trend line or error bars.

3.14.1 Lines

Line graphs have lines, which either join the data points or form a straight 'line of best fit' (or trend line) through the middle of the points. If a continuous smooth relation between X and Y is expected, then a trendline is appropriate, otherwise the points should be joined by a series of small lines. To join the points with lines: double-click on any data point, select the Patterns tab and click on Line-automatic. It is usually not helpful to have Excel draw a curved or smoothed line, as these curves can create spurious peaks or troughs.

3.14.2 Error Bars

If you are plotting means on a scattergram (see *Figure 352*) or on a bar graph then errors bars are an excellent way of illustrating the confidence of the data displayed on the graph. However, error bars are not a requirement of the Group 4 assessment criteria for Biology and their inclusion will not score any additional marks.

Small error bars suggest reliable data, whereas large error bars suggest less reliable data. A line of best fit should pass through all the error bars. Error bars usually show ± confidence interval, although they can also be plotted from the standard deviation or standard error.

To add error bars to show the confidence intervals on your graph, double-click on any data point to open the Format Series Dialogue box, and choose the Y Error Bars tab. Click in the Custom+box, and highlight the range of cells containing your confidence intervals. Repeat this process for the Custom-box, and then press OK.

Figure 352 shows a scattergram with error bars (displaying confidence intervals) where a curve has been drawn through the data, but a straight line would also pass through all of the error bars, so a linear relationship is not ruled out.

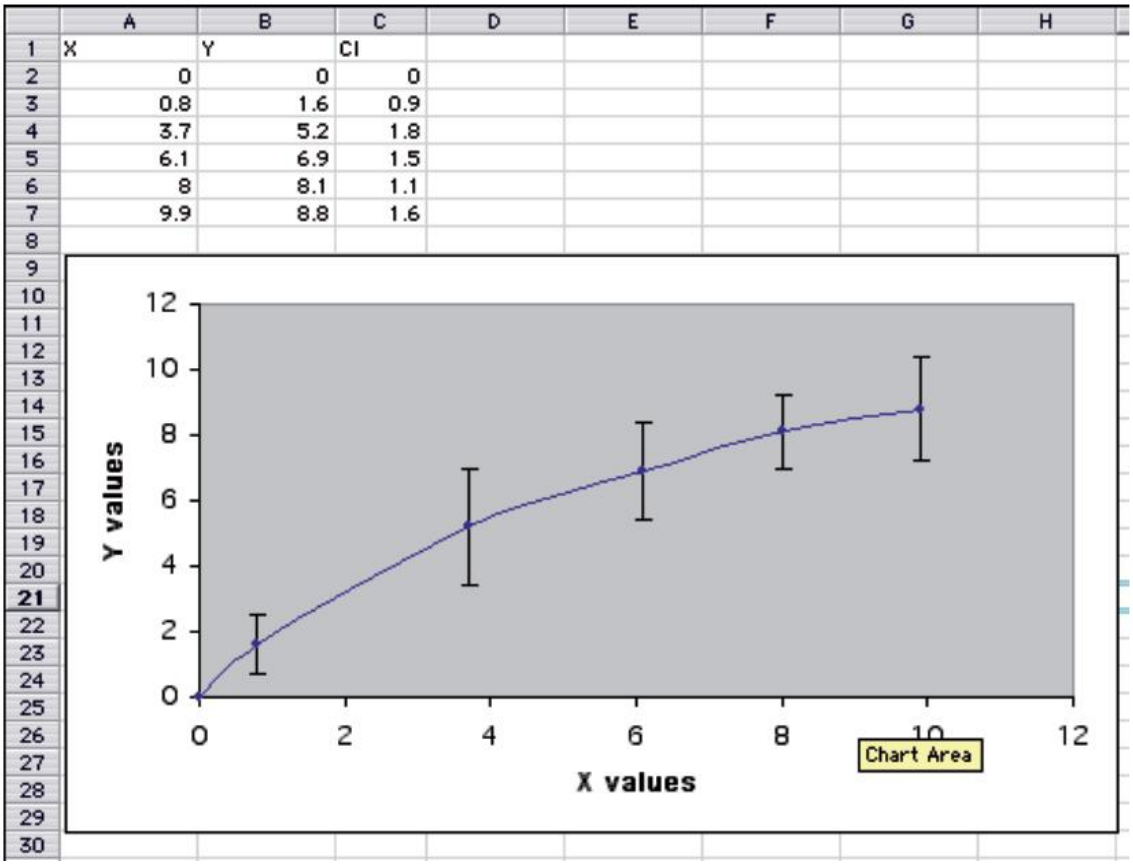


Figure 352 A scattergram

3.14.3 Association Statistics

A common task in the analysis of biological data is to investigate an association between two variables. This can take the form of a correlation to ascertain whether two variables vary together, or a regression to ascertain how one biological variable affects, or depends upon, another.

One common test for correlation is the Pearson product-moment correlation coefficient (r) for normally distributed data. The value of r can vary from +1 (prefect correlation), through 0 (no correlation) to –1 (perfect negative correlation).

In Excel, the Pearson coefficient can be found by two alternative formulae:

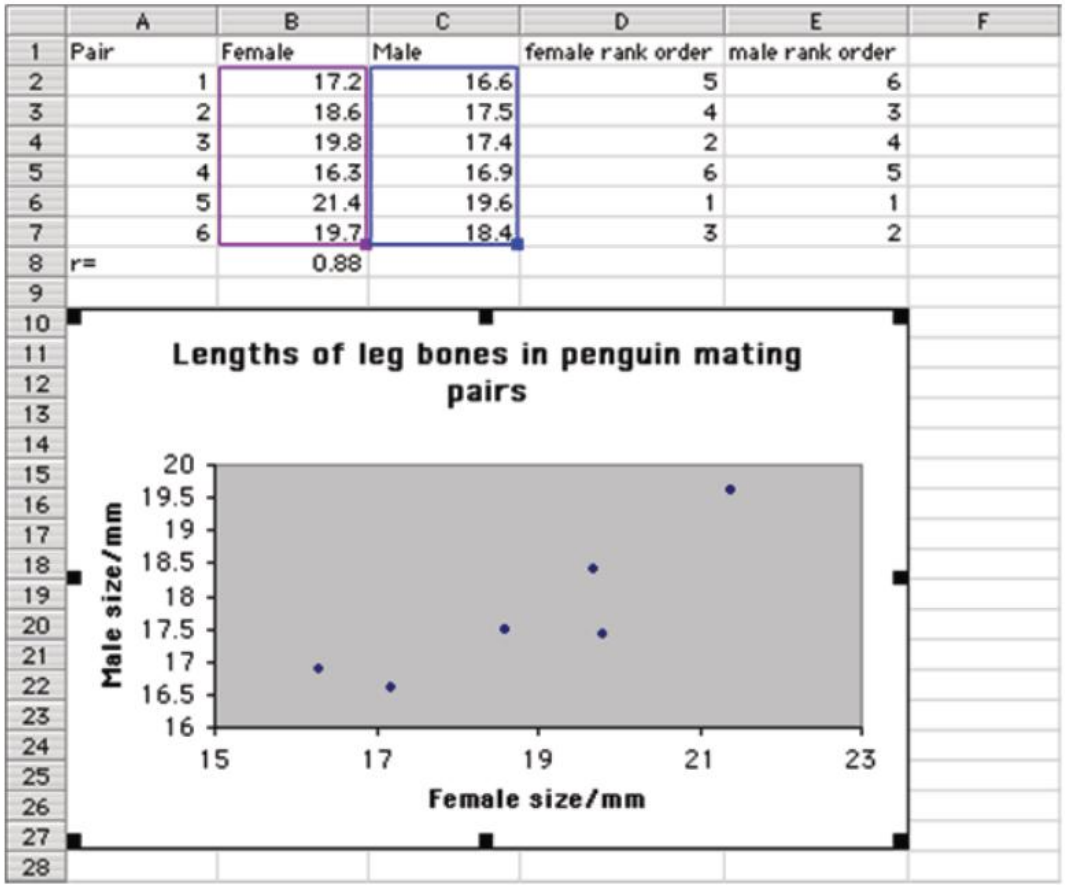
=CORREL (range 1, range 2)

or

=PEARSON(range 1, range 2)

The use of this formula is demonstrated below, using measurements of the size of breeding pairs of penguins, to establish whether there is a correlation between the sizes of males and females. The ranking of data seen in *Figure 353* can be done by hand or by the use of Excel's =RANK command as discussed below. However, the ranking of data is only required if you are going to calculate the Spearman rank-order correlation coefficient.

The RANK function returns the ranked position of a particular number within a set of numbers, and takes the arguments (number, reference, order). The number argument is the number for which you want to find the rank, reference is the range containing the data set, and order ranks the number as if it were in a ranking list in descending order. For example, to find out which ranking the score 16.6 has in the male data set in *Figure 353*, use the formula =RANK(16.6, C2:C7)). The value of the Pearson coefficient is given by the formula =CORREL(B2:B7,C2:C7) in cell B8. The value of 0.88 indicates a strong positive correlation, in other words, large females do mate with large males.



	A	B	C	D	E	F
1	Pair	Female	Male	female rank order	male rank order	
2	1	17.2	16.6	5	6	
3	2	18.6	17.5	4	3	
4	3	19.8	17.4	2	4	
5	4	16.3	16.9	6	5	
6	5	21.4	19.6	1	1	
7	6	19.7	18.4	3	2	
8	r=	0.88				

Figure 353 The lengths of a leg bone (in millimetres) in penguin mating pairs.

3.14.4 Correlation

The construction of a scattergram indicates whether there is a possible relationship between two variables. In particular, the closer the data points come to lying on a straight line, the closer the relationship and the higher the correlation. Conversely, the more scattered the data points, the weaker the relationship and the lower the correlation.

If the data points lie on a perfectly straight line, then the correlation is perfect; if the line slopes up from left to right the correlation is described as positive, and if the data points slope down from left to right, the correlation is described as negative (see *Figure 354*).

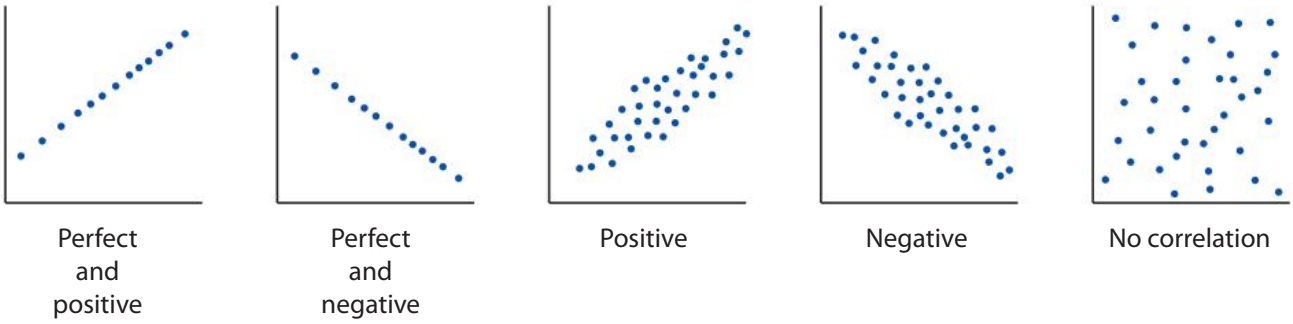


Figure 354 Simple scattergrams showing the common types of correlation

Be aware that a strong correlation does not necessarily mean causation. In other words, you cannot state definitely, that X causes the changes in Y (where X and Y are an dependent and independent variables). You may of course suggest in your conclusion that X causes changes in Y, or *vice versa*. However, you should not draw definite cause and effect conclusions based on correlation.

There are several reasons why you cannot make definite causal statements:

- You do not know the direction of the cause - does X cause Y, or does Y cause X?
- A third variable Z may be involved that is responsible for the correlation between X and Y.
- The apparent relationship may simply be due to chance.

A quantitative measurement of the strength of correlation known as the Pearson product-moment correlation (r), or simply the correlation coefficient, can be calculated.

3.14.5 Linear Regression

The technique of regression is applied when we think or know that a change in one variable causes the changes in the other. Experiments are frequently designed based upon an assumed causal relationship between two variables. A causal relationship between two variables implies that changes in one variable are directly responsible for changes in the other variable. The simplest type of causal relationship is a straight line or directly proportional relationship which can be analysed using linear regression.

The simplest way to perform linear regression with Excel is to plot a scattergram. Right-click on any data point on the graph, select Add Trendline, and choose linear. Click on the Options tab, and select Display equation on the chart.

You can also choose the intercept to be zero (or another value) and to display r^2 (the square of the Pearson Correlation coefficient). The full equation (in the form of $y = m\ x + c$, where m and c are the slope/gradient and intercept, respectively) with the slope or gradient and the intercept are now displayed on the graph.

Linear regression is demonstrated in *Figure 355*, which shows data obtained from counting a yeast cell suspension in a hemocytometer and in a colorimeter. We predict a linear causal relationship between cell density and turbidity and hence apply linear regression yielding an equation which allows us to predict the cell count for a given absorbance. The scattergram has a trend line with the regression equation and the square of the Pearson correlation coefficient displayed. In this example the intercept was fixed at zero since zero cells have zero absorbance.

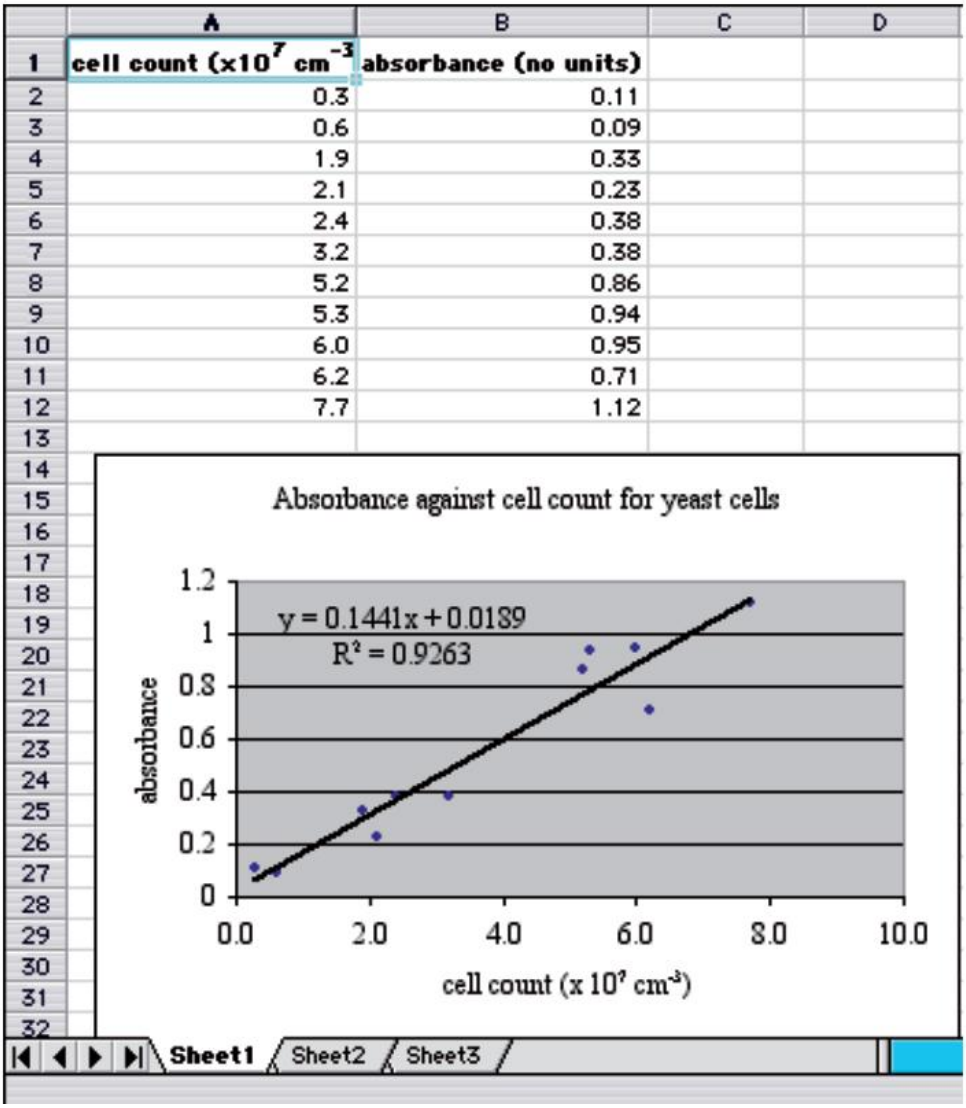


Figure 355 Linear regression

3.14.6 Comparative Statistics

Another common task during biological analysis is to compare two (or more) sets of data to determine whether they are basically the same (that is, whether they could come from the same population) or whether one set is significantly different from the others.

The means and confidence intervals for the two different groups should be calculated and bar charts should be plotted with confidence intervals represented as error bars. This approach gives an indication to the eye of how different the two groups are.

However, a statistical test is needed to test for significant differences between the two sets of data. The end result of such a test is a probability (P) that the null hypothesis, which always asserts that there is no difference between the two sets of data, is true.

In biology we accept differences as being significant if P is less than 5%. If P is less than 5%, then we assert that there is a significant difference between the two sets of data, that is, we cannot accept the null hypothesis – it is rejected.

If, however, P is greater than 5%, then we can assert that there is no significant difference between the two sets of data, that is, we accept the null hypothesis. (*The critical probability of 5% appears relatively low, but it reflects the fact that biological investigations are expected to produce quite varied results, due to random errors and natural variation*).

t-test

A common form of biological data analysis is to compare two sets of measurements to establish if they are the same or different. If the means of the two sets of data are very different, then it is relatively easy to decide that the two sets of data are different. However, if the two means are relatively close to each other it is more difficult to judge whether the two sets are the same or significantly different.

The t-test is used to compare two sets of normally distributed data. It returns a probability value (P) that there is no statistical difference between the two sets. This is called the null hypothesis.

In Excel, a t-test is performed by the formula

=TTEST(range1, range2, tails, type)

which returns P directly, where P varies from 0 (impossible) to 1 (certain). The higher the probability, the more likely it is that the two sets of measurements are the same, and that any difference between them are just due to random chance. The lower the probability the more likely it is that the two sets of measurements are significantly different and that any differences are real.

The value of the variable tails will be one for a one-tailed test or two for a two-tailed test. The variable type will either be one for paired data (when the two sets of data are from the same individual) or two for unpaired data (where the sets of data are from different individuals), and both are common.

Both types of t-test are shown in *Figure 356*. In the unpaired test (type 2) the yield of potatoes in ten plots treated with one fertiliser was compared to that in ten plots treated with another fertiliser. Fertiliser results in a larger mean yield, but the value of the t-test P indicates that there is an 8% probability that these two sets are the same. Since this value is greater than 5% we conclude that fertiliser B is not significantly better than fertiliser A.

In the paired test (type 1), the pulse rate of eight individuals was measured before and after a large meal. The mean pulse rate is a little higher after the meal, and the value of t-test P indicates that there is only a 0.01% probability that the data are the same. Hence, the pulse rate does rise significantly after a meal.

In *Figure 356*, the top of the diagram shows the unpaired or type 2 t-test. The bottom of the diagram shows the paired or type 1 t-test. The cells with the t-test probabilities were formatted as percentages (Format menu>cell>number tab>percentage). Although not shown it is also helpful to plot the means as a bar chart with error bars to show the difference graphically. The t-test formulae at cells B15 and C31 are =TTEST(B3:B12,C3:C12,2,2) and =TTEST(C21:C28,D21:D28,2,1).

	A	B	C	D	E
1		Yield of potatoes/kg			
2	Plot	Fertiliser A	Fertiliser B		
3	1	28	29		
4	2	21	20		
5	3	17	19		
6	4	19	22		
7	5	23	25		
8	6	20	21		
9	7	24	26		
10	8	22	28		
11	9	18	30		
12	10	20	22		
13	Mean	21.2	24.2		
14	95% CI	2.00	2.44		
15	t-test P	7.88%			
16					
17					
18					
19					
20		Subject	pulse rate before heating/bpm	pulse rate after heating/bpm	
21		1	106	110	
22		2	80	88	
23		3	80	87	
24		4	104	110	
25		5	88	91	
26		6	75	79	
27		7	74	79	
28		8	83	90	
29		Mean	86.25	91.75	
30		95% Confidence interval	8.58	8.41	
31		t-test	0.01%		
32					
33					

Figure 356 The two types of t-test.

Simpson's Diversity Index

The Simpson's Diversity Index is a statistical measure used to describe the richness of flora and fauna in a particular habitat. A high biodiversity index does not necessarily imply that a habitat or site should receive priority protection. For example, the Arctic, Antarctic and hot deserts contain many interesting and well-adapted species living in habitats of low biodiversity. The values calculated (see *Figure 357*) are not absolute, they are just used for comparison purposes; a higher value of the Simpson's Diversity Index suggests a higher biodiversity.

In practice, the Simpson's diversity index is frequently used as a measure of human interference in a habitat: a low value may indicate cultivation and/or pollution. However this practice is questionable unless the figures/indices are compared to those of sites that are similar, better still, identical, in all respects except the human interference. In other words, a control should be ideally employed.

simpson

	A	B	C	D	E
1	**Species**	**Number of plants, n1**	**n1*(n1-1)**	**Number of plants, n2**	**n2*(n2-1)**
2	1	36	1260	75	5550
3	2	15	210	21	420
4	3	14	182	4	12
5	4	13	156	2	2
6	5	9	72	1	0
7	6	8	56	0	0
8	7	7	42	0	0
9	8	4	12	0	0
10		106	1990	103	5984
11					
12		**Simpson's diversity index**		**Simpson's diversity index**	
13					
14		5.6		1.8	

Figure 357 Calculation of Simpson's Diversity index from plant data using Excel

Shown below are selected Excel formulae in the above spreadsheet:

- Cell Formula
- B10 =SUM(B2:B9)
- C10 =SUM(C2:C9)
- D10 =SUM(D2:D9)
- E10 =SUM(E2:E9)
- C2 =B2*(B2-1) (Use the Fill-down function to cell C9)
- E2 =D2*(D2-1) (Use the Fill-down function to cell E9)
- B14 =B10*(B10-1)/C10
- D14 =D10*(D10-1)/E10

Cells B14 and D14 are formatted to a precision of one decimal place.

3.15 Using Units In Calculations

Units should always be included during any calculation. The advantages of using units throughout a calculation include:

- an awareness that mathematical equations are not merely symbols, but express relationships between physical quantities.
- an in-built check on whether the correct equation has been used.

The following rules should be applied when performing arithmetic with units:

Addition and subtraction: the units do not change:

- for example, 2.0 g + 5.0 g = 7.0 g

Multiplication and division: the units multiply and divide too:

- for example, 3.0 cm × 3.0 cm = 9.0 cm^2; 10 kg × 9.8 m s^{-2} = 98 kg m s^{-2}
- for example, 16.7 cm × 10.0 cm × 6.00 cm = 1 002 cm × cm × cm = 1 002 cm^3

  (*The answer should be expressed as 1 000 cm^3 since the original measurements were given to three significant figures*).

As a consequence, the units may cancel.

- for example, 5.0 g/10.0 g = 0.5
- for example, 0.08 cm^3/2 s = 0.04 cm^3 s^{-1}

(formerly written as 0.04 cm^3 / s).

- for example, 5.0 mol/ 2 dm^3 = 2.5 mol dm^{-3}

(formerly written as 0.2.5 mol / dm^3).

where the symbol '/' is known as a solidus and pronounced as 'per'. Hence 2.5 mol dm^{-3} in the spoken form is '2.5 moles per cubic decimetre'. The notation with negative exponents, for example, 2.5 mol dm^{-3}, is the agreed notation.

3.16 Concluding

The conclusion is a final summation of the information contained in the interpretation section. The size and origin of any uncertainties assigned to any final numerical values must be discussed and justified. Where appropriate any numerical values or observations should be compared to the biological literature value or observations.

For example

The narrow range of preferred temperatures for species X in this study agrees with the findings for other genera of beetles (appropriate reference).

If the investigation produces a quantitative result then a percentage error should be calculated.

For example:

The iso-electric point of enzyme X was found to be 6.2. This is a 9% percent difference from the literature value of 6.8 (appropriate reference).

The conclusion also involves an assessment of the experiment(s), in terms of the stated aim, or verification or rejection/falsification of the hypothesis.

The results indicate that the hypothesis was verified: light is required for the process of photosynthesis.

This hypothesis has been verified.

The results indicate that the hypothesis was false: seeds of species X do not require light for germination.

This hypothesis has been falsified. You should include your reasoning for accepting or rejecting your hypothesis.

ASSESSMENT CRITERIA	EVIDENCE REQUIRED	WHAT YOU MUST DO
Recording raw data	Records sufficient and relevant quantitative and associated qualitative raw data	Records sufficient and relevant raw data (qualitative observations and/or quantitative data (usually tabulated). This may include data-logger print outs and digital photographs. Records data for the controlled variables. The data must allow a detailed and valid conclusion to the research question. This often implies data supporting a relationship between an independent and dependent variable.
	Records units and absolute uncertainties where relevant.	Records all measurements to the correct number of significant figures and records appropriate units, usually SI units (showing derivation where appropriate). Records the level of absolute uncertainty or precision for each quantitative reading.
Processing raw data	Processes the quantitative raw data correctly.	Raw data is subjected to relevant calculations (processing). Calculations are correct and accurate to the level necessary. Units are included in the calculations. Significant figures rules are stated and followed. Converts absolute uncertainties to percentage uncertainties and propagates percentage uncertainty calculations correctly. Converts tabulated data into graphical form as relevant. Extracts relevant quantities from the graph (ideally a linear graph) via extrapolation or interpolation or determination of the gradient. Makes appropriate choice of graph or chart. Simple statistics may be performed if relevant.
	Presents and interprets processed data appropriately and, where relevant, includes errors or uncertainties in calculations and graphs.	• Use of proper scientific conventions in tables (for example, units written once at top of columns), drawings of graphs and charts. • Sample calculations are shown and explained/justified; derived units are included in final calculations. • For graphs, labels and units are correct and scale is appropriate; error bars may be present. • For a graph a line or curve of best fit is drawn (if appropriate). • Final numerical answers are accompanied by an absolute uncertainty.

Figure 358 Details of what is required in the Analysis criterion

This criterion assesses the extent to which the student's report provides evidence of evaluation of the investigation and the results with regard to the research question and the accepted scientific context.

The descriptors in the following table will be used by your teacher to allocate a mark for your performance in this criterion:

MARK	DESCRIPTOR
0	The student's report does not reach a standard described by the descriptors below.
1–2	A conclusion is outlined which is not relevant to the research question or is not supported by the data presented. The conclusion makes superficial comparison to the accepted scientific context. Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are outlined but are restricted to an account of the practical or procedural issues faced. The student has outlined very few realistic and relevant suggestions for the improvement and extension of the investigation.
3–4	A conclusion is described which is relevant to the research question and supported by the data presented. A conclusion is described which makes some relevant comparison to the accepted scientific context. Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are described and provide evidence of some awareness of the methodological issues* involved in establishing the conclusion. The student has described some realistic and relevant suggestions for the improvement and extension of the investigation.
5–6	A detailed conclusion is described and justified which is entirely relevant to the research question and fully supported by the data presented. A conclusion is correctly described and justified through relevant comparison to the accepted scientific context. Strengths and weaknesses of the investigation, such as limitations of the data and sources of error, are discussed and provide evidence of a clear understanding of the methodological issues* involved in establishing the conclusion. The student has discussed realistic and relevant suggestions for the improvement and extension of the investigation.

Guiding Questions

- ***To what extent has the student discussed limitations and/or likely sources of error in their methodology?***
- ***To what extent has the student discussed the reliability of their data?***
- ***To what extent has the student demonstrated an understanding of the impact of experimental uncertainty on their conclusion?***
- ***To what extent has the student suggested relevant and feasible modifications to their methodology?***
- ***To what extent has the student suggested relevant and feasible extensions to the investigation?***
- ***To what extent has the student demonstrated an understanding of the implications of the conclusion?***

4.1 Interpreting Graphs

Interpreting a scattergram or line graph involves examining the graph to determine whether any trend is present. The presence of a trend may suggest some kind of relationship between the two variables.

4.1.1 Interpreting scattergrams

When interpreting a scattergram examine the distribution of the plotted points and decide whether it indicates a relationship or correlation between the two variables. The diagrams in *Figure 401* will help you interpret the three different types of plot distributions for scattergrams. Refer to *Figure 402*.

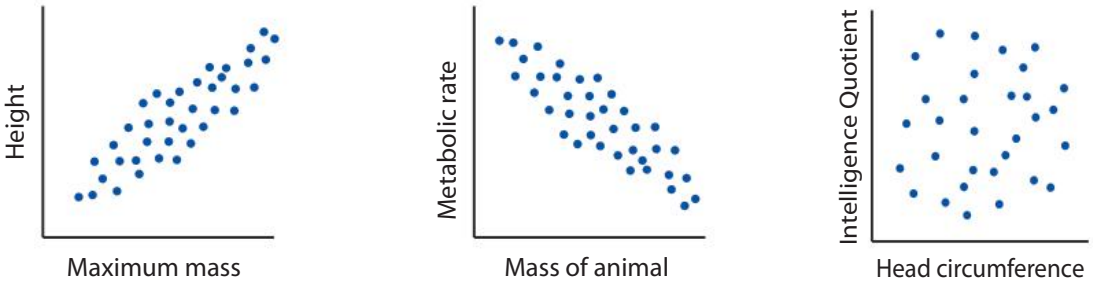


Figure 401 (a), (b) and (c) Plot distributions for scattergrams

FIGURE 401(a)	FIGURE 401(b)	FIGURE 401(c)
Interpretation	**Interpretation**	**Interpretation**
Heavier people tend to be taller and lighter people shorter.	Heavier animals tend to have higher metabolic rates and vice versa	People with large craniums do not necessarily have higher intelligence quotient scores and vice versa.
Conclusion	**Conclusion**	**Conclusion**
Height and weight are directly proportional.	Mass and metabolic rate are inversely proportional.	There is no relationship between intelligence quotient score and cranium size.
A positive correlation exists between the two variables.	A negative correlation exists between the two variables.	There is no correlation between the two variables.

Figure 402 Interpretation of scattergrams

There are also various statistical tests which can be used to determine the strength of correlation between two variables in a scattergram, for example, the use of Excel in determining the Pearson product-moment correlation coefficient (as found later in this Handbook.)

If a correlation does exist then it is possibly to mathematically determine a line of 'best fit' which can represent the relationship between the two variables.

Evidence for correlation between two variables does not prove that variable A caused the change in variable B, or vice versa. Strong correlation between two variables only suggests a connection between two variables, it does not confirm causation, that is, that one causes the change in the other.

4.1.2 Interpretation of line graphs

The initial interpretation of a line graph involves stating the trend in the slope of the graph. *Figures 403, 404 and 405* shows some common trends in line graphs of various biological phenomena, though bear in mind that your points will have some 'scatter' due to random errors.

Figure 403 (a) and (b)

FIGURE 403 (a)	FIGURE 403 (b)
Slope	**Slope**
Constantly rising slope.	Constantly decreasing slope.
Interpretation	**Interpretation**
Variable A causes variable B to increase in a linear manner.	Variable A causes variable B to decrease in a linear manner.

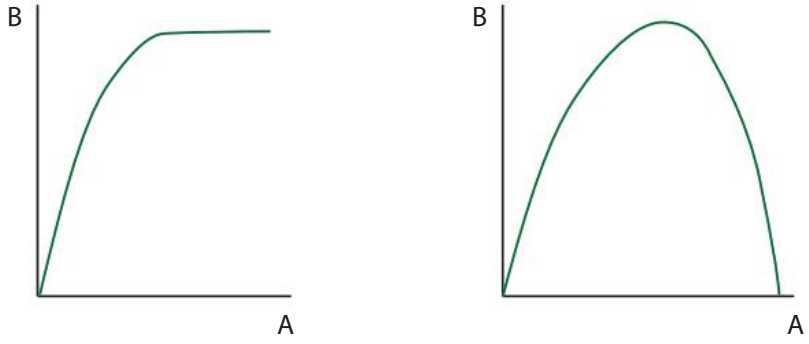


Figure 404 (a) and (b)

FIGURE 404 (a)	FIGURE 404 (b)
Slope	**Slope**
A rising slope which becomes level.	A rising slope which peaks and then decreases.
Interpretation	**Interpretation**
Variable A causes variable B to increase initially, but then has no effect.	Variable A causes variable B to increase to a maximum then to decrease.

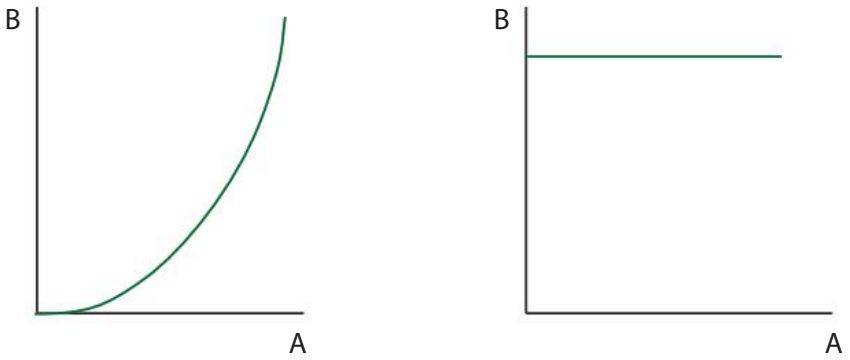


Figure 405 (a) and (b)

FIGURE 405 (A)	FIGURE 405 (B)
Slope	**Slope**
The slope get steeper and steeper.	No slope
Interpretation	**Interpretation**
Variable A causes variable B to increase in an exponential manner.	Variable A causes no change in variable B.

A complete and accurate interpretation of a line graph will include reference to numerical data from the graph. For example, in *Figure 406*, a suitable description might be: During the first three minutes the rate of photosynthesis increased slowly, then over the next two minutes the rate increased further. There was then a steep increase in the rate until a maximum of 60 units at 11.5 minutes. The rate remained constant for three minutes and rapidly decreased to zero at eighteen minutes at a constant rate of decline.

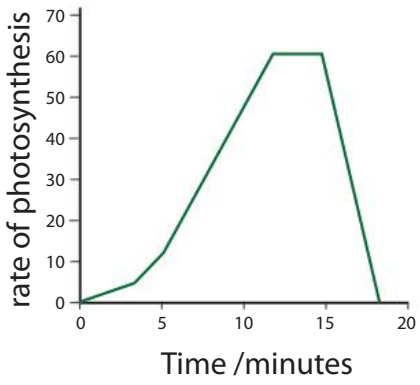


Figure 406 Interpretation of a graph displaying rate (arbitrary units) against time.

4.2 Using Graphs

4.2.1 Interpolation

Interpolation means determining a value which has not been plotted, but is within the range of your experimental data, by reading directly from the graph (a so-called graphical method) or by calculation. For example, consider the graph in *Figure 407* of osmotic pressure against concentration of sucrose. The dotted construction line illustrates how a student can graphically determine the osmotic pressure generated by a 0.25 mol dm^{-3} sucrose solution. The value is approximately 680 pascals.

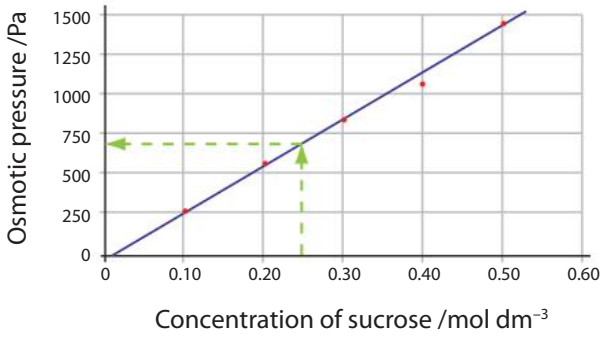


Figure 407 Osmotic pressure produced by sucrose solutions

4.2.2 Extrapolation

Extrapolation (see *Figure 408*) means the determining a value that it outside the range of your experimental data. It involves continuing the interpolated line or curve beyond the range of the plotted points (see *Figure 408*). This enables you to predict what might occur outside the range of measured values. The extrapolation should always be drawn as a dotted line so that that it contrasts with the continuous line or curve of the rest of the graph. (*Although curved graphs may be extrapolated this can lead to large errors since the curved can be smoothly extended by a number of curves. This is one reason why curved graphs should be transformed into straight line graphs*).

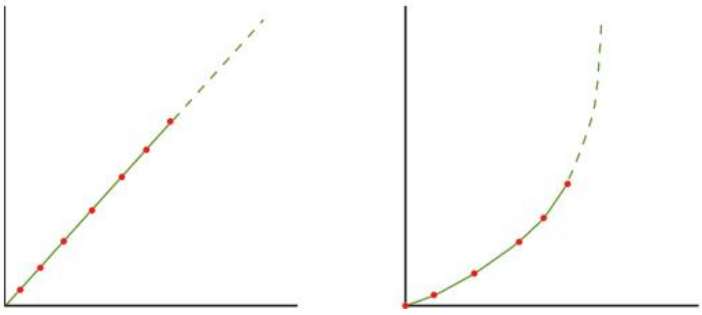


Figure 408 Extrapolation of straight line and curved graphs

On some occasions extrapolation may be justified, but often it can be misleading. For example, the rate of photosynthesis rises almost linearly with light intensity, but at high levels of light photo-saturation occurs and the graph (see *Figure 409*) becomes flat at very high levels of light intensity the graph will begin to slope downward as the chloroplasts in the palisade layer migrate downwards and damage to chlorophyll occurs. (Similar plots occur with plotting rates against a limiting factor).

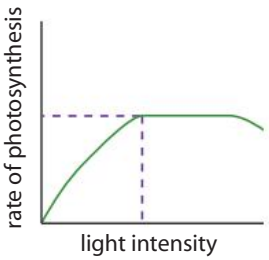


Figure 409 A graph of the rate of photosynthesis (at constant temperature) against light intensity

It is also unwise to extrapolate curved graphs in Biology, especially with enzyme-controlled reactions, since there is a tendency for the reaction to slow down or decrease rapidly after a general increase due to denaturation of the enzyme.

4.2.3 Measuring the Gradient of a Graph

The gradient or slope of a straight line graph is a measure of the steepness with which the graph line is rising.

The gradient of a straight line graph = $\frac{\text{y increase}}{\text{x increase}}$.

To calculate this ratio as accurately as possible the increases in x and y should be as large as possible. See *Figure 410*.

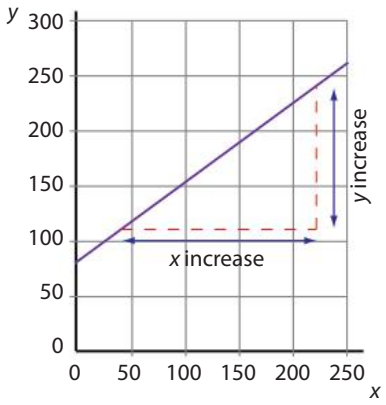


Figure 410 Measuring the gradient of a straight line graph

You may also have to determine the gradient or slope at a particular data point on a curved graph as shown below in *Figure 411*. This type of calculation is likely to be performed during an investigation into an enzyme-controlled reaction.

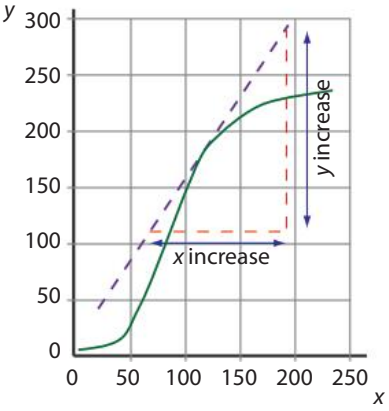


Figure 411 Measuring the gradient of a curved line graph

4.2.4 Displaying Errors on Graphs (Extension Material)

When carrying out experimental investigation it is impossible for you to record measurements with 100% accuracy. There will be an error or uncertainty in all your measurements which can be easily determined. For example your measurements of some seedlings obtained with a ruler might be to the nearest millimetre, so all your measurements should be reported as ± 0.05 mm.

This error or uncertainty can be indicated on a line graph (see *Figure 412*) by means of an error bar drawn above and below the data points on the graph. Often the data plotted are means so the standard deviation can be plotted as an error bar.

Although error bars are not a requirement of the Group 4 Assessment Criteria for Biology they do indicate to your teacher and the moderator that you are aware of the limitations of your apparatus or sampling method. Standard deviations can be readily calculated by Excel and error bars are easily added to an Excel-generated line graph.

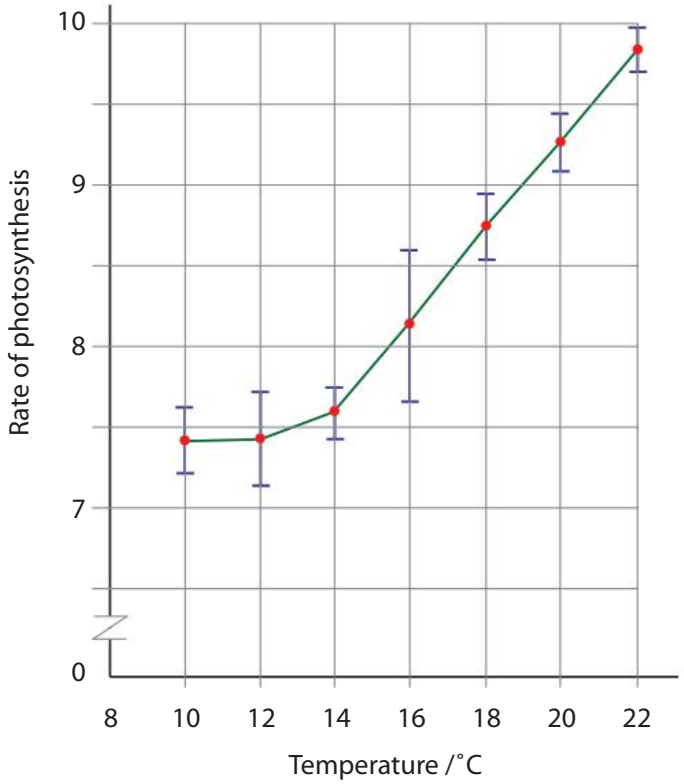


Figure 412 A line graph with associated error bars and their interpretation

4.3 Evaluating procedures

4.3.1 Evaluation

If, however, a conclusion was difficult or impossible to draw, then the hypothesis may not have been formulated correctly. It may then be necessary to reject the original hypothesis and formulate another.

Another possibility is that while the hypothesis is accepted, the data acquired during the investigation is rather limited. You may also have found that your techniques or materials were unsuitable or inaccurate and did not allow you to test your hypothesis.

A final possibility is that the results are too complex or numerous to analyse properly. In this case a family of statistical methods called ANOVA (analysis of variance) might be appropriate, but a detailed knowledge of such techniques is outside the scope of the current IB Biology Programme.

This section will also include identifying weaknesses inherent in the experimental techniques, or experimental design (for example, identification of uncontrolled variables) as well as suggestions for improving the method.

If your method involved sampling then the following approaches will increase the precision of the measurements:

- Increase the size of the experiments by adding more replicates or trials;
- Refine and improve the experimental technique, for example, by using measuring instruments of a higher precision;
- Handle the experimental material so that the effects of variation are reduced.

Other issues that should be raised, where appropriate, are suggestions for further practical work in that area. This should either extend the practical work or help to clarify areas of doubt in your results.

4.3.2 Evaluation of error

Precision

Precision indicates how close together the various experimental measurements on a sample are. It is the spread or variation in a set of measurements for several trials or samples of the same experiment. It may be assessed by calculating the mean, range or standard deviation and compared with the maximum experimental error calculated. Random errors affect precision, but it may be improved if a sufficient number of repetitive measurements is performed.

Accuracy

Accuracy indicates how close the experimental value is to the true or accepted value, if this is known. Systematic errors affect accuracy, but if the sample size is constant, they have no effect on precision. The concepts of accuracy and precision are illustrated below with darts thrown at a dart board (see *Figure 413*).

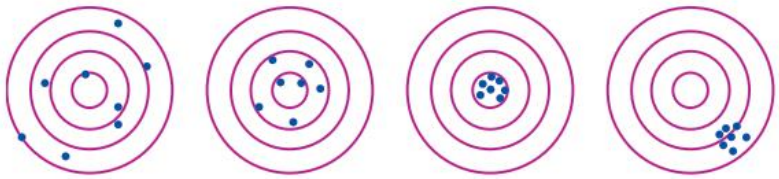


Figure 413 (a), (b), (c) and (d) Concepts of accuracy and precision

- (a) imprecise, and inaccurate
- (b) imprecise and accurate
- (c) accurate and precise
- (d) precise and inaccurate

An illustration of errors and concept of an experimental control

Shown below in *Figure 414* is a simple respirometer, which is used to measure the volume of oxygen taken up by a small organism, for example, an insect, during a given length of time. The oil droplet moves from right to left along the scale as the animal consumes oxygen.

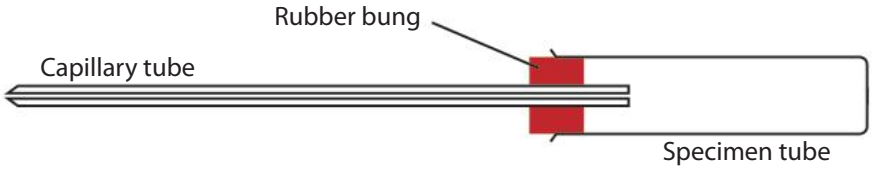


Figure 414 Simple respirometer

Random experimental errors inherent in the apparatus may arise from the following:

- Small temperature fluctuations may occur in the surrounding air during the experiment which will alter the pressure inside the respirometer and hence move the oil droplet.
- The pressure of a gas is directly proportional to its (absolute) temperature at constant volume. In addition, small variations in the values of measurements due to parallax error when reading from the capillary tube scale may occur.

Systematic errors inherent in the apparatus may arise from the following:

- The capillary tube may not be slightly titled away from the horizontal position and gravity may cause the droplet to move very slowly.
- The soda lime (a dry mixture of calcium and sodium hydroxides) may be exhausted and be unable to absorb any more carbon dioxide gas.
- The apparatus may not be air tight and gases may be leaving and entering via a small hole.

Control

The control would consist of an identical respirometer set up under identical experimental conditions of air temperature and pressure, except the animal should be excluded. Ideally, the experiment involving the control should be performed at the same time. If there is no movement of the oil droplet in the control apparatus the results of the experimental apparatus require no alteration.

If, however, changes in atmospheric pressure cause the droplet in the control tube to move, the distance should be recorded. If repetition of the experiment yields the same distance then a systematic error is inherent in the experiment.

This distance should be added to the distance measured in the experimental tube, if the droplet moves away from the specimen tube in the control, or subtracted if it moves towards the specimen tube. This is an example of compensating for a systematic error.

4.4 Improving the investigation

Your suggestions for improvements should be based on the weaknesses and limitations. Your modifications to the experimental techniques and the data range can be addressed here. your modifications proposed should be realistic and clearly specified. It is not sufficient to state generally that more precise equipment should be used. Refer to *Figure 415*.

TYPE OF ERROR	SOURCES OF ERROR	METHOD OF REDUCING ERROR
SYSTEMATIC ERROR	• Faulty apparatus or instrument.	• Regular maintenance and proper storage of apparatus or instrument.
SYSTEMATIC ERROR	• Incorrect calibration of instrument or apparatus (for example, the conditions under which calibration is carried out may be different from those in the school or college laboratory, or the wrong standard was used).	• Re-calibrate correctly, if possible (for example, a pH meter should be calibrated against a fresh buffer, rather than one that has expired or been contaminated with excess acid or alkali).
SYSTEMATIC ERROR	• Incorrect measuring techniques (for example, generation of a parallax error when consistently reading from a scale incorrectly).	• Use the correct technique when recording measurements.
SYSTEMATIC ERROR	• Problems involving the design of the method for the investigation (for example, no effective control of a particular variable, for example, temperature during an enzyme, respiration or photosynthesis investigation).	• Refine the design of the method for the investigation (for example, the use of a thermostatically controlled water bath instead of an un-insulated glass beaker of water for controlling temperature).
RANDOM ERROR	• Lack of precision on the instrument (for example, using a plastic ruler to measure an object smaller than 1 mm).	• Use an instrument with greater precision or one which can detect and measure smaller quantities (for example, using a micrometer screw gauge or vernier calipers).
RANDOM ERROR	• Interpolation between scale divisions (for example, recording readings of a solution level found between two graduations (markings) of a burette (Class B)).	• Use an instrument with greater precision (for example, use a burette (Class A). • Use a magnifying glass).
RANDOM ERROR	• Fluctuations in readings from external factors when measurements are recorded, for example draughts on balance pan causing the digital reading to fluctuate.	• Ensure that environmental conditions are stabilised before recording any measurements (for example, switch off the air conditioner).
RANDOM ERROR	• Small sample size	• Increase the sample size
RANDOM ERROR	• The chosen samples are not representative.	• Repeat measurements for more samples within the same locality and from different localities
RANDOM ERROR	• Variation in the consistency of the experimenter in recording measurements, for example, changes in the human reaction time when using the stop watch during an investigation in involving the time taken for a colour change to occur or for a task to be completed.	• Refine measurement technique (for example, practice recording measurements or use a data-logger in conjunction with an electronic timer/trigger).

Figure 415 A summary of systematic and random errors

4.5 Limitations of measurements and procedures

As mentioned previously, many sources of errors arise from the limitations of measurements and experimental procedures. Below is a summary of the various limitations that may be present in an experimental investigation. These should be addressed under the heading 'Conclusion and Evaluation' in the report or 'write-up' for your Individual Investigation.

Limitations of measurements:

- The lack of precision of the instruments used.
- Miscalibration of instruments or apparatus.
- Inconsistency in the recording of measurements.
- Fluctuations in the readings.
- Limitations of experimental procedures:
- Insufficient or small number of repeats or replicates of measurements.
- The experimental sample (if appropriate to the investigation) was too small.
- The range of the independent variable for a specific investigation was too narrow or too wide.
- The intervals between the independent variable were too large.
- There was a failure to control all the controlled variables.

Listed in Figure 416 is a summary of what you need to do to score well in the Evaluation criterion.

Assessment criteria	Evidence required	What you must do
Concluding	States a conclusion, that is described, justified and supported by the data.	Analyse and explain the data from the experiment and draws a valid conclusion which is relevant to the research question and its scientific context (background information that may include a hypothesis, competing hypotheses and a scientific model). The conclusion must be supported by the raw and processed data, (though it may be tentative and subject to some statistical uncertainty). If a graph is present, the correct graphical relationship is stated and numbers quoted to support the relationship. The graph may be used to obtain a gradient or intercept or be used for extrapolation or interpolation. If appropriate, uses the graph to identify any anomalous data points. Where appropriate, compares the experimental result with the accepted result: calculates absolute and percentage errors from the expected or literature value. Compares results obtained by repetition, or against the chemical literature, and comments on the reliability of the values obtained. Some simple statistics may be included if large numbers of repeated random measurements are recorded.
Evaluating methodology and data	Evaluates strengths and weaknesses, such as limitations of data and sources of error	Outline any limitations to the accuracy/reliability/amount/range of data that you have obtained. States simplifying assumptions that were made which may affect the accuracy of the results. Discusses any limitations of the methodology used. Identifies and quantifies limitations due to the precision and accuracy of the equipment. Performs error propagation with random errors. Identifies possible systematic errors or other unanticipated factors. Strengths may involve control of variables, reduction of random errors and identification of systematic errors. Weaknesses may involve inability to control or monitor important controlled variables, biological variation, large random errors or large percentage errors in small measurements.
Improving and extending the investigation	Suggests realistic improvements in respect of identified weaknesses and limitations.	Suggests modifications to improve the existing investigation to reduce random errors and to identify possible sources of systematic error. Suggests alternative methodology to improve the investigation, perhaps by better control of controlled variables and more precise measurements of the dependent variable. Suggests alternative equipment or apparatus (with higher sensitivity) if applicable. Suggests how to extend the experiment, for example, collecting additional and more precise data outside the current data range

Figure 416 Summary of the Evaluation criterion.

This criterion assesses whether the investigation is presented and reported in a way that supports effective communication of the focus, process and outcomes.

The descriptors in the following table may be used by your teacher to allocate a mark for your performance in this Communication criterion:

MARK	DESCRIPTOR
0	The student's report does not reach a standard described by the descriptors below.
1–2	The presentation of the investigation is unclear, making it difficult to understand the focus, process and outcomes. The report is not well structured and is unclear: the necessary information on focus, process and outcomes is missing or is presented in an incoherent or disorganized way. The understanding of the focus, process and outcomes of the investigation is obscured by the presence of inappropriate or irrelevant information. There are many errors in the use of subject-specific terminology and conventions*.
3–4	The presentation of the investigation is clear. Any errors do not hamper understanding of the focus, process and outcomes. The report is well structured and clear: the necessary information on focus, process and outcomes is present and presented in a coherent way. The report is relevant and concise thereby facilitating a ready understanding of the focus, process and outcomes of the investigation. The use of subject-specific terminology and conventions is appropriate and correct. Any errors do not hamper understanding. * e.g. incorrect/missing labelling of graphs, tables, images; use of units, decimal places. For issues of referencing and citations refer to the academic honesty section.

Guiding Questions

- *To what extent is the student work concise, clear, and structured in a logical sequence?*
- *How well does the reporting of the methodology allow the investigation to be successfully repeated by others?*
- *How well does the report allow the process of data analysis to be followed?*
- *To what extent are graphs, tables, and images presented unambiguously?*
- *To what extent is appropriate subject-specific terminology used throughout the investigation?*
- *To what extent is appropriate subject-specific notation used throughout the investigation?*
- *To what extent has the student used correct conventions for presentation of quantitative data, including appreciation of decimal places, significant figures, and uncertainties where appropriate?*

For maximum marks the report for your Individual Investigation must be biologically and mathematically correct, as well as clear and easy to follow. It must be well structured and follow conventional formats for referencing and the processing and presentation of raw and processed data. Your report must centre your research into an appropriate Biological context and must not include material that it is not appropriate or relevant.

5.1 Organization of the written report

The following is a suggested layout for the written report for the Individual Investigation. It is *not* mandated by the IBO and other presentations may also be acceptable.

5.1.1 Formatting a report

Title and Research Question

List the title or aim of the experiment or meaningful name for your research report followed by your Research Question, for example,

An investigation into the importance of surface area to volume ratio in model cells.

To investigate the effect of surface to volume ratio on the absorption of sodium hydroxide acid by simple diffusion into an agar cube (filled with hydrochloric acid and phenolphthalein indicator).

This could be in the form of a cover page that also includes your full name (as registered with the IBO), your school's name and your IB candidate number.

Contents page

The contents page will list the contents and page numbers for ease of location or cross-referencing. All pages must be numbered throughout the report. Hotlinks to the pages may also be useful.

Abstract

The abstract is a brief summary or description of the investigation and should 'stand alone' which means that one of your fellow biology students should be able to read only your abstract and understand the basic nature of your Individual Investigation. For this reason, a good abstract clearly identifies the purpose of the investigation and the important results.

Avoid comments such as, '*this experiment helped me learn about cell size and shape*' or '*the goal of this experiment was to learn about the importance of surface area to volume ratio*.' Although those are important aspects and goals of the laboratory experience, the abstract of the research report should focus only on the data and results.

Background information on the relevant biological theory or applications of your investigation belongs in the Introduction section. Avoid referencing any other sources or parts of the report, because the abstract should 'stand alone.'

The abstract must be able to stand by itself, it must be brief, and it must include the significant numerical results. Generally its structure consists of three parts: what did you do; what were your results; what the results indicate and how they relate to the Research Question. One approach to learn how to write a good abstract is to read published abstracts from biology or educational journals.

Introduction

The introduction section explains what focused biological Research Question is being addressed. It includes general background material and a brief historical perspective on the topic being investigated. It presents brief summaries, with references, of previous work. Any relevant biological laws, theories, models and hypotheses must also be included.

An effective introduction directs the person reading from a larger area of biological research, through examples of progress in that biological field to a clear statement of the Research Question and approach being addressed in the Individual Investigation Report. Annotated diagrams and graphs, digital photographs, structural formulae, balanced equations, etc. must be included as appropriate.

Downloading directly from the Internet or copying directly from books may suggest to your Teacher and the IB moderator that you have not understood the biology involved and may be considered as plagiarism. It is always best to put everything into your own words.

Here is a part of a sample introduction for a simple investigation involving diffusion into agar cubes:

Diffusion is a purely physical process by which substances such as nutrients (for example, glucose), water, oxygen and urea are exchanged between living cells and their environment.

Simple diffusion occurs when a chemical moves from a region of high concentration to a region of low concentration. Temperature, molar mass of the molecules or ions, magnitude of the concentration gradient and surface area to volume ratio are the factors that affect the diffusion rate.

For simple diffusion there is a directly proportional relationship between surface area and the rate of diffusion. There is a linear relationship between concentration and the diffusion rate and an exponential relationship between temperature and diffusion rate. Ficks' first law of diffusion states that the diffusion rate is proportional to the difference in concentration. That is, if C_a and C_b are the concentration at two points a and b, diffusion rate from a to b is (some constant) × (C_a - C_b).

My investigation will help me explore the relationship between diffusion and cell size by experimenting with model 'cells' composed of agar (a soluble protein) containing hydrochloric acid and phenolphthalein. Phenolphthalein is an acid/base indicator that turns pink in the presence of a strong base such as sodium hydroxide, NaOH. Thus the surface of the agar cubes will turn pink immediately when put into NaOH solution. The NaOH will continue to diffuse through the cube and gradually turn the inside of the cube pink.

The proximal independent variable is the surface area to volume ratio of the agar blocks, the proximal dependent variable is the rate of diffusion of sodium hydroxide and the important controlled variables are temperature of the hydrochloric acid, concentration of hydrochloric acid and agar cube composition (hydrochloric acid and phenolphthalein concentrations).

My hypothesis is the cube with the greatest surface area to volume ratio will be the one that stains pink the most quickly.

The Introduction indicates the focus of the experiment or investigation but does not indicate results. It does not have to stand by itself and can refer to later parts of the Individual Investigation report such as a diagram or a graph. Such reference should be to a numbered figure or graph

Method

The experimental section should provide all the necessary detail for someone to be able to reproduce your work and obtain the same results (within experimental error). There should also be a 'Planning' section you explain what various options were open and why one technique was chosen rather than an alternative. Any statistical aspects in the design must be outlined and justified.

Here is a part of a sample introduction for a simple investigation involving diffusion into agar cubes:

Using a scalpel I will cut agar cubes of different sizes: 3.00 cm × 3.00 cm × 3.00 cm, 2.00 cm × 2.00 cm × 2.00 cm, 1.00 cm × 1.00 cm × 1.00 cm and 0.50 cm × 0.50 cm 0.50 cm. I will use a metal ruler which measures to the nearest millimetre.

I will place the cubes in a beaker and cover with the diffusing solution: 0.100 mol dm-3 sodium hydroxide. I will then place the beaker into a water bath thermostatted at 25 °C. I will record the temperature of the solution with a mercury in glass thermometer *(range - 10 to 110 degrees Celsius ; uncertainty +/- 0.5 degrees Celsius).*

I will start an electronic stopwatch and leave the agar cubes for 5 minutes.

I will pour off the solution. I will rinse the cubes in a little cold water and blot the surfaces of each cube dry with a paper towel.

I will time how long it takes for the alkali to change the colour of the indicator in each agar block. If the alkali does not penetrate the largest block in the time available, I will cut the block with a scalpel and measure how far it has penetrated in the time. To help follow the penetration of alkali I will illuminate the cubes with a light and stand the beaker on white paper.

I will also take digital photographs of the cubes and their sliced interiors after five minutes.

I will use a control leave a cube in water, or on a piece of filter paper so I can be sure the change in indicator is due to diffusion of the surrounding solution inwards, not the effect of time on the cubes.

Safety issues: *I will take care with the solutions used: wear eye protection and disposable gloves. I will rinse splashes off the skin.*
Ethical issues: *Since living organisms are not used in this investigation there are no ethical issues in this investigation.*
Environmental issues: *The sodium hydroxide solution should be poured into the sink and diluted with cold tap water.*

Results

This section should include a summary of your raw data (preferably in tabular form) and important observations (qualitative data). Major calculations may be included in this section, or in as separate Interpretation section or in an Appendix. A description of the mathematical equations used in your calculations must be presented.

All quantities should have units and be expressed using the correct number of significant figures and decimal places and random uncertainty. Scientific notation should be used when appropriate. For values less than unity, use a leading zero. Avoid writing values having too many zeros; use scientific notation. Error propagation must be performed.

Examples:
'0.15 cm^3' not '.15 cm^3'
'2.5 × 10^{-5} mol dm^{-3}' not '0.000025 mol dm^{-3}'

Important experimental conditions should be listed as footnotes, especially when the table includes data obtained under different experimental conditions. All tables, figures and graphs should be numbered sequentially and must be mentioned in the text.

Here are some blank results table for a simple investigation involving diffusion into agar cubes:

I plan to record raw data and display the processed data in the two data tables outlined below.

A =A Length of side of agar cube (cm)	B = A^2 Area of one side of cube (cm^2)	C = 6B Total surface area of cube (cm^2)	D = A^3 Volume of cube (cm^3)	E = A/2 Shortest distance from edge to middle of cube (cm)	F =C/D Surface area to volume ratio	G Time taken for NaOH to diffuse to centre of cube (s)/ ±0.5 s	H Distance NaOH solution diffuses in 5 minutes (cm)	I =H/5 or E/G Rate of NaOH diffusion ($cm\ s^{-1}$)
2.0 ±0.1	4.0 ±0.4	24.0 ±2.4	8.0 ±1.2	1.0	3			
1.0 ±0.1	1.0 ±0.2	6.0 ±1.2	1.0 ± 0.3	0.5	6			
0.5 ±0.1	0.25 ±0.1	1.5 ±0.6	0.125 ±0.075 [3]	0.25	12			

A =A Length of side of agar cube (cm)	B = A^2 Area of one side of cube (cm^2)	C = 6B Total surface area of cube (cm^2)	D = A^3 Volume of cube (cm^3)	E = A/2 Shortest distance from edge to middle of cube (cm)	F =C/D Surface area to volume ratio	J Volume of cube that did not turn pink (cm^3)	K E-J Volume diffused = total volume – volume not pink (cm^3)	L (K/A^3) × 100 % diffusion = volume diffused/ total volume × 100
2.0	4.0	24.0	8.0	1.0	3	5.1	2.9	36
1.0	1.0	6.0	1.0	0.5	6	0.5	0.5	50
0.5	0.25	1.5	0.1	0.25	15	0.0	0.1	100

I will plot the following graphs: surface area (C) (X axis) against volume diffused (K) (Y axis) and distance diffused (H) (in 5 minutes) (X axis) against the time for the cube to go pink (G) (Y axis).

I will also plot a graph of log H against log G. I expect a line of best fit with a gradient of approximately two.

Discussion

A discussion section should take the form of an analysis of your results and whether you have answered your Research Question.

Comment on the purpose of the experiment. What do the results indicate? What are sources of random and systematic error (experimental uncertainty/precision)? What additional experiments could help address any unresolved issues? Do the results agree with what other researchers have found? Do the results support a biological model, law or hypothesis?

Here is a part of a discussion for a simple investigation involving diffusion into agar cubes:

In this investigation, it would have been more accurate if the dimensions of the agar blocks was measured to the hundredth of a centimetre. Also, it would have been better if the agar can be cut into a more perfect cube. I used a scalpel to cut the agar blocks, but if I had used a sharp cork borer and a pair of vernier calipers then I would have had more accurate data (although I would then have cylinders, rather than cubes).

Also, the smaller cubes were very hard to measure the amount of sodium hydroxide diffused, because the pink colour was so faint, the measurements might not have been accurate. To obtain more accurate results and reduce the effect of percentage error, larger agar cubes should have been used.

When interpreting the behaviour of substances in the model (in this instance, the movement of hydrochloric acid by simple diffusion) the limitations of the model must be taken into account. For example one limitation is that the agar cubes do not have a selectively permeable boundary such as a cell membrane. The validity of the model should also be assessed. For example, the lack of a cell membrane and an energy source suggests that no active transport can take place, indicating that only diffusion, a passive process, is being investigated.

I suggest the following improvements to the investigation: to extend the range of cubes to be tested to include cubes with lengths of 3.0, 4.0 and 5.0 cm as well as the following cubes within the current range of data: 1.5 cm, 2.5 cm and 3.5 cm lengths.

I plan to extend the experiment to include the effect of shape and to test a range of cuboids with the same indicator under the same conditions. I also plan to extend the experiment to include the effect of temperature on the diffusion rate by performing the experiment in a thermostatted water bath. Another investigation would involve changing the concentration of sodium hydroxide via dilution.

Conclusion

Summarise your results and discussion with a short conclusion that is more than simply a repetition of your results. Phrase it in terms of the Research Question addressed in the Introduction.

Here is a part of a conclusion for a simple investigation involving diffusion into agar cubes:

The experiment shows that cube size and the surface area to volume ratio are inversely proportional if the surface area volume ratio increases the diffusion rate of hydrochloric acid increases. So the larger surface area volume ratio causes an increase in the hydrochloric acid diffusion rate.

These results suggest is that a group of smaller cells can transport soluble materials in and out at a faster rate than one cell equal of the same total volume. A cell could eventually reach a size at which materials could not diffuse in fast enough to meet requirements, and waste products, such as urea, could build up to toxic levels.

These are general statements, but your own conclusions must be supported by specific reference to numerical and perhaps statistical results.

References

Citations of the literature used in the previous sections.

Appendices

Photographs of the apparatus and results may appear here, along with lengthy mathematic or statistical calculations or additional material not needed when reading through the report, e.g. preparation of solutions or buffers and storage of specimens. The risk assessment and safety information, e.g., CLEAPSS hazcards or MSDS data sheets may also appear in the Appendix.

5.1.2 Sentence style and writing style

The following guidelines are designed to improve the quality of your reports for experimental work and bring it close to the standard of a published biological paper. The key guiding principle is clear communication. You will not lose any marks for poor English phrasing unless the meaning is unclear or incorrect. The IBO is aware that many IB Diploma students have English as a Second Language (ESL).

Be clear, straight-forward and concise: use short sentences and get to the point. Be dispassionate: report the facts and ensure your writing is free of bias and personal opinion. Try to ensure your writing is grammatically correct and free of spelling mistakes. Avoid redundancy, in other words, do not repeat statements. Define any uncommon Biological terms or abbreviations that appear in your 'write-up'.

Try not to use the first person, 'I', 'we' etc. when writing the method. Use the passive past tense since the Investigation has already been performed, unless it is an experimental design exercise.

For example:

The leaf was boiled with alcohol (ethanol) to remove the photosynthetic pigments.

A reading was taken not *we took a reading.*

Do not write your method as a list of instructions. Avoid the second person, that is, avoid using the word 'you'.

Every sentence must have a subject and a verb. In the example above the leaf is the subject and was boiled is the verb.

Be aware that data is plural while datum is singular and that the word species is used both as singular and as plural.

Ensure your 'write-up' is divided into a number of paragraphs correctly. In a 'write up ' or part of a 'write-up', for example, the method, should not be one long whole paragraph.

Numbers should be written as numerals when they are greater than ten or when they are associated with measurements; for example, '7.2 mm' or '2.0 g', but 'two explanations of four factors affecting the photosynthetic rate'.

Words and phrases such as "a little", "easy", and "very accurately" have no definitive meaning in the Scientific context and are therefore inadequate.

Avoid colloquial phrases, slang and contractions, for example, write … 'do not' .. as opposed to 'don't'.

- If the subject is singular (or plural) the verb must match appropriately.

e.g. *Our results indicate (not indicates) the significance of pH in controlling salivary amylase activity.*

- Affect is a verb and effect is a noun.

e.g. *The effect of drug X was noticeable* and *magnesium concentrations affect the level of photosynthesis*.

If you use the words 'precise' and 'accurate' then employ them correctly. The term 'accuracy' typically refers to how close your value is to the accepted or known value from the Biological literature and 'precision' typically refers to how close together your results are.

- Significant is usually only used for a discussion of statistical tests.

e.g. Increases in enzyme rates were substantial is better than *Increases in enzyme rates were significant*.

- 'Among' refers to three or more objects or groups and 'between' refers to exactly two groups.

e.g. *There were no significant differences among the five treatment groups. There were significant differences between the males and females*.

- Avoid any ambiguity in your writing.

e.g. The sample was incubated in mixture X minus Y plus C. Does the mixture lack both X and Y or lack Y and contain Z?

- All scientific names (genus and species) must be italicised if your 'write-up' is word-processed. If you are using a typewriter or writing by hand then underline but the italicised presentation is preferred. Genus names are capitalised but species names are not (Genus species).

e.g. *Homo sapiens*, *H. sapiens* or, if necessary, Homo sapiens.

- If you have performed a statistical analysis then present the information in parentheses at the end of a sentence stating in words whether a trend or not was observed.

e.g. *The chance …. did not depend significantly …* (χ^2=0.48, *N*=67, *p*>0.05)

- Since Biologists know that all results contain errors, they almost never give definite answers.

e.g. *'It is likely that ...*', or, *'It is probable that ...*' than to give a precise answer.

- Avoid beginning a sentence with a symbol, numeric value or equation.

Incorrect:

317.8 mg of sodium chloride was added to the solution, which was then heated to 55 °C by means of a thermostatted water bath.

Correct:

After the addition of 317.8 mg of sodium chloride, the solution was heated to 55 °C by means of a thermostatted water bath.

- Check that a modifier phrase or the pronoun 'it' actually refers to the intended subject.

Incorrect:

After transferring to a larger round bottomed flask, the solution of alcoholic chlorophyll was heated to a boil.

Correct:

The solution of alcoholic chlorophyll was transferred to a larger round bottomed flask and heated to a boil.

Equations

Mathematical equations typically appear in italics as a separate line from the text and are numbered sequentially throughout the manuscript. Equations can then be referred to by number.

$$Q_{10}\ value = \frac{activity\ of\ enzyme\ at\ (T+10\ degrees\ Celsius)}{activity\ of\ enzyme\ at\ T\ degrees\ Celsius}$$

Spaces

There should be one space between a quantity and its units and between a quantity or word and subsequent parenthetical phrase.

6.626 kJ

298.5 °C

45.00 cm^3

456.0 nm

Grammar

Personal pronouns

By tradition, scientists avoid using the personal pronouns 'I', 'we' and 'you' in most scientific communications. The use of third person instead of first person is preferred when reporting results.

First person: *I heated the solution Vitamin C at 100 °C for 1 hour and I noticed that it turned pale red.*

Third person: *When heated at 100 °C for 1 hour, the Vitamin C solution turned pale red.*

Subject-verb agreement

Based on whether the subject is singular or plural, use the correct verb tense. A quantity used is a singular subject, even when that quantity is in a plural form of units.

Incorrect: *1.20 g of solid sodium hydroxide were added to the Tris buffer…*

Correct: *1.20 g of solid sodium hydroxide was added to the Tris buffer…*

Verbing a noun

Do not turn verbs into nouns.

Incorrect: *the reaction mixture was centrifuged to separate the colloidal DNA*

Correct: *the colloidal DNA was separated from the reaction mixture using a centrifuge*

Abbreviations, Formulas and Numerals

Use standard abbreviations, for example, h = hour, min = minute, s = second and °C = degrees Celsius.

Chemical formulas and names

Use subscripts, superscripts, parentheses, and symbols appropriately in chemical formulae.

Examples

Fe^{3+} (aq), NaCl (s), CO_2 (g), HCO_3^- (aq)

IUPAC names should be used with trivial names:

Examples

Ethanol (alcohol)

Glycine (amino ethanoic acid)

Lime water (calcium hydroxide solution)

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid)

However, complex molecules are usually referred to by trivial names, for example, beta-carotene, chlorophyll, and alpha-D-glucose.

Chemical names should not be capitalized unless named after a person, for example, Benedict's reagent or Knop's solution or an abbreviation, for example, DCPIP.

5.2 Referencing

Referencing is a standardised method of acknowledging the sources of information you have consulted for writing your Individual Investigation report. Words, paragraphs, quotes, figures, tables, theories, ideas, facts–originating from another source and used in your Individual Investigation report must be referenced (i.e. acknowledged). Referencing is done for the following reasons:

- to avoid plagiarism.
- so that your Assessor can verify quotations.
- so that your Assessor can follow up on the original author's thinking by consulting the source you used.

There are many ways to acknowledge sources of information, for example, MLA (Modern Language Association), and none is mandated by the IBO. This publication recommends a Bibliography at the end of the Individual Report together with in-text referencing. The style adopted is the MLA (Modern Language Association) format. However, what is important is that the method used is consistent. Do not switch from one method to another. Familiarise yourself with the format and terms (*Figure 502*) that your school or IB Biology teacher expects you to use.

Paraphrasing	This is explaining in your own words what the original source wrote.
Quoting	"To repeat (words) exactly from (an earlier work, speech or conversation), usually with an acknowledgement from the source" (Collins Paperback English Dictionary, 1998, p. 665) Put in quotations everything that comes directly from the text especially when taking notes. (Collins Paperback English Dictionary, 1998, p. 136).
Citing or In-Text Referencing	"1. To quote or refer (a passage, book or author). 2. To bring forward as evidence" When citing your sources, you are telling your IB Biology Teacher who/what the original source. You are giving credit where credit is due.
Bibliography	This is a list of cited works.
Common Knowledge	These are facts that are located in several sources and probably known by many biologists.

Figure 502 Important referencing terms

Terms to Know

In-text referencing

In-text referencing is when you provide information about the source in the text of your Individual Investigation report. The bibliography at the end of your report shows the reader which sources were researched but sometimes that is not enough. That is when you use in-text referencing inside your essay. Usually the author's last name and a page reference are enough to identify the source. With this information, the reader can find the complete publication information in your citation list at the end of your essay.

Tips for in-text referencing

When you find a useful resource for your Individual Investigation Report, note down all the details required in your bibliography (e.g. author, title, publication details, date of access, URL etc) before you start taking notes.

When reading a resource highlight key words, main ideas or make bullet point notes that you might want to include in your essay.

If you use the exact words from the writer put them inside quotation marks so you do not accidentally plagiarise, and if possible note down the page number.

Examples of In-Text Referencing

Signal Phrase: introduces where the idea or quote comes from and usually has the author's name in the text.

According to Anthony *Methylacidiphilum fumariolicum* SolV bacterium, a methanotroph (methane consumer) found in Italian volcanic mudpots, relies on lanthanides to survive (Anthony 68-69).

Signal phrase = According to Anthony

Paraphrase or Summary: the idea in your own words or the main ideas only.

Current research has shown that certain species of bacteria that metabolise methane found in Italian volcanic mud need rare earth metals (lanthanides) to survive.

Direct Quote: show these are the exact same words used by the author in the source with quotation marks.

'*This fascinating work has important implications for studies of most other methanotrophs growing on methane or methanol*' (Anthony 47).

Citation in Bibliography: this is the source with the full publications details.

Homewood, Jon "The effect of global warming on amphibians". *New Scientist.* 1 October 2009: 38-41.

Because Internet sources typically have no page or paragraph numbers, and Web sites often list no author, students are often confused about how to refer to these sources within their papers. The answer is to cite the author's name whenever possible, and use the source's title otherwise (or a shortened version of the title). If no page or paragraph number is provided, leave that portion of the citation blank. Keep in mind that the primary purpose of an in-text citation is simply to point readers to the correct entry on the "Works Cited" page. Also, as web sites change, give the date it was referenced so, if necessary, cached versions may be retrieved.

Bibliography

How to cite sources

Books

Author's name (put family name first). Title. Place of publication: Publisher, Year of Publication.

Note: titles can be underlined or put into italics

e.g.: Andrew, John. Chemistry in Focus. United Kingdom: Hodder and Stoughton, 1999.

Two authors (note the order of names for the second author)

McKissack, Patricia, and Frederick McKissack. Modern Biology. United Kingdom: Oxford University Press, 1995.

Three or more authors

Adams, Roger et al. Encyclopedia of Science. New York: Consolidated Press, 1994.

Encyclopedia article

Article title". Title of Encyclopedia. Year of Publication.

Note: put title of article in speech marks

e.g.: "Ozone layer". World Book Encyclopedia. 2009.

Interview

Name of the person interviewed. The kind of interview (personal, telephone, email). Date or dates of interview.

e.g.: Martin, J. K. Email interview. 8-12 May 2008.

Magazine Article

Author. "Article title". Magazine title. Date of Magazine: Pages.

Note: the use of speech marks and underlining

e.g.: Churchman, Deborah. "Global warming: the sceptic's view". New Scientist. March1999: 28-31.

Remember also that:

citations should not be numbered.

citations should not be separated into different formats (e.g. books, websites, interviews, etc.).

citations should be in alphabetical order by the main entry (e.g. author's surname, title, article title, etc.). Ignore a, an, the.

Website

Author (if available). "Title of the article." (in speech marks) Title of whole site. Date of visit to site. *<URL of Page>*.

e.g.: "Using MLA Format." Purdue University Online Writing Lab. January 23, 2006. *<http://owl.english.purdue.edu/handouts/research/r_mla.html>*

Online Encyclopedia Article

Author. (family name first) "Title of article". Magazine title . Date of Magazine: Page numbers. Product Name. Date researcher visited site. *<Electronic Address, or URL, of the source>*.

e.g.: Churchman, Deborah. "Be a Nature Detective". Ranger Rick March 1999: 28-31. MasterFILE Premier on-line. EBSCO Publishing. 30 Feb. 2004.

<http://www/epnet.com/ehost/login.html>.

There are several on-line bibliography makers. Try them out but you are advised to use the MLA version and take care with the data you enter otherwise you will get a bad outcome. Try Landmarks Citation Machine and Bibme.org for example:

<http://citationmachine.net/>

<http://www.killerstartups.com/Web20/bibme-org-the-quickest-way-to-build-a-bibliography>

Additional Materials for Writing Lab/Research Reports

Davis, Martha *Scientific papers and presentations* San Diego: Academic Press, 1997

Dodd, Janet S. (ed.) *The ACS style guide: a manual for authors and editors* ACS, 1997.

Eisenberg, Anne "Strategies five productive chemists use to handle the writing process." J. Chem. Educ. 1982, 59, 566.

Potera, Carol "The Basic Elements of Writing a Scientific Paper: The Art of Scientific Style" *J. Chem. Educ.* 1984, 61, 247.

Spector, Thomas "Writing a Scientific Manuscript: Highlights for Success" *J. Chem. Educ.* 1994, 71, 47.

5.3 Use of units

5.3.1 SI Base Units

The six basic quantities or base units of the SI system commonly used in Biology are: the metre for measuring length, the kilogram for measuring mass, the second for measuring time, the mole for measuring the amount of substance, the kelvin for measuring temperature and the ampere for measuring electric current (*Figure 503*).

Dimension	Symbol	SI unit name and symbol
Length	*L*	metre, m
Mass	*m*	kilogram, kg
Time	*t*	second, s
Temperature	*T*	kelvin, K
Amount of substance	*n*	mole, mol
Electric current	*I*	ampere, A

Figure 503 The six commonly encountered physical quantities in Biology

One physical quantity that is very important in Biology, is volume, which is derived from length and expressed in metres cubed, m^3. However, the usual unit used in Biology is the decimetre cubed (1 dm^3), which is commonly called a 'liter' in North America (symbol L) and 'litre' in Europe. There are 1000 dm^3 in one metre cubed (1 m^3). Each decimetre cubed (dm^3) can be divided into 1000 centimetre cubed (cm^3). Note that milliliter (mL) is widely used in North America and can be virtually regarded as being identical to cm^3 (1 litre = 1.000028 dm^3).

Strictly speaking, measurements of length should be expressed in metres and masses should be expressed in kilograms. However, often such measurements are expressed in centimetres (cm) and grams (g), where 100 cm = 1 m and 1000 g = 1 kg.

The size of the units given above is not always the most suitable for certain measurements and decimal multiples and fractions are frequently used as shown below. A set of common SI prefixes and associated symbols is given in *Figure 504*.

Fraction	Prefix	Symbol	Multiple	Prefix	Symbol
10^{-1}	deci	d	10^{3}	kilo	k
10^{-3}	milli	m	10^{6}	mega	M
10^{-6}	micro	μ	10^{9}	giga	G
10^{-9}	nano	n	10^{12}	tera	T

Figure 504 SI Factors

5.3.2 SI Derived Units

A large number of additional SI units exist and are widely used during the IB Biology Program, for example, the joule for energy. The joule and other so-called derived units can be expressed in terms of base units.

Commonly used SI derived units and their symbols for a number of physical quantities relevant to the IB Biology program are given below in *Figure 505*, together with a 'useful' relationship between these and the definition in terms of SI base units. (The term specific in front of a physical quantity has the meaning 'per unit mass').

Quantity	Definition	Unit	Relationship to other quantities	Basic definition
Molar concentration (formerly molarity, M)	mole per decimetre cubed	mol dm^{-3}		mol dm^{-3}
Frequency	reciprocal second	Hz		s^{-1}
Wave number	reciprocal metre	m^{-1}		m^{-1}
Volume	metre cubed	m^3		m^3
Force	newton	N	J m^{-1}	kg m s^{-2}
Pressure	pascal	Pa	N m^2, J m^{-3}	kg m^{-1} s^{-2}
Charge	coulomb	C		A s
Potential difference (voltage)	volt	V	J s^1 A^1	kg m^2 s^{-3} A^{-1}
Density	kilogram per metre cubed	kg m^{-3}		kg m^{-3}
Heat capacity	joule per kelvin	J K^{-1}		kg m^2 s^{-2} K^{-1}
Specific heat capacity	joule per kilogram per kelvin	J kg^{-1} K^{-1}		kg m^2 s^{-2} kg^{-1} K^{-1}

Figure 505 SI derived units and their symbols

The base and derived SI units are a coherent system of units. This means that all the units for the derived physical quantities are obtained from the base units by multiplication or division without the need for the introduction of numerical factors. This simplifies many calculations.

For example, if we consider the ideal gas equation, $PV = nRT$, we can rearrange it to make P the subject, namely,

$$P = \frac{nRT}{V}$$

We can then substitute numerical values for the volume of gas (V), the amount of gas (n), the molar gas constant (R) and the absolute temperature (T), in coherent SI units, namely, m^3, mol and J K^{-1} mol^{-1}, respectively and calculate the numerical value of P which will also be in coherent SI units, namely pascals.

Example

If R = 8.31 J K^{-1} mol^{-1}, T = 300 K, V = 6.34×10^{-3} m^3 and n = 0.250 mol.

Then,

$$P = \frac{nRT}{V} = \frac{0.250\ \text{mol} \times 8.31\ \text{J K}^{-1}\ \text{mol}^{-1} \times 300\ \text{K}}{6.34 \times 10^{-3}\ \text{m}^3} = 9.83 \times 10^4\ \text{Pa}$$

Note that the more familiar unit of volume the decimetre cubed is not a base or fundamental SI unit and would not yield a pressure in pascals.

Rules For The Use of SI units

1. Units may be written out in full, e.g. 5 coulomb, or by using the agreed symbol, e.g., 5 C, and printed in upright Roman type.
2. A full stop is not written after symbols, except at the end of a sentence.
3. Values of quantities are expressed in SI units using Arabic numerals (i.e. 1, 2, 3,… etc) and the symbols for the units. e.g., m = 5.0 g but not m = five gram or m = five g.
4. A space should be inserted between the numerical value and the unit's symbol. e.g., a 5.00 g copper cube but not a 5.00g copper cube.
5. The digits of numerical values having more than four digits on either side of the decimal marker can be separated into groups of three using a space. e.g., 15 739.012 53 is better than 15739.01253

6. Avoid abbreviations, such as sec (for either s or second) or cc (for either cm^3 or centimetre cubed).
7. Avoid mixing unit symbols and unit names, for example, kg/m3 or, better still, kg m^{-3} is acceptable, but not kilogram/m^3.
8. Those symbols named after a person have a capital for the first letter. When the name of the unit is written in full it has a small letter, even when commemorating a person, for example, pascal, symbol Pa.

Common Mistakes with SI Units

- The rules listed previously may seem minor at best, and at worse pedantic, but it is good practice to follow them. Below are outlined some major and common mistakes frequently made by IB Biology students.
- It is not acceptable to record measurements with a mixture of units, for example, 5 min 10 s and 1 kg 10 g. These should be expressed as 310 s and 1.01 kg, respectively.
- Another common mistake is to confuse weight and mass. When using a balance, masses should be recorded in kilograms (kg) or grams (g). Weight is a force (due to gravity) and should therefore be expressed in newtons (N). On Earth a l.0 kg mass has a weight of approximately 9.8 N. (Weight on Earth varies slightly according to latitude and height above sea level). (However, terminology is not always logical since a 'weighing bottle' should logically be termed a 'massing bottle').
- In addition, no plurals, are added to SI names or unit, for example: 5 gram not 5 grams. This is, in part, to avoid confusion with the symbol s for seconds, but units do not have a plural form. Strictly speaking, all temperatures should be expressed as thermodynamic temperatures in kelvin. (Often expressed in the common but less correct absolute temperatures).
- However, unless gas law calculations are being performed, then temperatures are often expressed in degrees Celsius.
- The numerical value of a Celsius temperature expressed in degrees Celsius is given by: $t/°C = T/K - 273.15$ where t is the numerical value of a Celsius temperature and T is the absolute or thermodynamic temperature in kelvin.
- It follows that the degree Celsius is equal in magnitude to the kelvin, thus, temperature differences or intervals may be expressed in either the degree Celsius or the kelvin using the same numerical value.
- The specific heat capacity of water is approximately 4.18 J g^{-1} $°C^{-1}$ or 4.18 J g^{-1} K^{-1}.
- When using the unit of the mole a common mistake is to write, for example, 'number of moles of sodium chloride = 2.5 moles'. The correct approach is to write 'amount of sodium chloride = 2.5 mol'.
- Number of moles is a dimensionless number, e.g., 7, but amount is a physical quantity with an associated unit, e.g., 7 mol.
- Be careful not to use the word 'amount' in the everyday sense of the word where it is used in a much more wide ranging and 'loose' way and can refer to a number, a volume or even a weight. In the SI system the term 'amount' refers only to the mol.
- When referring to an amount (in mol) ensure that there is no ambiguity. Quote a formula or systematic name, for example, 1.0 mol of lead chloride might refer to lead(II) chloride, $PbCl_2$, or lead(IV) chloride, $PbCl_4$; 1.0 mol of oxygen might refer to 1.0 mol of oxygen atoms or 1.0 mol of oxygen molecules.
- The relative molecular mass is a dimensionless number, e.g., M_r (H_2O) = 18.02, but the molar mass is a physical quantity, e.g., M (H_2O) = 18.02 g mo^{l-1}.

The term molecular mass should not be used to describe ionic substances such as sodium chloride, NaCl, since the lattice does not contain covalently bonded clusters of sodium and chlorine atoms. Instead, the lattice consists of a very large number of sodium and chloride ions (in a 1:1 ratio) each of which is bonded to several (theoretically all) other ions of opposite charge. The terms formula mass (g mol-1) or molar mass (g mol-1) should be used to describe ionic substances.

- When labelling graphs or tables with SI units, then the symbol of the unit is followed by a solidus(/), for example, V/ dm^3 and *T*/ K. This approach converts quantities to numbers by dividing the quantity by its unit.
- Note that the solidus notation is avoided within SI units, and scientific notation is preferred for example, mol dm^{-3} rather than mol/dm^3.
- A double solidus is forbidden, for example, is a/b/c meant to be (a/b)/c or a/(b/c)?

For example, the units of specific heat capacity should be written as J kg^{-1} K^{-1}. The equivalent expression using the solidus notation is J/(kg K), but its use is not encouraged. Another example is illustrated by a second order rate constant which has units of cubic decimetre per mole per second, which is expressed symbolically as dm^3 mol^{-1} s^{-1}, or dm^3/ (mol s) but not as dm^3/mol/s.

- Spaces, not commas, are used in numbers greater than 1000, for example, 101 325 Pa and not 101,325 Pa.
- All units in calculations should be expressed in SI base units (or units derived from them), for example, an energy calculation should give an initial answer in joules, which can then be expressed in kilo joules (kJ) (a multiple SI unit).

Always make sure that terms on either side of an equal sign are capable of being equal, for example, strictly speaking, you cannot write 100 °C = 373.15 K, just as 27 apples cannot equal 1.5 dollars. The correct approach to writing the above temperature conversion is: *T*/K = *t*/°C + 273.15. Therefore, *T*/K = 100 °C/°C + 273.15. Therefore, *T* = 373.15 K. However, many students (and IB Biology teachers) ignore this pedantry and simply write 100.00 °C = 373.15 K.

5.3.3 Non-SI Units

Several non-SI units may be encountered during your IB Biology programme. They may arise from the measuring device you are using or from data that you have obtained from the literature. Ideally any raw data expressed in non-SI units (see *Figure 506*) should be converted to SI units before data processing.

Many non-SI units are now defined exactly in terms of SI units; some can only be related to SI units via fundamental constants and the relationship is therefore restricted by the precision to which the constants are known. Exact values are printed in bold type.

Non-SI unit	Unit type	SI conversion	Notes
Bar	Pressure	1 bar = 10^5 Pa	
Atomic mass unity (u)			Approximately equal to the mass of a proton or neutron; also known as a Dalton or amu
Minute (min)	Time	1 min = 60 s	
Hour (h)	Time	1 = 60 min = 3600 s	
Electronvolt (eV)	Energy	1 eV = 1.602×10^{-19} J	
Millimetre of mercury (mmHg or Torr)	Pressure	1 mmHg ≈ 133.322 Pa	
Atmosphere (atm)	Pressure	1 atm = 101.325 kPa	
Calorie (Cal)	Heat energy	4.184 Cal = 1 J	
Degree Celsius (°C)	Temperature	1 °C = 1 K	
Debye (D)	Dipole moment	3.336×10^{-30} C m	
Degree Fahrenheit (°F)	Temperature	5/9 K	

Figure 506 Non-SI units

Listed in *Figure 507* is a summary of what you need to do to score well in the Communication criterion.

Assessment criteria	Evidence required	What you must do
Writes a well structured and clear report (write-up)	Structured report	The report must be well structured into different clearly designated sections and the English must be clear, correct and accessible. It should resemble the form of a scientific paper but must present relevant information on focus (the Research Question and introduction), process (the methodology), my
	Relevant and concise report with correct conventions and referencing	The report must be relevant and concise (10 pages). It should not contain irrelevant or tangential issues (those not directly relevant to your Research Question). The data collected should be relevant to the Research Question and support a justified conclusion. Subject Specific terminology and conventions, for example, referencing and labelling of all data tables, graphs, charts and digital images, or the use of the passive voice, should be appropriate and correct. Data should be processed and displayed with the correct type of graph or chart. All literature consulted should be referenced and cited according to well known conventions. Any errors in the report should be minor and not hamper understanding of the investigation.
	Units and calculations	Calculations should be accompanied by appropriate units, usually SI. Calculations should be carried out according to the rules of significant rules and final values reported to the correct number of significant figures. Error propagation should be performed and the working shown in the report. It may also be appropriate to show how the final units of a calculation are derived. Some statistics may be relevant.

Figure 507 Summary of the Communication criterion

6.1 Common biological techniques

Your IB Biology teacher will use some of your investigations to assess your manipulative skills, that is, your ability to correctly, accurately and safely use Biological apparatus and equipment. Adopting these practices will also help reduce random errors and prevent systematic errors. Your IB Biology teacher may have a tick list covering key points. Some simple examples of what they might be looking for are outlined below.

6.1.1 Test-tube techniques

Adding reagents

When using plastic teat pipettes hold the pipette so that the jet is just above the mouth of the test tube. If the pipette jet touches the inside of the test tube it may carry reagent back to the stock bottle. Hold the bulb of the teat between the thumb and forefinger. Place your second third fingers under the pipette tube. This technique will allow you to add drops of reagents steadily while holding the pipette in the vertical position. When used correctly, a dropping pipette will deliver a constant drop volume, which can be useful in quantitative investigations. It is also good practice to retain a plastic teat pipette in a beaker of distilled water when it is not in use.

Mixing

This can be achieved by gently tapping the tube against a finger, gently shaking the tube from side to, swirling the tube using the wrist or stirring the mixture with a clean glass rod. It will be necessary to mix reagents together, such as an enzyme and its substrate, or food samples and food test reagents.

Heating

Do *not* point your heated test tube at any other students. Gently agitate the tube during the heating of liquids and solutions. Look out for thick, dense precipitates, since they may cause excessive 'bumping' and eject the hot contents of the tube. A safer way of heating is to use a beaker as a water bath. The beaker, ideally a metal beaker, should be heated on a tripod with a gauze, using a Bunsen burner. A thermometer may be used to monitor the temperature, which can be maintained at an approximately constant level by alternate gentle heating or via the addition of small pieces of ice. An ideal alternative is the use of a thermostatically controlled water bath.

6.1.2 Measuring Mass

In nearly all practical situations encountered during your IB Biology practical program you will be 'weighing' Biological specimens or samples and recording their mass. The term fresh mass, should therefore be used rather than the term fresh weight. Plant material mass is generally measured after drying – dry biomass.

The mass of a Biological specimen or chemical is usually determined by an electronic balance that gives a direct digital reading to a particular degree of precision – usually to the nearest 0.01 of a gram. Before using the balance it should read zero, you may need to use the 'tare' button. Failure to calibrate the balance in this way may result in a systematic error.

You then need to take into account the mass of the container holding the chemical or Biological specimen being weighed. The mass of the container should be determined *before* the object itself is weighed. The first mass (the container) can then be subtracted from the second (the container and the object) to find the true mass of the object. This approach to weighing is known as weighing by difference.

It is *not* good practice to place chemicals or Biological specimens, especially if they are wet, onto the pan of the balance and weigh them directly. In addition, no attempt should be made to weigh hot samples since the resulting convection currents will cause the pan and hence the mass reading to fluctuate.

6.1.3 Measuring Temperature

Measurement of temperature is particularly important in enzyme experiments since a relatively small change in temperature has a relatively large effect on the rate of the reaction. Temperature is also important in experiments to study the cooling effects of perspiration and transpiration. The temperature values obtained from Biological experiments should be expressed in degrees Celsius (°C) or kelvin (K) to the appropriate number of significant figures.

Here are some simple but essential rules to help maximise the accuracy of temperatures recorded with a liquid-in-glass mercury or alcohol thermometer:

- Ensure that the temperature covers the temperature range you require.
- Ensure that the thread of liquid has no gaps in it; it must be unbroken.
- Carefully stir or shake the liquid or solution before recording the temperature.
- Hold the bulb of the thermometer in the 'body' of the liquid or solution, away from the sides and bottom of the container.
- Place your eye level with top of the mercury meniscus to minimise parallax errors . The use of a magnifying glass will help you improve the precision of your measurement.
- Do *not* use thermometers to stir liquids or solutions since you may shatter the bulb.
- *(If a mercury thermometer breaks you must inform your Biology teacher immediately. They will cover the mercury with powdered sulfur to prevent it evaporating and becoming a hazard. Working safely and minimising the environmental impact of your investigation are important components of the Group 4 assessment criteria).*
- To maximise the precision of your reading you will need to estimate the marks on the scale.
- The bulb of the thermometer must be fully immersed in the liquid throughout the time it takes you to record the temperature. Do *not* lift the thermometer out and up to your eye to read the scale, since the thermometer will cool. The thermometer should be immersed in the liquid for 30 seconds to allow the mercury inside the thermometer to reach the temperature of the liquid.

  (*This procedure is only possible with a medical or clinical thermometer that has been specially designed to be read after removal from the patient's mouth. These thermometers are designed so that the stem acts as a magnifying glass to help you observe the fine mercury thread*)

  A clinical thermometer has a greater precision than a typical laboratory thermometer which makes it more suitable for measuring small changes in temperature.

6.1.4 Measuring Time

Timing in Biological experiments is frequently undertaken with an electronic stopwatch (typically one that measures times to within 0.01 seconds). Some biological 'events' can be quite brief, for example, the movement of a bubble in the capillary tube of a potometer, so the accuracy of a timing measurement is often determined by the speed at which the stopwatch is turned on and off by the operator.

The precision of the measurement can therefore be improved by familiarity with the controls, and also by anticipating the beginning and end of the biological event being timed. The random errors inherent in such measurements can be minimised by repeating the experiments, both individually and collectively. Mean times can then be calculated.

In human physiology experiments, the human subject should preferably *not* be involved in the recording of times because this can affect the accuracy of the result. Examples of such experiments include the measurement of pulse rate and reaction times. It should be noted that every human recorded measurement of time is associated with a systematic error due to the length of time needed by a person seeing an event and then moving the muscles in the hand to start or stop the stopwatch. This length of time is known as the 'reaction time' and for a typical adult is in the order of 0.3 seconds.

Reaction time varies with:

- Age
- Degree of tiredness
- Size of person
- Hand being used
- Alcohol consumption
- Medicine consumption
- Practice

6.1.5 Measuring pH using a pH Meter (glass electrode type)

Take care to rinse the electrode thoroughly by rinsing it with distilled water from a wash bottle every time you change solutions. Do *not* leave the electrode un-immersed for longer than you need or let the electrode dry out: salt deposits will form and interfer with the electrode response. Standardise the meter (as shown in the instructions or by your IB Biology Teacher) at a particular pH and temperature using the buffer(s) provided.

The buffer provided should reflect the nature of solutions whose pH values you are going to measure. For example, if you are investigating acidic solutions then an acidic buffer solution (for example, pH 4.0) should be provided. However, calibrating with two buffers allows the 'slope' of the pH meter to be set so that it reads correctly at all pH values. Check to ensure that the buffer solutions have not "expired".

Do *not* touch the electrode or move about near to it whilst a reading is being taken. If you are using a magnetic stirrer, switch it *off* when you take the readings. The electrode needs time to reach equilibrium in solution, so do *not* rush to record the measurement, instead wait patiently until the meter reading becomes steady (see *Figure 501*).

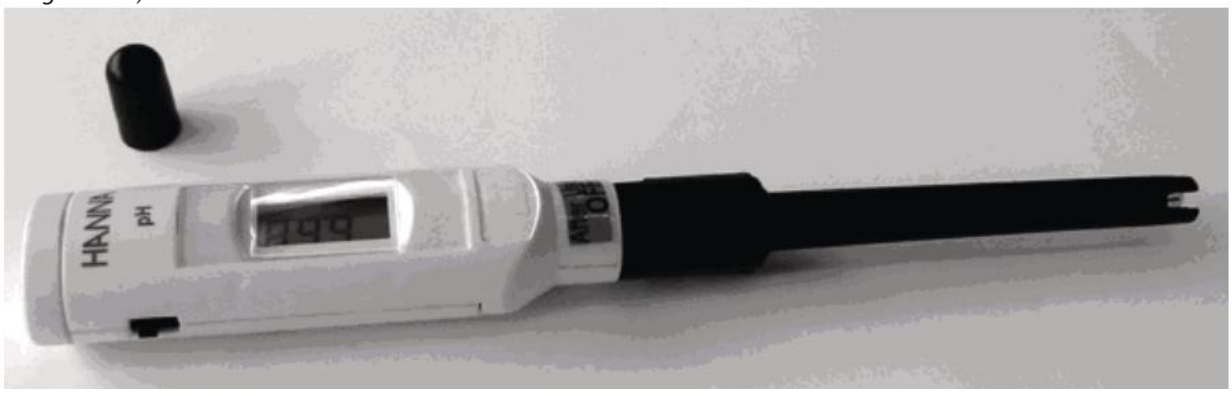


Figure 501 A pH meter (manufactured by Hanna)

(Photography by Robert Balcer, Overseas Family School, Singapore).

6.1.6 Measuring volumes of liquids or solutions

Measuring cylinders, graduated and volumetric pipettes and syringes

These pieces of apparatus are all used for measuring volumes of liquids or solutions. A pipette filler should always be used when filling graduated pipettes. Solutions and liquids, even distilled water, should *never* be sucked up by mouth.

Prior to using a graduated pipette, look carefully to see how the scale is orientated. Some pipettes read from zero to full volume, whereas others start at full volume and read zero. A volumetric pipette is used in the same manner as a graduate pipette except it has a single calibration mark and can only deliver a fixed volume.

In addition, some graduated pipettes are designed to deliver a specific volume when allowed to empty naturally (via the action of gravity), but others will only deliver the specified volume when completely empty. The latter type of pipette will have 'Blow out' printed at the top of the scale.

When reading the volume of water or an aqueous solution in a measuring cylinder or graduated pipette, you should look directly at the bottom of the meniscus (curved surface) and read the volume from there (see *Figure 602*).

If a graduated pipette is being used to prepare a series of serial dilutions, from a stock solution, then it is important to use a clean pipette at each stage, to avoid any carry-over of liquids or solutions.

These pieces of apparatus will come in a variety of sizes; ensure you choose the most appropriate size. For example, if you are to measure 5.0 cm^3 of solution then use a 5 cm^3 measuring cylinder, as opposed to a 25 cm^3 or larger measuring cylinder. This will improve the precision and accuracy of your volume measurement.

Figure 602 shows the correct use of reading the volume of a liquid in graduated apparatus, such as measuring cylinder (which should be placed on a smooth level surface) or a pipette.

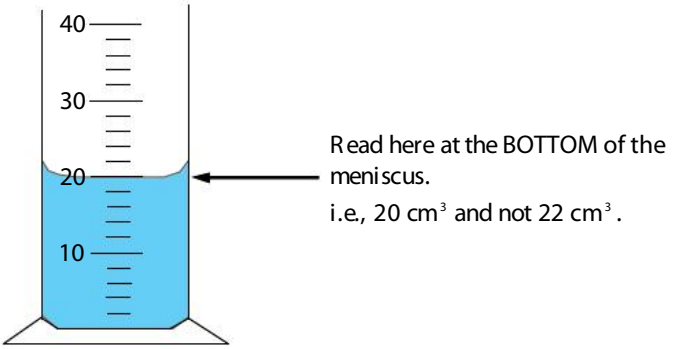


Figure 602 Reading a measuring cylinder

6.1.7 Measuring gas volumes

In a simple but inaccurate method, the reaction, for example, the decomposition of hydrogen peroxide, is allowed to proceed in the presence of detergent within a measuring cylinder. The volume of gas produced is estimated by measuring the volume of froth accumulating in the measuring cylinder (see *Figure 603*) over a given time period.

A more accurate procedure it to set up the apparatus as shown in *Figure 604*. This method allows volumes of insoluble gases to be measured via the displacement of water in an inverted measuring cylinder.

Higher precision can be achieved by connecting the reaction vessel to a datalogger and appropriate pressure sensor.

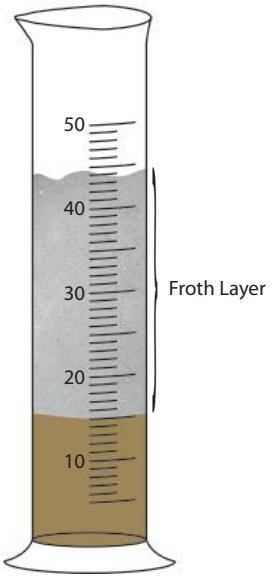


Figure 603 Measuring gas volume

Micropipettes (pipettors or autopipettors)

These may be made available during investigations involving enzymes or genetic engineering. They are relatively expensive but can quickly and accurately dispense relatively small volumes of liquids or solutions. Two types are available: fixed volume, those that can deliver a single pre-set volume, and, adjustable, which can deliver a volume within a set range. It is most important that the micropipette is used with the appropriate disposable plastic tip.

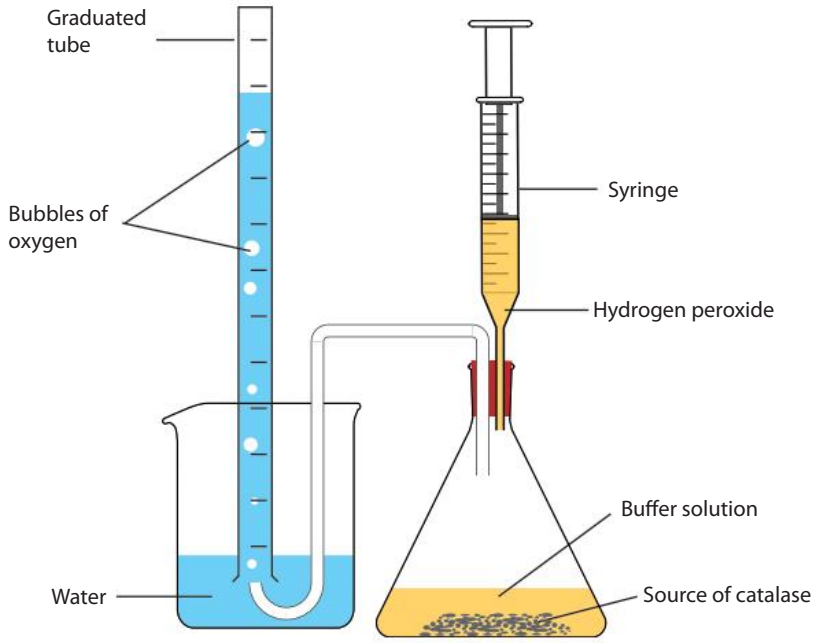


Figure 604 Apparatus for gas collection

6.1.8 Measuring Length

The unit used to measure length will be determined by the scale of the object being measured. For small measurements a ruler will be frequently used. However, since the end of the ruler is often damaged you will get a more accurate result when you measure, for example, from the 10 cm line and subtract 10 cm from your measurement. This avoids introducing a small systematic error.

For example, distances along a transect in ecology will probably be expressed in metres (m), the heights of small shrubs are best expressed in centimetres (cm) and the length of a leaf in millimetres (mm).

If the objects to be measured are extremely small then a microscope should be used. This process is known as micrometry. If the measurement is to be made to an accuracy of 0.1 mm then a micrometer screw gauge or vernier calipers should be used (see *Figure 605*).

Figure 605 Micrometer screw gauge and vernier calipers
(photography by Robert Balcer, Overseas Family School and College, Singapore)

Measurements to within 1 mm can be made with an ordinary ruler and longer measurements can be made with a metre ruler. Longer distances can be measured using a long tape measure. Accuracy becomes critical in measuring small distances, since even small errors can make a significant difference. In measuring any length, you must look straight down at right angles to the ruler and object to avoid any parallax error. As shown in *Figure 606*, person (a) sees 23 mm but person (c) sees 22 mm and person (b) sees 24 mm.

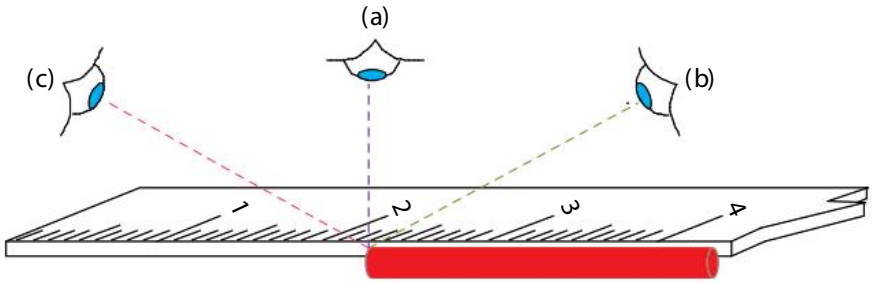


Figure 606 Illustration of parallax error with a ruler

Use of Graduated Scales

Graduated scales are often found in measurement devices commonly used in IB Biology investigations, for example, liquid-in-glass thermometers and glassware such as measuring cylinders, pipettes and burettes.

If the level being measured does not line up exactly with the markings on the graduation scale, it will be necessary to estimate the value being measured. In effect, this means imagining a more detailed scale is located between the actual markings.

There are occasions when a scale (see *Figure 608*) has to be read the 'wrong way around', for example, in an inverted syringe; as shown in *Figure 607*. Note that the measurement is from the bases of the meniscus for water and from the top of the meniscus for mercury. The **accuracy** of the measurement is partly determined by the instruments, and partly by the skill of the student taking the measurement.

Figure 607 Measuring and estimating using an inverted graduated scale (syringe)

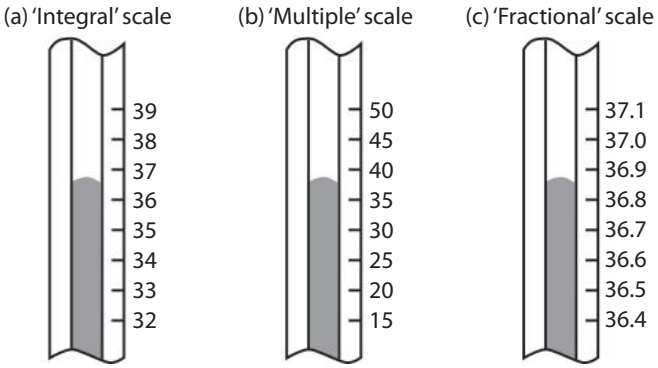


Figure 608 Comparison of integral, multiple and fractional graduated mercury thermometers, all reading the same temperature.

6.2 Microscopy

6.2.1 Using a Light Microscope

A typical light microscope is shown in *Figure 609*. In order to achieve suitable illumination (assuming a lamp is being used), the following steps should be followed:

- Swivel the low power objective of the microscope into the correct position for viewing by rotating the nosepiece in a clockwise direction, until a slight 'click' is heard.
- Place a prepared slide on the stage of the microscope so that the specimen is immediately below the selected objective lens.
- Looking at the stage, use the coarse focus adjustment knob and carefully move the objective lens so that is just above the surface of the slide.
- If necessary, adjust the lamp and the mirror (if present) so that light shines up through the tube of the microscope and the field of view is evenly illuminated. The brightness of the field of view can be adjusted by opening or closing the iris diaphragm situated below the condenser.
- Using the coarse focus adjustment knob (in the opposite direction) enables the specimen to brought into focus at low power, without the risk of breaking the slide and damaging the lens.
- To use the high power objective, the nosepiece should be rotated in a clockwise direction until the selected high power objective lens 'clicks' into place above the slide.
- It should only be necessary to use the fine focus adjustment knob to bring the specimen into focus and see it clearly.
- When viewing or drawing specimens on slides it is good practice to have both eyes open, since this avoids eye strain.
- When viewing of the slides is finished, it is good practice to return to the low power objective before removing the slide from the stage of the microscope.

Figure 609 A light microscope

When using light microscopes in the Biology laboratory, there are a number of precautions which need to be observed to prevent damage to the microscope and the lens:

- Only clean and dry slides should be used.
- Lenses should be kept clean using special lens cleaning tissues – no other material, for example, paper tissue should be used, since it will scratch the lenses.
- The microscope should be supported underneath on the base when it is being lifted or carried.
- Specimens on slides should be observed under low power first, before switching to higher powered objectives.
- Objectives should be changed by moving the nosepiece in a clockwise direction.
- If an oil immersion lens is used (to increase the resolution) then after use, both the lens and the slide should be cleaned carefully using lens tissue.
- Digital microscopes are very similar and usually enable the image to be photographed quite easily.

6.2.2 Mounting specimens for viewing

All specimens should be mounted on glass microscope slides and covered with a coverslip. Specimens should *never* be placed directly onto the stage. To make a temporary wet mount the following procedure should be used:

- Place the specimen in the centre of a clean, grease-free microscope slide.
- Cover the specimen with a small drop of water, or other mounting solution, using a teat pipette.
- Using the thumb and forefinger of the left hand (if you are right handed), hold a clean coverslip so that its bottom edge just comes into contact with the left-hand side of the drop on the microscope slide. The coverslip needs to be held at an angle of between 45 and 60 degrees
- Supporting the coverslip at its upper edge with a mounted needle held in the right hand (if you are right -handed), gently lower it on the drop of mounting solution containing the specimen, see *Figure 610(a)*.

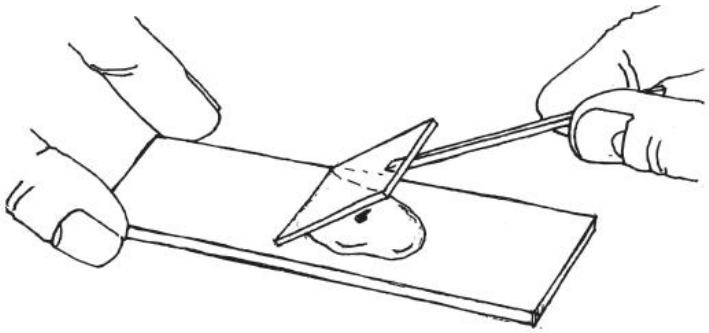


Figure 610 (a) Making a temporary wet mount

The method described should help avoid or minimise the inclusion of any air bubbles in the mounting. Any excess solution or liquid can be gently removed using a tissue or filter paper.

Frequently you may wish to stain a specimen in order to highlight particular tissues, or cell components. If the specimen has already been mounted, a drop of the stain can be placed at one side of the coverslip and drawn through by capillary action by means of a piece of filter paper placed at the opposite side. Once the stain has permeated the specimen, a drop of water can be drawn through in an identical manner to remove the excess stain, see *Figure 610(b)*. When viewing swift aquatic protists, methyl cellulose or 'quieting solution' is used to slow their movement, so they can be better observed. In this case also it is much better to use a 'cavity slide' to avoid squashing the specimen.

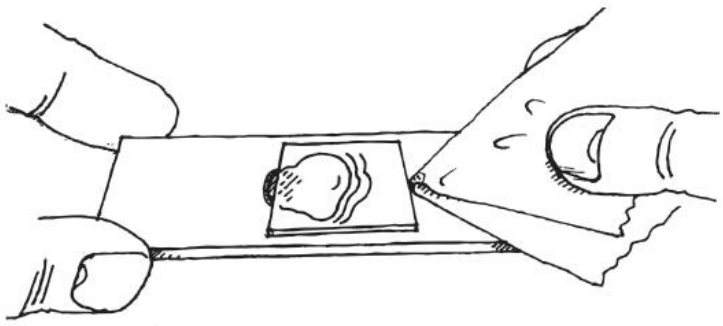


Figure 610(b) Irrigating a slide

Identifying faults

If good definition of the specimen is not obtained, check the following:

- Is the slide clean?
- Is the objective lens centred?
- Are the lenses clean?
- Is the source of illumination satisfactory?
- Is the condenser focused?
- Is the diaphragm adjusted correctly?

Improving the resolution of the light microscope

The resolution of the microscope is a measure of its ability to distinguish between two small objects that are very close to each other. There are two possible approaches to improving the resolution of a light microscope:

- By decreasing the value of the wavelength of light. This can be achieved by using blue light which has a shorter wavelength relative to the average wavelength of white light.
- By increasing the refractive index of the glass lenses. In practice, this is achieved by applying a small drop of immersion oil on the cover slip above the specimen. When using the high power objective lens, the oil layer fills up the air space between the objective lens and the cover slip, reducing the intensity of light being reflected in addition to improving the resolution. This technique is known as oil immersion. The immersion oil has been formulated so that it has a refractive index identical to that of the glass used in the objective lens.

Digital microscopes

Digital microscopes are now commonly used in class rooms. They contain a camera for viewing images of live or preserved specimens. Digital microscopes allow you to capture and save images and many cameras include software for making measurements or adding text to images. Refer to *Figure 611* below.

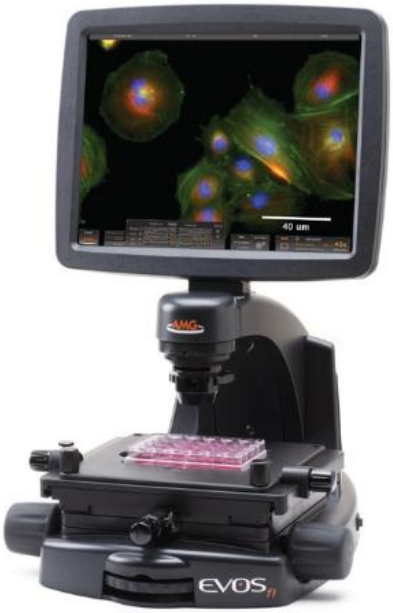


Figure 611 An example of a digital microscope
(Source: /wikipedia/commons/8/8e/Evos_fl_microscope.jpg)

6.3 Preparation of Solutions

- Dilution techniques are a very useful set of Biological laboratory procedures. They provide simple and accurate methods for:
- Changing the concentration of a solution,
- Indirectly 'weighing' a solute whose mass is above or below the limits of electronic balances,
- Determining the quantity of bacteria in culture.

6.3.1 Simple Dilution

A simple dilution is one in which a particular volume of a liquid or solution is mixed with an appropriate volume of a solvent to achieve the desired concentration. The dilution factor is the total number of unit volumes in which your liquid or solution will be dissolved. The unit volumes can be cm^3, dm^3, uL etc.

For example: a 1:5 dilution of an enzyme stock solution involves combining one unit volume of diluent (the liquid or solution to be diluted) with four unit volumes of the solvent. Hence, the dilution factor is 5, that is, (1 + 4) and the enzyme solution is now $\frac{1}{5}$ as concentrated as it was before. Therefore, in a simple dilution, *add one less unit volume of solvent than the desired dilution factor*.

6.3.2 Serial Dilution

A serial dilution is a series of simple dilutions which amplify the dilution factor. The source of dilution material for each step comes from the diluted material of the previous dilution. In a serial dilution the *total dilution factor* at any point is the *product* of the individual dilution factors in each step up to it.

Final dilution factor = Dilution factor 1 × Dilution factor 2 × Dilution factor 3 ... etc.

For example: A two step $1:10^4$ serial dilution of a bacterial culture.

The first step combines one unit volume (10 µL) with 99 units of nutrient broth (990 µL) resulting in a 1:100 dilution. In the second step, one unit volume of the 1:100 dilution is combined with 99 unit volumes of broth (990 µL) now yielding a total dilution of 1:100 × 100 = 1:10,000. The concentration of bacterial is now ten thousand times less than in the original sample.

6.3.3 Making volumes of specific concentrations from liquid reagents

During an IB Biology Investigation you may need to prepare a specific volume of a solution of known concentration.

The formula shown below is quick method to calculating such dilutions:

Original solution **Diluted solution**

$$\mathbf{V_1 \times C_1} = \mathbf{V_2 \times C_2}$$

Where V is volume and C is concentration. The formula is applicable for any units of volume and concentration but they must be consistent.

For example: you have 3 cm^3 of a stock solution of 100 mg cm^{-3} enzyme solution and you want to prepare 200 µL of solution having a concentration of 25 mg cm^{-3}.

V_1 = unknown

C_1 = 100 mg cm^{-3}

V_2 = 200 µL

C_2 = 25 mg cm^{-3}

$$V_1 = \frac{V_2 \times C_2}{C_1}$$

Rearranging:

$$V_1 = \frac{V_2 \times C_2}{C_1}$$

So you would take 0.05 cm^3 or 50 µL of enzyme stock solution and dilute it with 150 µL of distilled water to obtain the 200 µL of 25 mg cm^{-3} solution.

$$V_1 = \frac{(0.2\ cm^3 \times 25\ mg\ cm^{-3})}{100\ mg\ cm^{-3}} = 0.05\ cm^3 = 50\ \mu L$$

6.3.4 Percent Solutions

Many Biological solutions are prepared as percent concentrations. When working with a dry solid it is dissolved as dry mass (g) per volume where the number of grams per 100 cm^3 is equivalent to the percent concentration.

For example: if you want to prepare a 1% sodium chloride solution you would add sufficient water or other solvent to one gram of sodium chloride make a total volume of 100 cm^3 of solution. This is known as the weight- to-volume (w:v) method.

Most Biological solutions used in laboratory work are relatively dilute. Therefore the accuracy of most solution preparation is not significantly affected if a previously weighed mass of solute is dissolved in the required volume of water or other solvent because dilute solutions do not appreciably change in volume after the addition of relatively small quantities of solution.

Hence a 1% solution of sodium chloride could also be prepared by dissolving one gram of sodium chloride in 100 cm^3 of water or other solvent. This solution will weight 11.0 g, but the volume will only be slightly greater then 100 cm^3. If the amount of solute added to the water or solvent is relatively large, this assumption is no longer true and the volume increases measurably leading to a systematic error.

When using liquids, the percent concentration is based upon volume per volume, namely, number of cm^3 per 100 cm^3. For example: if you want to prepare a 60% ethanol solution you would mix 60 cm^3 of pure or 100% ethanol with 30 cm^3 of water (or the equivalent for the volume you desire).

To convert from a percent solution to molarity, multiply the percent solution value by ten to obtain grams per decimetre, then divide by the molar mass.
For example: convert a 6.5% solution of a solution of a chemical with a molar mass of 325.6 g mol^{-1} to a molarity.

$$\text{Molarity} = \frac{\left(\frac{6.5\ g}{100\ cm^3} \times 10\right)}{325.6\ g\ mol^{-1}} = 0.1996\ \ mol\,dm^{-3}$$

To convert from molarity to percent solution, multiply the molarity by the molar mass and divide by ten.

For example: convert a 0.0045 mol dm^{-3} solution of a chemical with a molar mass of 178.7 g mol^{-1} to a percent solution.

$$\text{Percent solution} = \frac{(0.0045\ mol\ dm^{-3} \times 178.7\ g\ mol^{-1})}{10} = 0.08\%$$

6.3.5 Indirect 'weighing' of solutes

For example: Suppose you want to prepare a solution having a solute concentration of 0.001 mg cm^{-3}. Since 0.001 mg is 1/100 of 0.1 mg, dissolving 0.1 mg in 100 cm^3 would give the required concentration.

Determining the quantity of bacteria in a culture (refer to Option B: Biotechnology and Bioinformatics).

For example: suppose that after plating out 1 cm of a 1:2000 dilution of an original culture containing *x* cells per millilitre, you find that 400 bacterial colonies develop.

Calculate how many bacteria were originally in the culture.

$$\frac{400 \text{ bacteria}}{1 \text{ cm}^3 \text{ plated}} \times 2000 = 800,000 = 8.0 \times 10^5 \text{ bacteria/cm}^3$$

in the original suspension.

Suppose you find 400 colonies on a plate. The volume you plated was 0.2 cm^3 (or 1/5 cm^3). The dilution was 1:5000.

Determine the original number of bacteria in the original suspension.

400 bacteria × 5000 × 5 = 10000000 = 1.0×10^7 bacteria/cm^3

in the original suspension, or $\frac{400 \text{ bacteria}}{0.2 \text{ cm}^3} \times 5000 = 1.0 \times 10^7 \text{ bacteria/cm}^3$

6.4 Specialised biological experimental techniques

6.4.1 Fermenters

Fermenters (see *Figure 611*) are used to perform fermentations with yeast or bacteria. These may be undertaken during a study of *Option B: Biotechnology and Bioinformatics*. The sequence below summarises a typical sequence of steps undertaken during investigations into alcoholic fermentation:

- sterilise all equipment and culture media
- assemble the fermenter, including syringes and three-way taps
- set up the systems for agitation (stirring) and aeration (introduction of air)
- allow for inputs, for example, glucose
- control variables, such as temperature or pH
- monitor progress of reaction or growth of yeast or bacteria population
- collect products
- dispose of waste at end of fermentation

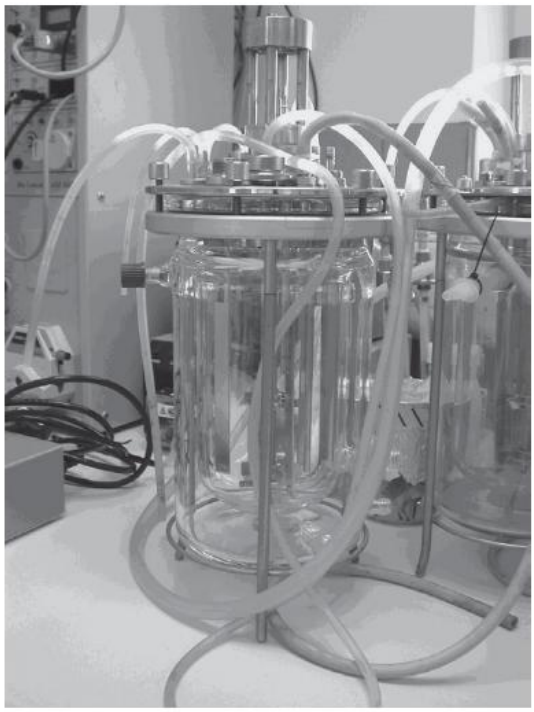


Figure 611 Pilot brewery (Photograph courtesy of Dr Robert Muller, Brewing Research International.)

Sterilisation of equipment and culture media is carried out by high temperature steam in an autoclave. Aseptic technique should then be used at all stages when transferring media and cultures. If aeration of the fermenter is required then ensure incoming air passes through a filter to ensure it is sterile and passes through a second filter when leaving the fermenter to prevent contamination of the atmosphere. The growth of bacteria or yeast can be determined by removing samples and either making a direct count of cells with a hemocytometer or estimating the turbidity (cloudiness) with a colorimeter. An alternative method of monitoring the metabolic activity of the bacteria or yeast is to collect and measure the volume of carbon dioxide released.

6.4.2 Microbiological Assay

A microbiological assay is a technique used to determine the concentration of a chemical (e.g. an antibiotic) by its effect on the growth of bacteria. In the agar diffusion assay nutrient agar is inoculated with a suitable strain of bacteria, and the active substance is allowed to diffuse from a solution in a reservoir into the agar. After incubation in an oven, a clear zone of inhibition is formed, which is proportional to the concentration of the antibiotic. The diagram in *Figure 612* illustrates the principle of a diffusion assay and shows how the zone diameter is measured. This technique can be used to investigate the antibacterial properties of antibiotics, antiseptics, disinfectants and extracts of natural products from terrestrial plants or marine animals.

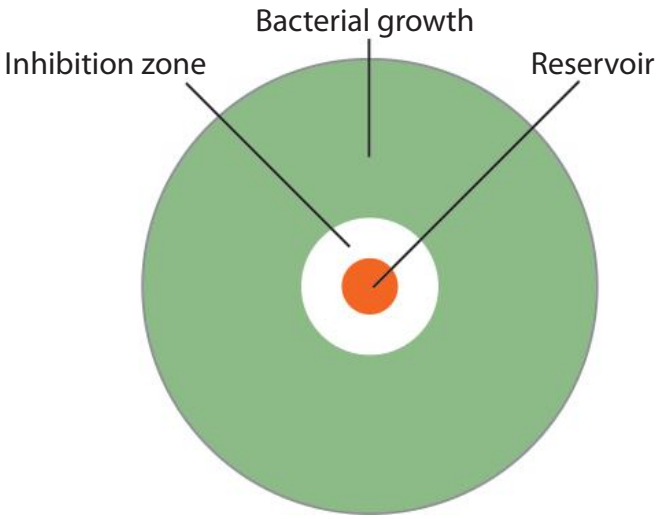


Figure 612 A zone of inhibition

Typically as the concentration of the antibacterial substance increases, the diameter of the zone of inhibition increases. Using solutions of known concentrations, a dose-response curve can be plotted, from which it is possible to determine the concentration of an unknown solution of the same substance. A graph of mean or average zone diameter against the logarithm of the concentration is linear.

Figure 612 shows the outward diffusion of an antibacterial substance from a reservoir into a layer of inoculated agar medium in a Petri dish to create a zone of inhibition.

A specialised microbiological assay can also be used to detect chemicals that cause quorum quenching, that is, prevent quorum sensing exhibited by certain strains of bacteria. *C. violaceum* is a motile bacterium that can protect itself with the antibiotic violacein, which has a purple colour. The production of violacein is regulated by a quorum sensing molecule. A mutant strain of *C. violaceum* (CV026*)* is used which cannot produce the quorum sensing molecule. Therefore colonies of the bacterium cannot produce violacein and are white when cultured. By adding the quorum sensing molecule to a CV026 culture the bacterium produces violacein since the quorum sensing gene is the only deficient gene in the mutant and this can be used for detection of quorum sensing molecules. If the extract is bactericidal then a clear halo will be observed in a purple background. True quorum quenching will result in the formation of a white halo, but with small colonies of bacteria growing within the halo. *Figure 613* shows a photograph of cultured *C. violaceum* plate showing quorum quenching for the chemicals present in fraction 3 (duplicates labeled 3A and 3B). The mixture of chemicals was extracted from the marine cyanobacterium of the species *Lyngbya majuscula*.

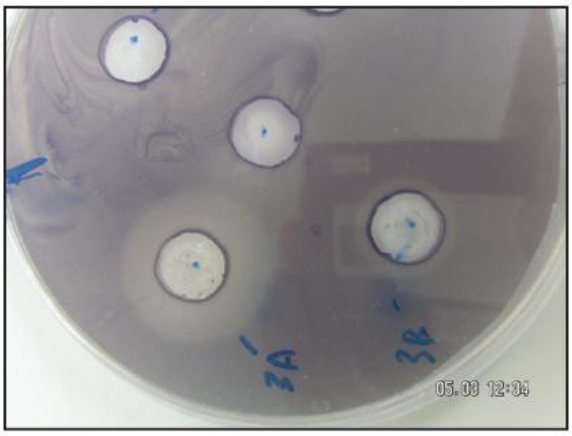


Figure 613 Photograph of cultured C. violaceum plate showing quorum quenching

6.4.3 Gel Electrophoresis

Gel electrophoresis (see *Figure 614*) is a technique used for separating fragments of DNA or proteins. The technique depends on the movement of charged molecules in an electric field. The gel is used as the support medium and is contained in a tank. The relative rate of movement depends on the relative size of the DNA or protein molecule, with the smallest moving the furthest in a given time.

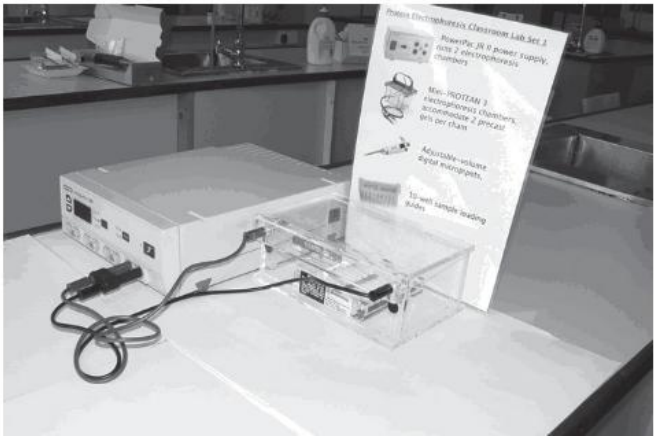


Figure 614 Protein electrophoresis kit (from Carolina Biological)

For protein separations, a buffer is employed often coupled with a powerful detergent, for example, SDS (sodium dodecyl sulfate). A pattern of bands is formed following staining (see *Figure 615*). For DNA separations, it is common to include in the buffer a denaturing agent (for example, urea) to keep the DNA in single-stranded form. The patterns of bands are compared to each other, or with known samples of proteins or DNA fragments. The pattern should be recorded by drawing or photography since the stains used fade with time.

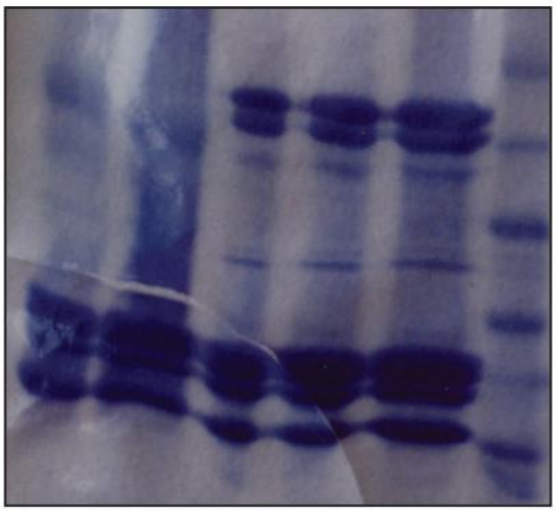


Figure 615 Histone preparations from pig thymus and the salt-tolerant alga Dunaliella run on15% SDS polyacrylamide gel.

6.4.4 Extraction and isolation of DNA

Relatively simple methods can be used for isolating DNA from plant material. A detailed protocol from a research paper or commercial kit should be referred to. However, a summary of typical steps and their rationale are given below:

- Break up the plant tissue mechanically, using a grinder and/or blender – this disrupts cells.
- Degrade the membranes using powerful detergents.
- Separate and remove cell fragments by filtration through filter paper, followed (if possible by centrifuging).
- Degrade the proteins which remain associated with the DNA by adding protease.
- (If commercial proteases are not available try using a meat tenderiser, pineapple juice or contact lens cleaning solution).
- Precipitate the DNA in ice cold ethanol (DNA is insoluble in ethanol).
- The extracted DNA can be pulled out on a glass rod or centrifuged into a pellet.
- Store the isolated crude DNA in a fridge or freezer in buffer solution.

Note that the crude extract contains nuclear DNA together with mitochondrial and chloroplast DNA. RNase enzymes may also be employed to remove messenger and ribosomal RNA. Quantitative estimates of DNA are carried out by determining the absorbance of ultra violet light (with suitable safety precautions). Simpler semi-quantitative methods can also be devised based on the density of colour developed with stains (see *Figure 616*), for example, methylene blue or using a set of standards made up from known quantities of DNA.

Quantitative polyacrylamide gels can also be run where a small amount of known volume from the DNA extract is run on a gel next to known amounts of DNA. After staining with methylene blue, the band size and intensity can be compared with known samples to make an estimate of the DNA in the original sample.

Figure 616 DNA stain kit (produced by Carolina Biological).

6.4.5 Transformation with DNA

Transformation of DNA provides a way of introducing new genes into a cell or organism. The term transformation refers to the uptake and expression of 'foreign' DNA by a living cell or organism. It occurs naturally in some strains of bacteria. When transformation is coupled with antibiotic selection techniques, bacteria can be induced to uptake certain DNA molecules, and those bacteria can be selected for that incorporation.

Transformation is frequently performed on bacteria using plasmids as the vector. Bacterial strains that do not naturally undergo transformation can be made 'competent' by treatment with calcium chloride. As the ions enter the cell, water molecules accompany the charged particles. This influx of water causes the cells to swell and is necessary for the uptake of DNA. The exact mechanism of this uptake is unknown.

A detailed protocol from a research paper or commercial kit should be referred to when performing bacterial transformation with plasmids. However, a summary of typical steps for transformation of *E.coli* and their rationale behind them are given below:

- Competent cells of *E. coli* are prepared by treatment with calcium chloride - this can be thought of as making holes in the cell membrane.
- Mix the suspension of competent *E. coli* cells with the plasmid DNA.
- Incubate the mixture on ice followed by heat shock - this treatment promotes uptake of the DNA. A set of genes are expressed which aid the bacteria to survive at such temperatures. This set of genes is called the heat shock genes. The heat shock step is necessary for the uptake of DNA. At temperatures above 42 °C, the bacteria's ability to uptake DNA becomes reduced, and at extreme temperatures the bacteria will die.
- Add broth containing nutrients which allow the bacterial cells to grow and expression of the genes to be carried out by the plasmid.
- Plate out the cell culture on to agar plates which have been set up to detect whether transformation has occurred. A common approach utilises antibiotic resistance. The plasmids used for the cloning and manipulation of DNA have been genetically engineered to contain the genes for antibiotic resistance. For example, if the bacterial transformation is plated onto medium containing the antibiotic ampicillin, only bacteria which possess the plasmid DNA will have the ability to metabolise ampicillin and form colonies. In this way, bacterial cells containing plasmid DNA are selected.

6.4.6 Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an *in vitro* method of amplifying (copying many times) a specific section from a sample of DNA. PCR can produce many copies of a single DNA sequence, whereas gene cloning in bacteria using plasmids would take several days.

The target length of DNA to be copied is selected using two primers: short lengths of artificially synthesised oligonucleotides. The primer oligonucleotide sequences are complementary to the sequences located at each end of the target DNA.

The PCR reaction mixture contains the sample of DNA (template), an excess of appropriate primers, a heat stable DNA polymerase, the four deoxynucleoside triphosphates required for DNA synthesis, (dCTP, dATP, dGTP and dTTP) and buffer, which maintains a pH suitable for PCR.

The PCR cycle (see *Figure 617*) involves heating the reaction mixture to separate the strands of DNA (via denaturation) and allowing the mixture to cool so that the primers anneal (via hydrogen bonding) to their respective strands. The temperature is then raised to the optimum of the polymerase enzyme, to allow a fresh DNA strand to be synthesised from each template strand, extending in each case from the primer position. After the extension reaction, the reaction mixture is further heated to separate these newly formed strands of DNA. The exact temperatures used depend upon the base sequence and the length of the primer. The cycle is repeated many times and each cycle results in a doubling of the number of copies made of the target length of DNA.

DNA samples for PCR - regardless of preparation method - are generally run in duplicate in order to provide a control for the relative quality and purity of the original sample. The essential criteria for any DNA sample are that it contain at least one intact DNA strand encompassing the region to be amplified and that any impurities are sufficiently diluted so as not to inhibit the polymerisation step of the PCR reaction.

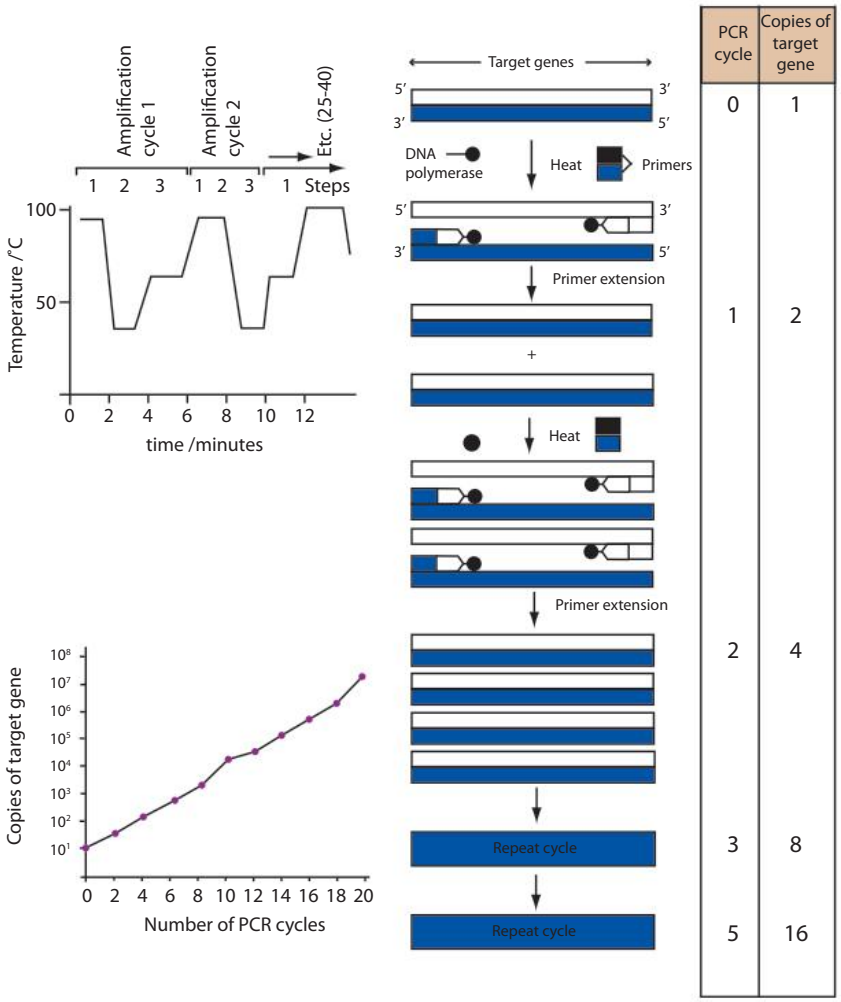


Figure 617 Temperature changes during each PCR cycle and a summary of the PCR progress, illustrating the exponential growth in the amount of DNA

6.4.7 Hemocytometer

A hemocytometer consists of a glass slide with an accurately ruled, etched grid of precise dimensions (see *Figure 618*). It was originally developed for counting red blood cells, but can also used for counting microorganisms in a liquid medium.

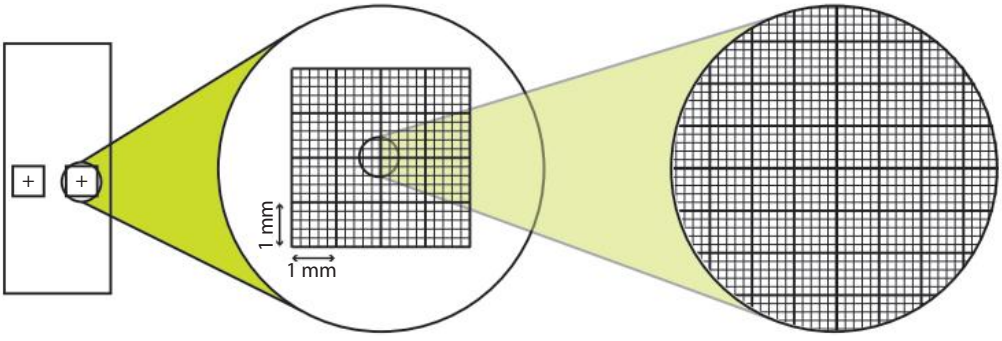


Figure 618 The improved hemocytometer, viewed at magnifications of × 05, × 10 and × 35

Unless special staining techniques are used, it is not possible to distinguish between living and dead cells, therefore this method of direct counting gives the total number of cells, including both living (viable) and dead (non-viable) cells. The Improved Neubauer hemocytometer has two counting grids, each with a central area of 1 mm × 1 mm, divided into twenty five large squares. Each large square is edged by triple-ruled lines and consists of sixteen small squares. There are therefore 25 × 16 = 400 small squares in the counting grid.

When the cover slip is positioned over the counting grid, the depth of the chamber is 0.1 mm, and the volume over one small square is therefore 1⁄400 mm^3. The number of cells are counted in five large squares, which is equivalent to 80 small squares. The total number of cells present per cubic centimetre is given by the following formula:

Number of cells per $mm^3 = \frac{N}{80} \times 4000$, where *N* represents the number of cells counted in 80 small squares.

For best results the cell density should be at least 10^5 cells per millilitre. Common errors occur by improper mixing of the cell suspension prior to sampling and/or by allowing the cells to settle in the pipette prior to loading the hemocytometer counting chamber. Avoid the counting of multiple cell aggregates; the presence of aggregates indicates incomplete dissociation which may require further optimisation of the isolation parameters. A single cell suspension provides the best results.

6.4.8 Colorimetry

A colorimeter is an instrument that measures the intensity of light which passes through, or is absorbed by, a coloured solution. Colorimeters normally have two inversely related scales:

- Absorbance, an exponential (logarithmic scale) from zero to infinity
- Transmittance, a linear scale from zero to one hundred, giving the percentage transmittance

The absorbance scale is normally used since there is a direct relationship between the absorbance of a coloured solution, and the concentration of the coloured substance (provided it is present at low concentration). The diagram in *Figure 619* shows the principle of a colorimeter.

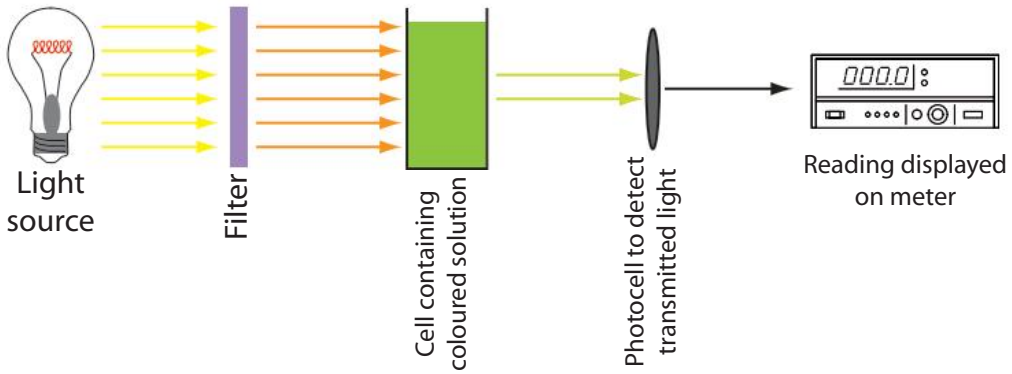


Figure 619 Principle of a colorimeter

Colorimeters can be used to determine the concentration of a coloured substance in solution. Some substances, such as chlorophyll, carotene, hemoglobin, myoglobin, and anthocyanins (plant pigments), are all strongly coloured, but with some other substances, a colour is produced by adding an appropriate reagent. For example, the concentration of starch in aqueous solution can be determined by adding iodine solution and the resulting blue-black colour of the starch-iodine complex can then be measured colorimetrically.

Determination of the concentration of a substance requires the use of a calibration curve. This is prepared by plotting a graph of absorbance of a range of standard solutions against their concentrations. The concentration of the test solution is then determined by reading a value from the calibration curve. If the absorbance of the test solution is located outside the maximum value on your calibration curve, then the appropriate dilution must be made.

Guidelines for the use of a colorimeter

- Select an appropriate filter for the colour of your solution. A colour wheel (see below in *Figure 620*) may be used to predict the filter colour which is diametrically opposite to the observed colour. Hence, a green colour implies that red light is absorbed by a solution. Red and green are complementary colours.
- Prepare a range of standard solutions, including a blank - typically distilled water, for establishing your calibration curve.
- Ensure that the glass or plastic cuvette is clean and avoid touching the optical surfaces. Use the same cuvette for all readings rinsing between each solution.
- Set the absorbance reading to zero using the blank and then record the absorbance of the dilute standard solutions.

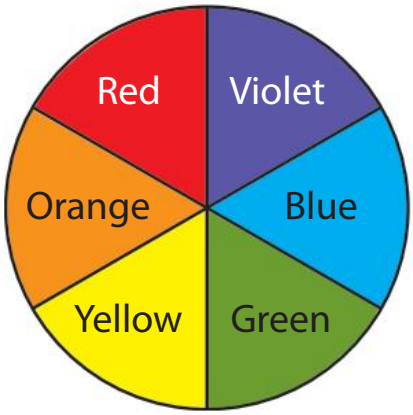


Figure 620 Colour wheel

- Read and record the absorbance of the unknown solution. Dilute if necessary.
- Plot a calibration line for the standard solutions. It may be necessary to draw a smooth curve through points at high concentration.
- Determine the concentration of the unknown solution from the calibration graph. If a dilution has been made, then remember to multiply this value by the dilution factor.

6.4.9 The spirometer

A spirometer can be used for investigating a person's breathing movements and oxygen consumption. This may be used during a sports science investigation. A small hand-held spirometer is shown in *Figure 621*.

Figure 621 A hand-held spirometer (Photography by Robert Balcer, Overseas Family School, Singapore).

However, another type of spirometer commonly used in schools and colleges is shown in *Figure 622*. It consists of plastic lid, hinged to a tank of water. The lid encloses a chamber, which is connected to the subject by a rubber mouthpiece at the end of a flexible breathing tube. As the subject breathes in and out, the lid moves up and down in time with his or her breathing. An inlet tube at the side can be used for filling the chamber with oxygen. A canister of soda lime (a mixture of calcium oxide and sodium or potassium hydroxide) is placed in the course of the breathing tube to ensure that all the carbon dioxide in the subject's expired air is removed before the subject breathes again.

A two-way tap controls the flow of air into and out of the chamber. By rotating this tap in the appropriate direction the spirometer chamber can be opened to the atmosphere (or to a subject if the breathing tube is connected to the mouth) or closed. The lid is counterbalanced by a moveable mass whose position should be set so that the lid falls very slowly when the spirometer chamber is open to the atmosphere. When the chamber is closed, the lid should remain stationary; if it falls, there is a leak in the system. Changes in the volume of oxygen in the spirometer chamber can be read off the scale attached to the side of the lid. Movements of the lid can be recorded by a pen writing on a kymograph drum or chart recorder. The recording paper should be calibrated for volume and time. To calibrate for time you need to know the speed at which the recording paper moves. Knowing the speed, you can make a series of vertical lines on the recording paper corresponding to one minute intervals. Alternatively, the spirometer can be attached to an appropriate data logger.

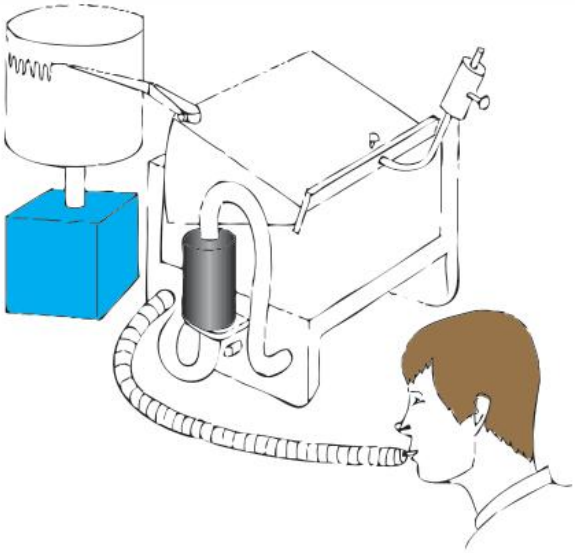


Figure 622 A spirometer

6.4.10 Potometer

A potometer is an instrument, which measures the uptake of water by a leafy shoot. However, of the volume of water taken up by a plant, only a very small percentage is used by the plant in processes such as photosynthesis (as a reactant), hydrolysis reactions (for example, hydrolysis of starch) and maintaining the turgor of cells. Almost all of the water which is taken up is lost by the process of transpiration, in which water evaporates from cells into intercellular spaces, then diffuses, as vapour out of the plant.

There are many different designs of potometer, but they generally work on the same principle, that is, the water is taken up by a suitable leafy shoot, connected to the apparatus. As water is taken up, a small air bubble is drawn along a horizontal capillary tube, so that the rate of movement of the bubble corresponds to the rate of water uptake by the shoot. One type of potometer, known as Ganong's potometer, is illustrated in *Figure 623*.

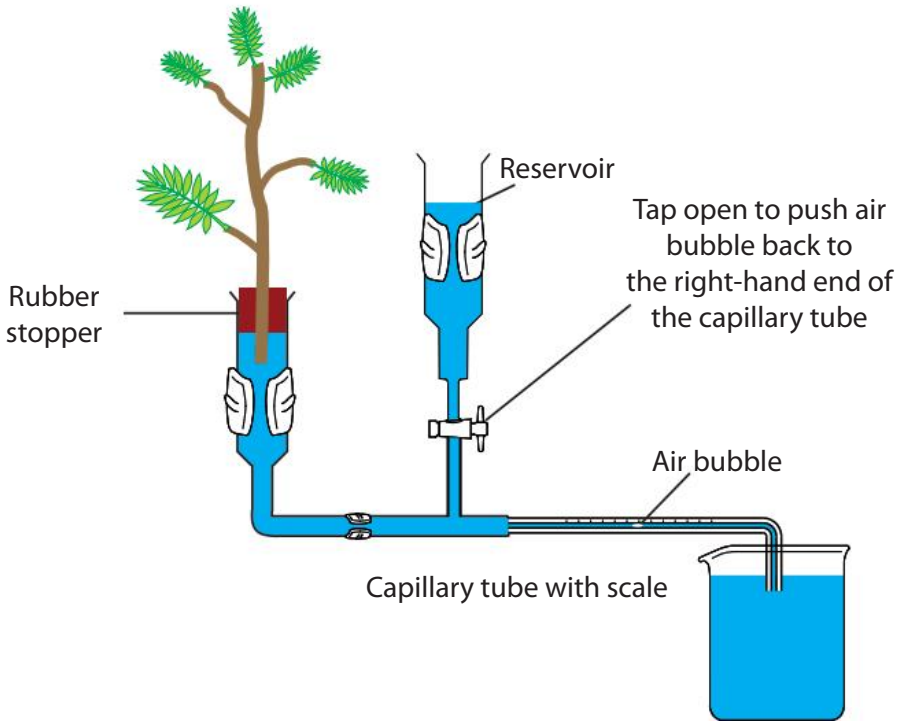


Figure 623 Ganong's potometer

There are a number of precautions which need to be taken when using a potometer to ensure reliable results. As soon as the root is cut off, the cut end should be placed in a suitable container of water, such as a bucket. In the laboratory, a second cut is made, under water near the end of the shoot. These precautions are to prevent air locks in the xylem. The potometer should be assembled in a sink full of water. After the shoot is fitted through the hole in the rubber bung, the last few centimetres of bark should be removed. This prevents any sap from the phloem from blocking the xylem. To introduce a bubble in the capillary tube, the tube is lifted out of the water in the beaker, blotted with a paper towel, then replaced into the water when an air bubble has entered the end of the tube. When the potometer is set up and the air bubble is moving at a steady rate, record the movement of the bubble along the scale, at suitable time intervals.

6.5 Ecological Sampling Methods

When considering any study of a whole ecosystem, or even a part within, for example, a mark-release-recapture investigation on one population, we have to accept that we cannot study it all. It is usually impossible or impractical to go and count each and every one of a species present in that location.

We therefore need to take sample sets of results (or samples) from the ecosystem, trying to ensure that these samples represent the whole location or population we are studying.

(*You also need to balance the need for field study sampling and environmental damage, but you should also note that if an ecosystem was not studied you could not assess its value, for example, its biodiversity, or how to conserve it, if appropriate. Such considerations are assessed by the group 4 assessment criteria.*)

Samples are usually taken using a standard sampling area of some kind. This ensures that all of the samples represent the same area of the habitat each time. The sampling area is frequently a quadrat, used for studying a small number of sites within the whole site being studied. The most frequently used being a metre squared quadrat.

Sampling with a quadrat is particularly suitable for sampling low-growing vegetation, for example, grassland, weeds, or lichens and mosses on rock surfaces. Sessile seashore animals living on rocks (small, immobile animals, such as, barnacles and limpets) are best sampled by quadrats. The shape and size of the quadrat used should be stated in any 'write-up' or report.

The choice of quadrat area depends to an extent on the type of survey being conducted. The use of relatively small quadrats allows the survey to be completed more quickly, while larger quadrats require more time and effort to examine properly. A balance is therefore necessary between what is ideal (from a crude statistical viewpoint about 30 readings per sample are required for a t-test) and what is practical.

To record percentage cover of species in a quadrat, look down on the quadrat from above and estimate the percentage cover occupied by each species. The range of species often overlap and there may be several different vertical layers. The percentage cover may therefore add up to well over one hundred percent for an individual quadrat.

The estimation can be improved by dividing the quadrat into a grid of one hunded squares each representing one percent cover. This can be done by actually dividing the quadrat by means of string or wire attached to the frame at standard intervals. This is only practical if the vegetation in the area to be sampled is very short, otherwise the string or wire will prevent the laying down of the quadrat over the vegetation.

Quadrats are most often used for sampling, but are not the only type of sampling units.

If you are sampling aquatic micro-organisms or analysing water samples, then you will most likely collect water samples in standard sized bottles or containers.

Plankton and other pelagic organisms (organisms living on the surfaces of open oceans or seas) are often sampled using a net trawled behind a boat moving at a standard speed for a standard length of time. Birds, which are highly mobile, are usually sampled by taking a standardised walk of a given length at a given speed at a given time of the day through the relevant habitat and recording sightings.

The sampling of insects is performed in a number of ways depending on their habitat and mobility. Ground dwelling species are caught in pitfall traps and sweep nets can be used for sampling flying insects. A standardized walk of 100 metres can be made using a standardised number of 'swishes' of a suitable net in vegetation. Nocturnal flying insects, such as moths, can be caught in light traps set for a fixed number of hours.

A D-vac machine (akin to a vacuum cleaner) is often used to perform insect sampling, but again a fixed area is sampled for a given period of time at a particular time of the day. This approach allows you to 'control' the variables to some degree and to try and ensure weather conditions are similar since temperature and wind can both affect insect catch-ability due to effects on emergence and preferential flying conditions.

If the insect, or other small invertebrates, live in leaf litter or soil then a top-heated funnel (Tullgren funnel) can be used to extract the organisms from a standardised volume. Animals active on the soil surface can be caught from pitfall traps and this approach also allows the use of the mark-release-recapture method. However, the method employed to mark the animals should not affect their chance of recapture or increase their risk of predation. Similarly, the animals should not become 'trap happy' and learn to associate the traps with free food.

If you are investigating parasites on fish, then an individual fish will most likely be your sampling unit. Similarly, studies of small leaf dwelling insects would probably involve collecting individual leaves as sampling units. In these last two cases, the sampling units will not be of standard size. This problem can be overcome by using a weighted mean, which takes into account different sizes of sampling unit, to arrive at the mean number of organisms per sampling unit.

There are three main approaches to taking samples:

- Random sampling.
- Systematic sampling (which includes the line transect and the belt transect methods).
- Stratified sampling.

6.5.1 Random Sampling

Random sampling is usually carried out when the area under study is fairly uniform, very large, and/or there is limited time available to carry out the investigation. When using random sampling techniques, large numbers of samples are taken from different positions within the habitat. A quadrat frame is most often used for this type of sampling. The frame is placed on the ground and the animals, and/or plants inside it counted, measured, or collected, depending on the nature of the investigation. This is performed many times at different points within the habitat to give a large number of different samples.

In the simplest form of random sampling, the quadrat is thrown to fall at 'random' within the chosen site. However, this is usually unsatisfactory because a personal element inevitably enters into the throwing and it is not truly random. True randomness is an important element in ecology, because statistics are used to process the results of sampling. Many of the common statistical techniques employed are only valid when applied to data that is truly randomly collected. This technique is also only possible if quadrats of small size are being used. It would be virtually impossible to throw anything larger than a one metre square quadrat. and within habitats, such as woodlands or tropical rainforests, it is also often not possible to physically lay quadrat frames down, because trees get in the way. In this case, an area the same size as the quadrat has to be measured out instead and the corners marked to indicate the quadrat area to be sampled.

A better method of random sampling is to map the area and then to lay a numbered grid over the map. A random number table (generated by a computer) is then used to select which squares to sample in. In some habitats it may be difficult to set up numbered grids (for example, in a woodland or rain forest) and in these a 'random walk' may be used. In this method, each sample point is located by taking a random number between 0 and 360, to give a compass bearing, followed by another random number which indicates the number of paces which should be taken in that direction.

Many ecological surveys are carried out over extended periods of time, with sampling taking place at regular intervals within a particular habitat. In such cases, it is necessary to estimate the number of samples which should be taken at each sampling period. The minimum number of samples which should be taken to be truly representative of a particular habitat, can be ascertained by graphing the number of species recorded, as a function of the number of samples examined. Once the graph begins to 'level out' further sampling becomes unnecessary and further sampling will merely waste time and duplicate results.

6.5.2 Systematic sampling

Systematic sampling occurs when samples are taken at fixed intervals, usually along a line. This normally involves carrying out transects, where a sampling line is set up across areas where there are clear environmental gradients. For example you might use a transect to show the changes of plant species as you move from grassland into woodland, successional changes across a dune system or to investigate the effect of a pollutant radiating out from a particular source on species composition.

Systematic sampling should also be employed if you are attempting to correlate two variables. For example, consider an investigation into the relationship between water velocity and the number of a particular aquatic insect species. Random sampling may give sampling positions in slow-moving areas. A systematic approach would involve identifying the slowest and fastest section, measuring their velocities and then selecting sample sites at intermediate velocities.

6.5.3 Line transect method

A transect line can be made using length of rope or string marked and numbered at 0.5 m, or 1 m intervals, all the way along its length. This is laid across the area you wish to study. The position of the transect line is very important and it depends on the direction of the environmental gradient you wish to study. It should be thought about carefully before it is placed. You may otherwise end up without clear results because the line has been wrongly placed. For example, if the source of the pollutant was wrongly identified in the example given above, it is likely that the transect line would be laid in the wrong area and the results would be very confusing.

A line transect is carried out by unrolling the transect line along the gradient identified. The species touching the line may be recorded along the whole length of the line (continuous sampling). Alternatively, the presence, or absence of species at each marked point is recorded (systematic sampling). If the slope along the transect line is measured as well, the results can then be inserted onto this profile.

6.5.4 Belt Transect Method

This is similar to the line transect method but it gives information on the abundance as well as the presence, or absence of species. It may be considered as a widening of the line transect to form a continuous belt, or series of quadrats.

In this method, the transect line is laid out across the area to be surveyed and a quadrat is placed on the first marked point on the line. The plants and/or animals inside the quadrat are then identified and their abundance estimated. Sessile animals can be counted, or collected, while it is usual to estimate the percentage cover of plant species. The cover is the area of the quadrat occupied by the above-ground parts of a species when viewed from above. The canopies of the plants inside the quadrat will often overlap each other, so the total percentage cover of plants in a single quadrat will frequently add up to more than one hundred percent.

Quadrats are sampled all the way down the transect line, at each marked point on the line, or at some other predetermined interval (or even randomly) if time is limited. It is important that the same person should do the estimations of cover in each quadrat, because the estimation is likely to vary from person to person. If different people estimate percentage cover in different quadrats, then an element of personal variation is introduced which will lead to less accurate results.

6.5.5 Stratified sampling

Stratified sampling is used where there are small areas within a larger habitat which are clearly different. For example, scrub patches within a heath land area, or areas of bracken in a grassland. Sampling would be carried out either randomly, or systematically within each of the separate 'stratum' identified. This approach recognises major differences within the communities before sampling begins.

6.5.6 Choosing a sampling method

Stratified sampling

Stratified sampling is the process of identifying areas within an overall habitat, which may be very different from each other and which need to be sampled separately. Each individual areas separately sampled within the habitat is termed a stratum. This is unnecessary if the habitat being sampled is relatively uniform.

Random sampling	Systematic sampling (transects)
Employed whene the habitat being sampled is relatively uniform, for example, the interior of a woodland.	To show zonation of species along some environmental gradient, for example, down a sea shore or across a woodland edge.
To remove observer bias in the selection of samples.	Employed when there is continuous variation along the line.
Employed when statistical tests are to be used which require randomly collected data.	To sample linear habitats, for example, a roadside verge.
Employed if time is limited and/or where a large area needs to be sampled rapidly.	Employed when physical conditions demand it, for example, sampling a vertical rock face.

Systematic sampling – Line or Belt?

Line transect	Belt transect
Employed when time is limited. A line transect can be performed more rapidly than a belt transect.	A belt transect will supply more data than a line transect and will supply data on the abundance of individual species at different points along the line, in addition to their range.
Supplies data showing how species change along the line.	A belt transect supplies data showing how the abundance of each individual species changes within its range.
Supplies data showing the species range along the line.	A belt transect will allow the relative dominance of species along the line to be determined.

What interval should be used?

Transects can either be continuous with the whole length of the line being sampled, or samples can be taken at particular points along the line.

For both line and transect belts, the intervals at which samples are taken will depend on the individual habitat, as well as on the amount of time available for the investigation.

- Too large an interval may mean that many species actually present are not recorded, as well as obscuring subtle zonation patterns due to lack of data.
- Too small an interval can render the sampling very time consuming, as well as yielding more data than is needed. This can make it more difficult to identify patterns of zonation.
- It is vital to ensure that the interval chosen does not happen to coincide with some regularly occurring feature of the habitat.

For example, if sampling a freshly ploughed field with ridges and furrows, the interval should not be such that all samples are taken on a ridge or in a furrow, unless the purpose of the investigation is to identify any differences between ridges and furrows.

The diagrams in *Figure 624* represent the different types of sampling. Each rectangle represents a field and each small square a quadrat within the field.

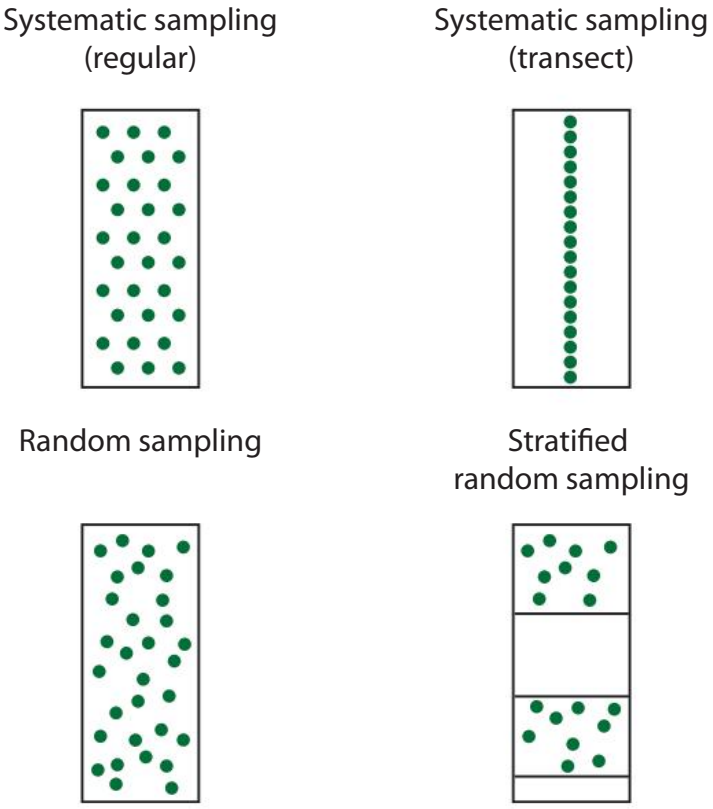


Figure 624 Different Types of Sampling

Area

In biology, shapes are often irregular and the area is estimated, often using some form of regular grid for reference. For example, the area of leaves can be found by tracing the outline onto graph paper (see *Figure 625*). The total surface area of leaves on a tree is then estimated by counting the number of leaves and multiplying this by the (average) surface area of one leaf.

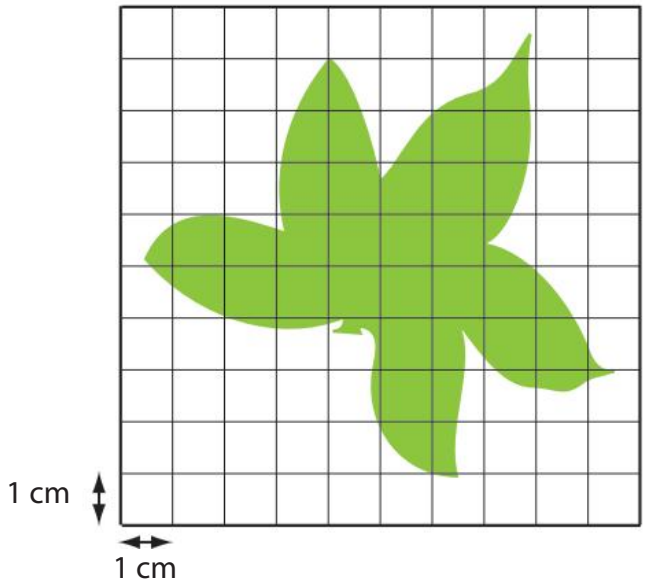


Figure 625 Estimating leaf area

Percentage cover

One common method to determine the percentage cover of vegetation is to use a quadrat. (This is not strictly an area measurement). These are typically square frames of a particular size which are laid on top of the vegetation of the habitat being investigated. The areas of percentage cover are determined by a combination of estimation (see *Figure 626*) and measurement. Quadrats may be further subdivided into grids for more accurate estimates.

The S.I. unit of area is the square metre (m^2) but other commonly used units are may be mm^2 and cm^2. 100 or 1×10^2 $mm^2 = 1$ cm^2; 1000 or 1×10^4 $cm^2 = 1$ m^2. A common unit used in ecological studies in the hectare (ha), where 1 Ha $= 10^4$ m^2.

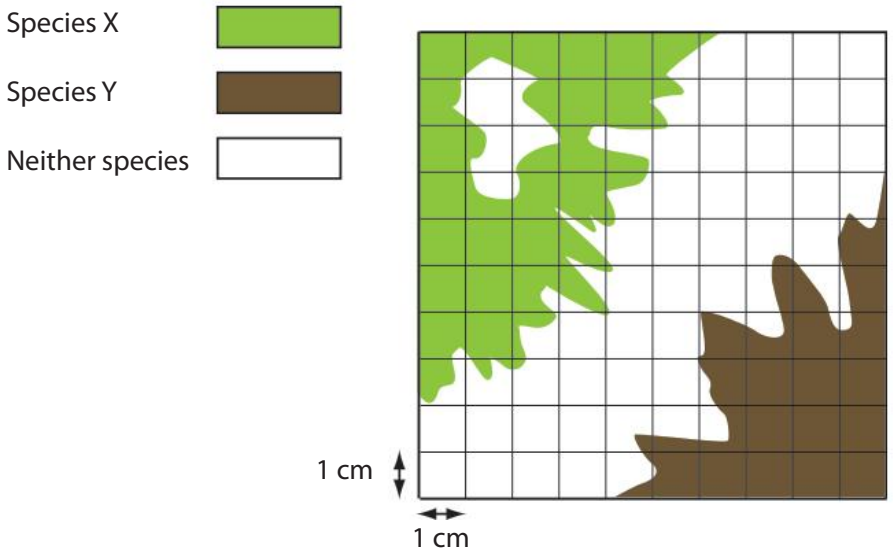


Figure 626 Diagram showing percentage cover for two plant species, X and Y

6.5.7 Environmental Measurements

An ecological investigation of an ecosystem will likely involve sampling the abiotic (non-living or physical) components. A summary is given in *Figure 627* of the various techniques and measurements that you may employ depending on the nature of the ecosystem.

MEASUREMENT	MEASURING DEVICE
Temperature	Liquid-in-glass mercury thermometer, maximum-minimum thermometer or thermistor
Light	Photographic light meter
Relative humidity	Wet-and-dry-bulb hygrometer
Precipitation (rain fall)	Rain gauge
Air movement (wind)	Wind vane (wind direction); cup anemometers (wind speed)
pH (of soil and water)	pH probe and meter (or universal indicator solution), barium sulfate solution and distilled water
Concentration of inorganic ions (in soil and water)	Various chemical kits (often involving colour changes)
Drainage rate/permeability/ porosity (of soil)	Funnel, stop watch and measuring cylinder
Humus/organic content (of soil)	Furnace, desiccator and balance
Air content of soil	Measuring cylinders
Water/moisture content of soil	Oven, tin can sampler (with perforated end) and balance
Soil structure	Soil auger
Soil salinity	Conductivity meter
Water velocity	Flow meter or float
Dissolved oxygen	Dissolved oxygen meter or Winkler titration
Hardness/calcium ion concentration	Conductivity or titration

Figure 627 Summary of the measurement of environmental factors

Due to space limitations pictures of the apparatus and a description of how to use them is not given. Please consult your Biology Teacher or the manufacturer's instructions. Many of these measurements could be monitored and measured via the technique of data-logging.

6.6 Safety

For the majority of the practical work that you perform during your time in the IB Biology programme, you will be likely to be following instructions from a worksheet or from your IB Biology teacher. Any hazards associated with the chemicals you will handle or the practical techniques that you will undertake will have already been assessed by an experienced Biology teacher, and measures put in place to minimise any risk involved.

(*Basic safety instructions are described in the IBID Press Biology Volumes of Investigations and individual chemical or biological hazards are clearly identified in every Investigation.*)

However, there will be a number of occasions during your IB Biology programme when you will have to design your own experiments as part of a design exercise, or when you participate in the Group 4 Project or perform an extended investigation for your Extended Essay.

When planning such an activity, one of the most critical stages is to perform a risk assessment on the chemicals and techniques you are proposing to use. Although this is an important skill for you to develop as an IB Biology student, the ultimate responsibility for risk assessment rests with the employer of the staff in your school or college.

Some common safety risks are involved with:

- **Heating at high temperatures** - burning and scalding may occur when heating, spilling hot water from a water bath or accidentally touching hot apparatus.
- **Manipulating sharp instruments** - scalpels and seekers (used in dissections), knives and razor blades (used in microscopy) are potential hazards.
- **Using hazardous chemicals** - these may be inflammable, corrosive (damage unprotected flesh), toxic, carcinogenic (cause cancer) or lead to allergic reactions. *Figure 628* shows a list of the common chemical hazards involved in typical investigations performed in a laboratory.

(N.B. You must consult your teacher before using any of these reagents and follow the safety rules, regulations or guidelines of your school or college.)

NAME OF CHEMICAL	HAZARD
Hydrogen peroxide, H_2O_2	Irritant (at low concentrations); corrosive (at high concentrations).
Acids/alkalis	Irritant (at low concentrations); corrosive (at high concentrations).
Trypsin	Irritant
Methylene blue	Irritant
Dichlorophenol indophenol (DCPIP)	Irritant
Bile salts	Irritant
Bromothymol blue	Toxic
Triphenyl tetrazolium chloride	Corrosive; toxic (poisonous)
Petroleum ether	Flammable
Ethanal	Harmful
Industrial methylated spirit (IMS)	Flammable

Figure 628 List of commonly used hazardous chemicals and their effects

There are several stages in performing a risk assessment:

- First, make a list of all the chemicals (including quantities (masses or volumes) and/or concentrations and the apparatus that you plan to use.
- Next, identify the hazards associated with each of the chemicals on your list. They will include explosive, oxidising, harmful, toxic, irritant and corrosive.
- Finally identify any control measures to deal with small spillages, for example, water is used to deal with small hydrogen peroxide spills.

Sources of information (in the United Kingdom) are CLEAPSS *Student Safety Sheets,* CLEAPSS *Hazcards* and the SSERC *Hazardous Chemicals Manual*. Sources of information in North America are the *Materials Safety Data Sheets*, MSDS. There should be similar publications or a safety manual in your school or college. You can also examine labels on chemical bottles or find hazard information in Biological texts and chemical supplier's catalogues. Remember to look up the hazards associated with any products of a reaction as well as the reactants.

In order to reduce the risks associated with a particular procedure you should consider the following control measures to reduce the risk involved. Consider whether replacing a hazardous chemical by a less hazardous one would reduce the risk. Alternatively, if possible, reduce the quantities or concentrations of the hazardous chemical being used and carry out the practical on a micro or small-scale basis. You may also have to consider modifying the experimental procedure to make it less risky.

Finally, list all the safety precautions that you will need to take in view of the hazards you have previously identified. These may include wearing of appropriate protective clothing – eye protection and gloves. Include the Risk Assessment in your written plans for a Design investigation or Group 4 Project, and discuss it with your IB Biology Teacher, but remember if you change your plans, you may have to change your Risk Assessment. If you feel that the risk cannot be reduced sufficiently, you may to take the decision to abandon your Investigation completely.

6.6.1 Working with micro-organisms

If you are performing an investigation that involves the culturing of micro-organisms, such as bacteria or fungi, there are certain safety procedures that have to be followed, such as aseptic technique. The micro-organisms have to be handled correctly to prevent the contamination of your experimental cultures by micro-organisms from external sources and to prevent the contamination of yourself or your Biology laboratory with any micro-organisms.

Listed below are some of the basic rules, but you must consult your teacher before working with bacteria or fungi and follow the safety rules, regulations or guidelines of your school or college.

- Any open cuts or broken skin must be covered with antiseptic cream and a water-proof plaster.
- Disinfect the bench before you commence practical work.
- Loops are used to transfer micro-organisms and these should be sterilised by heating the loop in a flame until the wire is red hot.
- Discarded glassware, loops etc, should be placed in a container of disinfectant.
- When opening a bottle containing a sterilised solution or cultures of micro-organisms, prevent cross contamination by sterilising the neck of the bottle using a hot flame. Use a sterilised inoculation loop to remove some of the contents and then stopper the bottle. Do not contaminate the bottle by placing the plug or lid on the bench.
- The recommended maximum temperature for the incubation of bacterial cultures is 30 °C, as this is an ideal temperature for the growth of many bacterial strains. Cultures should not be incubated at 37 °C, as this is an ideal temperature for the growth of many pathogenic species.
- At the end of the session, the bench should be disinfected once again and your hands thoroughly washed with disinfectant soap and warm water.

The information in the following table provides some indication of how the Extended Essay will be assessed. Individual teachers and schools may provide additional information as they wish.

Assessment criterion	Highest mark for criterion	Description of highest achievement level for each criterion.
A: Research question	2	The research question is clearly stated in the introduction and sharply focused, making effective treatment possible within the word limit
B: introduction	2	The context of the research question is clearly demonstrated. The introduction clearly explains the significance of the topic and why it is worthy of investigation.
C: Investigation	4	An imaginative range of appropriate sources has been consulted, or data has been gathered, and relevant material has been carefully selected. The investigation has been well planned.
D: Knowledge and understanding of the topic studied	4	The essay demonstrates a very good knowledge and understanding of the topic studied. Where appropriate, the essay clearly and precisely locates the investigation in an academic context.
E: Reasoned argument	4	Ideas are presented clearly and in a logical and coherent manner. The essay succeeds in developing a reasoned and convincing argument in relation to the research question
F: Application of analytical and evaluate skills appropriate to the project.	4	The essay shows effective and sophisticated application of appropriate analytical and evaluative skills.
G: Use of language appropriate to the subject	4	The language used communicates clearly and precisely. Terminology appropriate to the subject is used accurately, with skill and understanding.
H: Conclusion	2	An effective conclusion is clearly stated; it is relevant to the research question and consistent with the evidence presented in the essay. It should include unresolved questions where appropriate to the subject concerned.
I: Formal presentation	4	The formal presentation is excellent
J: Abstract	2	The abstract clearly states all the elements listed above. The requirements for the abstract are for it to state clearly the research question that was investigated, how the investigation was undertaken and the conclusion(s) of the essay.
K: Holistic judgement	4	The essay shows considerable evidence of such qualities (qualities that distinguish an essay from the average, such as intellectual initiative, depth of understanding and insight).

The Extended Essay in Biology

The Extended Essay is one of the core subjects of the IB Diploma Programme. The Extended Essay is an academic study of a focused biological research question. It is intended to give you the experience of working on a research project under the guidance of a teacher who acts as your supervisor. They will meet with you a number of times and comment on the final draft of your Extended Essay. He or she will also conduct a short interview, known as a *viva voce*, after your Extended Essay (4000 words) is complete. The Extended Essay is graded on a scale from A (highest) to E (lowest).

Extended Essays in biology may involve the following broad research areas: microbiology, cell transport (osmosis, diffusion and active transport), animal physiology, plant growth, enzymes, animal behaviour, embryology, genetics, bioinformatics and ecology. The scope of the project is open-ended and the topic or phenomenon under study may not appear in the IB biology syllabus, e.g., allelopathy: the release of harmful chemicals from plants.

The Extended Essay may involve the collection and analysis of secondary data from the published literature. However the majority of Extended Essays involve the collection and analysis of primary data often using relatively simple apparatus or techniques. In other words, you carry out investigations in the laboratory or field and collect raw biological data.

The most successful Extended Essays in biology are those based on a small number of clearly defined and easily manipulated independent variables and a quantifiable and easily measured dependent variable.

For example, you may be investigating the rate of photosynthesis in *Scenedesmus quadricauda* algae immobilized in calcium alginate (see *Figure 701*).

Independent variables affecting the rate of photosynthesis will be dissolved carbon dioxide concentration, temperature of the water, intensity of light and frequency of wavelength.

Light intensity may be an independent variable and rate of photosynthesis (as measured by bubble production) a dependent variable. The temperature of the water and dissolved carbon dioxide are controlled variables.

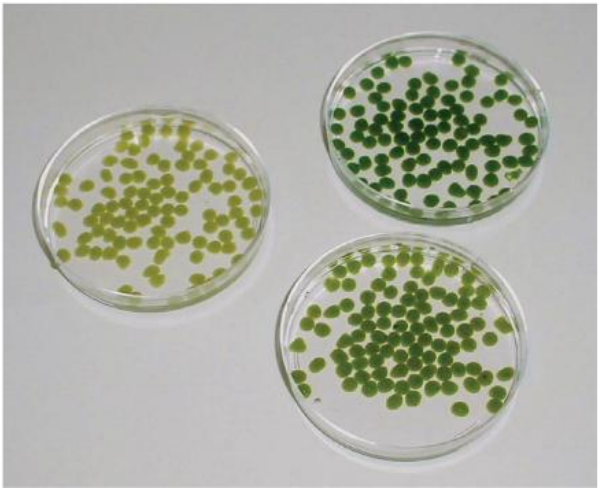


Figure 701 Immobolised green algal balls

A hypothesis and a prediction can then be generated. A linear relationship between rate of photosynthesis and light intensity may be predicted (until carbon dioxide becomes a limiting factor). It should be testable and falsifiable which means that your results should either support the hypothesis or show it to be false.

A hypothesis must be quantifiable which means you must be able to measure changes in the independent and dependent variables and obtain quantitative data (numerical results).

You are advised to avoid Extended Essays that involve surveys and focus strongly on ethical or moral issues. An Extended Essay should have a strong biological or biochemical focus often centred on one of the topics in the IB biology syllabus.

Any experiments on animals, including humans, must comply with the ethical guidelines by the IBO known as the IB Animal Experimentation Policy. Informed consent must be given if you wish to carry out tests on people. The drawing of blood is a breach of these guidelines.

Criterion A: The Research Question

A focused Research Question is the first step in a successful Extended Essay in biology. It must be clearly focused on biological principles and capable of being answered within the word limit and time limitation (40 hours) of an Extended Essay. You may need some assistance from your supervisor to help formulate a research question from a topic you are interested in.

The Research Question does not have to be a question; it could be a statement which outlines what you are trying to discover. A Research Question clearly states the variables that are to be manipulated, measured and analysed.

An example of a Research Question related to photosynthesis:

To establish the relationship between the intensity of white light and rate of photosynthesis (as measured by oxygen bubble production per unit time) in algae (Scenedesmus quadricauda) immobilized on calcium alginate (at constant temperature and carbon dioxide concentration).

An example of a Research Question related to allelopathy:

Do different aqueous extract concentrations of crude leaf extract (0%, 20%, 40%, 60%, 80% and 100% by volume) from Impatiens glandulifera have an effect on the germination of Trifolium repens seeds (as measured by radicle length)?

Research Questions can come from a number of different sources. They may be the result of casual observations from watching animals or examining plants. They may be formulated after carrying out an investigation (as part of your Internal Assessment) or watching a teacher demonstration.

The Research Question must also appear on the Title Page, Abstract and in the Introduction. The Research Question must be identical in all the places where it occurs in your Extended Essay. The Research Question may or not be the title of the Extended Essay.

The Research Question should allow you to apply your IB knowledge of biology and analysis of data in a personal way. You should not choose a Research Question that is an extension of your internal assessment or can be easily answered by looking at an IB Biology Textbook.

For example, you may have investigated the effect of changing sugar concentration on the mass of potato tissue during an IA. Studying the effect of changing sodium chloride concentration on the mass of onion tissue by the same technique may not be a wholly suitable topic for an Extended Essay.

There are a number of publications that can be sources of ideas or topics for investigation by a Biology Extended Essay: School Science Review (*Figure 702*) *<http://www.ase.org.uk/journals/school-science-review>* published by the Association for Science Education (ASE), Biology Review published by Philip Allan *<http://magazines.philipallan.co.uk/Holding-Page1.html>*.

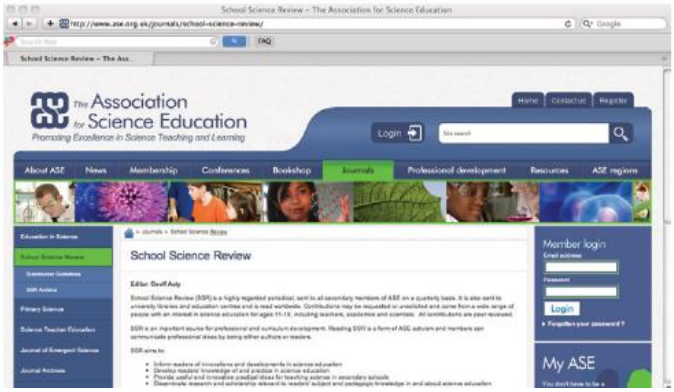


Figure 702 Screenshot of the ASE web site

EBSCO *<http://www.ebscohost.com>* and Science Direct *<http://www.sciencedirect.com>* (produced by Elsevier) are on-line databases subscribed to by many International schools. Find out from your Library Supervisor whether your school has access. There are also two CDs available from the on-line IB shop *<https://store.ibo.org/diploma-programme>*, titled '*50 Excellent Extended Essays*', which contain useful exemplars, for supervisors and students.

Criterion B: Introduction

The introduction should include a background to the study, which means any theoretical or previous experimental work or observations that led to the Research Question. Background information will include references to published work in books, papers and on-line web sites. It may also include a brief review of competing hypotheses or interpretations of data. You should also discuss any personal motivation you have in selecting your biological topic.

There are three aspects to this criterion: the biological context, the biological significance and the importance of investigation. In order to score the highest mark all three aspects must be addressed. In order to demonstrate the biological context and significance of the research question you need to present a summary of the literature (typically published papers and reviews) and other sources, such as book chapters, that you have consulted.

For example, your Extended Essay may involve the study of allelopathy of a named plant species. Allelopathy is defined as the direct or indirect biotic interaction of a plant on another plant by releasing chemicals called allelochemicals into the environment. This release causes a harmful effect on plants in the same habitat. The plant may be an invasive and foreign species introduced into your country decreasing the county's biodiversity and affecting its soil. Allelopathic chemicals may also find use as pesticides.

Criterion C: investigation

This criterion covers both data collected from printed sources as well as data collected by the candidate (through doing experiments or field work). You will be judged about the range and appropriateness of the data you have collected as well as the method used to obtain the raw data. You must show strong evidence of planning and exploratory investigative work. You should explain how information from your sources (on-line and written) helped you decide on your approach.

Your method must be reproducible and full details of all apparatus and instrumentation should be given including the random uncertainty (tolerance) and range, where appropriate.

One common problem when collecting and analyzing biological data is the confounding effect. Confounding effects occur when the variable of interest in the investigation is closely correlated with some other variable which is not of interest. This kind of correlation must be controlled for in the design of the investigation or by using suitable techniques during the analysis. If this is not done an incorrect conclusion may be drawn. An experiment that fails to take a confounding variable into account is said to have poor internal validity.

For example, imagine that you want to compare the amount of insect damage on leaves of Tree Species A (which are susceptible to a plant virus) and closely related Tree species B (in the same genus), a species of tree that is resistant to the viral disease. You find 30 Tree A and 30 Tree B and sample 50 leaves from each and measure the area of each leaf that was eaten by insects. Imagine that you find significantly more insect damage on Tree A than on Tree B.

It could be that the genetic difference between the types of trees directly causes the difference in the amount of insect damage. However, there are likely to be some confounding variables, such as age, fungicide treatment, amount of fertilizer added, water and pruning (cutting).

Criterion D: Knowledge and understanding of the topic studied

You can display knowledge and understanding of the topic by presenting relevant background information and explaining how this relates to the Research Question. You can demonstrate understanding by referring to the variables that may affect the investigation and by referring to the significance of the outcomes. You should provide explanations and justifications for your apparatus and methodology and choice of techniques to process, present and analyse your data. You should also explain why alternative approaches were considered but not adopted.

You should also demonstrate appropriate chemical and mathematical knowledge. You should have a firm grasp of chemical and biochemical concepts such as acidity, basicity, pH, serial dilution, buffers, denaturation of enzymes, conductivity, hydrogen bonds and lock and key principle.

Criterion E: Reasoned argument

You should have a convincing argument in relation to your Research Question. You should set your ideas clearly and logically and analyse the strengths and weaknesses of your claims. You should not arrive at a conclusion to your experimental work without questioning any assumptions or possible competing explanations.

Criterion F: Application of analytical and evaluative skills

Criterion F requires you to apply appropriate biological analytical and evaluative skills. This includes deductive reasoning (generalizing from examples), graphical analysis and statistical analysis (where appropriate).

Statistical methods will help you decide the level of confidence you can have in what the results are suggesting. This is often a problem in a biology extended essay where you are often dealing with variable organisms, tissues or cells and where it is difficult to control the controlled variables. This means that you get a set of results that do not precisely match what is predicted from your hypothesis or where you are not sure whether the set of results obtained from one experiment is *statistically significant* from the set of results you obtained from another experiment.

Descriptive or summary statistics include: mean, mode and median, standard deviation, variance, standard error (for a mean) and confidence interval (for a median). Be aware that you need to be cautious about using means since they assume a Gaussian or normal distribution of data. A histogram will show whether the data approximates to a Gaussian or normal distribution.

Statistics include the chi-square, t test and the Mann-Whitney test.

The use of statistics means that any conclusion must not be expressed in definite terms. Many tests of significance use a probability of 5% as the threshold of significance. This is an arbitrary value and leaves you with a 1 in 20 chance of rejecting the null hypothesis incorrectly (falsely accepting there is a difference in trend when there is not).

Criterion G: Use of language

There are two aspects to this criterion: the use of clear and precise language and the use of terminology appropriate to the biological topic. You need to have a clear and precise style and show an understanding of and fluency in the main technical terms associated with the topic.

For example, if your Extended Essay is concerned with investigating biodiversity then ecological terms and concepts, such as food chains, food webs, ecosystem, community, Simpson biodiversity index etc. are likely to be present in the write-up. The more technical and unfamiliar terms should be defined before use, perhaps in the form of a glossary in the Appendix. You should also use correct chemical formulas and IUPAC names, for example, ethanol (alcohol), C_2H_5OH, where appropriate.

There is no requirement to write in the passive voice, for example, a transect was placed across the rocky shore line at high tide. You can use the first person singular, active voice, for example, I placed a transect across the rocky shore line at high tide. However your style must be formal, consistent and use key terms accurately.

Criterion H: Conclusion

In an effective conclusion you should restate the research question and outline the extent to which it has been answered, dealing also with issues that have not been resolved. You should not overstate your findings and always be tentative in your conclusions. Use statements similar to: *The graph suggests a possible relationship between variables X and Y.*

Criterion I: Formal presentation

You should follow a well known format for correct referencing, for example, MLA (Modern Language Association). You should not use the appendix as a way of keeping the word count below 4000. New information should not be introduced into the appendix since the Extended Essay examiner is not required to read them.

Examples of correct referencing:

Anaya, A. (1999) Allelopathy as a tool in the management of biotic resources in agroecosystems. *Critical Reviews in Plant Sciences*, 18: 697-739

Hannaway, D.B., Cool, M., (2004) White Clover (Trifolium repens L.) Date accessed: 12.9.14

<http://forages.oregonstate.edu/php/fact_sheet_print_legume.php?SpecID=29&use=Soil>

Rizvi, S. J. H., and Rizvi, V. (1992) Allelopathy: Basic and Applied Aspects. *Chapman and Hall*, London.

Criterion J: Abstract

Writing an abstract is a difficult requirement for many students. It is suggested you consult any reputable journal and examine abstracts from published biological papers. The use of the on-line database *Pubmed* is described in *Guide to IB Biology Coursework* published by IBID Press.

An abstract should include the following: the Research Question, an outline of the method including any controls, a summary of the data collected and its analysis (graphical and statistical), a discussion of important assumptions and errors and the conclusion.

8.1 Use of ICT

Information Communication Technology (ICT) is an explicit area of all Group 4 subjects, including the IB Biology Programme. The use of data-logging is one of the recommended technologies as are spreadsheets.

Data-logging is an electronic method of gathering and recording physical measurements; electrical sensors provide signals which are calibrated and recorded by a computer system. Data-logging software not only automates the process of data collection but also provides tools that help in the process of analysing and interpreting the data.

Probes commonly used in Biological data-logging experiments include:

- pH (concentration of hydrogen ions)
- Light intensity
- Infra-red and ultra violet radiance
- Temperature (via a thermocouple)
- Sound
- Dissolved oxygen
- Relative humidity
- Pressure (via a manometer)
- Heart rate (via voltage)
- Conductivity
- Strain gauge (position)
- Oxygen and carbon dioxide sensors

Experiments and processes that are amenable to data-logging given the appropriate software and probes include:

- Monitoring of enzyme activity (via light absorbance or gas pressure)
- Monitoring transpiration (via pressure)
- Monitoring of heart rate
- Soil and ecology measurements (via temperature, light intensity, pH, humidity and dissolved oxygen)
- Photosynthesis (via oxygen concentration (for terrestrial plants) or dissolved oxygen (for aquatic plants))
- Respiration (via carbon dioxide or oxygen sensors)
- Plant growth (via a position sensor)

Once data has been collected and a graph drawn by the software then a process of inquiry should begin. For example, some or all of the following questions may be appropriate:

- For each part of the graph, what was happening during the investigation?
- What caused that peak?
- What are the highest and lowest values?
- How large was a particular change and how long did it take?
- How quickly are the values changing?
- What is the underlying trend?
- What sort of pattern is present in the results?
- How does one variable seem to depend on another?

8.1.1 Data-logging in your Investigations

You may use data-logging software to perform a traditional experiment in a new way. Use of data-logging software is appropriate with respect to assessment if you decide on and input most of the relevant software settings.

For example, an investigation could be set up to monitor a person's heart rate while on a tread mill using an ECG sensor linked to a calculator-based data logger in which you control the level of exercise (speed or workload). Data-logging software that automatically determines the various settings and generates the data tables and graphs would be inappropriate with regard to assessment because the your input required to investigate the heart rate data would be minimal. If your experiment is suitable for assessment the following guidelines must be followed for the DCP criterion.

You may present raw data collected using data-logging as long as you are responsible for the majority of software settings. Your numerical raw data may be presented as a table, or, where a large amount of data has been generated, by graphical means. For example, you should set the duration and rate of the sampling, and the generated data in the form of lists of measurements from the calculator or computer could be downloaded by you into a computer spreadsheet. You must organise the data correctly, for example, by means of table or graph titles, columns or graph axes labelled with units, indications of uncertainties, associated qualitative observations, and so on. The number of decimal places used in your recorded data should not exceed that expressed by the sensitivity of the instrument used. In the case of electronic probes used in data logging, you will be expected to record the sensitivity of the instrument.

Use of software for graph drawing is appropriate as long as you are responsible for most of the decisions, such as:

- what to graph
- selection of quantities for axes
- appropriate units
- graph title
- appropriate scale
- how to graph, for example, linear graph line and not scatter. A computer-calculated gradient is acceptable

In an investigation to monitor heart rates, you could process data by drawing a graph in Excel and measuring the heart rate from the data. By inspecting the graph or spreadsheet data, the maximal and minimal heart rate values could be identified and used to calculate the mean heart rate at rest. The mean heart rate per minute and recovery rate after exercise could also be calculated.

Data-logging software can enhance data collection and transform the sort of investigations possible. In this case fully automated data-logging software is appropriate with regard to assessment if it is used to enable a broader, complex investigation to be undertaken where you can develop a range of responses involving independent decision-making.

For example, a design task could be set to investigate a factor that affects the rate of respiration in aerated yeast in the presence of glucose. If an oxygen sensor with automatic pre-programmed software to monitor the amount of oxygen absorbed by yeast is used, you could use the program to develop a broader, complex investigation, for example, comparing rate of respiration in different strains of aerated yeast at different temperatures.

7

8.2 Use of ICT in Practical Work

It is not necessary to use ICT in assessed investigations but you will be required to use each of the following software applications at least once during the IB Biology course.

- Data-logging in an experiment
- Software for graph plotting
- A spreadsheet for data processing
- A database
- Computer modelling/simulation

8.2.1 Spreadsheets

A spreadsheet is an excellent tool where a large number of repetitive calculations need to be performed on a set of numerical data. This is especially true when the data can be presented graphically.

The most suitable areas for using spreadsheets in modelling chemical reactions lie in evolution, molecular biology and ecology. It is acceptable for you to develop a spreadsheet model as part of your Group 4 Project. An example is shown in *Figure 801*.

The reasons for this are:

- Numerous equations to manipulate.
- Repetitive calculations where a variable is incrementally changed, gives useful information/trends.
- Graphical representation of the data is useful as a learning tool.

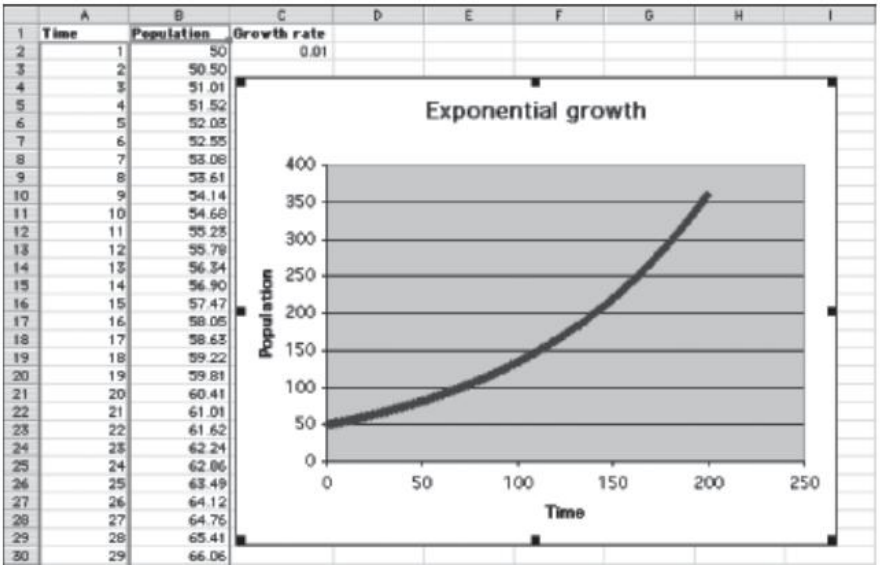


Figure 801 Simulation of exponential growth

There are a number of very useful simulations available on various websites although of course these are liable to change. You can do your own Internet search if you wish.

8.3 Retrieving scientific papers from the Internet

During your IB Biology Practical programme you might want to find out about previously published Scientific research. This can be done by performing a search on a public database known as PubMed (see *Figure 802*). PubMed is a bibliographic database maintained by the National Center for Biotechnology Information in the United States. It is also known as Medline or Entrez.

To search PubMed use your browser to navigate to <*http://www.ncbi.nlm.nih.gov/entrez*> on the Internet.

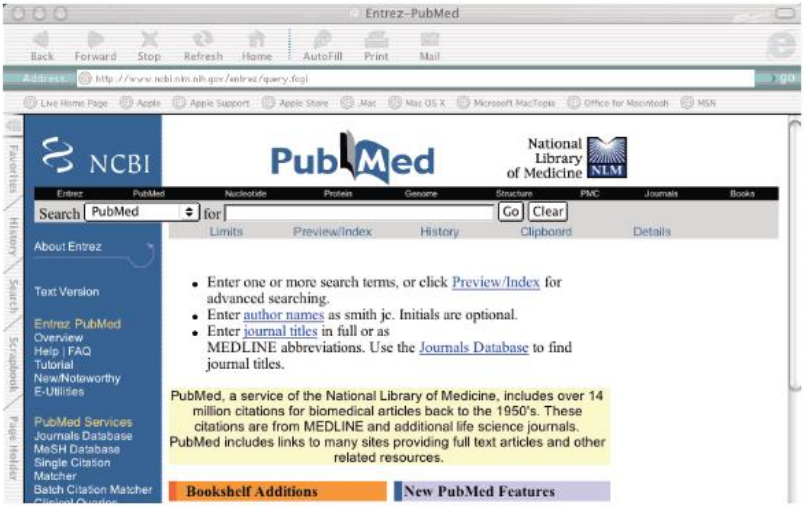


Figure 802 The initial PubMed search window

Then type in a name, for example ATPase which is the name of an enzyme type. Very rapidly a Results list is generated on the screen (see *Figure 803*).

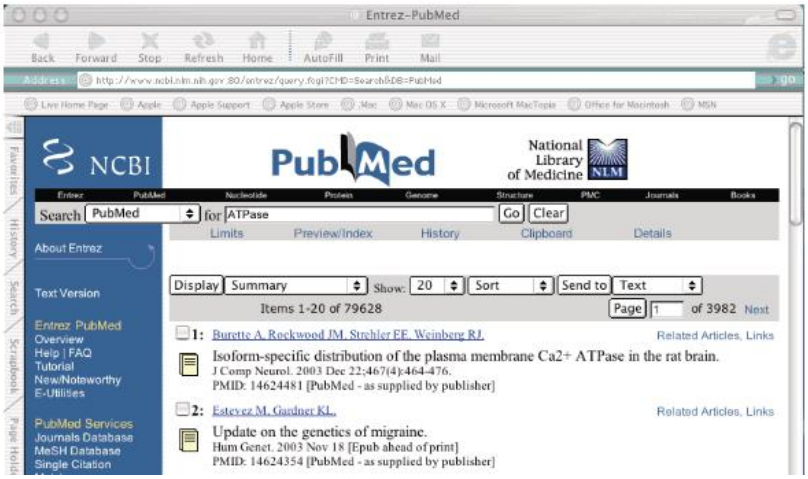


Figure 803 The initial result of a standard Pub Med search for ATPase

To move through the Results pages, simply click Next located in the top right of the screen.

The authors' names are clickable hyper links. Clicking on them will display a summary of the selected research paper (see *Figure 804*).

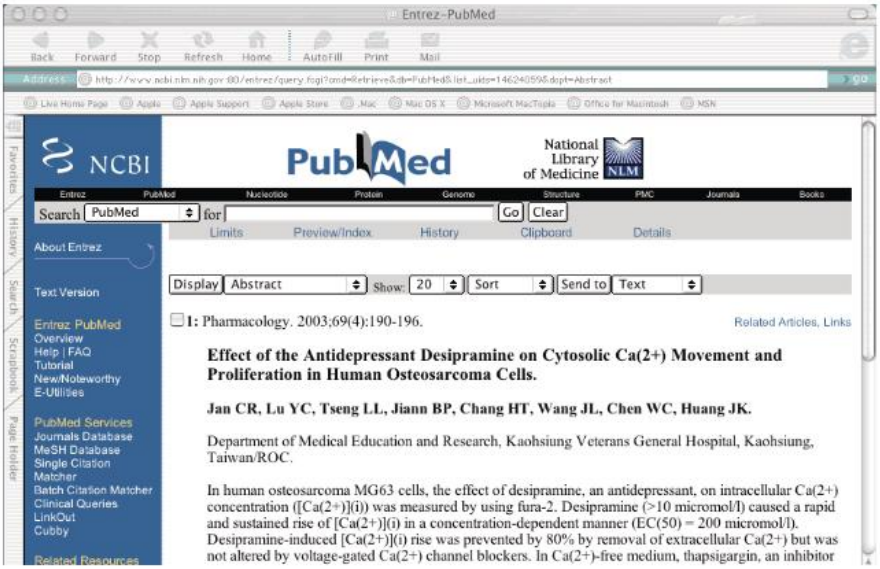


Figure 804 A summary of a research paper selected from the Results list

You can save the files on to your computer's hard drive by choosing your browser's File → Save As option.

You can also search Pub Med by using authors' names as shown in *Figure 805*.

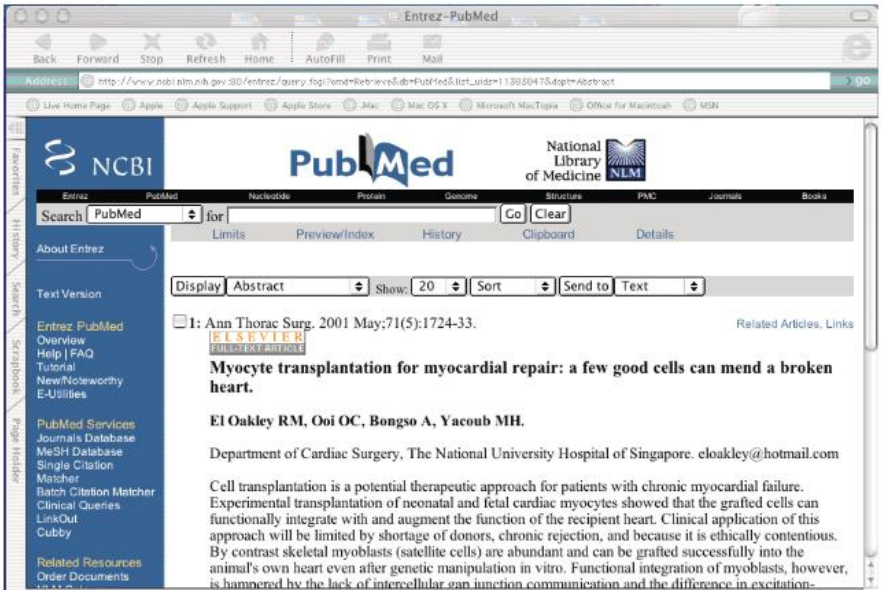


Figure 805 The results of a standard PubMed search for 'Bongso'

You can narrow your search down to a geographical area, for example, typing in SARS [AB] Singapore [AD].

The [AB] field means that PubMed will only display abstracts, that is short summaries of papers and the [AD] field means that the address of the laboratory will be included (see *Figure 806*).

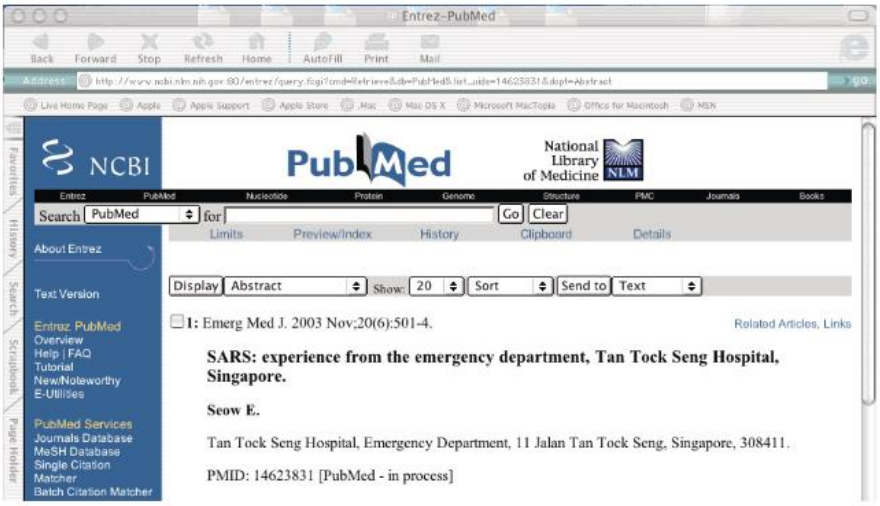


Figure 806 Searching for SARS researchers in Singapore

Queries to PubMed can be narrowed down by predefining different attributes in different fields before running your search. If you want to limit your PubMed search to recent review articles about the enzyme dUTPase then follow these steps:

- Type in *dUTPase* in the 'For' window of PubMed.
- Do not press the Go button, but click on *Limits* located just below the arrows of the pull-down menu of the search window.
- The limits search window appears.

Figure 807 shows how you can limit a search for *dUTPase* to recent review articles in English. Review articles are summaries of research findings from a number of research papers.

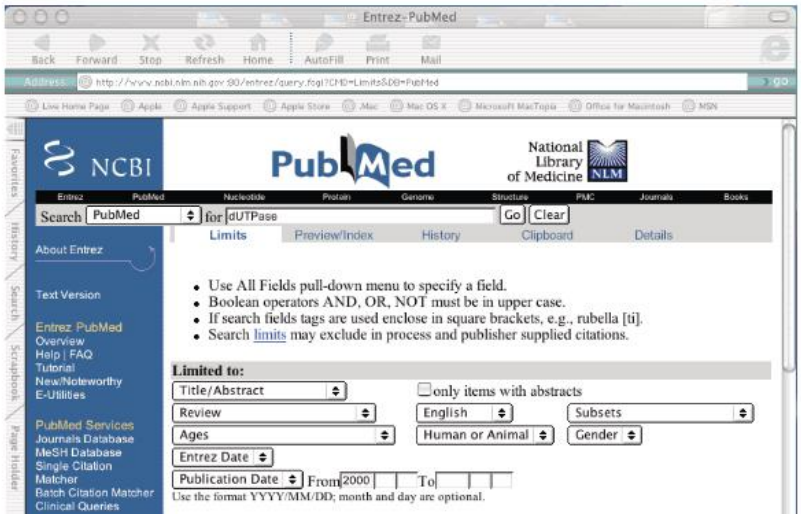


Figure 807 Limiting a search

8.4 Retrieving amino acid sequences of proteins

If you want to retrieve a protein sequence from the World Wide Web then you need to access the SWISS-PROT protein sequence database hosted by the Expasy server. You might want to examine and compare protein sequences if you are studying some of the Option Topics.

For example, if you want to retrieve the sequence of the ATPase enzyme in the bacterium *Escherichia coli* (abbreviated to *E. Coli*). Use your browser to navigate to <*http://tw.expasy.org/sprot/*> on the Internet (see *Figure 808*).

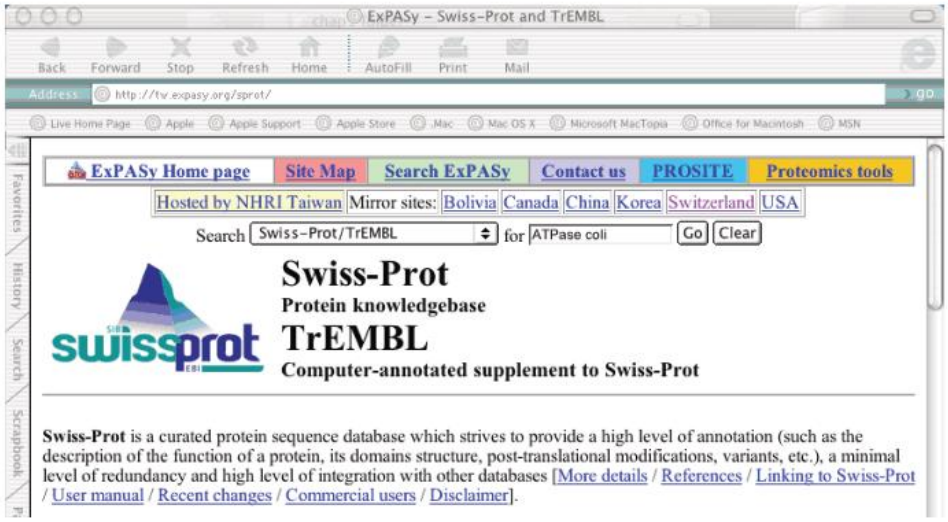


Figure 808 The Search Window at the top of the SWISS-PROT home page.

Type '*ATPase coli*' in the Search Window (see *Figure 809*) and then press Go.

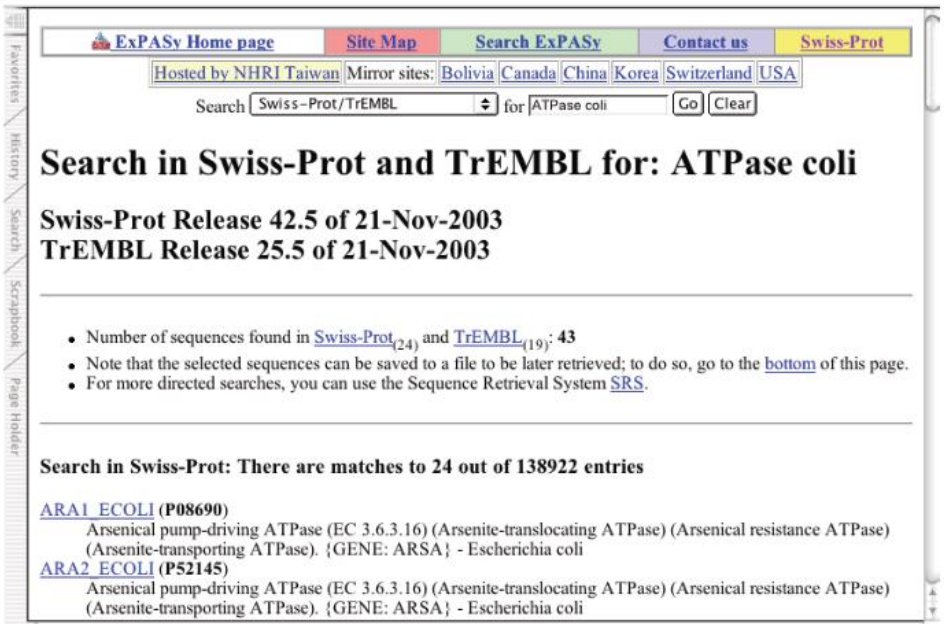
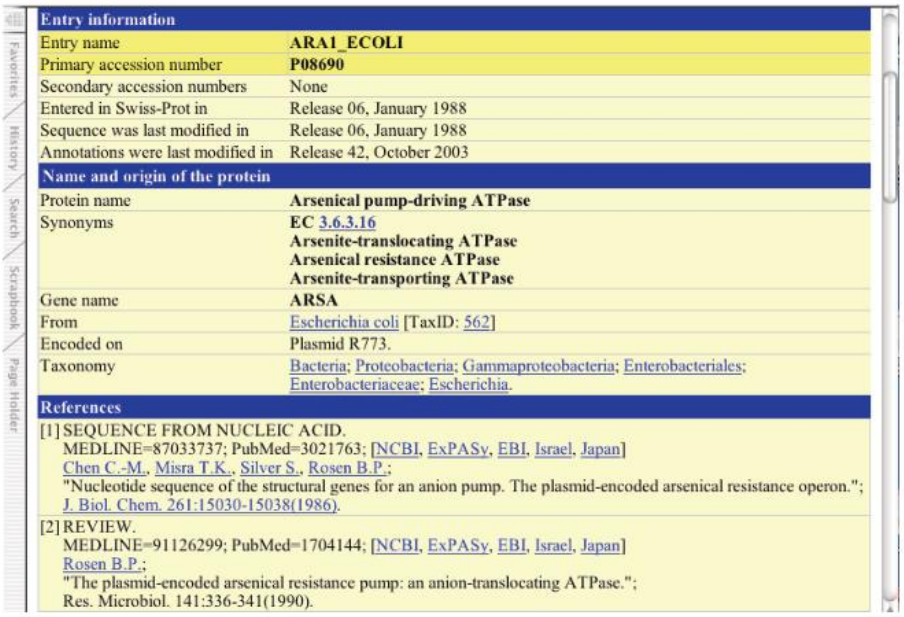


Figure 809 Part of the search results for the E. coli ATPase protein.

Select the first match and double click to display the SWISS-PROT entry. The first part of the section is displayed in *Figure 1009*

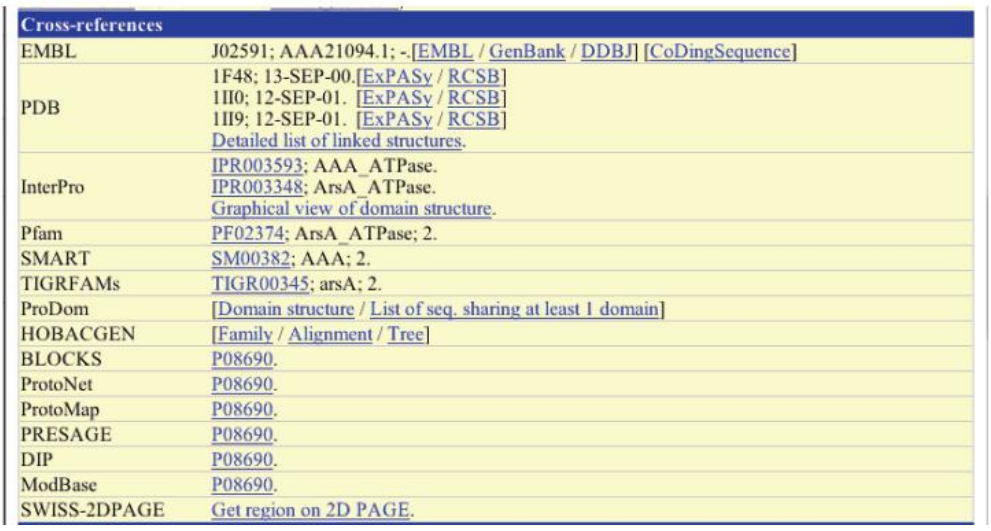


Entry information	
Entry name	**ARA1_ECOLI**
Primary accession number	**P08690**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 06, January 1988
Sequence was last modified in	Release 06, January 1988
Annotations were last modified in	Release 42, October 2003
Name and origin of the protein	
Protein name	**Arsenical pump-driving ATPase**
Synonyms	**EC** 3.6.3.16 **Arsenite-translocating ATPase** **Arsenical resistance ATPase** **Arsenite-transporting ATPase**
Gene name	**ARSA**
From	Escherichia coli [TaxID: 562]
Encoded on	Plasmid R773.
Taxonomy	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia.
References	
[1] SEQUENCE FROM NUCLEIC ACID. MEDLINE=87033737; PubMed=3021763; [NCBI, ExPASy, EBI, Israel, Japan] Chen C.-M., Misra T.K., Silver S., Rosen B.P.; "Nucleotide sequence of the structural genes for an anion pump. The plasmid-encoded arsenical resistance operon."; J. Biol. Chem. 261:15030-15038(1986).	
[2] REVIEW. MEDLINE=91126299; PubMed=1704144; [NCBI, ExPASy, EBI, Israel, Japan] Rosen B.P.; "The plasmid-encoded arsenical resistance pump: an anion-translocating ATPase."; Res. Microbiol. 141:336-341(1990).	

Figure 810 First part of the SWISS-PROT entry for the E. coli ATPase

- The top of the entry (*Figure 810*) contains the entry name ARA1_ECOLI on the first line and a unique identifier P08690 (known as the primary accession number). They are used to cross reference related entries in other databases.
- The middle section (*Figure 811*) has a biochemical description of the enzyme, its International Enzyme Committee (EC) number, as well as some synonyms: equivalent names.
- The bottom section has a list of bibliographical references related to the sequence of the protein or studies of how the enzyme works (so-called functional studies).

The middle section has a number of links to various functional classification schemes.



Cross-references	
EMBL	J02591; AAA21094.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PDB	1F48; 13-SEP-00.[ExPASy / RCSB] 1II0; 12-SEP-01. [ExPASy / RCSB] 1II9; 12-SEP-01. [ExPASy / RCSB] Detailed list of linked structures.
InterPro	IPR003593; AAA_ATPase. IPR003348; ArsA_ATPase. Graphical view of domain structure.
Pfam	PF02374; ArsA_ATPase; 2.
SMART	SM00382; AAA; 2.
TIGRFAMs	TIGR00345; arsA; 2.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
HOBACGEN	[Family / Alignment / Tree]
BLOCKS	P08690.
ProtoNet	P08690.
ProtoMap	P08690.
PRESAGE	P08690.
DIP	P08690.
ModBase	P08690.
SWISS-2DPAGE	Get region on 2D PAGE.

Figure 811 Second part of the SWISS-PROT entry for the E. coli ATPase.

The last section (*Figures 812 and 813*) displays the amino acid sequence of the enzyme.

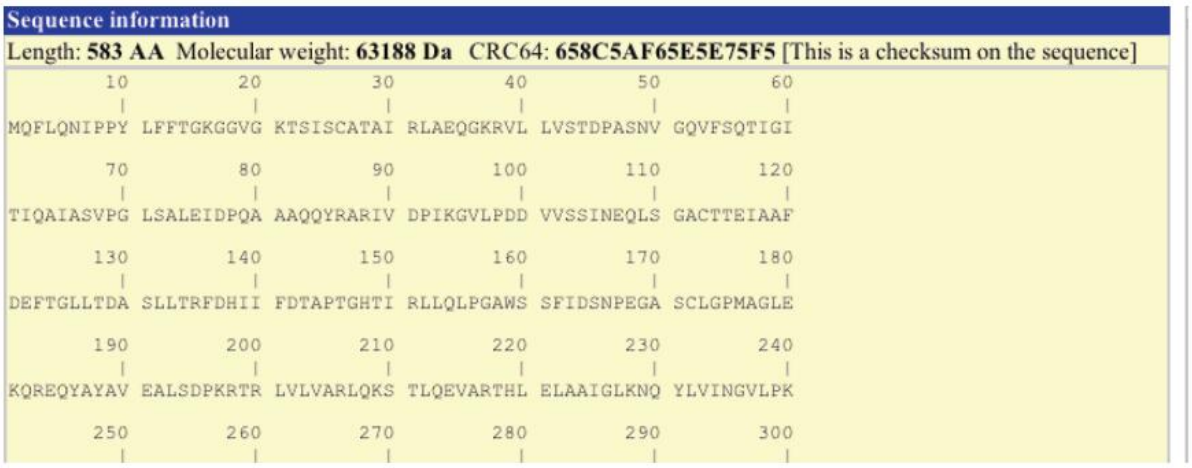


Figure 812 Top part of the amino acid sequence of the E. Coli ATPase enzyme

You can see that *E. coli* ATPase is composed of 583 amino acid (AA) residues with a relative molecular mass of 63188 Daltons (Da). The sequence shown here uses the one-letter code format for describing amino acids.

However, if you want to analyse this sequence then you need to obtain the amino-acid sequence in FASTA format. To obtain the FASTA format, click the FASTA format button, on the extreme right of the very bottom of the entry, see *Figure 814*.

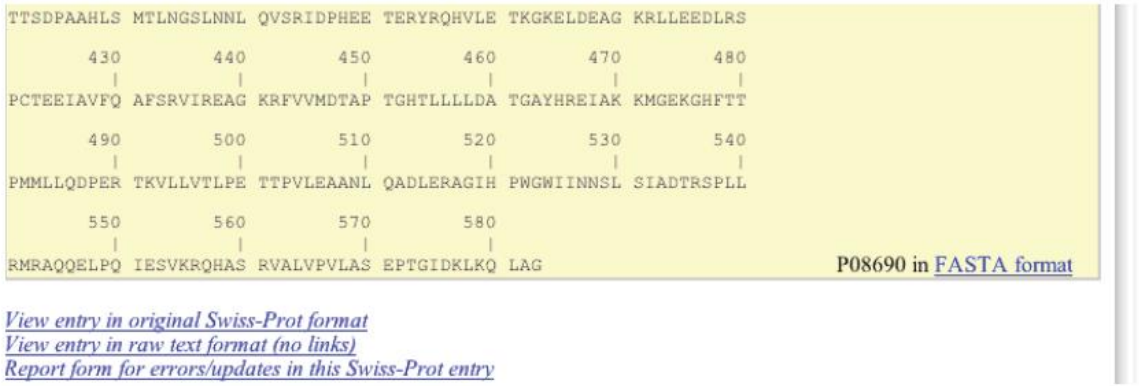


Figure 813 Bottom part of the amino acid sequence of the E. Coli ATPase enzyme.

>sp|P08690|ARA1_ECOLI Arsenical pump-driving ATPase (EC 3.6.3.16) (Arsenite-translocating ATPase) (Arsenical resistance ATPase) (Arsenite-transporting ATPase) – Escherichia coli.

MQFLQNIPPYLFFTGKGGVGKTSISCATAIRLAEQGKRVLLVSTDPASNVGQVFSQTIGITIQAIASVPGLSALE
IDPQAAAQQYRARIVDPIKGVLPDDVVSSINEQLSGACTTEIAAFDEFTGLLTDASLLTRFDHIIFDTAPTGHTI
RLLQLPGAWSSFIDSNPEGASCLGPMAGLEKQREQYAYAVEALSDPKRTRLVLVARLQKSTLQEVARTHLELAAI
GLKNQYLVINGVLPKTEAANDTLAAAIWEREQEALANLPADLAGLPTDTLFLQPVNMVGVSALSRLLSTQPVASP
SSDEYLQQRPDIPSLSALVDDIARNEHGLIMLMGKGGVGKTTMAAAIAVRLADMGFDVHLTTSDPAAHLSMTLNG
SLNNLQVSRIDPHEETERYRQHVLETKGKELDEAGKRLLEEDLRSPCTEEIAVFQAFSRVIREAGKRFVVMDTAP
TGHTLLLLDATGAYHREIAKKMGEKGHFTTPMMLLQDPERTKVLLVTLPETTPVLEAANLQADLERAGIHPWGWI
INNSLSIADTRSPLLRMRAQQELPQIESVKRQHASRVALVPVLASEPTGIDKLKQLAG

Figure 814 The amino acid sequence in FASTA format of the E. Coli ATPase enzyme.

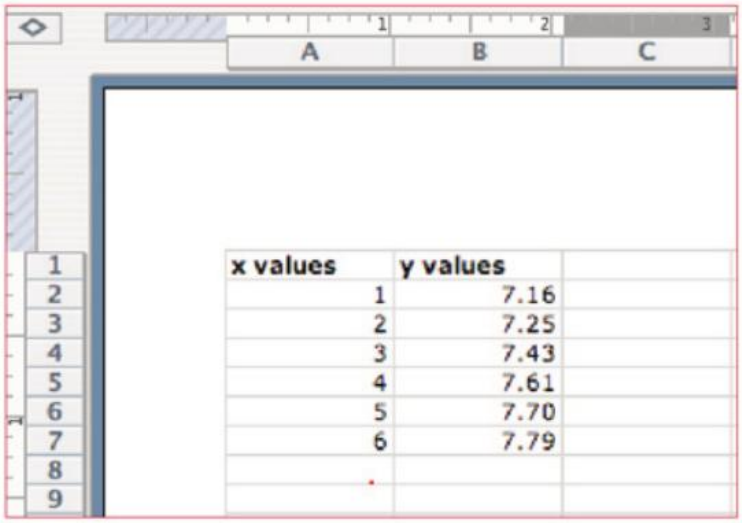
To save the FASTA file (with Internet Explorer) choose Edit → Select All and then choose Copy. Open Microsoft Word, choose Edit → Paste and then reformat the whole document with a Courier font (size 8 or 10) to realign the sequence. Save your file with a suitable name in text only (.txt).

8.5 Spreadsheets

A spreadsheet is the most commonly available software application suitable for analyzing and displaying of data. The data may be added manually via the keyboard or imported from files. One of the most important uses of a spreadsheet is that it allows its data to be analysed graphically. Two or more sets of corresponding data can be plotted as histograms or as simple scatter graphs.

8.5.1 Using Excel to Present Processed Data

The tutorial below with associated screenshots show how to plot a line graph with error bars and perform a least squares regression analysis using Excel. The least squares approach allows you to draw a line of best fit through experimental data. On the spreadsheet enter the name of your x–variable into cell A1 and the name of your y–variable into cell B1. Enter your x–values into the remaining cells of column A and the corresponding y–values into column B (*See Figure 815*).



	A	B	C
1	x values	y values	
2	1	7.16	
3	2	7.25	
4	3	7.43	
5	4	7.61	
6	5	7.70	
7	6	7.79	
8			
9			

Figure 815 Six pairs of x and y values in Excel

Highlight both columns of numbers with the mouse. From the Insert menu, select Chart. In the first window of the Chart Wizard, select XY(Scatter) as your chart type, and compares pairs of values (the first box in the first column) as your chart sub-type. Click on Next. In the second window of the Chart Wizard, in the Data Range screen, ensure the series in Columns is selected and then click on Next. In the third window of the Chart Wizard, type in a suitable title for your graph. Type in labels for your x and y axes, and remember to include units using the solidus notation. It may be helpful to click on major x and y gridlines. Click on Next. In the fourth window of the Chart Wizard, select As New Sheet. Click on Finish. A graph should appear on a new sheet in your work book.

To perform least squares analysis click on the line itself so that the points of the line become highlighted. Pull down the Chart menu and select Add Trendline. In the Type window, select Linear as your trend/regression type. Click on the Options tab at the top of the window, and select Display equation on chart and Display r-squared value on chart in the Options window. Click on OK. A straight line and an equation in the form and a value should appear (*Figure 816*) '*r*' is the correlation coefficient of your least squares determined line. The closer is to 1, the better the fit of your data points to a straight line.

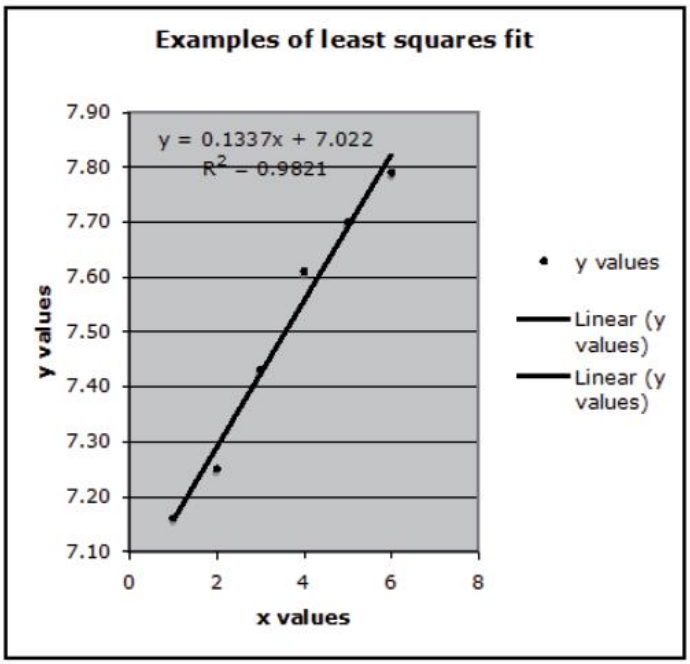


Figure 816 An example of an Excel generated least squares fit

8.5.2 Plotting a Graph with Error Bars using Excel

To plot a graph with error bars follow these steps: Enter the data and highlight both columns with the mouse. From the Insert menu, select Chart … In the first window of the Chart Wizard, select XY(Scatter) as your chart type, and data points not connected by lines (the first box in the first column) as your chart sub-type. Click on Next. To add the error bars, highlight all the data points by clicking. From the Format menu, click Selected data series and then select the Y error bars tab and enter the appropriate value for the uncertainty.

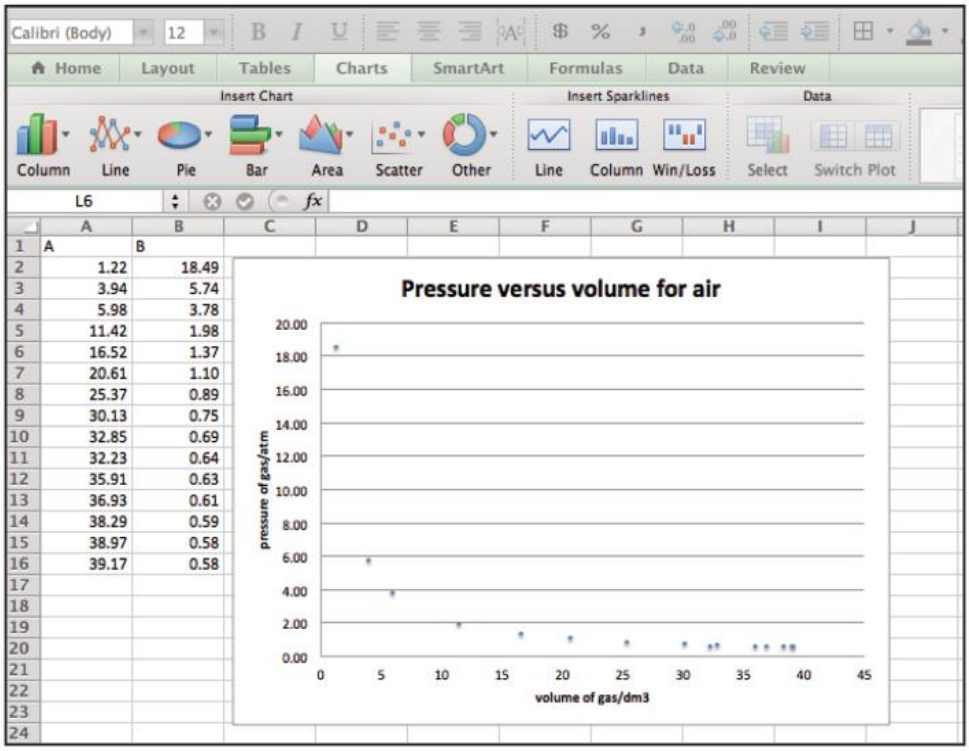
8.6 Using Excel functions

8.6.1 Plotting graphs

Graphing data on Excel

Type in your data, with your X axis data (independent variable) in the left-hand column, and your Y axis data (dependent variable) in the right column. Highlight your data (*Figure 811*). To the data is displayed to two decimal places Place your cursor over one of the data cells. Control click. In the drop-down menu that appears, choose "Format Cells." "Number" tab, and choose "Number" in the scroll-down menu. Type in "2".

With data still highlighted, choose the Chart Wizard icon in the menu bar (looks like a bar graph). Choose "Scatterplot" as your type of graph, and choose the version that has no line on it. Click At Step 2 (Source Data), click "Next." On Step 3 (Chart Options), give your graph a suitable title (your dependent variable versus your independent variable), and label your X and Y axes, making sure you include suitable units of measurement. Click "next," and on Step 4 (Chart Location), save graph as a "New Sheet." Change the name from "Chart 1" to a title that describes your graph (*Figure 812*). Click on "Finish."



	A	B
1	A	B
2	1.22	18.49
3	3.94	5.74
4	5.98	3.78
5	11.42	1.98
6	16.52	1.37
7	20.61	1.10
8	25.37	0.89
9	30.13	0.75
10	32.85	0.69
11	32.23	0.64
12	35.91	0.63
13	36.93	0.61
14	38.29	0.59
15	38.97	0.58
16	39.17	0.58

Figure 812 Gas law data plotted

8.6.2 Formatting graphs

To add a trend line and equation put your cursor directly over a data point, so that the point coordinates pop up. Control-click on this point and choose "Add Trendline" from the drop-down. For Trendline Type, choose the type of line that appears to best match the pattern your points make. This may be trial-and-error – you may have to do this more than once to find the best-fitting, but in this example a power trend line is appropriate. Click on the "Options" tab, and click on the "Display equation on chart" and "Display R-squared value on chart" at the bottom of the spreadsheet (*Figure 813*).

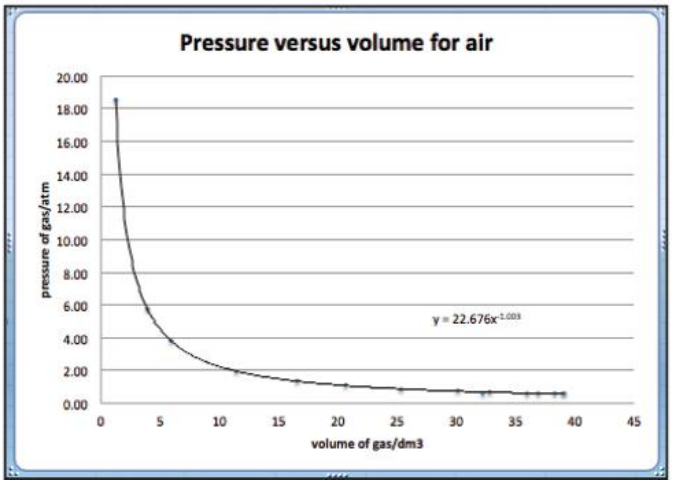


Figure 813 Gas law data plotted with power trendline

If you need to read X or Y values that are off the axes of your graph, you can forecast the trendline on your graph forwards or backwards to reach those values. Control-click on your trendline and choose "Format Trendline." Then, under "Options," you can forecast forwards or backwards however many units are needed. Then click "OK."

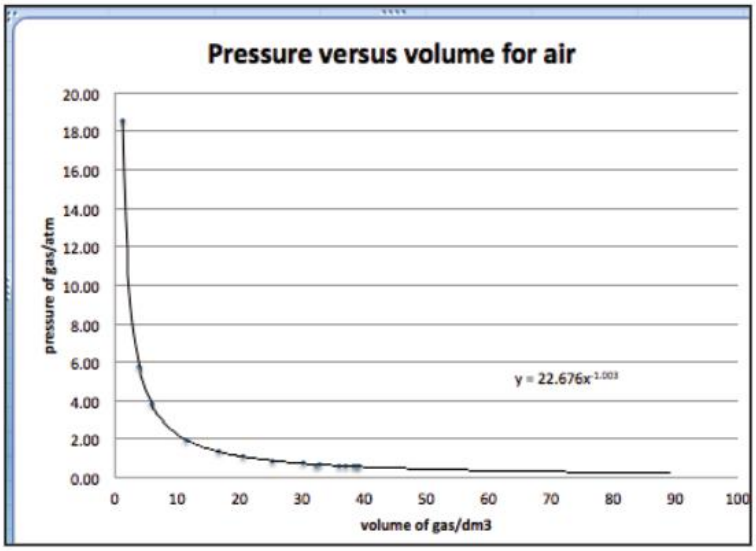


Figure 814 Gas law data with a forward forecast of 50 units

You can change the numbering system on your axes and add extra tick marks to your axes by formatting each axis. Put your cursor on the axis you want to format and Control-click, and choose "Format axis." See *Figure 814*.

You can choose to add additional tick marks to your scale by clicking on the "Colors and Lines" tab and clicking on the "Minor tick mark type" button of your choice. You can also choose the manner in which the tick marks are labeled. You can change the way the axes are labeled by choosing the "Scale" tab.

8.7 Using Microsoft Word functions

8.7.1 Formatting tables

To insert a table using this method, simply click the Insert Table toolbar button when your cursor is positioned at the place in your document where you would like the table to begin. A grid will pop up allowing you to select how many rows and columns you would like your table to contain. Simply use your mouse to select the number of rows and columns by highlighting the boxes (text at the bottom of the grid will indicate what your selection is). When you have specified the correct number of rows and columns, simply click once, and your table will be inserted.

You can still customize your table after it is inserted by right-clicking on the table handle (the double-headed arrow at the top left corner of the table) and using the options on the shortcut menu to make changes.

Group	Ultra violet intensity/lux	5 days	10 days
Control	10	70.3 ± 2	90 ± 10.5
Test	10	60.4 ± 1.5	78 ± 7.9
Control	17	75.7 ± 8	100 ± 23
Test	17	52.2 ± 2	81 ± 26.7

Figure 808 Concentration of chemical X in sample after treatment

Look at the results in *Figure 808*. Two columns of data have been placed in the same cell, with the data arranged using the space bar.

Tables should be created with the correct number of rows and columns. You can also add new rows and columns to an existing table by right clicking on the table, selecting 'Insert' and choosing to insert new rows and columns above or below the existing rows or columns.

It is also possible to insert multiple rows/columns to a table by highlighting the number of rows/columns you require on existing rows/columns. For example, if you would like to add three columns to the left of your table, highlight the first three columns, right click and choose "Insert Columns to the Left".

8.7.2 Inserting symbols

When writing the report for your Individual Investigation you may need to use a number of special symbols from Microsoft Word. They can be accessed by selecting the Insert pull down menu and then choosing Symbol. Click on the symbol you want, then on 'Insert' and 'Close.'

8.7.3 Inserting charts and graphs

It is very easy to insert charts and graphs from Excel into Word. Select the Excel Spreadsheet so that it is highlighted and copy it by clicking "Ctrl+C." Mac users click "Cmd+C." In the Word document, click where you want the chart to appear. Paste the spreadsheet into the document by holding down the Ctrl key and hitting "V." On a Mac OS, click "Cmd+V."

With your cursor next to the data, click "Paste Options." To input the spreadsheet as a Word table, click "Keep Source Formatting." The chart will look like it did in Excel. Click "Match Destination Table Style" if you want the new graph to look like others you are using in the document.

8.7.4 Creating short cuts

You will probably have to write cm^3 a number or times for your reports. You type in cm^3 and then highlight the three and change into a superscript by selecting Format, Font and then Superscript. This is quite a long process and can be avoided by using a short cut:

Type cm^3 and format it to cm^3. Highlight it and choose Tools and AutoCorrect options. cm^3 will be present in the 'with box'. Type cm^3 in the 'Replace box' and select the 'Formatted text' circle. The cm^3 in the 'with' box will change to cm^3. Click 'Add' and 'OK'. Now when you type cm3 it will be automatically changed to cm^3. The short cut can be easily deleted.

8.7.5 The Equation Editor

If you want to include an equation in your report that includes division then you need to use the Equation Editor (*Figure 810*). Select the Insert pull down menu and then choose Object followed by Microsoft equation.

A special toolbar will also appear on your screen. Use the toolbar to select symbols, brackets, etc. to place in the box. You may also type numbers and letters into this box. At the top of the screen, a simplified toolbar lets you select font size (including subscript and sub-subscript), style, and alignment.

Figure 810 A screenshot of the Microsoft Equation Editor

Here is an example of a physical chemistry equation (Graham's law) written and then inserted using the Microsoft

Equation Editor: $\frac{rate\,1}{rate\,2} = \sqrt{\frac{molar\,mass\,1}{molar\,mass\,2}}$.

For ten hours of your IB Biology course you are required to participate in a Group 4 Project. The intention of the Group 4 Project is that students from all the different Group 4 subjects (Chemistry, Physics, Biology and Environmental Systems and Societies) work together to investigate or solve a common problem (see *Figure 701*). It is a compulsory component of the IB Diploma Programme and is usually completed in the first year of the IB Biology Programme.

This aim is to provide all students with the opportunity to appreciate both the implications of using science and the limitations of scientific study, in a local context. The main philosophy of the Group 4 Project is to emphasise interdisciplinary co-operation and the processes involved in a scientific investigation rather than the products of such an investigation.

Schools differ in the arrangements they make for the Group 4 Project but essentially it is separated into three distinct phases: planning, action and evaluation.

9.1 Planning

TOPIC/THEME/ CONTEXT	CHEMISTRY	PHYSICS	BIOLOGY
SCHOOL SWIMMING POOL	Determination of chlorine content via redox titration; study of the decomposition of chlorine water in the presence of sunlight; study of chemicals used to chlorinate pool water; relationship between free chlorine and pH; effect of urine in pool water.	Determination of physical properties of chlorinated water, e.g., density, specific heat capacity, refractive index; surface tension, boiling point, melting point; change in pressure with depth, heat loss due to evaporation; measurement of heat gain during the day, heat loss at night.	Culturing of bacteria, algae (effect on pH) and fungi; effect of changes in chloric(I) acid concentration and pH on microorganism growth; pathogens present in polluted pool water from faeces; the diver's 'bends'.
KEEPING COOL	Determination of the amount of sodium chloride and urea in sweat; investigation of the reactions in cool packs. Study of chemicals used in air conditioners: CFC, HFC, impact on global warming.	Investigating the ability of different surfaces and colours in reflecting solar energy; studying the design and effectiveness of a cool box, air conditioner and thermos flask.	Investigation of the changes that occur in the souring of milk or red wine; measuring the transpiration rate under different environmental conditions.

Figure 901 Some ideas for Group 4 Projects

9.2 Action

About six hours is devoted to the action stage. You can investigate your topic or theme either in mixed subject groups or in a Biology group. Some schools spread this time out over one or two weeks, others complete it in a day or weekend. Remember the aim is on the process not the product, in other words, you might not obtain reliable results or they may not prove amenable to analysis. You may plan to do experimental work but it is perfectly permissible and sometimes more efficient to get your data from elsewhere. If you do perform your own practical work then you must pay attention to safety, ethical and environmental considerations. If you are studying two science subjects for your IB Diploma then you do not need to carry out the action stage of the Group 4 Project twice.

9.3 Evaluation

The time allotted to this is usually about two hours. The emphasis during this stage is on sharing your findings, both successes and failures, with other IB Diploma students. There are many ways in which you might do this. Some schools devote a morning, afternoon or evening to a symposium where all students, as individuals or as groups, give brief presentations, perhaps involving PowerPoint presentations. Your school may invite parents. Others take the form of a science fair where students circulate around posters summarising the results of each group or all the students in the school could contribute their findings to a specially designed webpage on the school's Intranet.

9.4 Self motivation

Your self motivation may be assessed by your Teacher. Listed below are some ways by which you can demonstrate your self motivation and perseverance.

9.4.1 Planning Stage

- arriving to meetings or brainstorming sessions on time.
- coming to meetings or brainstorming sessions prepared. Once you have discovered the general theme of your Group 4 project you are expected to conduct some research about the topic.
- contributing positively to the brainstorming session. Be supportive of ideas.
- staying focused during the meeting. When you are not speaking, be an active listener. Take down notes if you hear something important.
- submitting the planning sheet on time (if applicable).

9.4.2 Action Stage

- Ensuring that the apparatus chosen and methodology for the project will give precise and reliable results.
- Presenting a creative approach to the problem.
- Making sure that you know and understand the methodology to be performed during the investigation. This includes being able to identify the data to be collected and how these data are to be collected.
- Finding and suggesting ways to solve the problem if the team is encountering difficulties with the project.
- Adapting to new circumstances. If an unexpected result is obtained, then you must try to make sense of this result or perform additional measurements to verify the reliability of this result.

9.4.3 Evaluation

- Approaching the Project with integrity. This includes acknowledging resources that were used and not altering the data to fit a preconceived **hypothesis**.
- Presenting or contributing positively towards an effective method to present your findings, successes and failures to other teams.
- Being available to other teams to help them better understand your Group 4 Project.

9.5 Working within a team

You may be assessed on how well you work as part of a team during the Group 4 Project. A good team will work more effectively than individuals.

9.5.1 Planning Stage

During the Planning Stage of the Group 4 Project, your ability to work with a team can be demonstrated by:

- Arriving to the meetings and planning sessions on time. This suggests that you value the time of your team mates.
- Responding to a different idea in a supportive fashion. Look at the strength of the idea first before stating its perceived weaknesses. This shows you value your team mates' ideas and this can encourage further exchange of ideas.
- Readily sharing ideas and asking questions.
- Listening to and not interrupting the speaker.
- Accepting and following the collective decision of the team even if you do not agree with it.
- Taking the initiative to contact other members of the team to update yourself should you miss a planning session.

9.5.2 Action stage

During the Action Stage of the Group 4 Project, your ability to work with a team can be demonstrated by:

- Leading by example.
- Completing the task assigned to you to the best of your ability and, if necessary, seeking assistance from other members of the team.
- Readily assisting other members of the team to the best of your ability.
- Acting responsibly and not performing any action that will place the other members of the team or the other teams in unnecessary danger. This includes seeking assistance from your IB teacher in matters involving a breach of safety.

Due to some personal differences, it possible that your team or a few members of the team do not work well together. This will prevent your team from completing the Group 4 project well. It is suggested that you take time to discuss the problem as a team. During the discussion, it is important that the team stick to the issues and avoid personal attacks. Everyone should be courteous, respectful of other people's opinions, and open to other people's ideas. If a problem concerns one or two individuals only, then someone can act as a moderator and discuss the issues privately with this individual(s).

As much as possible, the team must resolve the issues amongst themselves quickly. Discussing the problem with your IB teacher should be the last resort. Also, requesting to be transferred to another group suggests poor collaborative skills on your part.

9.5.3 Evaluation Stage

During the Evaluation stage of the Group 4 Project, your ability to work with a team can be demonstrated by:

- recognizing the strengths of the team and of the individual members of the team. This can be done by contributing positively to the presentation of the team's successes.
- Suggesting ways and methods by which the weaknesses of the team is eliminated or reduced. This includes proposing ways which will prevent the same mistakes being committed again.

9.6 Self-reflection

You might be asked to reflect and write a self evaluation report. If this is the case, below are suggested questions that can help you conduct a thorough self-reflection.

9.6.1 Planning Stage

- Did you contribute positively towards the planning of the investigation? If yes, what preparations did you do? Could you have contributed more? What prevented you from doing so? How can you overcome these obstacles?
- If you did not contribute positively, what prevented you from doing so? How can you overcome these obstacles next time?
- Were you able to accept differing ideas easily? Did you attempt to look at both the strengths and weaknesses of a proposal? Did you analyse the proposal objectively? If not, what prevented you from doing so? How can you overcome these obstacles?

9.6.2 Action stage

- Did you have the necessary skills to conduct the investigation efficiently and safely? Which skills were you good at? Which skills do you need to improve on? What do you need to do to improve these skills?
- Did your actions help promote effective collaboration amongst the different members of the team? What could you have done better for the team to improve collaboration amongst the members?
- What went well for the team? Why did the team encounter such difficulties? What could you have done better for the team to help it complete the investigation effectively?
- Did you help the team manage the time effectively?

9.6.3 Evaluation

- What insight(s) did you gain from the conclusions obtained?
- Was the team able to present its findings accurately and clearly? If yes, how was team able to do it? If not, what prevented the team from doing so?
- Did you contribute positively towards the team's presentation? What could you have done better to help the team present its findings more accurately and clearly?

A sample of an Individual Investigation Report

An outline or beginnings of a relatively simple open-ended experimental Investigation 'write-up' or report is reproduced to illustrate the standards of work expected from IB Biology students. The investigation begins with the brief written instructions presented to the student, the context of the investigation followed by an edited student 'write-up' (using actual student results and Excel drawn graphs). Common student mistakes or omissions are also highlighted. You will have less guidance than this when you carry put your Individual Investigation. However, the write-up and comments are a useful guide to the standard expected.

Investigation

Investigate the activity of an enzyme in the presence of metal ions. You will be assessed on your abilities to design, collect data and present and process data and conclude and evaluate.

Context

(The Higher Level Biology students had previously studied enzymes and had performed some simple illustrative experiments on the breakdown of starch solution using aqueous iodine (to detect the complete hydrolysis of starch) and saliva as a source of amylase. They were also familiar with the concept of inhibition and the observation that a number of metal ions acts as inhibitors or activators of enzymes.)

Research Question

To find the effect of changing the concentration of aqueous copper(II) ions on the rate (as the reciprocal of time) of hydrolysis of starch to maltose by amylase (as measured by the time for the blue-black starch-iodine complex to be converted to brown iodine).

A clearly stated Research Question has been given by the student. A Research Question is required by the Group 4 Assessment Criteria. The relevant variables are also identified.

Hypothesis

It is predicted that the greater the concentration of aqueous copper(II) ions, the longer the reaction would take to go completion, until saturation and probably complete inhibition occurs. This is because an increasing degree of inhibition occurs as a greater proportion of binding sites of the amylase are involved. This is accounted for by the expectation that copper(II) ions will act as an inhibitor of amylase and decrease the rate of reaction. Many heavy metal ions act as enzyme inhibitors. The mechanism of inhibition is expected to occur as a result of an ionic interaction between the aqueous copper(II) ions and the amylase, possibly involving the functional groups of the active site or other binding site. This would prevent formation of enzyme-substrate complexes and hence catalysis.

A testable hypothesis, a prediction and a justification with references to Biological and Chemical concepts have been formulated independently by the student. An equally acceptable and testable hypothesis would have been for the student to suggest copper(II) ions would act as an activator of amylase and accelerate the rate. This would have been falsified by the data, thus allowing the student to conclude that the copper(II) ions act as an inhibitor.

Biological Background Knowledge

The enzyme amylase hydrolyses the α1-4 glycosidic bonds present within starch, a polysaccharide, to release maltose, a disaccharide. The reaction can be followed by the use of aqueous iodine: starch forms a deep blue-black complex with aqueous iodine solution but in the presence of maltose, the brown colour of iodine is observed. The starch-iodine complex is formed by iodine molecules being 'trapped' inside the helical starch molecule. This reaction is very sensitive and can detect very low concentrations of starch.

This section would have benefited from the inclusion of structural formulas to illustrate the structures of starch and maltose as polymers and dimers of glucose, respectively.

Exploration

I propose to prepare a number of solutions of aqueous copper(II) sulfate with different molarities or molar concentrations. Appropriate calculations involving the mole concept will ensure that the range of concentration of copper(II) ions is centered on the concentration of amylase solution.

Appropriate volumes of starch solution and amylase solution will be mixed with an appropriate volume of aqueous copper(II) sulfate solution and the reaction mixture will then be placed in a thermostatically controlled water bath.

Small samples of the reaction mixture will then be extracted at regular time intervals and tested with aqueous iodine. The time taken for the brown colour of iodine to be observed will be recorded. The reaction will then be repeated with a range of copper(II) sulfate molarities. A control of amylase and starch solutions, without aqueous copper(II) sulfate, will be employed. A 'trial and error' approach will be employed to establish the precise concentrations and molarities for the reaction to occur at a measurable rate.

A very brief outline plan has been prepared – it is similar to a previous practical performed by the students with two differences: saliva is not the source of amylase and aqueous copper(II) ions, a putative inhibitor, are present. It is important that evidence of forward planning is presented in your 'write-up' and that the research question and method have not been explicitly given to you by your IB Biology teacher.

Variables

The **dependent variable** is the concentration of aqueous copper(II) ions and the **independent variable** is the time required for the complete hydrolysis of starch (as indicated by the formation of free iodine).

The **controlled variables** include:

- the concentration of the amylase solution.
- the concentration of the starch solution.
- the concentration of the aqueous iodine solution.
- the temperature of the reaction mixture.
- the extent of stirring or mixing.

Although this section is perhaps implied in the plan above, it is preferable to have a section in your 'write-up' that explicitly focuses on outlining the nature of the control as well as identifying and classifying variables. This, again, is one of the critical aspects of the Group 4 assessment criteria for Exploration.

A frequent student mistake is to include volume as an additional variable for the three solutions: a change in volume has no effect on the rate of reaction since the collision rate is unchanged. One important variable omitted is pH or hydrogen ion molarity which typically has a significant effect on the rates of many enzyme reactions, often by causing a key functional group in amino acid residue to change its state of ionisation. Mixing is not usually regarded as a variable, it is assumed that the reaction mixture is homogeneous. However, with large volumes of solution, mixing should be carefully performed and quantified.

Safety Precautions

Consulting *Hazcards* reveal that copper(II) sulfate is harmful if swallowed and solutions greater than or equal to 1.00 mol dm^{-3} should be labeled as harmful. It is also irritating to the eyes and has been known to sensitise the skin. Safety glasses and disposable rubber gloves will be worn at all times. Small quantities of copper(II) salts may be disposed of by flushing down the drain with copious amounts of water.

You should consider and minimise the environmental impact of your investigation.

(*Hazcards are published by the CLEAPPS organisation based in the United Kingdom. Hazcards list which chemicals are prohibited by law in schools and those which are allowed in schools and the associated hazards. Sources of information in North America are the Materials Safety Data Sheets, MSDS. Equivalent safety publications should be available in your school laboratory.*)

Method

20.0 cm^3 of 2% by volume amylase solution and 20.0 cm^3 of 2% by volume starch solution were placed into separate glass boiling tubes by means of a 10 cm^3 plastic syringe and then placed into a water bath maintained at a temperature of 40 °C. Both tubes were left for ten minutes to equilibrate. The tubes of starch and amylase solutions were mixed. This tube was retained in the water bath and every minute a drop of the mixture was removed by means of a glass rod and placed with a drop of 0.01 mol dm^{-3} aqueous iodine in a spotting tile. The time was taken with an electronic stopwatch for a brown colour to be formed. This will act as a control. The method was repeated using 10.0 cm^3 volumes of 0.02, 0.04, 0.06, 0.08 and 0.10 mol dm^{-3} copper(II) sulfate solution.

Calculations showing how the copper(II) sulfate molarities were derived and justified from the amylase and starch concentrations have been omitted. An appendix at the end of the 'write-up' might be the most appropriate location for such information. Any apparatus should be described accurately, their use justified and their precision quoted. Full experimental details of how the amylase and starch solutions were prepared should be given.

Analysis

Qualitative results

All the samples of the reaction mixture initially turned blue-black, indicating the presence of starch. Then at different times each solution became light brown, indicating that all of the starch had been hydrolysed to maltose by amylase.

The recording of relevant qualitative results is frequently omitted by students but it is an important requirement of the Group 4 Assessment Criteria.

Quantitative Results

Data Table 1

AQUEOUS COPPER(II) SULFATE, $CuSO_4$ (aq)/mol dm^{-3}	MEAN TIME TAKEN FOR APPEARANCE OF BROWN COLOUR/minutes
0.00	19
0.02	23
0.04	25
0.06	27
0.08	29
0.10	32

Figure 1016 The effect of copper(II) sulfate concentration on the time required for complete hydrolysis of starch by amylase

It would have been preferable for the student to record the times in the SI units of time, namely, seconds. Students often misuse minutes and seconds as decimals.

The student has been consistent with the number of decimal places used to record the molarities of copper(II) sulfate, namely two, and significant figures used to record the times, namely, two.

However, the student has failed to display the raw data – a very serious omission. It is not acceptable to simply present the processed data, namely, the mean times.

The student should have included the implied uncertainties or errors in the headings associated with the molarities and times, namely, ± 0.005 mol dm^{-3} and ± 0.0005 s (given that electronic stop watches typically have a precision of 0.001 s). However, given the size of timing errors (due to the reaction times) the student is probably justified in recording his times with an uncertainty of 0.5 s. The first entry in the data table is the control.

Data Table 2

AQUEOUS COPPER(II) SULFATE $CuSO_4$ (aq)/mol dm^{-3}	RATE (1/time)/minutes^{-1}
0.00	0.052
0.02	0.043
0.04	0.04
0.06	0.037
0.08	0.034
0.10	0.031

Figure 1017 The effect of copper(II) sulfate concentration on the rate of starch hydrolysis by amylase

The third entry in the rate column should be presumably written 0.040 assuming the times from the previous data table were all recorded to the same degree of precision. A common mistake is to omit the leading zeros in the rate values. The rates are correctly presented to two significant figures since the times are only expressed to two significant figures. A common student mistake is to write down all the numbers displayed by an electronic calculator for the rate values.

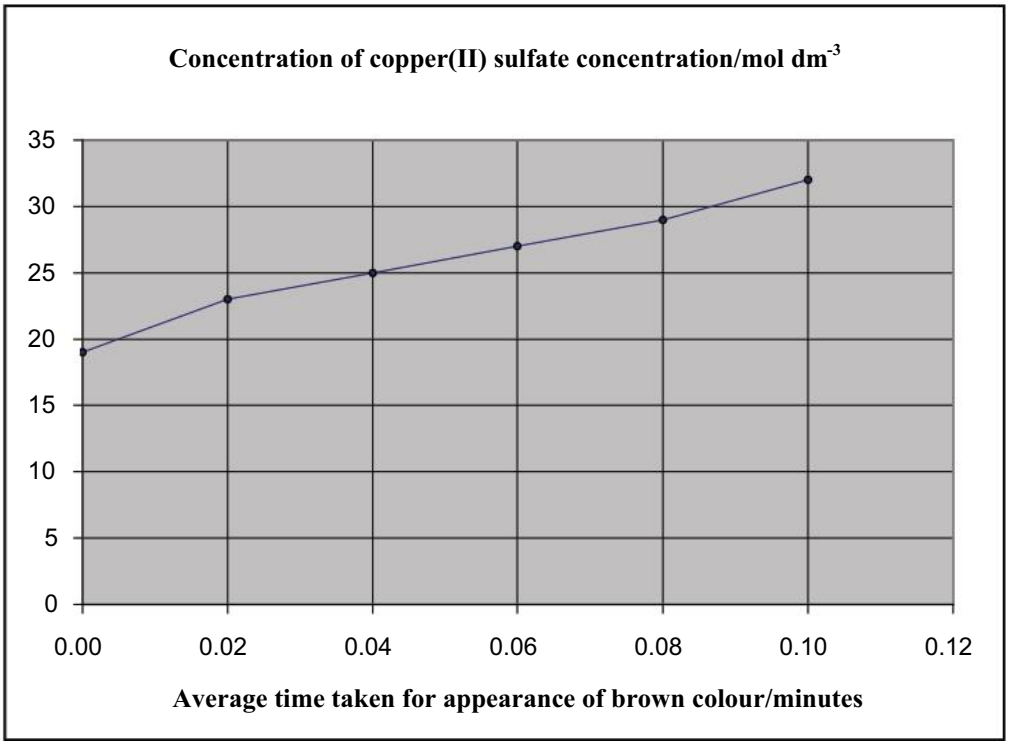


Figure 1018 The effect of concentration on reaction time

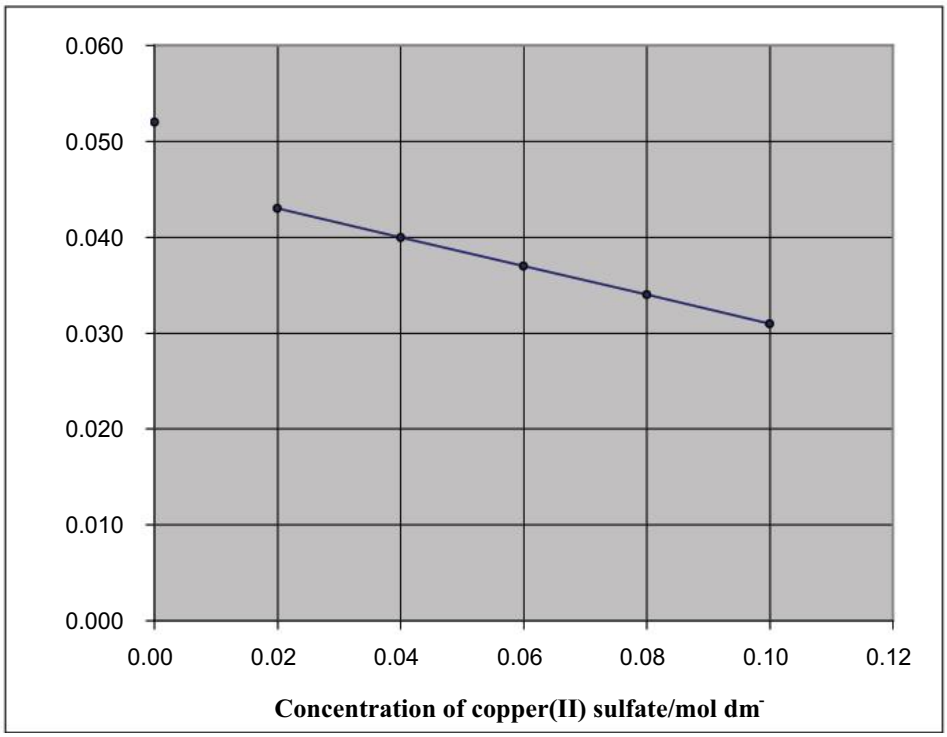


Figure 1019 The effect of concentration on starch hydrolysis

Conclusions

If *Figure 1018* is examined it can be seen that as the concentration of copper(II) sulfate solution increases, the time required for the starch to be completely hydrolysed by amylase increases. The graph indicates an approximate directly proportional relationship between the time taken for hydrolysis and the concentration of aqueous copper(II) ions. If *Figure 1019* is examined it can be seen that as the concentration of copper(II) sulfate concentration increases, the rate of reaction decreases. between the rate of hydrolysis and the molarity of aqueous copper(II) ions. These results are presumably due to the interaction between amylase and copper(II) ions decreasing the rate of formation of enzyme-substrate complexes.

Copper(II) ions actually reduce the concentration of enzyme-substrate complexes not the rate of their formation. That is, the ions affect the equilibria and not the rate of formation. Actual numbers should be quoted to support suggested relationships.

Evaluation

My results suggest that my hypothesis is verified, namely, that aqueous copper(II) ions act as an inhibitor of amylase and the rate of reaction decreases with increasing concentration of aqueous copper(II) ions.

However, there was one apparent **anomalous result**: the value for the control in *Figure 1018* did not lie on the straight line that passes through the other five data points. The value seems anomalously high. Time permitting, this trial should have been repeated and a mean result calculated.

The following sources of error are noted:

The 'end point' of the colour change involving the aqueous iodine was slightly difficult to judge. Hence, there is a random error inherent in all of the timing measurements, but this was reduced by repeating each experimental trial three times and calculating a mean time.

As pointed out previously, the raw data was not presented.

Random errors are inherent in the preparation of all the solutions by our school technician. These are derived from parallax errors when using glassware and weighing errors due to convection currents when using the electronic balance.

The use of a human operator in measuring times will lead to the introduction of a systematic timing error. This was minimised by practice.

None of the chemicals were 100% pure.

The solutions may not have been mixed and hence were not homogeneous.

The size of the drops of the reaction mixture on the glass rod will have varied slightly.

However, since the concentrations are constant this should not introduce a random error.

The following limitations are noted:

Time permitting, I could have made up further solutions of aqueous copper(II) sulfate to extend the range of concentrations as well as provide concentrations intermediate between those used.

The present set of results do not include a concentration of copper(II) sulfate sufficiently high to achieve complete inhibition of amylase.

Further repetition of each experiment (including the control) five times involving particular concentrations of copper(II) sulfate could also have been performed, as well as recording the colour for a shorter time interval than one minute.

This would have further reduced random errors and improved the accuracy of the mean times.

The investigation could have been extended to establish the nature of inhibition operating, namely, competitive or non-competitive.

The student should indicate how this could be achieved, namely, altering the concentration of the starch substrate.

The precision of the method could have been improved by the use of a colorimeter to measure the intensities of the colours formed by measuring the amount of light absorbed.

The investigation could have been widened to include the effect of other transition metal ions, for example, aqueous zinc and iron(II) and iron(III) ions, as well as other variables, such as pH and temperature of the reaction mixture.

Purer chemicals could have been employed and more thorough and rapid mixing could have been achieved by using an ultrasound bath.

The pH was not controlled, copper(II) sulfate is slightly acidic. A suitable buffer should have been employed.

Absolute error

An absolute error is an error expressed in physical units.

Accuracy

A measure of the total error in your measured value. The accuracy of a measurement depends on the experimental techniques and equipment used. Accuracy can be improved by removing or minimising error.

Anomalous data

Data with unexpected values that does not match the relationship predicted by the hypothesis. Anomalous results can be due to experimental error.

Calibration

Standardisation of the measurement scale of an instrument or apparatus.

Chi-squared test

A statistical test used for comparing expected and observed frequencies, or for comparing observed frequencies with theoretically predicted values.

Conclusion

A conclusion is a concise interpretation of experimental data. The conclusion should, if possible, show whether the data supports or rejects the hypothesis.

Control

A standard of comparison for checking the results of an experiment.

Controlled variable

Controlled variables are the variables which are controlled and not allowed to vary during the investigation.

Dependent variable

It is the variable that is measured during an investigation.

Error

An error in a measurement is the difference between the measured value and the true value.

Evaluation

This involves the consideration of all errors, random and systematic, which may affect the results, identifying weakness and limitations in the method, calculating the total error present in the results and explaining how the errors can be minimised.

Extrapolation

To estimate (a value of a variable outside a known range) from values within a known range by assuming that the estimated value follows logically from the known values.

Fair test

A test in which one variable is manipulated or changed.

Hypothesis

A tentative explanation and prediction for an observation, phenomenon, or chemical problem that can be tested by further investigation. A null hypothesis is used in statistical tests. The null hypothesis is the opposite of what you expect, for example, there will be no difference.

Independent variable

It is the variable that is manipulated or changed during an investigation.

Inference

An inference is a tentative conclusion drawn from a series of observations. It may lead to the formulation of a hypothesis.

Interpolation

To estimate a value of between two known values, frequently on a graph.

Law

A scientific law is a generalisation that Scientists make from their research findings. A useful Scientific law can be used to accurately predict what will happen in many situations.

Limitations

The restrictions of a particular experimental technique or set of apparatus. Limitations encountered during an investigation could influence the results and would need to be addressed in the evaluation.

Literature value

A value from the chemical literature of a physical constant or experimental measurement.

Normal distribution

A frequency distribution in which the shape of the curve is symmetrical about the mean.

Observation

Observations are any changes that can be measured or described during an investigation.

Precision

The precision is the total amount of random error present in a measurement. This is dependent on the experimental technique and the apparatus selected during the investigation.

Percentage error

A percentage error is an error expressed as a percentage of the value measured or the true value.

Prediction

Predictions are a consequence of a hypothesis and descriptions of the results you expect to obtain from an investigation.

Processed data

Raw data which has been ordered and/or mathematically or graphically transformed.

Propagation of errors

Calculating the overall error from a series of mathematical operations.

Qualitative data

Qualitative data refers to observations made without measurements.

Quantitative data

Quantitative data refers to numerical measurements.

Reliability

A measure of the confidence that can be placed in a set of observations or measurements. The reliability of a set of observations or measurements depends on the number and accuracy of the individual observations or measurements. Reliability can be improved by replicating observations and measurements.

Replication

This involves repeating a test a number of times.

Random error

A random error is an error which is present every time a measurement is recorded. Their effects can be reduced by repeating the measurement and averaging.

Raw data

This is unanalysed data which not yet been processed or analysed.

Risk assessment

A consideration of the possible safety hazards that could be encountered during an investigation.

Sensitivity

The ratio between the change in measurement to the change in measured quantity.

Significant figures

The significant figures in a number are those that are meaningful.

Standard deviation

A measure of dispersion, providing an estimate of the average deviation of data points from the mean.

Systematic error

An error which biases your measurements in some predictable but perhaps unknown or unrecognised way. Systematic errors cannot be reduced by repeating the measurement and averaging.

Tests

Investigations are usually composed of a number of tests where one variable is manipulated or changed.

Theory

A set of statements or principles devised to explain a group of facts or phenomena, especially one that has been repeatedly tested or is widely accepted and can be used to make predictions.

Trend

The general direction, tendency or patterns shown by a set of measurements or observations.

Trueness

Refers to the closeness of agreement between the average value obtained from a large series of test results and an accepted true.

t-test

A statistical test used to compare the means of two independent samples from a normally distributed population.

Uncertainty

An uncertainty is the range that will likely contain the true value of whatever is being measured.

Validity

A measure of the confidence that can be placed in a conclusion. The validity depends on factors such as the range and reliability of observations and measurements. Statistical tests may be used to place a value on the reliability of data.

Variable

The conditions or factor that can vary and may be varied during an experiment. As far as possible only one variable should be changed or manipulated at a time.

Variability

The degree to which the observations or measurements differ from one another.