

Teachers' guide to A2 Units

**Edexcel Advanced GCE in Biology 9040 and
Biology (Human) 9042**

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PRACTICAL WORK IN ADVANCED BIOLOGY AND BIOLOGY (HUMAN)

Introduction

Practical work is an integral part of the Specifications and should be encouraged wherever possible. One of the assessment objectives (Experiment and investigation, AO3), for both AS and Advanced GCE, relates specifically to the assessment of candidates' experience of practical activities. This assessment objective states that candidates should be able to:

- Devise and plan experimental activities, selecting appropriate techniques
- Demonstrate safe and skilful practical techniques
- Make observations and measurements with appropriate precision and record these methodically
- Interpret, explain, evaluate and communicate the results of their experimental activities using biological knowledge and understanding and employing appropriate specialist vocabulary.

Further details of the experimental and investigative skills are given on pages 9 - 10 of the Specifications.

Assessment Unit 6, Synoptic and practical assessment, includes two assessment components:

- **Paper 01** **T2 Practical assessment of coursework**
- **Paper 03** **Synoptic Paper**

Candidates may take Paper 02 (W2 Written alternative test) as an alternative to this Paper.

For Paper 01, students will carry out and present a report of an individual study and will be required to apply and understand the significance of an appropriate statistical test. The teacher in the centre will carry out assessment of the planning and implementing, and Edexcel will mark each written report.

The aims of this booklet are

- To outline the practical activities included in Units 4 and 5 of the Specifications (Indicated by □)
- To give suggestions and guidance on the development of individual studies (Indicated by •)
- To give guidance on the choice of appropriate statistical tests.

Note that some of the suggestions for individual investigations included in *the Teachers' guide for Advanced Subsidiary GCE in Biology and Biology (Human)* (Publication Code 007577) could be developed to form the basis for individual studies.

Unit 6: Paper 01 T2 An Individual Study

This section of the assessment is intended to give students the opportunity to plan and carry out a scientific investigation, and to write a report of it in a style similar to that used in scientific journals. The study must be based on a quantitative investigation and may be linked to any part of the Specifications. Students will be required to apply and understand the significance of an appropriate statistical test.

Each student will submit for assessment the written report of the practical work carried out for the individual study. The teacher in the centre will carry out assessment of the implementing and planning of the study and Edexcel will externally assess the written report. Teachers or tutors responsible will be required to authenticate the work and may be asked for details as to how it was carried out.

The practical work may be carried out by students in different situations: for example, in a laboratory of the school or college, or as part of fieldwork. It is expected that the topic being investigated will be an extension of the normal experimental work carried out in the study of the Specifications and should be related to the biological principles covered in the Specifications. Whilst it is accepted that students may address similar areas of the Specifications, it is expected that they will have the opportunity to investigate individual hypotheses. The practical work must involve making measurements and thus yield quantitative results. Reviews of literature or qualitative observations are not acceptable for the study.

The length of the written report is expected to be about 3500 words. Students will not be penalised for presenting longer reports but they should realise that they may have spent excessive time on the practical work or the writing of the report, and not gained any more marks by doing so. Before starting the individual study, students should be guided into devising an investigation that is likely to give meaningful results and can be carried out with the resources available.

The marks allocated to each section of the individual study are shown below.

<i>Planning</i>	<i>4 marks</i>
<i>Implementing</i>	<i>4 marks</i>
Introduction	4 marks
Methods	3 marks
Analysing evidence	6 marks
Discussion and evaluation	8 marks
Style	3 marks

Total 32 marks

Sections in Italics are to be assessed internally by teachers.

Full details of the scheme of assessment and practical skills criteria are included in the Specifications.

Progression from AS to A2

It will be evident from the practical skills criteria that, whilst there is considerable overlap with the requirements for Unit 3 (T1), there are significant additional requirements. In particular, candidates are expected to consider the nature and quantity of data they are to collect at the planning stage, with a view to subsequent statistical analysis, discuss and analyse their findings in greater depth, and pay particular attention to the format and presentation of their reports.

Help and guidance

It is expected that candidates will need some guidance in selecting an investigation, which will enable them to meet all the criteria. Ensuring that students avoid the use of multiple hypotheses, or simple repetition of standard experiments, would be acceptable without penalty. Where further advice or guidance was necessary it would be expected that annotations on the final report would explain the extent to which this had been given and how this had been taken into account when awarding marks. For some students, limited intervention at this early stage with a reduction in marks in one section would be preferable to allowing them to continue with investigations, which would not provide opportunities to meet all the criteria.

Working in groups

It is not acceptable for students to work together to produce an individual study. This inevitably makes it impossible to assess the skills of individual candidates. It is expected that fieldwork will continue to provide many excellent investigative opportunities, but centres need to be aware that examiners will award only minimal marks where studies show strong similarities in areas such as sampling methods, data presentation and analysis.

Where investigations are carried out at field study centres, it remains the responsibility of the candidates' home centre to ensure that the requirements of the practical

assessment criteria are fully understood and that the nature of any help or guidance is carefully recorded.

The single most important factor in successful investigations is careful, detailed planning supported where possible by simple pilot experiments.

Past experience has shown that high-scoring studies:

- Have one, straightforward, testable hypothesis
- Have detailed plans, which consider how all the important variables are to be controlled and how safety requirements are to be met
- The plans also describe how suitable data will be collected, and refer to the use of an appropriate statistical test to verify the hypothesis
- Consider interesting questions rather than repeating standard ‘textbook’ experiments
- Give students opportunities to vary their own methods, and display and analyse their data in an individual way
- Have short pilot experiments to establish a workable and reliable method
- Show clear progression from AS coursework
- Limit the use of biological theory to that which is relevant to the hypothesis being tested
- Pay careful attention to the use of SI units in all tabulation and data presentation
- Display data in an appropriate format which aids analysis of results
- Analyse the limitations of the techniques used rather than suggesting practical incompetence in using simple apparatus.

A note on safety

Teachers should be aware of their obligations under the Health and Safety at Work Act, Control of Substances Hazardous to Health (COSHH) Regulations, and the Management of Health and Safety at Work Regulations.

When planning the Individual Study, students should be encouraged to carry out their own risk assessments to identify hazards and plan suitable ways of reducing the risks arising from them, before carrying out practical work.

Unit 4 Respiration and coordination and Options

Practical work listed in the specification for this Unit includes:

- ☐ **Experiments to illustrate the role of hydrogen acceptors using a redox indicator**
- ☐ **Reaction time experiments**
- ☐ **Microscopic examination of the histology of the spinal cord**

Option A Microbiology and biotechnology

- ☐ **Use of Gram staining in the identification of bacteria**
- ☐ **Preparation and sterilisation of media, agar plate pouring and inoculation using sterile wire loops, pipettes and spreaders**
- ☐ **Investigation of the use of different carbon and nitrogen sources for growth using cultures on agar plates or in liquid culture**
- ☐ **Measurement of culture growth to investigate the growth of a microorganism in liquid culture**
- ☐ **A study of the optimal conditions necessary for yoghurt production, or for fermentation by yeast in brewing or dough production**

Option B Food science

- ☐ **Determination of the calorific values of simple foods using a calorimeter**
- ☐ **Estimation of subcutaneous fat by skinfold measurements**
- ☐ **Investigations of the perception of sweetness in drinks or foods**
- ☐ **Quantitative estimation of sugars and ascorbic acid in fruits at various stages of storage**
- ☐ **Weight loss in packaged foods**
- ☐ **The resazurin test, methylene blue test and turbidity test in relation to milk of different ages and the effectiveness of pasteurisation and sterilisation**
- ☐ **Quantitative investigations of the changes in foods during the process of fermentation.**

Option C Human health and fitness

- ☐ Study of prepared slides of cardiac tissue, lung tissue and striated muscle tissue
- ☐ The effect of ATP on the contraction of muscle fibres
- ☐ The effects of physical activity on pulse rate and blood pressure
- ☐ Use of simple apparatus to estimate vital capacity and variation in breathing with physical activity
- ☐ Investigation of the effect of a training programme
- ☐ Estimation of percentage body fat.

Experiments to illustrate the role of hydrogen acceptors using a redox indicator

A method for investigating the role of an artificial hydrogen acceptor, (TTC, 2,3,5-triphenyltetrazolium chloride) is described in *Cell Biology and Genetics* and *Respiration and Coordination*. This method involves incubating an actively respiring suspension of yeast with a TTC solution and noting the time taken for the suspension to turn a standard pink colour, as the TTC is reduced.

Methylene blue may also be used as an artificial hydrogen acceptor, using pasteurised milk as a source of respiring microorganisms. Prepare a 5.0 % solution of acetaldehyde and add a few drops of phenolphthalein indicator, followed by a dilute solution of sodium carbonate until the mixture just turns pink. Add 1.0 cm³ of this solution to 5.0 cm³ of pasteurised milk in a test tube and add 1.0 cm³ of 0.01 % methylene blue solution. Shake carefully, then stopper the tube and incubate in a water bath at 40 °C. Note the time taken for the methylene blue to become decolourised. It is important not to shake the tube during the incubation.

☐ **Reaction time experiments**

Electronic methods may be available for investigating reaction times to various stimuli, such as audio or visual stimuli. Reaction time to a visual stimulus can be measured conveniently using the 'ruler-drop' test, in which a metre rule is held with the 50 cm mark between the thumb and first finger of the subject and dropped without warning. The distance the ruler drops is then noted by recording the distance just above the index finger of the subject. Distance is then converted to time using the formula below.

$$t = \sqrt{\frac{2s}{g}}$$

Where t = time (in seconds)

s = distance dropped (in metres)

$g = 9.81$ (acceleration due to gravity)

Students could use this method to investigate, for example, differences in reaction times between males and females and the relationship between reaction time and age. Further details of the test are given in *Physical Education and the Study of Sport* (Third Edition), B. Davis, R. Bull, J. Roscoe and D. Roscoe, Mosby 1997, ISBN 0 7234 2642 2.

☐ **Microscopic examination of the histology of the spinal cord**

Prepared microscope slides and 35 mm transparencies of transverse sections of spinal cord are available, for example, from Philip Harris Education. Students may find it helpful to view a transparency before examining a section with the microscope, and to use diagrams or photomicrographs to help with the interpretation of the sections.

Some suggestions for individual studies

- Does reaction time vary with the time of day?
- Does smoking affect reaction time?

Option A Microbiology and biotechnology

Practicals for this Option are described in *Microorganisms and Biotechnology*, and *Respiration and Coordination*.

There are a number of practical protocols for microbiology and biotechnology, available as pdf files from the **National Centre for Biotechnology Education (NCBE)** website:

www.reading.ac.uk/NCBE

SAFETY NOTE

In all microbiological practical work, correct aseptic technique **must** be used. You should also adhere to Codes of Practice produced by Education Authorities, or by Governing Bodies of schools or colleges



☐ Use of Gram staining in the identification of bacteria

This is an important technique for the identification of bacteria, which are divided into two groups, Gram positive and Gram negative. Gram positive bacteria retain a crystal violet-iodine complex when treated with organic solvents and appear purple, whereas organic solvents decolourise Gram negative bacteria. Gram negative cells are counterstained using, for example, safranin or carbol-fuchsin and appear pink or red. It is essential to use fresh, actively growing cultures of bacteria for reliable results.

Prepare a heat-fixed smear of bacteria on a clean, grease-free microscope and stain the smear with crystal violet. Leave for 30 seconds, rinse with tap water then flood the slide with iodine solution. Leave for a further 30 seconds, then rinse off the iodine with tap water. Rinse the slide with alcohol until the washings are pale violet, rinse again with tap water, then counterstain with safranin for one minute. Rinse the slide with tap water and blot dry. Examine using an oil immersion objective lens.

Crystal violet and Gram's iodine solution are available from Philip Harris Education.

- ❑ Preparation and sterilisation of media, agar plate pouring and inoculation using sterile wire loops, pipettes and spreaders

Microbiological media are available in a variety of forms, including ready to use slopes, 15 cm³ or 100 cm³ lots in screw capped bottles, or as dry powders. Media which are useful for general microbiological work include china blue lactose agar, malt extract agar, nutrient agar, and potato dextrose agar. Microbiological media are available from Blades Biological and Philip Harris Education.

If making up media from dry powders, follow the instructions and dispense into suitable screw-capped bottles, such as universal bottles. As a guide, 15 to 20 cm³ of medium is sufficient for one standard 90 mm diameter Petri dish. After sterilisation in an autoclave, allow the media to cool to about 50 °C before pouring into sterile Petri dishes, following standard aseptic technique. Ready to use media should be melted in a boiling water bath, then allowed to cool before pouring.

Cultures of microorganisms, which are suitable for use in schools and colleges, are available from Blades Biological, the National Centre for Biotechnology Education, and Philip Harris Education.

After inoculation with sterile wire loops, glass spreaders, or pipettes, the lids of Petri dishes should be secured with adhesive tape before incubation, but do not seal all the way round as this encourages anaerobic conditions. All cultures should be autoclaved before disposal, preferably by placing in autoclave bags.

- ❑ Investigation of the use of different carbon and nitrogen sources for growth using cultures on agar plates or in liquid culture

Suggestions for investigating the effect of different carbon sources on the growth of yeast are included in *Microorganisms and biotechnology*. One method involves growing yeast in media containing a range of sugars, including glucose, fructose, galactose, sucrose and maltose, and measuring the production of acid by the yeast. There are a number of suggestions for practical work on this topic in *Practical Fermentation: a guide for schools and colleges* (NCBE).

- ❑ Measurement of culture growth to investigate the growth of a microorganism in liquid culture

For this investigation, a fermenter could be set up containing a culture of a suitable microorganism, samples removed at regular intervals and growth determined by an appropriate method, such as viable counts using pour-plate dilution, direct cell counting using a haemocytometer, or optical methods using a colorimeter or spectrophotometer. Suitable organisms for this investigation include yeast, *Chlorella*, and *Vibrio natriegens*. Details of a method for investigating the growth curve of *Vibrio natriegens* are included in *Practical Fermentation: a guide for schools and colleges* (NCBE).

- ❑ A study of the optimal conditions necessary for yoghurt production, or for fermentation by yeast in brewing or dough production

Simple methods, such as changes in viscosity, or changes in pH, could be used to investigate yoghurt production. The influence of factors such as temperature and the type of milk used can be investigated using a natural yoghurt starter culture. Add 1.0 cm³ of natural yogurt to 10.0 cm³ of milk in a boiling tube and cover with cling film. Incubate in a water bath and observe the changes in viscosity and pH.

To investigate fermentation by yeast in brewing, it is suggested that students could devise experiments to compare, for example, the growth of *Saccharomyces cerevisiae* and *S. carlsbergensis* using different sugars, such as glucose, sucrose and raffinose.

To investigate factors affecting dough production, students could devise methods to compare the effects of factors such as different strains of yeast, types of flour, presence of amylase, and ascorbic acid concentration on the rate of rising of dough. Mix 1 g of dried yeast with 50 cm³ of water, then add 75 g of flour and mix well. Place the dough in a 100 cm³ measuring cylinder and record the volume of the dough at suitable intervals.

Practical details and suggestions for further work are included in *Practical Biotechnology: a guide for schools and colleges*, and *Practical Fermentation: a guide for schools and colleges* (NCBE).

SAFETY NOTE

Food made in a laboratory **MUST NOT** be tasted



Some suggestions for individual studies

- Do microorganisms grow faster using monosaccharides, rather than disaccharides, as a carbon source?
- Does brewers' yeast ferment glucose more rapidly than bakers' yeast?
- What is the optimum temperature for yoghurt production?

Option B Food science

Practicals for this Option are described in *Food and Health*, and *Respiration and Coordination*. Teachers (and students) are also recommended to refer to *Practical Fermentation: a guide for schools and colleges* (NCBE) and the *Science and Plants for Schools (SAPS) website* (www.saps.plantsci.cam.ac.uk) for suggestions and details of practical activities.

☐ Determination of the calorific values of simple foods using a calorimeter

The use of heat of combustion apparatus, or a food calorimeter, is recommended for this practical. The heat produced by the burning food can be calculated by assuming that all the heat is transferred to the water surrounding the combustion chamber. Since 1 calorie is the heat energy required to raise 1 g of water by 1 °C, then the heat produced by the food = mass of water (g) × rise in temperature (°C). Convert this figure to joules using the relationship 1 calorie = 4.2 joules.

☐ Estimation of subcutaneous fat by skinfold measurements

The method for estimation of subcutaneous fat, using skinfold calipers, is described in *Food and Health*, and *Respiration and Coordination*. This method, however, may give unreliable results. More accurate data relating to body composition are obtained using the technique of bioelectric impedance analysis (BIA), which involves passing a small electric current through the body. The impedance to the flow of the electric current is related directly to the level of body fat.

☐ Investigations of the perception of sweetness in drinks or foods

An experiment to investigate the perception of sweetness in drinks is described in *Food and Health*, and *Respiration and Coordination*. This experiment involves the subjective perception of sweetness in a range of sugar solutions, such as sucrose, lactose, maltose, glucose and fructose. It is also suggested that 'threshold concentrations' and relative sweetness of artificial sweeteners, such as aspartame and saccharin, could be investigated.

SAFETY NOTE

If this activity takes place in a laboratory, ensure that the benches have been thoroughly cleaned and that the sugars are not contaminated. Do not store the sugars with other chemicals.

Use disposable plastic cups for the sugar solutions.



- ☐ **Quantitative estimation of sugars and ascorbic acid in fruits at various stages of storage**

Methods for the estimation of sugar content and ascorbic acid (vitamin C) content of foods are described in *Food and Health*, and *Respiration and Coordination*. Concentrations of reducing sugars can be determined semi-quantitatively using Benedict's reagent and a range of glucose standards. Make up a range of glucose solutions (2.0, 1.0, 0.5, 0.1, 0.05, 0.02 and 0.01 per cent) and add 3.0 cm³ of each to 5.0 cm³ of Benedict's reagent in separate, labelled test tubes. Heat in a boiling water bath for 8 minutes, then cool. The concentration of glucose can be determined using Diabur-Test® 5000 strips (available from pharmacists). These give quantitative values for glucose concentrations, over the range 0.1 to 5 per cent, or 5.5 to 280 mmol per dm³.

Ascorbic acid concentrations are determined using the standard DCPIP (phenol-indo-2:6-dichlorophenol) decolourisation method. Make up a 0.1 per cent solution of ascorbic acid and a 0.1 per cent solution of DCPIP. Transfer exactly 1.0 cm³ of the DCPIP solution to a test tube. Fill a 1.0 cm³ syringe, fitted with a needle, with the standard 0.1 per cent ascorbic acid solution. Keep the end of the needle below the surface of the DCPIP and carefully add the ascorbic acid solution until the DCPIP is decolourised. Do not shake the mixture, but stir gently using the needle. Record the volume of ascorbic acid solution required to decolourise the DCPIP.

Repeat the procedure with 1.0 cm³ of fresh DCPIP solution and use the syringe to add fruit juice. Record the volume of fruit juice required to decolourise the DCPIP.

CALCULATION

If the volume of standard ascorbic acid required to decolourise 1.0 cm³ of DCPIP solution is x cm³ and the volume of fruit juice required to decolourise 1.0 cm³ of DCPIP is y cm³, then the concentration of ascorbic acid in the fruit juice is $x \div y$ mg per cm³.

1.0 mg per cm³ of ascorbic acid is equivalent to a concentration of 0.1 per cent.
To convert values from mg per cm³ to a percentage, multiply by 0.1.

- ☐ **Weight loss in packaged foods**

For this practical, students investigate the effects of various packaging materials, such as cling film, PVC films and paper, on weight in various fruits and vegetables. Apples, mushrooms, small lettuces and carrots are suitable. The method is included in *Food and Health*. This practical could be combined with investigations into other changes in the stored material, including enzyme activity, texture, and ascorbic acid content.

There are a number of suggestions for practical activities with plant materials, such as fruits and vegetables, including post-harvest changes and enzyme assays, on the Science and Plants for Schools (SAPS) website:

www-saps.plantsci.cam.uk

- ❑ **The resazurin test, methylene blue test and turbidity test in relation to milk of different ages and the effectiveness of pasteurisation and sterilisation**

Resazurin is an indicator, which shows metabolic activity of bacteria, changing from blue, through pink to white and can be used to compare the bacterial content of different milk samples. Resazurin tablets are available from Philip Harris Education. One tablet is dissolved in 50 cm³ of distilled water and 1.0 cm³ of this solution is added to 10 cm³ of the milk sample in a sterile container, such as a universal bottle. Replace the lid, invert once to mix the contents, then incubate in a water bath at 37 °C. Changes in the colour of this mixture are compared with a control containing 10 cm³ of boiled milk plus 1.0 cm³ of resazurin solution.

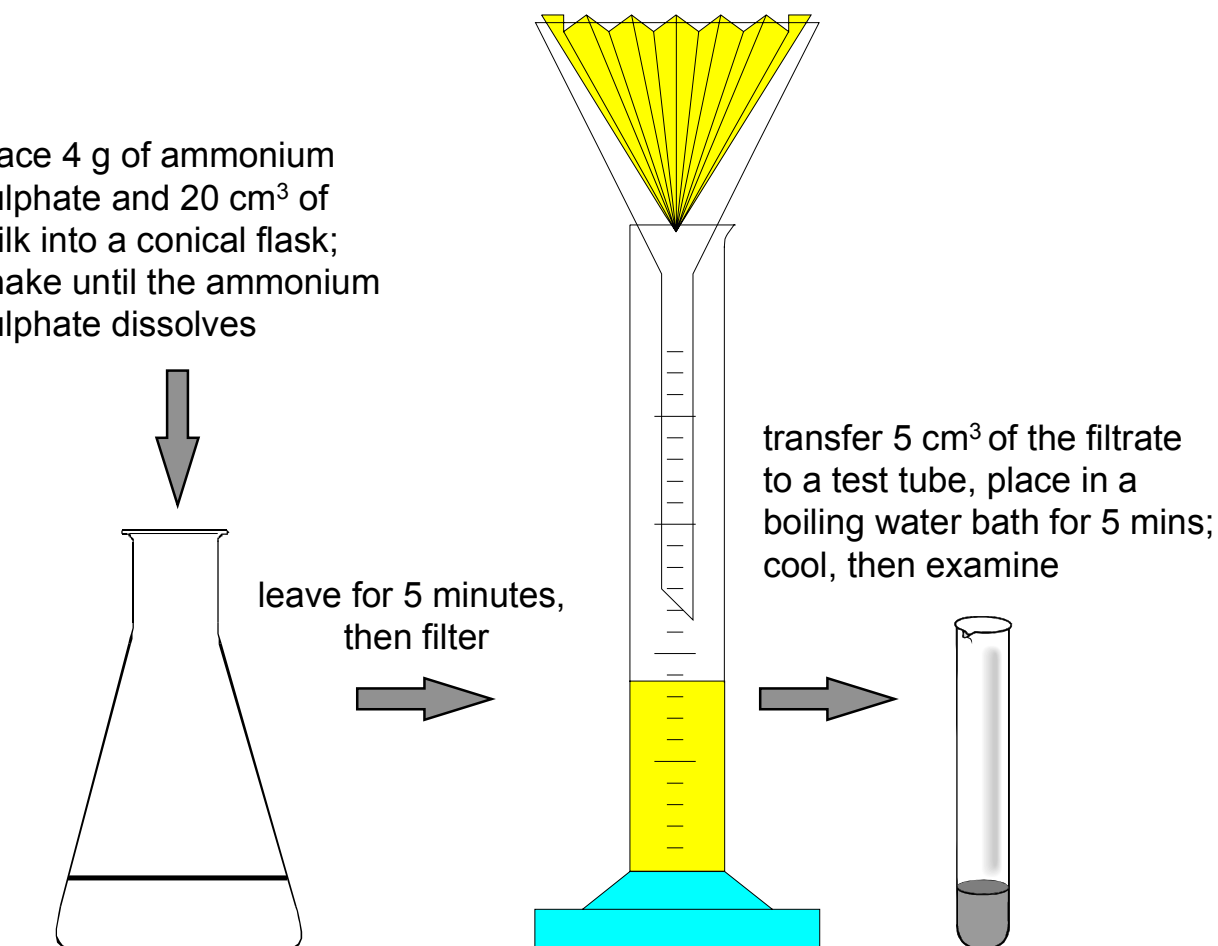
For the **methylene blue test**, prepare a 5 per cent ethanal (acetaldehyde) solution and add a few drops of phenolphthalein indicator, followed by a dilute solution of sodium carbonate until the mixture just turns pink. Transfer 5 cm³ of milk into a test tube, add 1.0 cm³ of the ethanal solution, followed by 1.0 cm³ of 0.01 per cent methylene blue solution. Mix by shaking the tube gently, cover with cling film, or aluminium foil, and stand the tube in a water bath at 40 °C. Note the time taken for the methylene blue to become decolourised. Compare results using other samples of milk.



ETHANAL

The turbidity test is used to check the efficiency of sterilisation in milk. Weigh out 4 g of ammonium sulphate and transfer to a conical flask. Add 20 cm³ of the milk sample to be tested and shake the flask for at least one minute until the ammonium sulphate dissolves. Leave the flask to stand for 5 minutes. Filter the contents of the flask into a measuring cylinder. Transfer 5 cm³ of the filtrate to a test tube and stand the tube in a boiling water bath. Leave for 5 minutes. Cool the tube in a beaker of cold water for a further 5 minutes, then examine the contents of the tube by placing in front of a bench lamp. Compare a sterilised milk sample with a pasteurised milk sample.

place 4 g of ammonium sulphate and 20 cm³ of milk into a conical flask; shake until the ammonium sulphate dissolves



The turbidity test

- ☐ Quantitative investigations of the changes in foods during the process of fermentation

A suggested approach to this practical is included in *Food and Health and Respiration and Coordination*. This involves measurement of changes in pH during the production of yoghurt. Transfer 10 cm³ of UHT milk to a boiling tube and add 1 cm³ of natural yoghurt as a starter culture. Record the pH of the mixture, then cover the tube with clingfilm and incubate in a water bath at 43 °C. Record changes in the pH and appearance of the contents at suitable intervals, such as 30 minutes, for up to 5 hours. This practical could be adapted to record the results with datalogging, using a pH sensor.

Further suggestions for practicals involving fermentation are available as pdf files from the **National Centre for Biotechnology Education (NCBE)** website:

www.reading.ac.uk/NCBE

SAFETY NOTE

Food made in a laboratory **MUST NOT** be tasted



Some suggestions for individual studies

- Does subcutaneous fat level increase with age?
- Does storing fruit in a refrigerator help to retain ascorbic acid content?
- Does the concentration of reducing sugars increase with the age of a fruit?

Option C Human health and fitness

Practicals for this Option are described in *Respiration and Coordination*.

- ☐ Study of prepared slides of cardiac tissue, lung tissue and striated muscle tissue

The aim of this practical is to familiarise students with the histology of cardiac tissue, lung tissue and muscle tissue and to consider the relationships between structure and function. Students should be encouraged to make annotated drawings of the preparations, showing the main histological features. The use of plastic resin embedded sections of lung tissue is recommended as these give better resolution and show more structural detail than conventional 5 µ sections.

Suitable prepared microscope slides are available from Philip Harris Education.

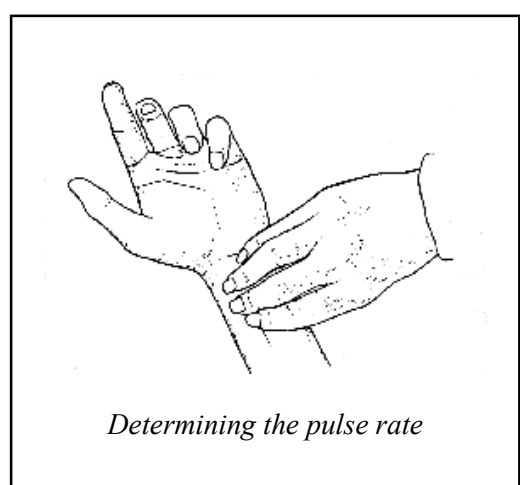
❑ The effect of ATP on the contraction of muscle fibres

Remove thin strands of muscle fibres, using blunt forceps, from fresh lean pork or shin beef. Place the strands in warm Ringer's solution (dissolve one quarter strength Ringer's solution tablet in 125 cm³ of warm distilled water). Place one muscle strand on a microscope and carefully straighten with a glass rod. Blot off the excess Ringer's solution, measure the length of the muscle then add one drop of ATP solution. Measure the length again.

Ringer's solution tablets and ATP solution (20 mg per cm³) are available from Philip Harris Education.

❑ The effects of physical activity on pulse rate and blood pressure

A number of digital pulse monitors and sphygmomanometers are available, which make these investigations straightforward to carry out. It is important to standardise the activity, for example, by using an exercise cycle or bicycle ergometer. As an alternative, step-ups at a fixed rate can be used as a method for standardising activity.



❑ Use of simple apparatus to estimate vital capacity and variation in breathing with physical activity

Students should be familiar with the principle of a spirometer and be able to interpret spirometer data. Satisfactory data can be obtained using, for example, lung volume bags, or pocket spirometers. As with the previous investigation, it is important to standardise the activity if meaningful, quantitative, relationships are to be determined.

❑ Investigation of the effect of a training programme

This is a relatively long-term investigation and should be spread over a minimum of two weeks. It is suggested that students jog with a partner so that a conversation can be held during the exercise, to avoid breathlessness and stress the aerobic energy systems. The resting breathing and pulse rates are recorded. After an initial jog of 1600 m, aiming to finish in ten minutes, the pulse rate is determined, then again after

a rest of 1 minute. These measurements are taken for a further three sessions, spread over a minimum of two weeks, as in the table below.

Approximate length of run / m	Time to aim for / min
1600	10
2400	15
3200	20
1600	10

Examples of training programmes are included in: *Physical Education and the Study of Sport*, 3rd edition, B Davis, R Bull, J Roscoe and D Roscoe, Mosby, ISBN 0 7234 26422.

☐ Estimation of percentage body fat by skinfold measurements

The method for estimation of subcutaneous fat, using skinfold calipers, is described in *Food and Health*, and *Respiration and Coordination*. This method, however, may give unreliable results. More accurate data relating to body composition are obtained using the technique of bioelectric impedance analysis (BIA), which involves passing a small electric current through the body. The impedance to the flow of the electric current is related directly to the level of body fat.

Some suggestions for individual studies

- Can aerobic training increase vital capacity?
- How does anaerobic conditioning affect muscle strength?
- Does resting pulse rate decrease with increased physical training?
- What is the relationship between body fat content and body mass index?

Unit 5B Genetics, evolution and biodiversity

Practical work in this Unit includes:

- ☐ Chromatography of chloroplast pigments
- ☐ Effect of light intensity and carbon dioxide concentration on the rate of photosynthesis
- ☐ Investigation of plant growth by water culture experiments
- ☐ Effects of hormones on plant growth
- ☐ Distribution of plants and animals and effects of abiotic factors
- ☐ Estimation of population size using the Lincoln index
- ☐ A breeding experiment to demonstrate the principles of inheritance

Details of practical activities in this Unit are included in *Genetics, Evolution and Biodiversity*, and *Tools, Techniques and Assessment in Biology: a course guide for students and teachers*.

☐ Chromatography of chloroplast pigments

A method for extraction and chromatographic separation of chloroplast pigments is included in *The Organism and the Environment*, and *Genetics, Evolution and Biodiversity*. The use of thin layer chromatography plates, coated with silica gel, gives much better separation than conventional chromatography paper. Particular care is needed in this practical because of the volatile and highly flammable nature of the solvents used.



Silica gel coated chromatography plates are available from Philip Harris Education.

Methods for chromatography of plant pigments are available on the **Science and Plants for Schools (SAPS)** website:

www-plantsci.cam.ac.uk

❑ **Effect of light intensity and carbon dioxide concentration on the rate of photosynthesis**

The use of a simple photosynthometer is described in *The Organism and the Environment*, and *Genetics, Evolution and Biodiversity*. Photosynthetic activity could be investigated using datalogging, with an oxygen electrode and oxygen sensor. *Elodea* is usually available from pet shops and aquarium and pond suppliers. As an alternative to *Elodea*, *Lagarosiphon* is recommended for use in datalogging experiments.

❑ **Investigation of plant growth by water culture experiments**

The aim of this practical is to investigate the effects of mineral deficiency on the growth of plants. Seedlings are grown in solutions containing a range of mineral salts, including those which lack phosphate, nitrate, calcium, potassium, magnesium, iron, and sulphate, plus the complete medium.

Suitable seedlings, which can be used for this investigation, include maize (*Zea mays*), castor beans (*Ricinus communis*), tomato (*Lycopersicon esculentum*) and cabbage (*Brassica oleracea*). Seeds are germinated in moist vermiculite, then transferred separately to the nutrient solutions. At weekly intervals, the plants are measured and any deficiency symptoms noted.

Suitable laboratory grade seeds and mineral deficiency water culture media are available from Philip Harris Education.

❑ **Effects of hormones on plant growth**

Suggestions for investigating the effect of selective herbicides are included in *Genetics, Evolution and Biodiversity*, and investigations on the use of hormones in the promotion of rooting in cuttings are described in *Applied Plant and Animal Biology*. A plant hormones set, including teaching notes, is available from Philip Harris Education.

SAFETY

When using the herbicide spray:

Keep off skin
Do not breathe the spray
Wash off any splashes
Wash hands and
exposed skin after use



❑ **Distribution of plants and animals and effects of abiotic factors**

Fieldwork techniques, including sampling methods and details of environmental measurements, are included in *Tools, Techniques and Assessment in Biology: a course guide for students and teachers*. Students are expected to study the distribution of plants and animals in at least one habitat and to investigate the influence of abiotic factors. The nature of the investigations will, of course, depend on the types of habitats available to study, but it is important to emphasise that worthwhile investigations can be carried out with relatively limited equipment and facilities, such as a quadrat and access to a garden, park, or school field.

❑ **Estimation of population size using the Lincoln index**

Details of this method, and suggestions for its use in fieldwork, are included in *Tools, Techniques and Assessment in Biology: a course guide for students and teachers*. It is also suggested that a laboratory model can be used to illustrate the principle of the capture-mark-recapture method and use of the Lincoln index to estimate population size. Larvae of the beetle *Tenebrio molitor*, known as mealworms, are recommended. Mealworms are usually available from pet shops, or may be obtained from Blades Biological or Philip Harris Education. A sample of mealworms is removed from the population, counted, and marked with a small dot using a permanent marker pen. These larvae are returned to the culture and left for a standard time to mix. A second sample is then removed, the number of larvae present in the sample is counted and the number of these, which are marked, is also noted.

CALCULATION

The formula for the Lincoln index is given below.

$$N = \frac{S_1 \times S_2}{R}$$

Where

N = the estimated total population size

S_1 = the number of organisms marked and released

S_2 = the number of organisms captured in the second sample

R = the number of marked organisms re-captured

- ❑ A breeding experiment to demonstrate the principles of inheritance

A breeding experiment using *Tribolium castaneum* is described in *Cell Biology and Genetics*, and *Genetics, Evolution and Biodiversity*. Other organisms which are suitable for practical investigations in genetics include: *Drosophila* and rapid cycling brassicas (*Brassica rapa*). Several different genetic strains of *Tribolium* are available from Philip Harris Education. Kits for breeding experiments with *Drosophila* and rapid cycling brassicas, including various genetic strains of the organisms, are available from Blades Biological and Philip Harris Education.

For further information, visit the **Blades Biological** and **Philip Harris Education** websites:

www.blades-bio.co.uk/home.htm

www.philipharris.co.uk

Unit 5H Genetics, human evolution and biodiversity

Practical work in this Unit includes:

- ☐ A breeding experiment to demonstrate the principles of inheritance
- ☐ Preparation of a karyotype from a print of human metaphase chromosomes
- ☐ Distribution of plants and animals and effects of abiotic factors
- ☐ A breeding experiment to demonstrate the principles of inheritance

A breeding experiment using *Tribolium castaneum* is described in *Cell Biology and Genetics*, and *Genetics, Evolution and Biodiversity*. Other organisms which are suitable for practical investigations in genetics include: *Drosophila* and rapid cycling brassicas (*Brassica rapa*). Several different genetic strains of *Tribolium* are available from Philip Harris Education. Kits for breeding experiments with *Drosophila* and rapid cycling brassicas, including various genetic strains of the organisms, are available from Blades Biological and Philip Harris Education.

For further information, visit the **Blades Biological** and **Philip Harris Education** websites:

www.blades-bio.co.uk/home.htm

www.philipharris.co.uk

- ☐ Preparation of a karyotype from a print of human metaphase chromosomes

Details of this practical are included in *Genetics, Evolution and Biodiversity*. Human chromosome analysis sets are available from Philip Harris Education. These include prints of metaphase chromosomes, from which students cut out the chromosomes and prepare their own karyotypes according to the banding patterns. The analysis set includes prints of metaphase chromosomes of normal male, normal female, Down's syndrome and Klinefelter's syndrome.

- ☐ Distribution of plants and animals and effects of abiotic factors

Fieldwork techniques, including sampling methods and details of environmental measurements, are included in *Tools, Techniques and Assessment in Biology: a course guide for students and teachers*. Students are expected to study the distribution of plants and animals in at least one habitat and to investigate the influence of abiotic factors. The nature of the investigations will, of course, depend on the types of

habitats available to study, but it is important to emphasise that worthwhile investigations can be carried out with relatively limited equipment and facilities, such as a quadrat and access to a garden, park, or school field.

Individual studies in Unit 5

There are a number of suggested practical activities in Unit 5, which, although valuable in demonstrating important techniques, do not lend themselves to providing opportunities to meet all the requirements of an individual study. In particular, chromatography of leaf pigments, and demonstrating the need for mineral ions by simple culture experiments, is unlikely to lead to high-scoring studies. Similarly, merely repeating a standard photosynthesis experiment is unlikely to offer sufficient opportunities for original planning. However, using the same technique to investigate different hypotheses could well be more productive. For example, repeating the standard light intensity investigation would gain little credit, but using the same apparatus to investigate the effect of different concentrations of common pollutants on the rate of photosynthesis could offer much more scope.

Investigations using standard genetic crosses can also be very limiting. Examiners will be unable to award more than minimal marks where individual studies repeat well-documented techniques to confirm simple Mendelian ratios. Past experience has shown that the use of such experiments has made it very difficult for candidates to demonstrate their individual practical skills.

Suggestions for individual studies

- Does hormone rooting powder lead to more rapid root formation in geranium stem cuttings?
- Is there a direct correlation between light intensity in a woodland habitat and the leaf area of *Dogs Mercury*?
- Does the abundance of swimming mayfly nymphs increase with the flow rate of a stream?
- Does the extent of exposure on a rocky shore change the height: width ratio of the common limpet?
- Is the distribution of *Calluna vulgaris* on a dune system influenced by the humus content of the soil?
- Is there a link between the abundance of common lichens and the aspect of the surface they are growing on?

Statistics in the individual study

The individual study must be based on a quantitative investigation and students are required to apply and understand the significance of an appropriate statistical test. Students should therefore decide on an appropriate method of statistical analysis at the planning stage of the study. The plan should include one clearly stated, concise hypothesis, which must be:

- ✓ Testable
- ✓ Predictive, so those students can say whether or not their data enables them to accept or reject their hypothesis.

As a guide, the types of statistical tests are likely to involve:

Differences:

- Mann Whitney *U* Test
- Wilcoxon Matched Pairs Test
- Student's *t*-test

Correlations:

- Spearman Rank Correlation Test
- Regression Analysis

Goodness of Fit:

- Chi-squared Test

Details of these statistical tests, including worked examples and Case Studies, can be found in *Tools, Techniques and Assessment in Biology: a course guide for students and teachers*.

Good practice when using statistics

- Consider the type of statistical test to be used in your detailed plan
- Link your chosen test to the hypothesis
- Distinguish carefully between tests for differences, correlations and associations
- Avoid the use of a chi-squared test unless you are certain it is appropriate
- Relate the statistical test to the type and number of measurements you intend to take
- If your method indicates that numerous repeated statistical tests will be needed to come to a conclusion, re-think your plan or selected test
- Use a 5 % confidence limit to accept or reject your null hypothesis
- Explain clearly how you have used the results of your statistical test in the analysis of your results.

Sids' stathisistical planner

Have you really got time to do this many samples? If so some form of **random sampling** is usually needed. Once you've got your data you will need to check to see if they are **normally distributed** - if they are use the **mean** as a summary and test for the significance of any differences using the **Students t-Test**. If the data do not fit a normal distribution **GO TO 25**.

Are you going to be looking for some form of **association** between two variables, for instance between the light intensity and the number of plants in a series of random quadrats or between the moisture content of soil samples and the percentage cover of a given plant species at sites along a transect. If yes **GO TO 37** if not **GO TO 51**.

Regression analysis is what you want. You need to be able to **control** the variable plotted as the **independent** variable on the x-axis and measure the uncontrolled or **dependent** variable plotted on the y-axis.

Some form of random sampling may be useful, your data can be summarised using the **median**. Use the **Mann Whitney U Test** to see if any differences are significant. Occasionally your data might be in **matched pairs** in which case you will need the **Wilcoxon Matched Pairs Test**

You are going to need to use **Analysis of Variance**. The maths are horrible you'll need to consult an advanced statistics book - it might be better to **GO BACK TO 15**.

WARNING - if you really want to **compare more than two sets of data** you need to check that you will have enough time to replicate all your measurements at least 6 times and you need some very complex maths - **GO TO 35**.

You will need at least **12 - 15** pairs of measurements. If you want to **correlate** two variables use the **Spearman rank correlation test** if you want to predict values of an uncontrolled variable from values of a controlled variable **GO TO 33**.

If you are planning on taking **6 - 15** measurements from each site, population or treatment **GO TO 25** if you think you can do more than fifteen **GO TO 26**.

It's probably best to keep things **simple** and stick to just **two sites, populations or treatments (GO TO 19)**. If you want to compare more than two - **GO TO 21**.

You will need to use the **CHI squared test (χ^2)**. This tests the **goodness of fit** of observed data to a set of expected values. It is most useful in genetics or choice chamber work and has **very limited use** for ecological studies.

If you don't repeat your measurements at least six times then you will have **too few replicates** and you will be unlikely to show any differences **GO BACK TO 5**.

Start Here - do you want to look at **differences** between measurements from two (or more than two) sites, populations or treatments? If yes **GO TO 5** if not ... **GO TO 8**.

Stathisitics if you don't get 'em right you'll end up in trouble!

You haven't really got a clue **GO BACK TO 1!**

Are you planning on **replicating** all your measurements at least **six** times at each site (or within each population or treatment)? If yes **GO TO 15** if not **GO TO 13**.

Are you going to want to see how your data compare with values predicted by some biological theory? - for instance to see how the results from a **genetics** experiment fit the ratios predicted by Mendel or to see whether there are any differences between **observed** and **expected** values in a choice chamber experiment - if yes **GO TO 43** if not **GO TO 30**.

Notes

- 1 Perhaps the most common form of investigation is to look for differences between two or more than two sets of samples
e.g. the number of animals found in fast and slow flowing sections of stream.
e.g. 2 - the number of bacterial colonies on two different types of agar plates or at two different temperatures.

- 5 Any measurements you collect need to be replicated. There is no guarantee that a single measurement of anything is typical. By taking a set of measurements you can obtain an idea of the average state. The more replicates that you take the more reliable that average is.

- 8 Occasionally you may have a biological theory which predicts a set of Expected values and you might want to see how close the agreement is between your Observed data and the predicted data. These situations are most likely to occur in Genetics (where an understanding of Mendel's laws of inheritance may give expected values) or in behavioural work (as in choice chamber experiments).

- 13 To show any difference between two or more than two sets of data there must be a sufficient level of replication. If you have less than six measurements in a set of data then many statistical tests will not allow you to make a decision about the significance of your results.

- 15 Time and resources are often limiting factors in investigations. If you keep things simple then it is more likely that you can replicate measurements adequately. Lets say you have allowed 6 hours to collect data for an ecological study. If it takes 30 minutes to collect one set of data from one quadrat then in 6 hours you can do 12 quadrats. If you want to compare 2 sites then you could do 6 quadrats at each site. If you wanted to look at 3 sites then you could only do 4 quadrats per site, for 4 sites it's 3 quadrats and so on.

- 19 With more than 15 measurements in each set of data then you might have enough information to show your data are normally distributed (see 26). With less than 15 it is unlikely that you could show this.

- 21 The more complex designs (i.e. where you want to compare more than two sets of measurements) do involve a lot of extra work. Remember all your measurements need to be replicated at least 6 times. To compare four sites or treatments you need 24 replicates in total. Have you really enough time and resources to do this?

- 25 To avoid any chance of bias in the collection of your data it might be a good idea to randomise your samples somehow. In ecological studies this is straightforward - a series of random coordinates could be used to position a set of quadrats within a sampling area. With lab studies it is perhaps less important so long as all extra variables are controlled properly.

With small sets of data (or data which are not normally distributed see 26) the mean is not necessarily the best summary of a set of data. The median is the better average to use.

Example - % cover of heather in 10 quadrats

11 11 12 13 16 16 17 24 25 50

Median = 16 Mean = 19.5 Mode = 11 or 16

Which is the best summary?

To be considered as matched pairs there must be a single unique way in which a measurement from one set of data is matched to a measurement in the second set of data. For instance if you were looking at the effect of exercise on pulse rate then a before exercise pulse rate for a person could be matched against their after exercise rate. If you are not sure if your samples are in matched pairs then it is safest to assume they are not.

- 26 Normal Distribution - if you measure the heights of a large number of people and plot the frequency with which people fall into different size classes then you would probably get a symmetrical bell shaped distribution like that below.

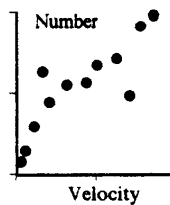


Class	Tally	Tally
1-3		
4-6		
7-9		
10-12		
13-15		
16-18		
19-21		
22-24		
25-27		
28-30		

To see if your data fit a normal distribution a rough tally chart is enough. Divide the range of values into 9-10 size classes and tally up the number of occurrences in each class. The first set of data couldn't be described as being normal, the second set would be.

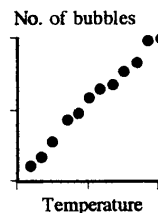
With small sets of data it can be hard to decide, so it's probably best to play safe and assume they're not normally distributed.

- 30 The other main type of investigation looks for an association between two variables.



For instance the relationship between water flow and the number of mayflies in a stream could be studied by looking at a number of sampling positions and, for each, recording the velocity and no. of mayflies caught.

- 33 In some instances you may be able to fix (or control) the increments of one of the variables and measure the effect of changes in this independent variable on a second dependent variable.



For instance you might want to look at the rate of photosynthesis at different temperatures. By using Regression Analysis you could fit a line to this graph and from the equation predict rates at intermediate or other temperatures.

- 35 Don't do it unless you're really sure!

- 37 If both your variables are uncontrolled and you simply want to correlate the two variables then this is for you. Be careful about putting in a best fit line. If you need to do this then it should really be fitted mathematically using regression analysis.

- 43 The Chi² test is the most **commonly misused** test. Its applications really are limited to tests of goodness of fit of data to expected values - e.g. as in genetics or behavioural work. Its only ecological application is in association analysis where presence/absence data from a large number of quadrats is being analysed.

A common error is to calculate an expected value from the two total values:-
 $(98 + 68) / 2 = 83$

Quadrat	Site A	Site B
1	1	12
2	3	13
3	2	9
4	4	11
5	1	14
6	87	9
Total =	98	68

(Mis)using the Chi² test you could show that the totals are significantly different from this value and conclude that whatever you've measured is more abundant at site A than site B yet it is clear that on average the thing is more abundant at site B.

- 51 Oh dear!

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Bibliography

The following texts in the Nelson Modular Advanced Science Series have been referred to in this document.

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Adds J, Larkcom E and Miller R *The Organism and the Environment* (1997) ISBN 017 448 2744

Adds J, Larkcom E and Miller R *Applied Plant and Animal Biology* (1998) ISBN 017 448 2701

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Adds J, Larkcom E and Miller R *Microorganisms and Biotechnology* Nelson (1998) ISBN 017 448 2269 8

Adds J, Larkcom E, Miller R and Sutton R *Tools, Techniques and Assessment in Biology; A Course Guide for Students and Teachers* (1999) ISBN 017 448 2736

Adds J, Larkcom E and Miller R *Genetics, Evolution and Biodiversity* (2001) ISBN 017 448 2965

Adds J, Larkcom E and Miller R *Respiration and Co-ordination* (2001) ISBN 017 448 2957

Introduction to W2

(Unit Test 6, Paper 02)

The written alternative paper, W2, is of **1 hour and 20 minutes duration** and carries a maximum of **32 marks**. There will normally be **two questions**, of which the first carries fewer marks.

This written paper may be substituted in place of T2 practical assessment. In making the decision to opt for this paper, centres will need to be aware of the fact that it is designed to test similar practical skills to those tested in T2.

Teachers and candidates are therefore strongly advised to consult the **Practical Skills Criteria** for Unit 6, on pages 64 – 70 of the subject specification (syllabus), since the Examiners use these when preparing questions for the written alternative paper.

Past experience has shown that candidates find this a very demanding theoretical exercise in the time available. This is especially true for students who have had little experience or training in planning investigations of the type required. Reference to recent Examiners' Reports on papers WTA1 and WTA2 will also provide a useful indication of the common errors and difficulties.

A brief summary of the main points is given below.

Question 1

(a) Tabulation

- Include all data required in one single table
- Accurate titles and units should be in the headings of rows and columns only
- Be consistent with significant figures

(b) Graphical presentation

- Ensure axes are correctly scaled and well-labelled with units
- Choose line or bar graph carefully
- Plot points carefully
- Decide whether it is appropriate to draw a line of best fit or to join the points – if you're not sure it is usually safer to join the points with ruled lines
- Do not extrapolate

(c) Conclusions

- Describe the trends and patterns in the data accurately
- Do not confuse theoretical assumptions with patterns shown by the data

Question 2

There are lots of marks available in each section, so it is important to include plenty of practical details.

(a) Planning

- Start by identifying as many variables as possible and give practical ways of controlling them. There is often no exactly 'correct answer', so credit will be given for sensible and scientific approaches
- Look back through past mark schemes for WTA1 and WTA2 to find examples of what is needed. There are always common themes such as control of biological variability in the sample, sensible sample numbers, or randomisation of samples, what exactly is being measured, and so on
- Avoid all vague terms such as 'about', 'approximately', 'a few days', and 'amounts'
- Do suggest sensible numbers to sample or measure, ensure that measurements are repeated and give appropriate times for such measurements
- Above all, think carefully about whether your suggested method will work practically and check carefully that it will actually test the hypothesis given in the question.

(b) Recording, presentation and analysis

- Check that any suggested tabulation actually matches what you are proposing in your method
- Check that the table accommodates all the readings and manipulated data
- Think about including means, where appropriate
- Sketch any suggested graph, making sure that you indicate the type of graph and the labels you would use for each axis
- Your named statistical test must be appropriate for the data
- Include details such as a suggested null hypothesis and confidence limits (normally 5 %).

(c) Limitations and further work

- Limitations must be genuine sources of error that would be encountered no matter how carefully the investigation was carried out, rather than descriptions of practical incompetence such as errors in reading a thermometer
- Suggestions for further work must be linked to the investigation in the question. Choose sensible extensions, without testing completely different hypotheses
- Try to give several suggestions to attain all the marks available.

Question 1

Question 1 on W2 is designed to test your ability to **organise and interpret experimental data**. You may be presented with an extract from a laboratory notebook, showing raw data. You could be asked to

- tabulate and organise the data
- plot a graph of the data
- suggest a suitable statistical test which could be used to analyse the data
- draw conclusions.

Tabulation of the data may require you to prepare a **tally chart**, or to prepare a table of derived data, such as **percentage changes**, or **means** of several replicated readings

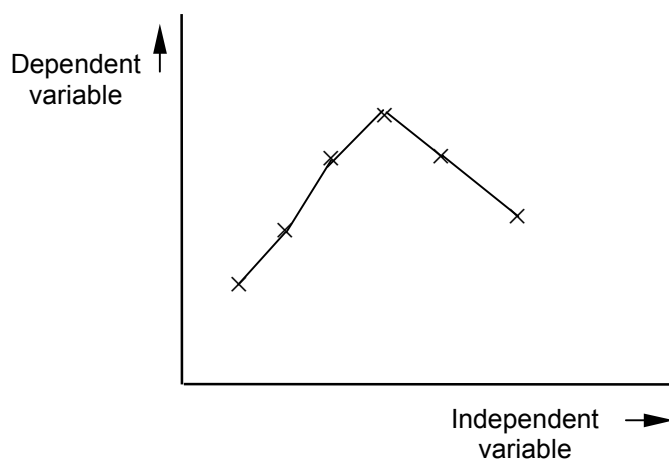
When plotting a graph, consider whether a **line graph** or **bar graph** is more appropriate.

Choosing which type of graph to plot

There are a number of different types of graphs, including

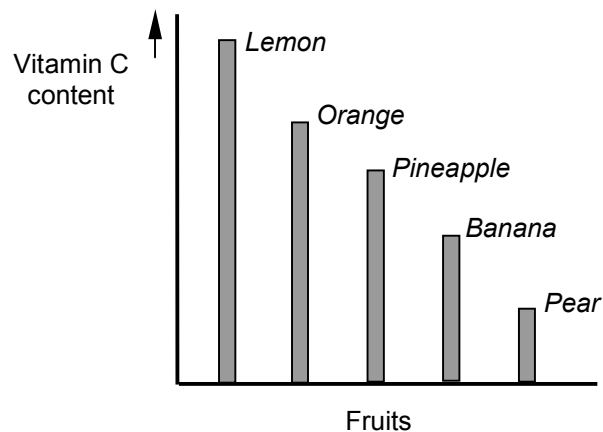
- ☐ line graphs
- ☐ bar charts
- ☐ histograms.

Line graphs are used to show the relationship between two variables, such as the effect of temperature on enzyme activity, or changes in the concentration of blood glucose against time.

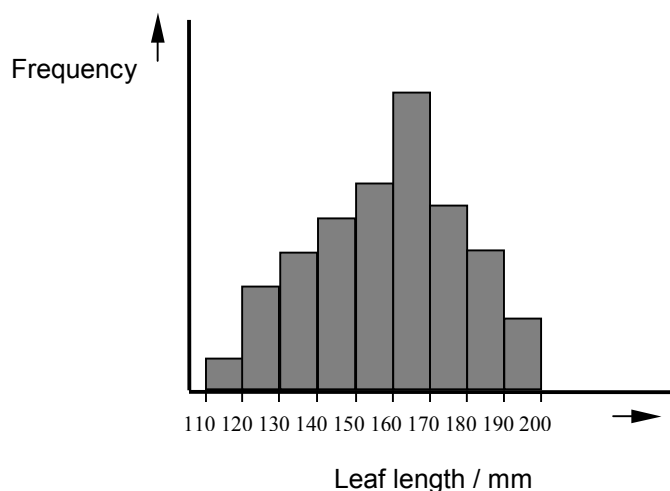


Hint: plot the points carefully, using crosses or a dot in a circle

Bar charts are used when one of the variables is not numerical. For example, a bar chart could be plotted to show the concentration of vitamin C (numerical) in different types of fruits (not numerical). By convention, the bars are plotted so that they do not touch. The bars can be arranged in any order, but it may be more appropriate to arrange them in order from the highest to the lowest, rather than in a random order.



Histograms are used to show frequency distributions with continuous data, for example, to show the frequency of leaves of different lengths (continuous data). When plotting a histogram, the blocks should touch.



To plot a histogram, the data must be *grouped*, for example, the leaves could be organised into classes of lengths:

110 to 119 mm
120 to 129 mm
130 to 139 mm
etc.

Name the type of graph which would be most suitable to show each of the following:

- ☐ The percentage of people in a population of blood groups A, B, AB and O
.....
- ☐ The relationship between the rate of reaction and substrate concentration for an enzyme-catalysed reaction
.....
- ☐ The relationship between body temperature of a lizard and environmental temperature
.....
- ☐ Changes in the pressure in the left ventricle during the cardiac cycle
.....
- ☐ The percentage frequencies of different species of flowering plants found in a meadow
.....
- ☐ Reaction times to a visual stimulus measured in 100 different people
.....
- ☐ The population densities of five major regions of the world in the year 2000
.....
- ☐ The numbers of different species of birds found in woodland
.....
- ☐ The effect of light intensity on the rate of photosynthesis
.....

❑ Example 1.1

The diagram overleaf shows a sample of 20 leaves collected at random from a tree growing in full sunlight. Measure the maximum width of each leaf, and complete the tally chart below.

Leaf widths / mm class intervals	Tally	Total
10 - 12		
13 - 15		
16 - 18		
19 - 21		
22 - 24		

To complete the tally chart, measure each leaf then draw a line in the appropriate box. For example, if a leaf measures 14 mm, draw a line in the 13 – 15 mm box. Continue like this until you have measured all the leaves, then write in the total for each class interval.

How to complete a tally chart

Measure each leaf and draw a line in the appropriate box.

Write the total for each class interval here

Leaf widths / mm class intervals	Tally	Total
10 - 12	<i>II</i>	2
13 - 15	<i>III I</i>	6

This is sometimes called 'gate scoring'

etc!



Use the grouped data to plot a histogram to show the distribution of widths in this sample of leaves.

Suppose that a second sample of 20 leaves was collected from the same species of tree growing in a shaded position. Name a suitable statistical test to investigate whether there was a significant difference between the mean widths of the two samples.

Answer

Hints on the choice of a suitable statistical test

The types of statistical test are likely to involve **differences**, **correlations**, or **goodness of fit**.

Differences

- Mann Whitney U test
- Wilcoxon matched pairs test
- Student's *t*-test

Correlations

- Spearman rank correlation test
- Regression analysis

Goodness of fit

- Chi-squared (χ^2) test

For advice on the choice of statistical tests, with worked examples, see:

Tools, Techniques and Assessment in Biology – A course guide for students and Teachers [ISBN 0 17 448273 6]

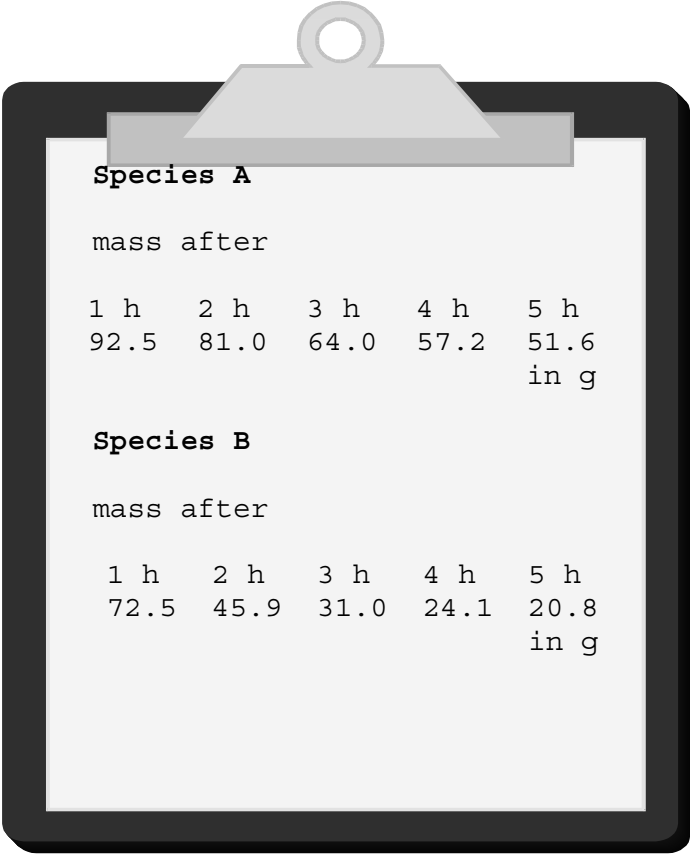
You are not expected to remember these statistical tests in detail, but you should be able to suggest which test would be appropriate for a given investigation and appreciate how to interpret the test, in terms of confidence limits.

❑ Example 1.2

A group of students carried out an ecological investigation into the distribution of two species of trees in a wood. They found that one species (A) was more common on dry, well-drained soils, whilst the other species (B) was more common where the soil was wet and poorly drained. They produced the hypothesis that one reason for this was that the leaves of species B lost water vapour more quickly than the leaves of species A.

To test this hypothesis they collected a sample of 100 g of leaves from species A and a 100 g sample of leaves from species B. They then hung each sample on a line to dry in identical conditions in the laboratory. Both samples were then reweighed each hour for five hours.

An extract from the records of this investigation is given below.



Species A					
mass after					
1 h	2 h	3 h	4 h	5 h	
92.5	81.0	64.0	57.2	51.6	in g

Species B					
mass after					
1 h	2 h	3 h	4 h	5 h	
72.5	45.9	31.0	24.1	20.8	in g

- (a) (i) Calculate the loss in mass compared to the original mass, for each sample every hour. Then organise the data in a suitable table so that the loss in mass for each sample can be compared. (4)
- (ii) Use the data in your table to present this information in a suitable graphical form. (4)
- (b) What conclusions can you draw from this investigation? (2)

(Total 10 marks)

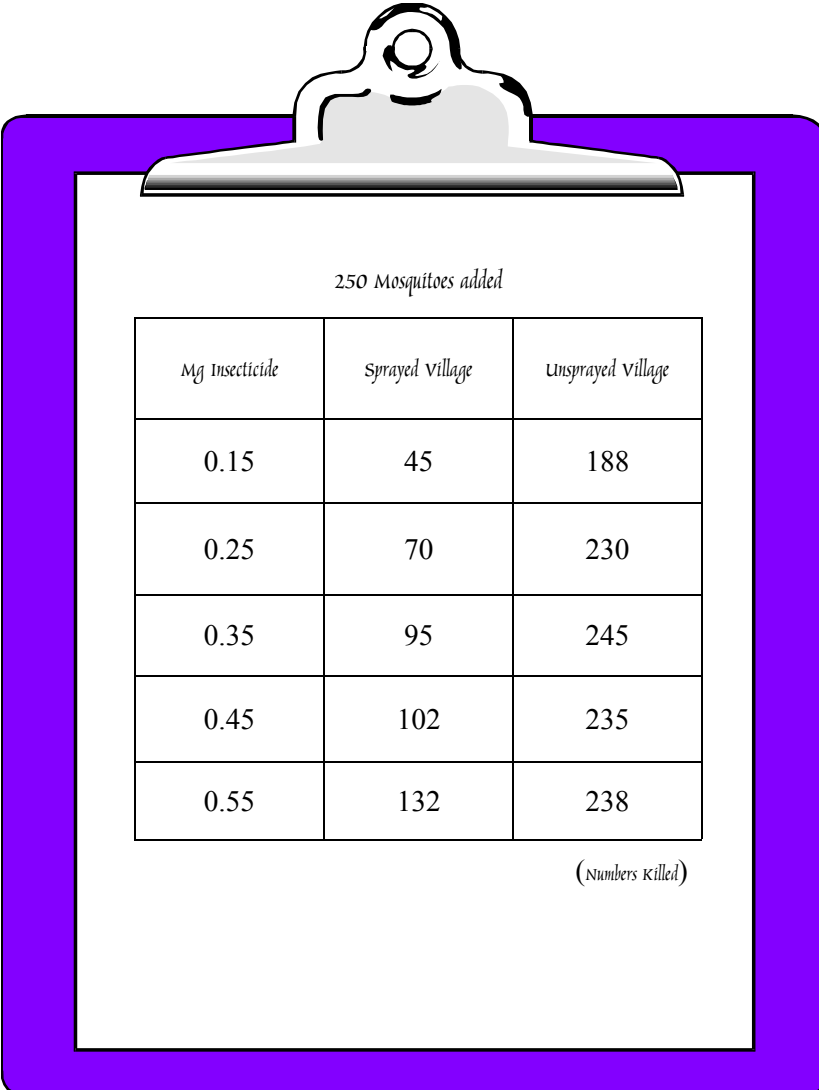
❑ Example 1.3

A long term investigation was carried out to test the hypothesis that populations of mosquitoes which are regularly sprayed with insecticide will become resistant to it.

A large sample of mosquitoes was randomly collected from a village which had been regularly sprayed with insecticide. 250 of these mosquitoes were placed in a sealed container and a measured dose of insecticide was added. After one hour, the total number of mosquitoes killed by this treatment was counted. The experiment was then repeated four times using fresh batches of mosquitoes and increasing the dose of insecticide each time.

A second sample of mosquitoes of the same species was then collected from a village which had never been sprayed with insecticide and tested in the same way.

An extract from the records of this investigation is shown below.



250 Mosquitoes added

<i>Mg Insecticide</i>	<i>Sprayed Village</i>	<i>Unsprayed Village</i>
0.15	45	188
0.25	70	230
0.35	95	245
0.45	102	235
0.55	132	238

(Numbers Killed)

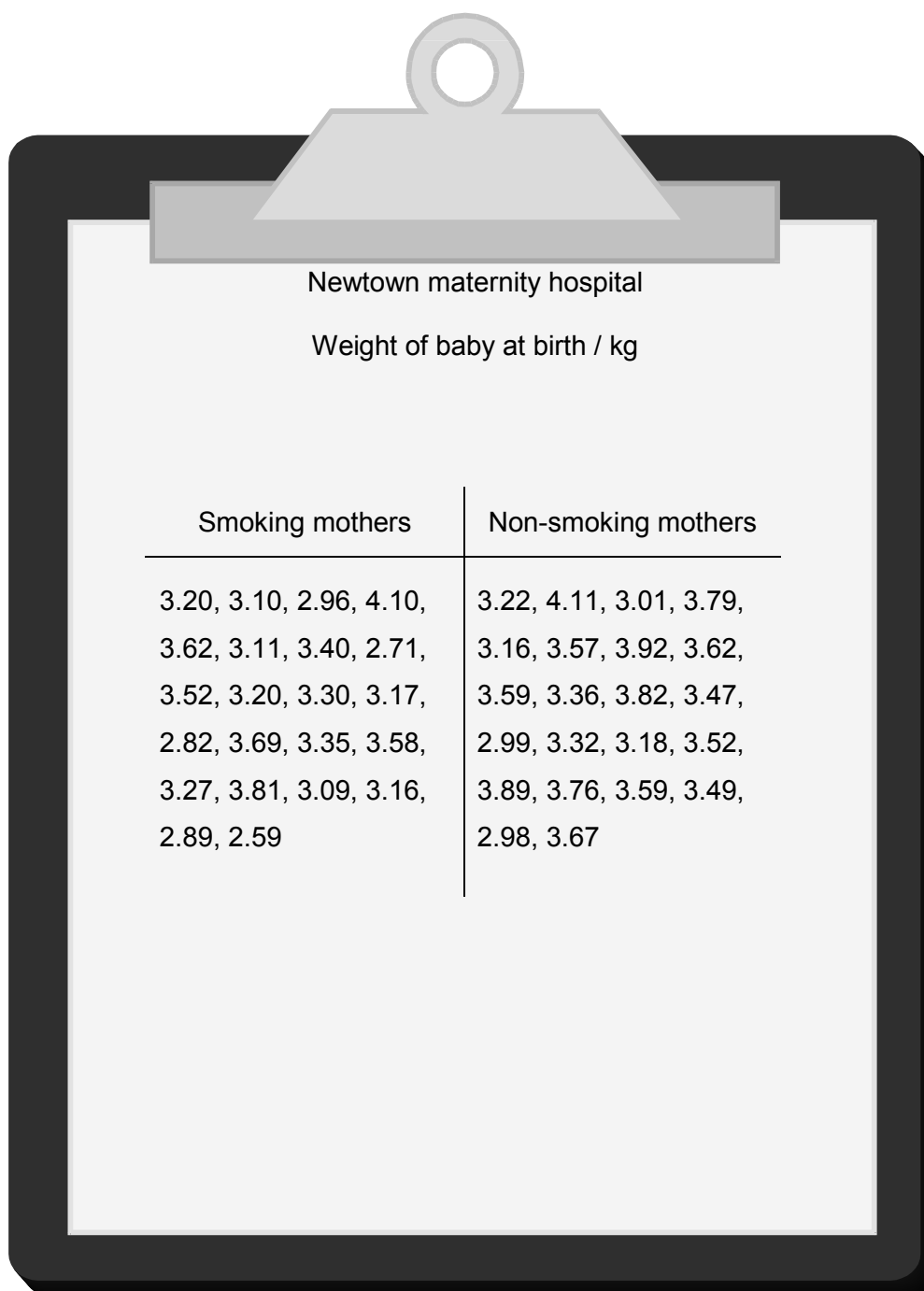
- (a) Calculate the percentage of mosquitoes killed by the different insecticide treatments for each village. Then organise the data into a suitable table so that the effect of increasing insecticide concentration for each village can be compared. (4)
- (b) Use the data in the table to present this information in a suitable graphical form. (4)
- (c) What conclusions can you draw from the results of this investigation? (2)

(Total 10 marks)

Now try the following examples. In each case, organise and tabulate the data so that suitable comparisons may be made. Use your tabulated data to present the information in an appropriate graphical form. You need to consider whether to use a **histogram** (for grouped data) or a **line graph** (to show the relationship between two variables).

What conclusions can you draw from the data?

❑ **Example 1.4 Smoking and non-smoking mothers and birth weight of babies**

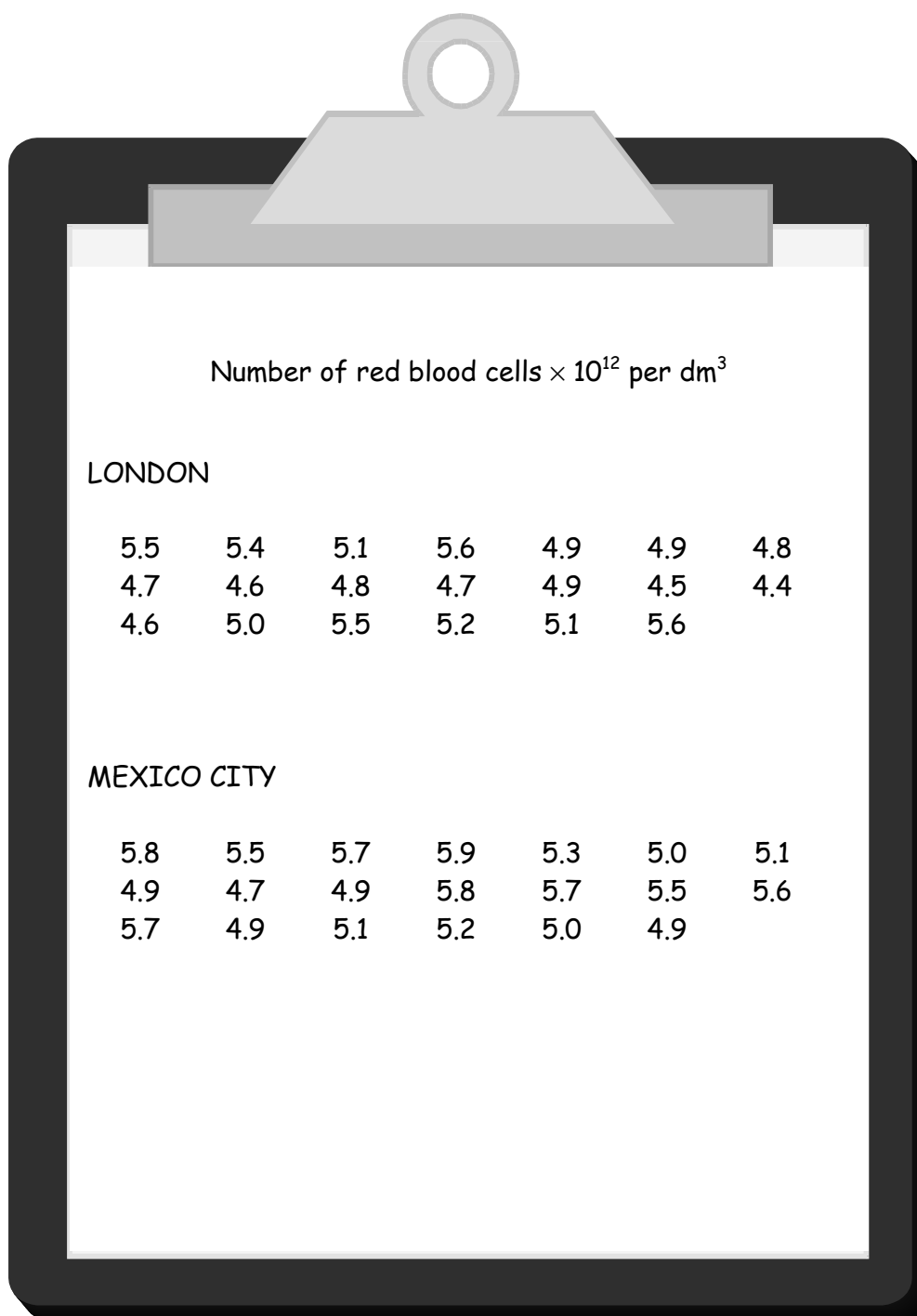


Newtown maternity hospital

Weight of baby at birth / kg

Smoking mothers	Non-smoking mothers
3.20, 3.10, 2.96, 4.10,	3.22, 4.11, 3.01, 3.79,
3.62, 3.11, 3.40, 2.71,	3.16, 3.57, 3.92, 3.62,
3.52, 3.20, 3.30, 3.17,	3.59, 3.36, 3.82, 3.47,
2.82, 3.69, 3.35, 3.58,	2.99, 3.32, 3.18, 3.52,
3.27, 3.81, 3.09, 3.16,	3.89, 3.76, 3.59, 3.49,
2.89, 2.59	2.98, 3.67

❑ **Example 1.5** Effect of altitude on numbers of red blood cells



Clipboard graphic with a silver clip at the top and a black border. The content is on a white sheet of paper.

Number of red blood cells $\times 10^{12}$ per dm^3						
LONDON						
5.5	5.4	5.1	5.6	4.9	4.9	4.8
4.7	4.6	4.8	4.7	4.9	4.5	4.4
4.6	5.0	5.5	5.2	5.1	5.6	
MEXICO CITY						
5.8	5.5	5.7	5.9	5.3	5.0	5.1
4.9	4.7	4.9	5.8	5.7	5.5	5.6
5.7	4.9	5.1	5.2	5.0	4.9	

❑ Example 1.6 Effect of training on pulse rates in trained and untrained swimmers

Some students carried out an investigation into the effect of sports training on the increase in pulse rate when running. They chose volunteers from the school swimming team who trained regularly and four volunteers who did not do any sports training. The volunteers in both groups were females of the same age and in good health.

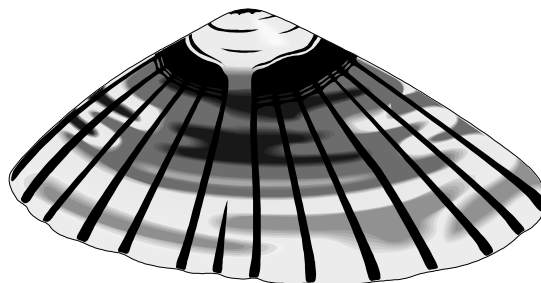
In the investigation, all the volunteers rested for two minutes and then their pulse rates were measured. They were then asked to run for one minute on a mechanical treadmill at a constant speed and their pulse rates were measured again. Each volunteer was tested at four different running speeds.

An extract from the experimental records is shown below.

Treadmill speed											
Trained swimmers						Untrained					
	Rest	1ms ⁻¹	2ms ⁻¹	3ms ⁻¹	4ms ⁻¹		Rest	1ms ⁻¹	2ms ⁻¹	3ms ⁻¹	4ms ⁻¹
Mary	56	84	115	150	183	Tracey	70	95	135	170	198
Jo	57	86	120	154	186	Carol	64	87	134	177	197
Faiza	54	84	126	155	182	Ann	64	101	132	169	199
Lynn	53	90	124	156	185	Kirsty	67	104	137	168	195

❑ Example 1.7 Comparison of limpets on exposed and sheltered shores

The diagram shows a limpet. Limpets are cone-shaped molluscs which are found attached to rocks on many seashores.



Some students carried out an investigation to compare the shape of limpets on a sheltered shore with that on a shore exposed to the action of waves. They measured the height and overall length of 15 limpets on each shore. They then used the ratio of height to length to describe the overall shape of the limpets. An extract from their field records is shown below.

<i>Limpet Investigation</i>	
<i>Sheltered Shore:</i>	
H/L Ratios	0.58, 0.57, 0.55, 0.64,
	0.65, 0.60, 0.57, 0.58, 0.59, 0.57, 0.54, 0.58
	0.58, 0.58, 0.56
<i>Exposed Shore:</i>	
H/L Ratios	0.55, 0.53, 0.45, 0.52,
	0.39, 0.53, 0.59, 0.54, 0.49, 0.47, 0.51, 0.51
	0.43, 0.51, 0.48

Mark Schemes for Questions 1 1, 1.2 and 1.3

Example 1.1

A *t*-test would be appropriate to compare the means of two distributions, assuming that the data are normally distributed.

Example 1.2

- (a)(i) suitable tabular format;
correctly labelled rows and columns (units in headings only) ;
loss in mass correct for species A / % loss in mass ;
loss in mass correct for species B ;
- (a)(ii) suitable axes labelled (must include zero time) ;
plot A correct ;
plot B correct ;
line graphs, correct key ;
- (b) species B lose water / mass more (rapidly) than species A ;
both rates of water / mass loss decreases with time / overall pattern of water loss is similar ;

Example 1.3

- (a)(i) percentages with spraying correct ;
percentages without spraying correct ;
tabular format ;
correct rows and columns, units in heading ;

Example of table

Insecticide dose / mg	0.15	0.25	0.35	0.45	0.55
Village with spraying % killed	18	28	38	40.8 (41)	52.8 (53)
Village with no spraying % killed	75.2 (75)	92	98	94	95.2 (95)

- (a)(ii) suitable axes labelled with units and correct scales ;
sprayed data correctly plotted ;
unsprayed data correctly plotted ;
line graph correctly keyed, accurately drawn and no extrapolation ;
- (b) sprayed village mosquitoes are more resistant / converse ;
% of sprayed village mosquitoes killed increases with increasing insecticide concentration ;
% of unsprayed village mosquitoes killed reaches maximum at *figure to match maximum from graph drawn, 0.3 to 0.35* ;

Question 2

Question 2 usually includes the words '*Plan an investigation which you personally could carry out to test the hypothesis that.....*'

The answer is under the following headings:

- (a) Plan of the investigation to be carried out (9 marks)
- (b) Recording of the raw data measurements, presentation of the results and methods of data analysis (7 marks)
- (c) Possible limitations of your method and an indication of further work that could be undertaken (5 marks)

Read through the following two examples (2.1 and 2.2) carefully, and then study the mark schemes which follow each question. You may find it helpful to make a note of *general points*, which appear in the mark schemes, particularly for the planning and presentation of results.

□ Example 2.1

Many fruits and seeds contain germination inhibitors, which delay germination until the inhibitors have been washed away by rain or become inactivated. In tomatoes, the inhibitors delay germination until the fleshy fruit has rotted away thus releasing the seeds.

It has been suggested that, in tomatoes, the inhibitor of seed germination is present in the fleshy tissues of the fruit rather than in the seed coats.

Plan an investigation, which you personally could carry out to test this hypothesis.

Mark scheme

- (a)
 - use seeds from same variety ;
 - reference to oxygen / air / water ;
 - keep at a suitable temperature (15 °C to 20 °C) ;
 - keep conditions for germination constant ;
 - use a suitable substrate for growth such as soil, compost, cotton wool etc. ;
 - remove seeds from a ripe tomato ;
 - count number germinated ;
 - wash tomato flesh in running water ;
 - check for germination in thoroughly washed seeds ;
 - use a large number of seeds in each batch ;
 - suitable replication ;
 - calculate rate of germination as percentage ;

Note: In mark schemes, a semi-colon (;) indicates separate marking points / indicates alternative marking points.

- (b)
- tabulate results ;
 - column for unwashed seeds ;
 - column for washed seeds ;
 - column for percentage germination ;
 - extra boxes for repeats ;
 - bar charts ;
 - correct axes ;
 - calculate means ;
 - calculate standard deviation ;
 - apply *t*-test ;
 - reference to interpretation of data (i.e. 5 % significance level) ;
- (c)
- tomatoes could vary in ripeness ;
 - initial treatments could vary ;
 - assumes inhibitor is water soluble ;
 - test effects on different strains ;
 - test for seasonal variation ;
 - test effects of extracts alone ;

❑ **Example 2.2**

Sugar beet is a root crop often grown as a commercial source of sugar. It has been suggested that the yield of the crop can be affected if weeds are allowed to grow amongst the rows of sugar beet plants.

Plan an investigation which you personally could carry out to test the hypothesis that the growth of weeds will reduce the yield of sugar beet.

Mark scheme

- (a)
- use one variety / species of beet ;
 - reference to uniform conditions / sterilised soil / compost ;
 - several plots of each ;
 - same size / area ;
 - plant beet seeds in each tray / plot ;
 - same density / distance apart ;
 - sow weed seeds / allow weeds to grow in one plot / half the plots ;
 - reference to control of weed density ;
 - method of keeping plots weed-free described ;
 - allow to grow for stated times ;
 - suitable method for assessing yield, e.g. fresh mass / diameter of root / sugar content ;
- (b)
- suitable table of raw data ;
 - correct headings of rows and columns ;
 - plot bar chart to compare yields ;
 - suitable axes stated ;
 - null hypothesis stated ;
 - application of *t*-test ;
 - comment on 5 % significance level ;

(c)

- possible uncontrolled variables ;
- fresh mass variable ;
- mass (or diameter) not necessarily related to sugar yield ;
- method of weed control might affect yield ;
- test effect of different weed species ;
- test other methods of weed control
- test different varieties of beet ;
- test effects of weeds in different parts of growth cycle ;
- test effects of varying weed density ;
- test effect of varying beet density ;

Now try the following examples.

❑ Example 2.3 Biology and Biology (Human)

Earthworms (*Lumbricus terrestris*) are annelid worms which are commonly found in a wide range of soil types.

Plan an investigation which you personally could carry out to test the hypothesis that more earthworms are found in the soil in woodland than in open grassland.

❑ Example 2.4 Biology and Biology (Human)

Aphids are insect pests of glasshouse crops, such as green peppers (*Capsicum* sp.)

Plan an investigation you personally could carry out to test the hypothesis that the presence of aphids significantly reduces the yield of green peppers grown in a glasshouse.

❑ Example 2.5 Biology and Biology (Human)

The coconut leaf roller, *Hedylepta blackburni*, is a moth whose caterpillars feed on the leaves of coconut palms and bananas.

One method of controlling such pests is to trap the male moths on a sticky surface using a chemical attractant called a pheromone. This prevents the moths from breeding.

Plan an investigation to determine the best position in which to place these traps to protect coconut or banana plants in a plantation.

❑ Example 2.6 Biology and Biology (Human)

Many areas of land around former mine workings are polluted with metals such as copper. As a means of reclaiming such areas, plants, which are able to grow in the presence of copper, can be planted.

You are provided with seeds collected from the grass *Agrostis tenuis*, which was found to be growing in small numbers in an area polluted with copper.

Describe how you would select and breed a variety of grass suitable for use in the reclamation of land polluted with copper.

❑ **Example 2.6 Biology (Human) only**

Synaptic transmissions can be affected by various drugs such as caffeine. It has been suggested that caffeine acts as a stimulant and so reduces the time taken to respond to a visual stimulus.

Plan an investigation, which you could personally carry out, to test this hypothesis.

❑ **Example 2.7 Biology (Human) only**

It has been suggested that learning is affected by background noise.

Plan an investigation, which you could personally carry out, to test the hypothesis that background noise reduces the ability to learn.

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