

# Teaching AS Biology Practical Skills

in preparation for 9700 papers 31 and 32

**Your attention is drawn to the section on Risk Assessment on page 17 of the Introduction to this booklet, and to the hazards indicated in Appendices 1 and 2. While all effort has been made to ensure that appropriate safety indications are given, CIE accepts no responsibility for the safety of these experiments and it is the responsibility of the teacher to carry out a full risk assessment for each experiment undertaken, in accordance with local rules and regulations. Hazard data sheets should be available from your suppliers.**

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## Introduction

You may have been teaching AS and A level biology for many years or perhaps you are new to the game. Whatever the case may be, you will be keen to ensure that you prepare your students as effectively as possible for their examinations. The use of a well-structured scheme of practical work will certainly help in this ambition. However it can do so much more. Scientists who are thoroughly trained and experienced in practical skills, will have a 'feel' for the subject and a confidence in their own abilities that is far greater above those with a purely theoretical background. It is true that there are branches of biology that might be described as purely theoretical but they are in the minority. Essentially, biology is a practical subject and we owe it to our students to ensure that those who pursue science further have the necessary basic practical skills to take forward into their future careers. Furthermore, the basic skills of planning, analysis and evaluation will be of great value to those who pursue non-science careers.

### Why should I read this booklet?

Some of you may be wondering why you should need a booklet like this. If your practical skills are of a high order and you feel confident teaching these skills to others, you probably don't need it; but you might find some of the exercises described in the appendices useful. However, if you are like the majority of us, a little help and support is likely to be appreciated. This booklet aims to provide at least some of this support.

It is designed for the teacher rather than for the student. Its objective is to provide a framework within which the practical skills of teachers can develop and grow. Experience shows that as a teacher's practical skills grow, so too do the confidence to teach such skills and the time that you will be prepared to spend on teaching practical work.

### How much teaching time should I allocate to practical work?

The syllabus stipulates that at least 20% of teaching time should be allocated to practical work. This is in addition to any time the teacher chooses to use for practical demonstrations to illustrate the theory syllabus. This emphasis on practical work is not misplaced. Consider the weighting given to assessment objectives in the syllabus: 24% is allocated to experimental skills and investigations and 30% is allocated to handling, applying and evaluating information. Taken together, 55% of the total award is related to a students' ability to interpret data, understand how this has been obtained, recognise limitations and suggest explanations; all of which lend themselves to investigative work involving practical experience. If the specific practical papers are considered in isolation, they still represent 23% of the AS and 24% of the A Level award.

In planning a curriculum, teachers should therefore expect to build in time for developing practical skills. If, for example, the time allowed is 5 hours per week over 35 weeks, then a minimum of 1 hour per week should be built into the plan, so that over the year, a minimum of 35 hours is made available. Bearing in mind the emphasis on assessment objectives that related to information handling and problem solving, a minimum of 2 hours per week might be more appropriate, which at 40% of the time is still less than the overall weighting for these assessment objectives.

### Can I use the practicals in these booklets in a different order?

It is assumed in these booklets that for A level candidates, the AS work will be taught in the first year of the course, with the A2 work being covered in the second year. If the linear A Level assessment route is used, care should be taken with regard to in the

order in which practical exercises are used, as the skills practiced in these booklet are hierarchical in nature, i.e. the basic skills established in the AS booklet are extended and developed in the A2 Level booklet. Thus, students will need to have practiced basic skills using AS exercises before using these skills to tackle more demanding A Level exercises.

The exercises in these booklets are given in syllabus order. A teacher may well decide to use a different teaching sequence, but the point made above, regarding AS/A2 exercises, still applies.

### **What resources will I need?**

For a practical course in A-level Biology to be successful, it is not necessary to provide sophisticated equipment. Some of the more advanced practicals in these booklets may require less easily obtainable equipment, but the vast majority can be performed using the basic equipment and materials in the lab. Alternative 'low-tech' exercises are also provided where possible.

A list of the basic resources required for assessment may be found in the syllabus. A more detailed list may be found in the booklet '*CIE Planning For Practical Science in Secondary Schools*', Appendix B.

### **Is there a limit to the class size?**

It is true that there is a limit to the class size that is manageable in a laboratory situation, particularly when students may be moving about. The actual size may be determined by the size of the room, but as a general guide, 15 - 20 students is the maximum that one person can reasonably manage, both for safety reasons and so that adequate support can be given to each student. Larger numbers can more easily and safely be accommodated with input from another person with appropriate qualifications / experience or splitting the class into two groups for practical lessons.

### **Why should I teach my students practical skills?**

Although this section is likely to be read once only, it is arguably the most important; for, if it convinces readers that practical work is an essential part of biology as a science and underpins the whole teaching programme, the aim of publishing this booklet will have been achieved.

### **Points to consider**

- It's fun! The majority of students thoroughly enjoy practical work. The passion that many scientists have for their subject grew out of their experiences in the practical classes. Students who enjoy what they are doing are likely to carry this enthusiasm with them and so be better motivated.
- Learning is enhanced by participation as students tend to remember activities they have performed more easily, thus benefiting their long-term understanding of the subject. Students who simply memorise and recall facts find it difficult to apply their knowledge to an unfamiliar context. Experiencing and using practical skills helps develop the ability to use information in a variety of ways, thus enabling students to apply their knowledge and understanding more readily.
- The integration of practical work into the teaching programme quite simply brings the theory to life. Teachers often hear comments from students such as "I'm glad we did that practical because I can see what the book means now." and "It's much better doing it than talking about it."

- Chemistry, physics and biology are by their very nature, practical subjects – both historically and in the modern world. The majority of students who enter careers in science need to employ at least basic practical skills at some time in their career. For all students, whether they regard themselves as scientists or non-scientists, the skills that they develop by doing practical work, hand-eye coordination skills, communication, numeracy and problem solving skills, will prove to be useful transferable skills throughout their future life.
- A practical course develops many cross-curricular skills including literacy, numeracy, ICT and communication skills. It develops the ability to work both in groups and independently and with confidence. It enhances critical thinking skills and it requires students to make judgements and decisions based on evidence, some of which may well be incomplete or flawed. It helps to make students more self-reliant and less dependent on information provided by the teacher.
- The skills developed are of continued use in a changing scientific world. While technological advances have changed the nature of practical procedures, the investigative nature of practical science is unchanged. The processes of observation, hypothesis formation, testing, analysis of results and drawing conclusions will always be the processes of investigative science. The ability to keep an open mind in the interpretation of data and develop an appreciation of scientific integrity is of great value both in science and non-science careers.
- Practical work is not always easy and persistence is required for skills and confidence to grow. Students often relish this challenge and develop a certain pride in a job well done.
- The more experience students have of a variety of practical skills, the better equipped they will be to perform well in the practical exams, both in terms of skills and confidence. While it could be argued that the required skills could be developed for papers 31 and 32 simply by practising past-papers, the all-round confidence in practical ability will be greatly enhanced by a wider experience. Similarly for paper 5, while it might be argued that planning, analysis and evaluation could be taught theoretically, without hands-on experience of manipulating their own data, putting their plans into action and evaluating their own procedures and results, students will find this section difficult and will be at a distinct disadvantage in the examination. Those students who can draw on personal experience, and so are able to picture themselves performing the procedure they are describing, or recall analysing their own results from a similar experiment are much more likely to perform well than those with limited practical skills.

## What are the practical skills required by this course?

This course addresses seven practical skills that contribute to the overall understanding of scientific methodology. In a scientific investigation these would be applied in the following sequence.

- 1 Planning the experiment**
- 2 Setting up / manipulating apparatus**
- 3 Making measurements and observations**
- 4 Recording and presenting observations and data**
- 5 Analysing data and drawing conclusions**
- 6 Evaluating procedures**
- 7 Evaluating conclusions**

The syllabus shows how these seven skills are assessed and the structure is common to all three sciences. The emphasis of the AS syllabus is on developing an understanding and practice of scientific procedures, the collection of data, analysis and drawing conclusions. It also starts to develop critical evaluation of procedures by suggesting improvements to experimental procedures. In general students find the performance of practical procedures and the collection of data more accessible than analysis, whilst evaluation is least readily accessed. To enable access to these more demanding skills, students need to understand why an experimental procedure is carried out in a particular way so that they can recognise sources of error or limitations which could affect the reliability of their results. Students will not be able to evaluate until they can critically review a practical procedure.

The A2 syllabus builds upon the skills developed in AS and its emphasis is on the higher level skills of planning, analysis and evaluating. In order to plan effectively, students need to be able to evaluate procedures and critically assess results. This is best achieved by the performance of practical exercises starting in AS with relatively straightforward and familiar contexts and developed in A2 by the use of more complex procedures and less familiar contexts. Data analysis again develops from AS into more complex treatments so that students need to be given opportunities to gather suitable data and perform the appropriate manipulations. The evaluation of conclusions and assessing procedures are very high order skills. Students who have not had sufficient opportunity to plan and trial their own investigations will find these skills difficult. Students are not expected to be able to plan perfectly, but to recognise weaknesses and make reasonable suggestions for improvement. The best learning tool to develop these skills is to devise a plan, carry out the investigation and then assess how well the planned procedure worked. The syllabus gives detailed guidance on the expected skills and learning outcomes.

In summary, as the syllabus clearly shows, skills 2-6 listed above will be assessed at AS level in papers 31 and 32. Skills 1 and 7 will only be assessed at A level in paper 5, which will also take skills 5 and 6 to a higher level.

The above list shows the seven skills in the order in which they would be used in an extended investigation. It is not suggested, nor would it be wise, to teach these skills in this order. Students who are new to practical work will initially lack the basic manipulative skills, and the confidence to use them. It would seem sensible, therefore, to start practical training with skill 2, initially with very simple tasks and paying attention to the establishment of safe working practices.

Once a measure of confidence in their manual dexterity has been established, AS students can move on to exercises that require skills 3 and 4 to be included. Extensive

experience in carrying out practical procedures allows students to gain awareness of appropriate quantities and become more organised in time management and the recording of data as it is collected.

It is likely that skill 6, Evaluating Procedures, will be the most difficult to learn at AS level. Critical self-analysis does not come easily to many people. 'My experiment worked well' is a frequent and inappropriate response. If students are to master this skill, they need to develop an appreciation of reliability and accuracy inherent in the equipment and procedure they are using. Only then will they be able to identify anomalous results, or results which fall outside of the 'range of uncertainty' intrinsic in the choice of apparatus used and so are considered to be inaccurate. Exercises with less reliable/accurate outcomes can be used to provide more scope for the evaluation of procedural, technique or apparatus errors.

Planning is arguably the most demanding of the seven skills. For it to be effective, students need to be very well grounded in skills 2-6, so that they can anticipate the different stages involved in the task, and can provide the level of detail required. It is for this reason that planning skills are not assessed at AS level but form part of the A2 assessment in Paper 5. Unless students use apparatus they do not develop an understanding of how it works and the sort of measurements that can be made using particular sorts of apparatus. Candidates cannot be taught to plan experiments effectively unless, on a number of occasions, they are required:

- to plan an experiment;
- to perform the experiment according to their plan;
- to evaluate what they have done.

The evaluation of conclusions, skill 7, is done by comparison of the outcome of an exercise with the predicted outcome, and so is also an A2 skill. It should be taught and practised as part of the planning exercises.

### Summary of each of the seven skills

Full details of the requirements for each of these skills may be found in the **Practical Assessment** section of the syllabus. What follows below is a brief summary of the skills involved.

#### 1 Planning

- **Defining the problem**  
Students should be able to use information provided about the aims of the investigation, or experiment, to identify the key variables. They should use their knowledge and understanding of the topic under consideration to make a quantitative, testable, prediction of the likely outcome of the experiment.
- **Methods**  
The proposed experimental procedure should be workable. It should, given that the apparatus is assembled appropriately, allow data to be collected without undue difficulty. There should be a description, including diagrams, of how the experiment should be performed and how the key variables are to be controlled. Equipment, of a level of precision appropriate for the measurements to be made, and quantities to be used should be specified. The use of control experiments should be considered.
- **Risk assessment**  
Candidates should be able to carry out a simple risk assessment of their plan, identifying areas of risk and suggesting suitable safety precautions to be taken.

- **Planning for analysis, conclusions and evaluation**

Students should be able to describe the main steps by which their results would be analysed in order that valid conclusions might be drawn. This may well include the generation of a results table and the proposal of graphical methods to analyse data. Also, they should propose a scheme for the interpretation and evaluation of the results themselves, and of the experimental procedure employed in obtaining those results. There should be an indication of how the outcomes of the experiment would be compared with the original hypothesis.

## 2 Setting up / manipulating apparatus

It is important that students are allowed sufficient time and opportunity to develop their manipulative skills to the point where they are confident in their approach to experimental science. They must be able to follow instructions, whether given verbally, in writing or diagrammatically, and so be able to set up and use the apparatus for experiments correctly.

## 3 Making measurements and observations

- **Measuring/observing**

Whilst successfully manipulating the experimental apparatus, it is crucial that students are able to make measurements with accuracy and/or to make observations with clarity and discrimination. Accurate readings of meters or burettes and precise descriptions of colour changes and precipitates will make it much easier for students to draw valid conclusions, as well as scoring more highly in the test.

- **Deciding on what measurements/observations to make**

Time management is important, and so students should be able to make simple decisions on the number and the range of tests, measurements and observations that can be made in the time available. For example, if the results of the first two titrations are in good agreement, there is no need to carry out a third.

Students need to be able to make informed decisions regarding the appropriate distribution of measurements within the selected range, which may not always be uniform, and the timing of measurements made within the experimental cycle. They should also be able to identify when repeated measurements or observations are appropriate.

The strategies required for identifying and dealing with results which appear anomalous should be practised.

## 4 Recording and presenting observations and data

An essential, but frequently undervalued, aspect of any experimental procedure is the communicating of the results of the procedure to others in a manner that is clear, complete and unambiguous. It is vital that students are well practised in this area.

- **The contents of the results table**

The layout and contents of a results table, whether it is for recording numerical data or observations, should be decided before the experiment is performed. 'Making it up as you go along' often results in tables that are difficult to follow and don't make the best use of space. Space should be allocated within the table for any manipulation of the data that will be required.



- **The column headings in a results table**  
The heading of each column must be clear and unambiguous. In columns which are to contain numerical data, the heading must include both the quantity being measured and the units in which the measurement is made. The manner in which this information is given should conform to 'accepted practice'.
- **The level of precision of recorded data**  
It is important that all data in a given column is recorded to the same level of precision, and that this level of precision is appropriate for the measuring instrument being used.
- **Display of calculations and reasoning**  
Where calculations are done as part of the analysis, all steps of the calculations must be displayed so that thought processes involved in reaching the conclusion are clear to a reader. Similarly, where conclusions are drawn from observational data, the key steps in reaching the conclusions should be reported and should be clear, sequential and easy to follow.
- **Significant figures**  
Students should be aware that the number of significant figures to which the answer is expressed shows the precision of a measured quantity. Therefore, great care should be taken with regard to the number of significant figures quoted in a calculated value. The general rule is to use the same number of significant figures as (or at most one more than) that of the least precisely measured quantity.
- **Data layout**  
Students should be able to make simple decisions concerning how best to present the data they have obtained, whether this is in the form of tabulated data or as a graph. When plotting graphs they should be able to follow best practice guidelines for choosing suitable axis scales, plotting points and drawing curves or lines of best fit. In drawing tables they should be able to construct a table to give adequate space for recording data or observations.

## 5 Analysing data and drawing conclusions

This skill requires students to apply their understanding of underlying theory to an experimental situation. It is a higher-level skill and so makes a greater demand on a student's basic understanding of the biology involved. Even when that understanding is present, however, many students still struggle. The presentation of a clear, lucid, watertight argument does not come naturally to most people and so much practice in this area is recommended.

- **Interpretation of data or observations**  
Once data has been presented in the best form for analysis of the results of the experiment, the student should be able to describe and summarise any patterns or trends shown and the key points of a set of observations. Further values such as the gradient of a graph may be calculated or an unknown value found, for example from the intercept of a graph.
- **Errors**  
Students should be used to looking at an experiment, assessing the relative importance of errors and where appropriate, expressing these numerically. Students should be aware of two kinds of error.
  - i The 'error' that is intrinsic in the use of a particular piece of equipment. Although we refer to this as an equipment error, we really

mean that there is a 'range of uncertainty' associated with measurements made with that piece of equipment. This uncertainty will be present no matter how skilled the operator might be.

- ii Experimental error, which is a direct consequence of the level of competence of the operator or of the effectiveness of the experimental procedure.

- **Conclusions**

Students should learn to use evidence to support a given hypothesis, to draw conclusions from the interpretation of observations, data or calculated values and to make scientific explanations of their data, observations and conclusions. Whatever conclusions are drawn, they must be based firmly on the evidence obtained from the experiment. At the highest level, students should be able to make further predictions and ask appropriate questions based on their conclusions.

## 6 Evaluating procedures

Arguably, this is one of the most important, and probably one of the most difficult skills for a student to develop. In order for the evaluation to be effective, students must have a clear understanding of the aims and objectives of the exercise, otherwise they will not be able to judge the effectiveness of the procedures used. They must be able to evaluate whether the errors in the data obtained exceed those expected due to the equipment used. If this is the case, they then need to identify those parts of the procedure which have generated these excess errors, and suggest realistic changes to the procedure which will result in a more accurate outcome. Students should also be able to suggest modifications to a procedure to answer a new question.

The evaluation procedure may include:

- i the identification of anomalous values, deducing possible causes of these anomalies and suggesting appropriate means of avoiding them,
- ii an assessment of the adequacy of the range of data obtained,
- iii an assessment of the effectiveness of the measures taken to control variables,
- iv taking an informed judgement on the confidence with which conclusions may be drawn.

## 7 Evaluating conclusions

This is also a higher-level skill, which will demand of the student a thorough understanding of the basic theory that underpins the science involved.

The conclusions drawn from a set of data may be judged on the basis of the strength or weakness of any support for or against the original hypothesis. Students should be able to use the detailed scientific knowledge and understanding they have gained in theory classes in order to make judgements about the reliability of the investigation and the validity of the conclusions they have drawn.

Without practice in this area, students are likely to struggle. In order to increase the confidence in drawing conclusions, it is recommended that practical exercises, set within familiar contexts, be used to allow students the opportunity to draw conclusions, make evaluations of procedure and assess the validity of their conclusions.

In the examination, students may be required to demonstrate their scientific knowledge and understanding by using it to justify their conclusions.

## Ways of doing practical work

Science teachers should expect to use practical experiences as a way to enhancing learning. Practical activities should form the basis on which to build knowledge and understanding. They should be integrated with the related theory, offering opportunities for concrete, hands-on learning rather than as stand-alone experiences. In planning a scheme of work it is important to consider a mosaic of approaches that include those that allow students to participate in their own learning.

- Some practical activities should follow the well established structure that includes a detailed protocol to follow. Such well-structured learning opportunities have a vital role to play in introducing new techniques, particularly in rapidly developing fields such as biotechnology. In these new areas of science, teachers will often find themselves leading practical work that they have not had the chance experience themselves as students.
- Other practical activities should offer the students the opportunity to devise their own methods or to apply to solving a problem the methods that they have been taught. The excitement generated by exposure to “new” and unfamiliar techniques provides a stimulus to engage a student’s interest and challenge their thinking.

Practical activities may be used as a tool to introduce new concepts – for example, introducing catalysis by experimentation, followed up by theoretical consideration of the reasons for the unexpected results obtained. On other occasions, practical work can be used to support and enhance the required knowledge and understanding – for example in building upon a theoretical consideration of the limiting factors of photosynthesis with a series of practicals investigating the effect of light intensity and hydrogen carbonate concentration on photosynthesis in water weed. In all cases, learning will be enhanced most effectively by practical work that encourages students to be involved, to think, to apply and use their knowledge, understanding and skills.

Practical work does not always have to be laboratory based. In classrooms, the use of models, role play and paper cut-outs to simulate processes can be equally valuable. Field studies also contribute greatly to a students’ appreciation of Biology and their motivation and enjoyment of the subject. No amount of reading or viewing videos can substitute for being exposed to an environment and the organisms living there. Even a carefully managed environment like a school lawn represents a challenge to recognise the species and to understand how they can survive.

There are a variety of strategies by which practical work can be integrated into a scheme of work. Teachers should use a variety of methods, enhancing a variety of subject specific skills and simultaneously developing a variety of transferable skills that will be useful throughout their future professional lives. Some of the ways of delivering practical work also enable the teacher to interact on a one-to-one basis with individual students. This allows a teacher to offer support at a more personal level and develop a greater awareness of an individual students needs.

Your choice of the specific strategy to use will depend on such issues as class size, laboratory availability, the availability of apparatus, the level of competence of your students, availability and expertise of technical support, the time available, your intended learning outcomes for the activity and safety considerations. The following are some possible strategies for delivery of practical work.

- **Teacher demonstrations**

These require less time than a full class practical, but give little opportunity for students to develop manipulative skills or gain familiarity with equipment. Careful planning can give opportunity for limited student participation. Teacher demonstrations are a valuable way of showing an unfamiliar procedure at the start of a practical session, during which students go on to use the method.

**Considerations** in choosing to do a demonstration **might include:**

- i **Safety** – some exercises carry too high a risk factor to be performed in groups.
- ii **Apparatus** – complicated procedures or those using limited resources
- iii **Time** – demonstrations usually take less time
- iv **Outcome** – some results are difficult to achieve and may be beyond the skill level of most of the students. A failed experiment may be seen as a waste of time.
- v **Students' attention** – a danger is that the attention of some students will drift.
- vi **Manipulative experience** – the teacher gets experience, the students' don't.

There are many good reasons for the teacher performing a demonstration but do be aware that most students have a strong preference for hands-on experimentation. So, where possible, do let them do it!

- **Group work**

**Whole class practical sessions.** These have an advantage in terms of management as all the students are doing the same thing. Students may be working individually, in pairs or in small groups. Integrating this type of practical is straightforward as lessons beforehand can be used to introduce the context and following lessons can be used to draw any conclusions are develop evaluation. Where specialised equipment or expensive materials are in short supply this approach may not be feasible.

**Small group work.** This can provide a means of utilising limited resources or managing investigations that test a range of variables and collect a lot of measurements. Although the same procedure may be performed, each student group collects only one or a few sets of data which are then pooled. For example, if five concentrations of the independent variable are being tested, each of which need to be measured at two minute intervals for thirty minutes, then a group of five students can each test one concentration. In biology, field studies also lend themselves to group activities as a lot of data has to be collected in a short period of time. The individual student has the opportunity to develop their subject-specific skills. Part of the role of the teacher is to monitor and maintain safety and also to enable and persuade reluctant learners to take part. Group work aids personal development as students must interact and work co-operatively.

**Considerations might include:**

- i **Learning** – successful hands-on work will reinforce understanding; also, students will learn from each other.
- ii **Confidence** – this will grow with experience

- iii **Awareness/insight** – should grow with experience
- iv **Team building** – a most desirable outcome.
- v **Setting out** – all students doing the same thing is easier for the technicians
- vi **Confusion** – incomplete, ambiguous or confusing instruction by the teacher will waste time while the instructions are clarified but may also compromise safety and restrict learning.
- vii **Opting out** – some students will leave it for others to do and so learn very little.
- viii **Safety** – this could be a serious issue and constant vigilance is essential.
- ix **DIY** – the urge to adapt their experiments, to ‘see what would happen if’, must be strictly dealt with.
- x **Discipline** – practical time must not be allowed to become ‘play time’.

Working in groups, whether as part of a whole-class situation or where groups are working on parts of a whole, is probably the preferred option for many students. At A level, it is highly desirable to include opportunities for students to work on their own, developing their own skills and independence. In Papers 31 and 32, a student’s practical skills will be assessed on an individual basis, so an individual’s experience, competence and confidence are of considerable importance.

- **Circus of experiments**

A circus comprises of a number of different exercises that run alongside each other. Individual or groups of students work on the different exercises and, as each exercise is completed, move on to the next one. These are a means by which limited resources can be used effectively.

There are two basic approaches. Most commonly, during a lesson a number of short activities are targeted at a specific skill. Alternatively, over a series of lessons, a number of longer practical activities are used, addressing a variety of skills. The circus arrangement may be more difficult to manage as the students are not all doing the same activity. This puts more pressure on the teacher as they have to cope with advising and answering questions from a variety of investigations. With circuses spread over a number of sessions, careful planning is needed to enable the teacher to engage each group of students, to maintain a safe environment. In these situations it is useful to have at least two of the circus activities that involve no hands-on practical work - using data response based simulations or other activities. In this way the teacher can interact with groups that need a verbal introduction or short demonstration and can monitor their activities more effectively.

- i **Apparatus** – if the amount of apparatus used in an exercise is limited, students are able to use it in rota.
- ii **Awareness** – students by observing their peers will become more aware of the pitfalls of the exercise and so will learn from the experience of others.
- iii **Safety** – different exercises may well carry different safety risks, all of which would need to be covered.
- iv **Setting out** –students doing different exercises will make it more difficult for the technicians

- v **Opting out** – some students may be tempted to ‘borrow’ the results of earlier groups.

- **Within theory lessons**

This option should be considered whenever it is viable. It is likely that the practical work would be by demonstration, as this would take less time. Given the power of visual images, the inclusion of a short practical to illustrate a theoretical point will reinforce that point and so aid the learning process. It is critical, however, that the practical works correctly, otherwise the flow of the lesson is disrupted and confidence in the theory may be undermined. The exercise should therefore be practiced beforehand.

- **Project work**

Projects are a means by which a student’s interest in a particular topic, which is not always directly on the syllabus, can be used to develop investigative skills. It can also be used to access parts of the syllabus that have little laboratory based investigation. For example, in gene technology students might use internet based research to find examples of genetic modification and present a poster display showing the implications. This sort of investigative work can be individual, or a group activity. Once the project is underway, much of the work can be student-based, outside the class room. Care is needed in selecting the topics and setting a time scale, so that the relevance is maintained to the syllabus context. The work can be directed at the production of posters, presentations to give to the group or reports from the group or individual.

### **Extra-curricular clubs**

The role that these can play is in stimulating scientific enquiry methods. There are a number of ways of using clubs. One way is to hold the club session during the teaching day so that all students can attend. In effect this becomes additional lesson time in which students can practice investigative skills, including laboratory work. Such lab work involves materials that have a cost, which must be planned for beforehand. If however the club is held outside the teaching day it may be voluntary. Syllabus specific activities should be limited and the most made of the opportunities for exciting work unrelated to syllabuses. After school clubs could be vehicle for project work that is related to science and of social or economic importance, for example, endangered species or local mineral resources. Students who do attend the club could be used as a teacher resource by bringing back their findings to a class room session.

### **Keeping records**

Students often find it a problem to integrate the practical work to the theory. This is particularly true when a series of experiments or a long term investigation or project is undertaken. Some potential issues include:

- Some students use odd scraps of paper in the laboratory, which are lost or become illegible as chemicals are spilled on them. One important criterion is that students are trained to record results immediately and accurately.
- Practical procedures may be provided, or students write their own notes from a teacher demonstration. These may be lost, so students end up with results but no procedure or context.
- When results take a period of time to collect, analysis becomes isolated from the context of the investigation and may not be completed.

The key to minimising these issues is to train students into good work practices. This is particularly important in colleges where students join at the start of their A levels from a variety of feeder schools. It is also vital for students with specific learning difficulties that affect their ability to organise their work such as dyslexia and Asperger's syndrome.

Students may be encouraged to integrate the practical in the same file as the theory. Alternatively, students may be encouraged to keep an entirely separate practical book or file. Loose leaf files make it easy to add to the file, but may make it easier to lose items. Exercise books can be used but students should be encouraged to glue provided protocols and their laboratory records into the book so that they are not lost. Depending on how they learn, individuals may vary in their preferred method. Whichever option is chosen, students need to be encouraged to relate their investigations to the appropriate theory and to regard it as something that needs to be thoroughly assimilated.

- Integrating the materials generated by practical work with the notes from learning of theory can be achieved by interspersing the records of investigations with the relevant section of theory. This may still require cross-referencing where several learning outcomes and assessment objectives are targeted by work.
- Keeping a separate practical book enables records of all the practical investigations to be kept in one place. Students need training to manage practical files effectively, particularly in keeping the contexts and cross referencing to the theory. If care is not taken to develop and maintain these skills, students may perceive practical as something different from theory.
- An intermediate between these two extremes is having a separate section for practical investigations with each syllabus section in each student's file and cross-referenced to the relevant theory.

## How is a practical activity organised?

Preparing for practical work needs thought and organisation. The practical work may be an activity that forms part of a lesson, it may comprise an entire lesson, or it may be an investigation designed to last for several lessons, but in every case, thorough preparation is a key prerequisite to success.

Practical and investigative work should be integrated into the programme of study. The scheme of work should identify appropriate practical investigative experiences for use at the most suitable time. In designing the scheme of work,

- the resource implications should be considered in terms of equipment and materials in stock,
- thought should be given to the seasonal availability of materials such as organisms, and the sometimes short shelf-life of thermo-sensitive substances such as enzymes or hygroscopic substances such as some salts,
- the time taken from order to delivery, potential for damage during despatch and cost of materials to be obtained from local, national or international suppliers should be considered,
- careful scheduling may be needed in Centres with a large number of students. It may be possible to permit several groups to do the work simultaneously or in quick succession, or it may be essential to re-order the scheme of work for different groups so that scarce resources can be used effectively,

- note must be taken of national or local health and safety regulations relating to chemicals, electricity, growing microorganisms etc. There may also be regulations controlling use of controversial materials such as genetically modified organisms.

Once the scheme of work has been established, the next stage is to consider each practical activity or investigation. In an ideal course, each of the following stages would be gone through in developing each practical exercise in a course. This is not always realistically possible the first time through a course, which is one reason for the existence of this booklet. It is better to get going and to get some practical work done with students than to hold out for perfection before attempting anything. Obviously, all practical work should be subject to careful and rigorous risk assessment no matter how provisional the rest of the supporting thinking and documentation.

- Decide on the aims of the work – the broad educational goals, in terms of the broad skill areas involved (e.g. planning) and the key topic areas (e.g. animal transport systems or unfamiliar material).
- Consider the investigative skills being developed. Reference should be made to the syllabus, which in the practical skills section, includes learning outcomes relating to practical skill. For instance, if the practical work intended is to be a planning exercise, which of the specific skills identified in the learning outcomes will be developed?
- With reference to the topics included, decide on the intended learning outcomes of the practical activity or investigation, again referring to the syllabus. For instance, which of the transport learning outcomes will be achieved? In a few cases during the course, the material on which the practical is to be based may be unfamiliar, in which case there may be no topic-related intended learning outcomes. Thus, A2 contexts may be used for AS practicals, and topic areas not on the 9700 syllabus at all may be used for AS or A2 practicals.
- In addition, it may be useful to assess any other context of the practical work investigation. For instance, is it intended as part of the introduction of a concept, or to support a theory, or to demonstrate a process?
- Produce a provisional lesson plan, allocating approximate times to introduction, student activities and summarising.
- Produce and trial a student work sheet. Published procedures or those produced by other teachers can be used. Alternatively produce your own. As a rule schedules produced by others need modifying to suit individual groups of students or the equipment available. It helpful to ask students or another teacher to read work sheets before they are finalised as they can identify instructions that are ambiguous or use inaccessible terminology.
- Refine the lesson plan in relation to the number of students for which the investigation is intended (whole class or a small group), the available equipment (does some have to be shared?) and materials. There are examples of lesson plans and student work sheets in appendix 2.
- Carry out a detailed and careful risk assessment (see below) before any preparatory practical work is done, and certainly well before students do any of the practical work. You should consider
  - the likelihood that any foreseeable accident might occur – for example, pupils putting glass tube through bungs are quite likely to break the tube and push it though their hand



- the potential severity of the consequences of any such accident – for example dropping onto a desk a plastic dropper bottle of  $0.01 \text{ mol dm}^{-3}$  hydrochloric acid will cause much less severe eye injuries than the same accident with a glass bottle containing  $5.0 \text{ mol dm}^{-3}$  hydrochloric acid.
- the means that can be taken to reduce the severity of the effect of any accident – for example, the teacher or technician preparing bungs with glass tubes before the lesson, or using eye protection such as safety spectacles during all practical work.
- Make an equipment and materials list. This may need to be in sections;
  - materials and apparatus per student or per group (chemicals and glassware)
  - shared equipment per laboratory (water baths, microscopes, pH meters)
  - any chemicals should include concentrations and quantities needed
  - any equipment should include number required
  - any hazard associated with specific chemicals or equipment should also be noted and cross referenced to the risk assessment. Sources of information about safety may be listed in the syllabus (and are reproduced below).
  - The location of storage areas for equipment and chemicals may be cross referenced to this equipment and materials list.
- Set up and maintain a filing system where master copies of the work sheets, lesson plans and equipment lists can be stored. It is helpful to have these organised, or at least indexed, by both their syllabus context and skills developed.
- Once an investigation has been used by a group of students it should be evaluated in relation to intended outcomes and the lesson plan. It is important to obtain feedback from the students about their perception of the work. For example,
  - was the time allocation appropriate,
  - were the outcomes as expected,
  - did the students enjoy the work,
  - did the students understand the instructions,
  - was the point of the work clear to the students?
 If necessary the work sheet and lesson plan should be revised.

## Risk assessment

All practical work should be carried out in accordance with the health and safety legislation of the country in which it is done. No activities should be attempted if they conflict with such legislation.

Hands-on practical work can be carried out safely in schools. If it is to be safe, then the hazards need to be identified and any risks from them reduced to insignificant levels by the adoption of suitable control measures. These risk assessments should be done for all the activities involved in running practical science classes including storage of materials, preparatory work by the teacher and by any technical support staff and the practical activities that are carried on in the classroom, whether demonstrations by the teacher or practical activities for the students. Such risk assessments should be carried out in accordance with the health and safety legislation of the country in which they are done.

Risk assessment involves answering two basic questions:

- 1 how likely is it that something will go wrong?** For example, pupils using a double sided razor blade to cut up carrots are quite likely to cut themselves.

- 2 how serious would it be if it did go wrong?** For example the consequences of a spark from an experiment landing in an open bottle of magnesium powder are likely to be serious, including spraying burning magnesium all over the laboratory, burning many pupils and setting the laboratory ceiling on fire (based on a real accident).

With the answers to these questions it is now possible to plan the practical activity to minimise the risk of an accident and to minimise how severe any accident might be. In our examples, this might include cutting up the carrot before giving to young pupils, or providing older pupils with an appropriate sharp knife, it might include bringing in to the laboratory only the amount of magnesium powder required for the activity.

How likely it is that something will go wrong depends on who is doing it and what sort of training and experience they have had. You would obviously not ask 11 year old students to heat concentrated sulphuric acid with sodium bromide, or to transfer *Bacillus subtilis* cultures from one Petri dish to another, because their inexperience and lack of practical skills makes a serious accident all too likely. By the time they reach post-16 they should have acquired the skills and maturity to carry such activities out safely.

Decisions need to be made as to whether an activity should be a teacher demonstration only, or could be done by students of various ages. This means that some experiments should normally only be done as a teacher demonstration or by older students. Perhaps with well-motivated and able students it might be done earlier, but any deviation from the model risk assessment needs discussion and a written justification beforehand.

There are some activities that are intrinsically dangerous, and, if included in the suggested activities, should always be changed to more safe modes of practice, for example, there are **no** circumstances under which mouth pipetting is acceptable – pipette fillers of some sort should **always** be used.

Teachers tend to think of eye protection as the main control measure to prevent injury. In fact, personal protective equipment, such as goggles or safety spectacles, is meant to protect from the unexpected. If you expect a problem, more stringent controls are needed. A range of control measures may be adopted, the following being the most common. Use:

- a less hazardous (substitute) chemical;
- as small a quantity as possible;
- as low a concentration as possible;
- a fume cupboard; and
- safety screens (more than one is usually needed, to protect both teacher and students).

The importance of lower concentrations is not always appreciated, but the following examples, showing the hazard classification of a range of common solutions, should make the point.

ammonia (aqueous)	irritant if $\geq 3 \text{ mol dm}^{-3}$	corrosive if $\geq 6 \text{ mol dm}^{-3}$
sodium hydroxide	irritant if $\geq 0.05 \text{ mol dm}^{-3}$	corrosive if $\geq 0.5 \text{ mol dm}^{-3}$
hydrochloric acid	irritant if $\geq 2 \text{ mol dm}^{-3}$	corrosive if $\geq 6.5 \text{ mol dm}^{-3}$
nitric acid	irritant if $\geq 0.1 \text{ mol dm}^{-3}$	corrosive if $\geq 0.5 \text{ mol dm}^{-3}$
sulphuric acid	irritant if $\geq 0.5 \text{ mol dm}^{-3}$	corrosive if $\geq 1.5 \text{ mol dm}^{-3}$
barium chloride	harmful if $\geq 0.02 \text{ mol dm}^{-3}$	toxic if $\geq 0.2 \text{ mol dm}^{-3}$ (or if solid)

Reference to the above table will show, therefore, that if sodium hydroxide is in common use, it should be more dilute than  $0.5 \text{ mol dm}^{-3}$ . The use of more concentrated solutions requires measures to be taken to reduce the potential risk.

### Material Safety Data Sheets (MSDS)

Your risk analysis should consider the hazards associated with the materials you propose to use. These risks are best assessed by reference to MSDS's appropriate to the chemical(s) in use. These are generally supplied by the chemical manufacturer and supplied with the chemical. If this is not the case then there are many internet sites that have this information freely available. These sheets also provide useful information on the actions to take following an accident, including first aid measures, and should therefore be considered essential for all practical experiments involving chemicals, as part of the risk assessment process.

### Hazard key

The following key applies.

**C = Corrosive substance**

**F = Flammable substance**

**H = Harmful or irritating substance**

**O = Oxidising substance**

**T = Toxic substance**

**N = Harmful to environment**

### Eye protection

Clearly students will need to wear eye protection. Undoubtedly, chemical splash goggles give the best protection but students are often reluctant to wear goggles. Safety spectacles give less protection, but may be adequate if nothing which is classed as corrosive or toxic is in use.

Your risk assessment should not restrict itself simply to the materials, procedures and equipment being used, but should have a wider remit, covering the time from when the class enter the room until they leave it.

Practical science can be - and should be - fun. It must also be safe. The two are not incompatible.

*Safeguards in the School Laboratory*, 10th edition, ASE, 1996

*Topics in Safety*, 2nd edition, ASE, 1988

*Hazcards*, CLEAPSS, 1998 (or 1995)

*Laboratory Handbook*, CLEAPSS, 1997

*Safety in Science Education*, DfEE, HMSO, 1996

*Hazardous Chemicals Manual*, SSERC2, 1997.

## AS Skills

AS skills will form the foundation on which A2 skills will be developed. Students will become competent in these skills through practical experience. They should be expected, during the AS course, to carry out as much practical work as possible, since this will develop both key practical skills and enhance their motivation as well as their understanding of the theory part of the course. The specific investigations to which references are made can be found in appendices 1 and 2. The syllabus clearly describes the skills that are to be assessed, and should be used to ensure that activities are appropriately targeted.

### Teaching students to manipulate, measure and observe

As part of their AS studies students will be expected to develop skills in manipulating and measuring using standard laboratory apparatus. These will form a basis on which more advanced manipulative skills will be developed in A2. During their AS course it is assumed that students will learn how to measure accurately and to manage their time effectively, so that they are confident in their use of apparatus.

- A good starting point to practice these skills is with microscope work (e.g. practicals 1 and 2). Students will be expected to be confident in the use of microscopes and be able to make temporary mounts of a variety of specimens. It is most important that they are capable of recording their observations by being able to make clear, well proportioned, labelled drawings of what they observe. This is not an easy skill to acquire and time should be spent to ensure that students develop their capability in this skill.
- Various investigations (such as practicals 5 and 6) will also allow students to collect data and make observations. This will require that students are able to follow a set of instructions and set up apparatus appropriately. They should then be able to collect data using a wide variety of means.
- Students should be able to make informed decisions about the number of times a reading should be taken and the range of readings that is required to collect reliable and valid data. Students should also be able to replicate readings or observations as necessary. Many of the practical investigations here offer such opportunities, but particularly open-ended investigations such as practical 16.

### Teaching students to present data and record observations

Many students do not find this an easy skill to master. It is important that students can record data so that it is capable of being understood by others. This requires skill in deciding how to present the data and what should be recorded.

- Students need to be able to present numerical data in tabular form and to decide on the structure of the table and what titles and units should be written in the column headers. They should produce the table so that readings from the investigation may be entered directly into the table as the readings are taken. Space should be allocated in tables as necessary for calculated values and deductions. Opportunities include practical 7 and 19.
- Students should ensure that all readings are taken with the same degree of accuracy and precision.
- Students often assume that everyone understands how they achieved their answers to questions or calculations without realising that this is not the case.

This is particularly true when answering examination questions. Examiners can only give credit for what they see and students may well receive credit for a correct method even if they reach the wrong answer or conclusion. However this requires that the students display their calculations and reasoning.

- Students should show the working in their calculations and the key steps in their reasoning.
- Students should also use the correct number of significant figures for calculated quantities.

Several of the practicals include such numerical work, for example practicals 2, 13, 14 and 20.

- Students should be able to choose a suitable method of presenting data obtained from an investigation for example quantitative data as graphs (e.g. practicals 6 and 7), qualitative data as tables (e.g. practicals 3 and 4) and cellular and histological data as drawings (e.g. practicals 1, 2, 13 and 14).
- When producing graphs, students should be able to select which variables to plot on the x and y axes. They should be able to plot with accuracy and follow the Institute of Biology recommendations for drawing lines on graphs.
- More information concerning the presentation of data and observations is provided in the syllabus.

### **Teaching students to analyse, draw conclusions and evaluate**

These are the hardest skills that have to be mastered by students. Evaluation in particular is found very difficult by most students as they are having to think in the abstract rather than handle real apparatus and materials. It is most important that the basics in these skills are mastered so that they can be further developed in the A2 part of the course.

- Students need to be able to interpret data or observations by describing patterns and trends shown by tables and graphs. In data such as highly curved graphs, the key patterns should be described (e.g. practical 5) and in data producing simple curves or straight lines, the trend of the data observed and described (e.g. practical 6).
- Students should be able to determine unknown values by extrapolation and interpolation of lines on graphs and be able to calculate the mean from replicated observations.
- It is most important that students are able to explain the degree of confidence they have in their conclusion and identify and explain possible sources of error in the investigation.
- Students should be able to say whether the data obtained supports the original hypothesis and use this to make further predictions.
- The ability to make simple evaluations should be practiced so that this skill can be further developed in A2. This enables students to suggest improvements to procedures so as to improve the reliability of the data obtained and to extend investigations into new situations or solve related problems. The more practice students have of this skill, the better. Ideally every investigation could be evaluated using a simple check list until it becomes an automatic response by a student.

## Appendix 1 - Designing a practical course for AS

### Outline List of Practical Experiments

Full details are provided for practicals 1 - 11

Number and title	type of practical	syllabus reference
1 Size and scale	obs	A
2 Plant tissue observation	obs	A
3 The identification of biological chemicals present in solutions	wet	B
4 The metabolism of different carbohydrates by yeast	wet	B
5 Effect of pH on enzymes	wet	C
6 Effect of inhibitors on enzymes	wet	C
7 Using beetroot to investigate cell membrane permeability	wet	D
8 Root tip squash	wet	E
9 The extraction of DNA from onions	wet	F
10 Effect of wind-speed on transpiration rate using a potometer	wet	G
11 Investigating the role of carbon dioxide in living organisms	wet	H
12 Size and scale	obs	A
13 Plant tissue observation	obs	A
14 Animal tissue observation	obs	A
15 Food tests	wet	B
16 Effect of temperature on enzymes	wet	C
17 Effect of substrate concentration on enzymes	wet	C
18 Effect of enzyme concentration	wet	C
19 Osmosis	wet	D
20 Plasmolysis and water potential	w&o	D
21 Chromosome observation - prepared root tip squash	obs	E
22 Stomata	obs	G
23 Effect of temperature and light on transpiration rate	wet	G
24 Investigate transport of water using celery	wet	G
25 Investigate tidal and vital capacity	wet	H
26 Effect of nicotine on heart rate of daphnia	wet	H
27 Bacteria and viruses	obs	I
28 Blood cell smear	w&o	J
29 Food chains and food webs	field	K
30 Nutrient cycling	wet	K

The route which teachers take through the AS syllabus may vary. The practical course follows the sequence of the syllabus, although some learning outcomes may well be grouped differently. Teachers can adapt the investigations as they wish as they wish. All of the investigations that are supplied in detail are intended to be completed within a one hour time span.

CIE 9700/03 practical papers are not intended to be used for formative development of practical skills. The questions set for the previous syllabus are written to different assessment objectives, although some of the practical components could be used as practice exercises. Teachers who choose to use past papers as a student's only learning experience are placing these students at a disadvantage.

**Syllabus section A – Cell Structure****Practical 1 – Size and scale 1**

**Learning outcomes (a) use a stage micrometer and graticule and (f) calculate linear magnification of drawings**

**Practical skills: Manipulation, measurement and calculation.**

The practical uses a stage micrometer to calibrate an eyepiece graticule so that specimen size can be determined. The candidates can also determine the scale of drawings that are made of the specimens under observation. It is suggested that the eyepiece lenses be fitted with the graticules prior to the investigation.

Full details of this practical are provided.

**Practical 12 – Size and scale 2** The investigation described in practical 1 uses a TS of *Lamium* stem, but the practical can and should be modified and form a template for a series of practicals to include other examples from a wide range of plant and animal tissues.

**Practical 2 – Plant tissue observation**

**Learning outcomes (f) draw plan diagrams of tissues. The practical can be extended to also cover (b) (e) and (g) distinguish between resolution and magnification and compare and contrast structure of plant and animal and prokaryotic and eukaryotic cells.**

**Practical skills: Observation and recording and interpreting observations.**

The practical emphasises and practices microscopic and drawing skills so that candidates are able to use a microscope and produce clear, well proportioned, labelled drawings in both low and high power. They also learn the difference between cellular and plan drawings and how best to represent the various tissues of a specimen under observation.

Full details of this practical are provided.

**Practical 13 – Plant tissue observations Practical 14 – Animal tissue observations** The investigation described in practical 2 should be modified and form a template for a series of practicals at appropriate points through the course, to include other examples from a wide range of plant and animal tissues.

**Syllabus section B – Biological Molecules**

**Practical 15 – food tests** – students may well have used food tests in their previous biology courses, but they should be given an opportunity to use Benedict's test for reducing and non-reducing sugars, biuret test for protein, iodine in potassium iodide solution for starch and the ethanol emulsion test for lipid. They should be given prepared solutions / oils to try, as well as liquid food materials such as milk and food materials that need grinding up and suspending such as bread.

**Practical 3 – Identification of biological chemicals present in solutions**

**Learning outcomes (a) carry out tests for reducing and non-reducing sugars, starch, lipids and proteins.**

**Practical skills: Manipulation, measurement, observation, recording of data, interpreting and drawing conclusions.**

The practical develops an understanding of the application of different biochemical tests to the problem of identification of unknown substances. The practical concentrates on developing skills of decision making, recording, drawing conclusions and identifying alternative strategies.

Full details of this practical are provided.

**Practical 4 – the metabolism of different carbohydrates by yeast**

**Learning outcomes (a), (b) and (d) form the context for this practical, making use of unfamiliar methods and materials and thus (e) use the knowledge gained to solve related problems in a new situation.**

**Practical skills: Manipulation, measurement and observation, presentation of data and analysing and forming conclusions.**

The practical investigates how efficient yeast is in metabolising different carbohydrates by observing the time taken for methylene blue to be discoloured. The ideas developed can include previous work done on cell membranes and the different ways that substances are transported into and out of cells.

**Syllabus section C – Enzymes****Practical 5 – Effects of pH on enzymes**

**Learning outcomes (c) follow the course of an enzyme catalysed reaction and (d) investigate and explain the effect of pH on the rate of enzyme catalysed reactions.**

**Practical skills: Manipulation, measurement and observation, presentation of data and analysing and forming conclusions.**

The practical investigates the effect of pH on enzymes and the effect on the hydrogen and ionic bonds that determine the shape of the active site. Candidates may determine the optimum pH and the point at which the enzyme has been denatured.

Full details of this practical are provided.

**Practical 6 – Effect of inhibitors on enzymes**

**Learning outcomes (c) follow the course of an enzyme catalysed reaction and (e) explain the effects of a non-competitive inhibitor on the rate of enzyme activity.**

**Practical skills: Manipulation, measurement and observation, presentation of data and analysing and forming conclusions.**

The practical investigates the effect of a non-competitive inhibitor, lead nitrate, on the hydrolysis of starch.

Full details of this practical are provided.



**Practical 16 – Effect of temperature on enzymes** The effect of temperature on enzyme catalysed reactions should be investigated. Candidates can carry out a simple enzyme catalysed reaction at different temperatures using provided troughs / plastic bowls at different temperatures as water baths. This works well using protease such as trypsin or bacterial protease and exposed, developed black-and-white photographic film, or using amylase and starch. The data collected can then be displayed graphically to assist in drawing conclusions. Experiments of this type lend themselves to be critically analysed, evaluated and improved.

**Practical 17 – Effect of substrate concentration on enzymes** **Practical 18 effect of enzyme concentration** These experiments can be further modified to investigate the enzyme concentration and the concentration of substrate on the rate of reaction. This would offer the opportunity to use other enzyme/substrate systems such as urea/urease (detect time taken for ammonia to be produced), yeast catalase/hydrogen peroxide (height of foam or count bubbles from delivery tube or collect gas over water and measure volume) or lipid emulsion/lipase (detect changes in pH). In addition this will also practice the skills of identifying and controlling variables. At this level, candidates should also be able to identify dependent and independent variables.

### **Syllabus section D – Cell Membranes and Transport**

#### **Practical 7 – Using beetroot to investigate cell membrane permeability**

**Learning outcomes (b) outline the role of membranes within cells, (c) describe the process of diffusion and (e) use the knowledge gained to solve related problems in a new situation.**

**Practical skills: Manipulation, measurement and observation, presentation of data and analysing and forming conclusions.**

The practical investigates the effect of temperature on the permeability of cell membranes. Candidates will need access to a colorimeter for this experiment although it could be modified so that diluted solutions were compared against a standard colour chart. Paint manufacturers produce a very wide range of standard colours for paints mixed 'in store' and a selected sample of these could be used for 'standards'.

Full details of this practical are provided.

**Practical 19 – Osmosis** This can be investigated in a variety of ways such as determining the water potential of potato tissue by placing samples of potato in different concentrations of sucrose solution and noting the gain or loss in mass of the tissue. The data can be displayed graphically and the concentration determined by interpolation on the graph.

**Practical 20 – Plasmolysis and water potential** Plasmolysis can be investigated in epidermal onion tissue. The water potential can be determined by finding incipient plasmolysis in cells placed in different concentrations of solution. This extends the work done in the previous practical on osmosis by including the skills of microscope technique in the practical. Students may well need training in order to identify cells with incipient plasmolysis.

**Syllabus section E – Cell and nuclear division****Practical 8 – root tip squash**

**Learning outcomes (d) describe with the aid of diagrams, the behaviour of chromosomes during the mitotic cell cycle.**

**Practical skills: Manipulation, observation, interpretation and recording observations.**

The practical involves preparing and staining cells from an active meristem in the roots of plants. The cell tissue is then 'squashed' and observed using standard microscopic techniques and different stages of mitotic cell division identified and drawn. It is advised that due to shortness of time within a one hour lesson, that candidates have previously done other microscope practicals so that they are familiar with and can use a microscope with skill and precision.

Full details of this practical are provided.

**Practical 21 – chromosome observation – prepared root tip squash** Practical 8 can be further extended and modified so that candidates are provided with ready made prepared cell tissue showing a full range of the different stages of cell division in order that they can become skilful in identifying and drawing them. This is good material for developing skills of measurement, observation and drawing as the structures are intra-cellular and therefore small.

**Syllabus section F – Genetic Control****Practical 9 – Extraction of DNA from onions**

**Learning outcomes (a) describe the structure and importance of DNA**

**Practical skills: Manipulation and making observations.**

The practical involves the extraction of DNA from living material such as onion and clearly and powerfully demonstrates to students the actual existence of DNA when it has probably been experienced by the student as a theoretical substance found only in textbooks.

Full details of this practical are provided.

**Syllabus section G – Transport****Practical 10 – effect of wind-speed on transpiration rate.**

**Learning outcomes (c) describe how to investigate experimentally the factors that effect transpiration rate.**

**Practical skills: Manipulation, data collection and analysis and drawing conclusions.**

The practical investigates transpiration in a leafy shoot using a potometer and the effect that wind speed has on the rate of transpiration. Wind-speed is determined by a fan that is set at different distances from the plant. Students come to realise that this does not involve the inverse square law but this idea can be developed to show how the inverse square law affects light and photosynthesis.

Full details of this practical are provided.

**Practical 23 – Effect of light and temperature on transpiration rate.** The investigation in practical 10 can be modified so that other variables such as temperature and light can be investigated to see how they affect transpiration rate.

**Practical 22 – Stomata** The structure of stomata can be observed by coating the lower surface of a leaf with clear nail varnish, which is then peeled off and made into a temporary slide for viewing through a microscope. This practical not only investigates stomatal structure but also reinforces microscope skills learnt earlier in the course. Leaves of different plants can be compared so that xerophytic adaptations can be studied.

**Practical 24 – Investigate transport of water using celery** Transport of water through plant tissue can be investigated using celery and broad bean seedlings. A stick of celery left to stand in a dilute solution coloured dye such as food colouring or ink can be used to show the presence of vascular bundles in roots, stems and petioles. Students can section the stem to show the distribution of the vascular bundles. The veins in the leaves of the celery and bean seedling will also become stained and thus show what happens to the vascular bundles when they enter leaves. This enables students to build up a whole picture of how the water is transported throughout the plant.

### **Syllabus section H – Gas Exchange**

**Practical 25 – tidal and vital capacity** Tidal and vital capacity can be investigated using spirometer. Schools that do not possess a spirometer can do a simplified version of this experiment by getting students to blow through rubber tubing placed in a 1000 ml measuring cylinder or larger, full of water and inverted into a large bowl. Measurements of expired air can be read directly from the measuring cylinder.

**Practical 26 – Effect of nicotine on heart rate of Daphnia** The effect of nicotine on the heart rate in daphnia can be investigated to show how different drugs can affect metabolism.

To make the nicotine solution, remove tobacco from a pack of 20 cigarettes and mix it with a beaker of water. Cover and allow to stand for at least 12 hours. Drain the supernatant liquid and mix one part solution to eight parts water. Different concentrations may be used to investigate the effect of concentration on heart beat.

### **Practical 11 – Investigating the role of carbon dioxide in living organisms**

**Learning outcomes (k) use the knowledge gained in this section in new situations or to solve related problems**

**Practical skills: Manipulation, observation, interpretation and planning.**

This practical investigates the carbon dioxide levels produced by living organisms using bicarbonate indicator solution. The students then use this information to plan an investigation to determine the unfamiliar interaction between respiration (producing carbon dioxide at a constant rate irrespective of light intensity) and photosynthesis (using carbon dioxide – dependent on light) in plants.

Full details of this practical are provided.

**Syllabus Section I – Infectious disease**

**Practical 27 – Bacteria and viruses** There are various ways of making practical material on these accessible, but the most satisfactory will be to use electron micrographs (downloaded from the web or in books) to make observations and measurements.

**Syllabus Section J – Immunity**

**Practical 28 – Blood cell smear** – this must be done using prepared slides. This is an important practical as the cells are small and not very easy to see, so they make an excellent context for experienced students to finely tune their skills of using apparatus, observation and drawing.

**Syllabus Section K – Ecology**

**Practical 29 – food chains and food webs** – students should have the opportunity to go out into the school grounds or a suitable local environment in which are found plants, animals that feed on the plants and predators (this might include trees, aphids and carnivorous beetles, or herbaceous plants, snails and snail-eating birds). Students should be enabled to identify, count (per unit area) and estimate biomass, preparatory to making food webs and attempting to make a simple estimated pyramid of biomass.

**Practical 30 – nutrient cycling** – students should set up small, sealed jars containing a few grams of soil, pond water and a small amount of shredded paper. These should be left in the light and observed for a few weeks. There will be a succession of changes in the microorganisms growing in the jars, but eventually it will settle down to a constant population of various photosynthetic and other species, demonstrating that they are recycling the limited amount of nutrients present.

## Appendix 2 – practicals for which full details are provided

### Practical 1 - Investigation into size and scale of microscopic tissues

This practical focuses on microscope technique and using graticules and stage micrometers to determine size and scale in biological cells and tissues.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Use a microscope fitted with an eyepiece graticule and stage micrometer
- Calibrate the eyepiece graticule using the stage micrometer
- Use the calibrated graticule to determine the actual size of microscopic specimens
- estimate the accuracy of a measurement
- Use the graticule to determine scales
- Understand the importance of repeating or validating set of results.

#### Safety Information

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- The measurement of specimen size with a microscope, is made by using an eyepiece graticule. This is a glass or plastic disc with 8 divisions etched onto its surface, which is inserted into the eyepiece lens.
- The size of the eyepiece graticule remains constant, despite the fact that the image viewed will change its size depending upon whether high- or low-power objective lenses are used. For example a cell viewed with the x40 objective will appear much larger than when viewed with the x10 objective. However because the graticule is in the eyepiece it will not change its size. Therefore the value of each of the divisions in the eyepiece graticule varies with the magnification of the objective lens.
- A stage micrometer is a very accurately etched glass or plastic ruler that is placed on the microscope stage so that the eyepiece graticule scale is superimposed on the stage micrometer scale. The scale is usually 1mm divided into 100 separate divisions so that each division equals 10 micrometres (10 $\mu$ m).
- It is necessary to calibrate the eyepiece graticule with the stage micrometer placed on the microscope stage for each objective lens used.

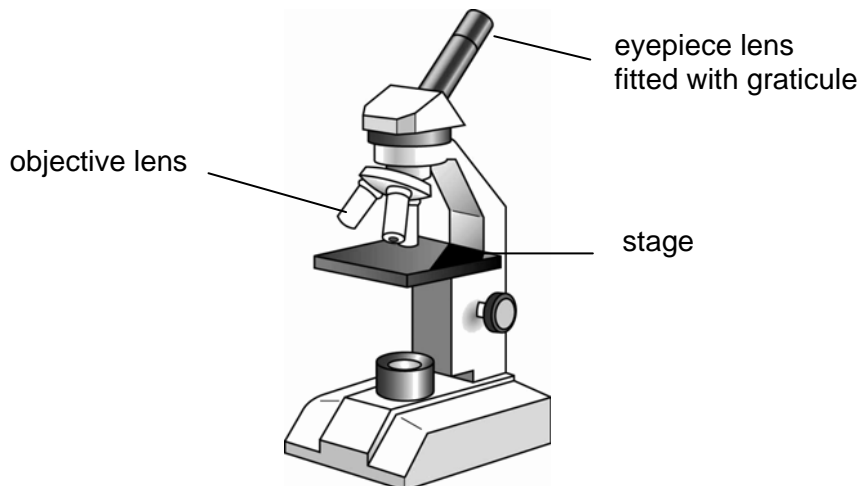
You will observe a TS of plant tissues through a microscope and use an eyepiece graticule and a stage micrometer to determine the size of some of the structures.

- Read the information above.
- Ensure that you understand the principles of using an eyepiece graticule and a stage micrometer before you continue with the investigation.

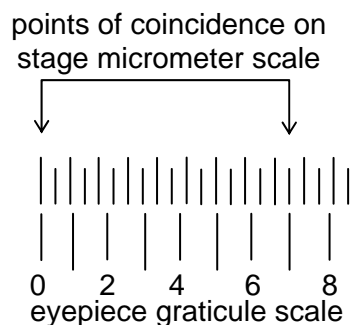
## Method

### Preparation

1. You have been provided with a compound light microscope with both low- and high-power objective lenses and an eyepiece lens that has been fitted with a graticule. You have also been provided with a stage micrometer.



2. You must now calibrate the eyepiece graticule. Place the stage micrometer onto the microscope stage and focus using the low-power objective lens so that the graticule scale becomes superimposed over the stage micrometer scale.



3. Move the stage micrometer until the start or zero line of each scale is coincident (lined up)
4. Look along the scale until another coincident point is found.
5. The relationship between the two scales can now be calculated

On the scale shown there are 17 divisions on the stage micrometer scale that line up with 7 divisions on the graticule scale.

Thus  $17 / 7 = 2.42857$  units.

Each unit on the stage micrometer scale is 10 micrometres ( $10\mu\text{m}$ ).

Therefore each division on the graticule scale is 24.2857 micrometres rounded to  $24.3\mu\text{m}$ .

6. Use the procedure described above to determine the size of each division on the eyepiece graticule using the low-power objective lens of your microscope.
7. Repeat the procedure to determine the size of each division when using the high-power objective lens.

### Making observations

1. You are provided with a stained transverse section through part of a dicotyledonous plant root.
2. Examine the specimen using the low-power of your microscope.
3. Make a large, **plan** drawing to show the distribution of tissues, labelling the stele (vascular bundle).
4. Use the eyepiece graticule to measure the width of the vascular bundle at its widest point in graticule units and then calculate the actual width of the vascular bundle in millimetres and in micrometres.
5. Draw a straight line on your drawing across the vascular bundle to show where you took your measurement. Write the dimension on your drawing next to the line.
6. Make a high-power drawing to show a group of **four** xylem vessels from inside the vascular bundle.
7. Use the eyepiece graticule to measure the width of the xylem vessel at its widest point in graticule units and then calculate the actual width of the vessel in micrometres, remembering to use the appropriate calibration of the eyepiece graticule for the high-power objective lens.
8. Draw a straight line on your drawing across the xylem vessel to show where you took your measurement. Write the dimension on your drawing next to the line.
9. Look at your two measurements and check on their accuracy. The actual size of the xylem vessel should be smaller than the size of the vascular bundle even though it looked larger using the high power objective lens.
10. You are now going to determine the magnification of your drawing of the xylem vessels. Use a ruler to measure the length of the line that you drew across the xylem vessel.

Use your knowledge of the actual size of the vessel to calculate the magnification of your drawing. Write your answer  $\times$ \_\_\_\_\_ at the bottom right hand corner of your drawing.

### Follow-up

- Compare your results with other members of the class and check for consistency of readings.
- Did any member of the class have anomalous results? What are the potential causes of such an anomalous result in this investigation?
- Write up your procedure including a discussion of the benefits of comparing your results with other students.

## Practical 1 - Lesson Plan

### Investigation into size and scale of microscopic tissues.

#### Context

A practical investigation set in the context of 9700 syllabus – Assessment objective C 2 and 3 – Cell structure.

#### Key aims of the lesson

This practical is designed to develop the skills of using an eyepiece graticule and a stage micrometer with a microscope to determine size and scale of microscopic tissues.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Use a compound light microscope with an eyepiece graticule
- Use a compound microscope with a stage micrometer
- Calibrate an eyepiece graticule with a stage micrometer
- Use the calibrated graticule to determine the actual sizes of cells and tissues using both low- and high-power objective lenses.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 10	<b>Context</b> – review the method for setting up and using a microscope with an eyepiece graticule and stage micrometer and how to calibrate the eyepiece graticule.
10 - 15	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns regarding breaking slide by focussing down with the objective lens.



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15 - 40	<b>Carrying out the practical</b> – students carry out the practical work.
40 - 50	<b>Obtain results</b> – Students compare their results with other students in the class
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained.

### **Practical 1 - Technical information**

#### **Investigation into size and scale of microscopic tissues.**

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 1 x prepared slide TS *Ranunculus* root
2. 1 x microscope with an eyepiece graticule fitted, suitable illumination and:
  - high power objective lens e.g. x40 (equal to 4mm or 1/6")
  - low power objective lens e.g. x10 (equal to 16mm or 2/3")
3. 1 x stage micrometer – a scale on a glass slide
4. suitable white paper, HB (medium-hard) pencil and rubber

#### **Safety Precautions.**

No specific hazards have been identified in this practical, however a risk assessment should be carried out as a matter of course.

### **Practical 2 - Microscopic observation of cells and tissues**

This practical focuses on microscope technique and making and recording observations in the form of biological drawings.

#### **Intended learning outcomes**

By the end of this practical you should be able to:

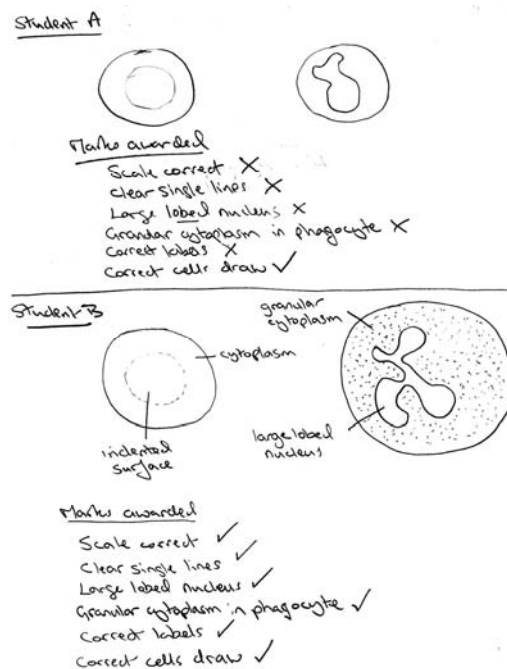
- Use a microscope with skill and precision
- Show all the structures that can be seen in the defined part of a specimen
- Make clear, accurate, labelled, scale drawings of specimens

#### Safety Information.

There are no particular hazards in this practical, however you must follow your laboratory rules.

### Background information

- Drawings should be done with a sharp HB pencil making clear single lines. Examiners do not give credit for sketchy lined drawings. A soft rubber can be used to correct errors.
- Always **draw what you see** and **not** what you expect to see from memory or textbook diagrams.
- Candidates often draw diagrams too small but rarely draw them too large. Ensure that your drawing is large enough to show all the detail.
- All parts of the drawing should be kept in correct proportions. In poor quality drawings, proportions changes as the drawing progresses.
- Biological drawings can be both high-power and low-power.
- Low-power drawings are usually plan drawings that do not contain cellular detail but do show the distribution of various tissues. When a plan drawing is requested, examiners may give credit for **not** drawing cellular detail.
- If more than one drawing of the same or different specimens or parts of a specimen are made, examiners may ask that they are drawn to the same scale (which means the same magnification). Credit is then awarded for this skill.
- Look at the following two sets of drawings of a red and white blood cell, made by different students and how marks would be allocated by an examiner.



- Student A would be awarded 1 mark. Student B would be awarded 6 marks.

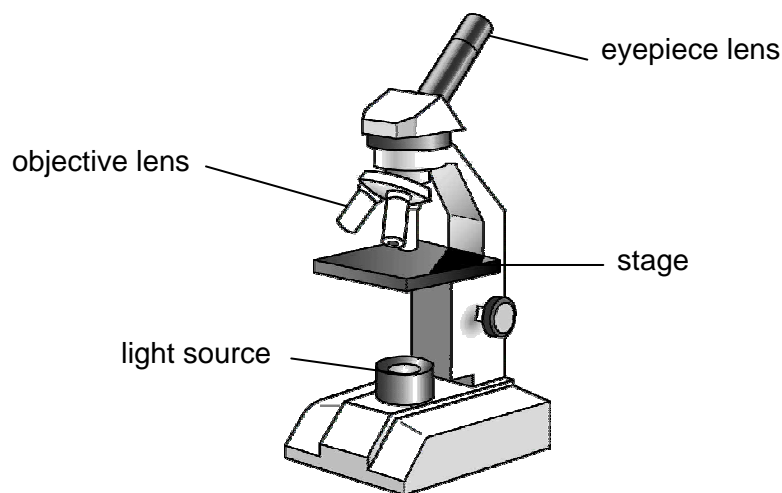
You will observe a TS of plant tissues through a microscope using both low and high power and draw appropriate structures.

- Read the information above.
- Read your textbook and look carefully at any drawings that have been made of biological material. Take care however; the quality of drawings in some textbooks is not all that could be desired.
- Write down the key features that are found in good biological drawings.

## Method

### Preparation

1. You have been provided with a compound light microscope with both low- and high-power objective lenses and a slide of a TS of a plant stem.



2. Place the slide onto the stage of the microscope.
3. Adjust the light source so that you can see a bright light when looking through the eyepiece lens.
4. With some microscopes it is possible to rack the objective lens so far down that it will break the slide. In order to prevent this it is good microscope technique to:
  - set the objective lens on low power.
  - not look through the eyepiece but to look at the side of the microscope and carefully lower the objective lens until it is nearly, but not quite touching the slide.
  - now look through the eyepiece and gradually raise the objective lens until the slide comes into focus.
5. You should now carefully move the slide around on the stage until you find the area that you wish to observe.
6. To change to high power, do not re-focus, but change the objective lens from low to high power. The slide should be almost in focus and only a fine adjustment to the focus should be necessary.
7. Practice focussing the slide on both low and high power until you are familiar with the technique.

### Making observations

1. You are provided with a stained transverse section through part of a dicotyledonous plant.

Examine the specimen using the low-power of your microscope.

Make a large, labelled, **plan** drawing to show the distribution of tissues.

2. Make a high power drawing to show a group of **four** cells from the region nearest the centre of the specimen.

### Follow-up

- State from which part of the plant the section was taken. Explain your answer
- Exchange your drawings with another student and mark their drawings using the following mark scheme.

### Mark scheme

#### Plan drawing

- Corner vascular bundles larger than other vascular bundles ✓
- No individual cells drawn ✓
- Four sided shape to plan ✓
- Both xylem and phloem correctly labelled ✓
- Parenchyma correctly labelled ✓
- Sclerenchyma on outer edge of vascular bundle labelled ✓
- Collenchyma in corners labelled ✓

#### High power drawing

- Good quality of drawing i.e. clear single lines** ✓
- 4 cells only drawn, similar in size and shape ✓
- between 5-8 sides to each cell ✓
- Air spaces shown between corners of cells ✓
- Thin cell walls shown either by a thin single line or two lines close together ✓
- Add up the marks out of 12 and return the drawings to the student.
- Write a list of all the reasons why you did not score full marks with your own drawings.

**Practical 2 - Lesson Plan****Microscopic observation of cells and tissues.****Context**

A practical investigation set in the context of 9700 syllabus – Cell structure and transport

**Key aims of the lesson**

This practical is designed to develop the skills of using a microscope and the recording and interpretation of observations by producing biological drawings.

**Intended learning outcomes**

By the end of the practical and the write-up the student should be able to

- Use a compound light microscope
- Make clear and accurate plan and cellular drawings of biological tissue
- Be able to interpret structures seen through the microscope

**Resources required**

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

**Planned activities**

<b>Timings/ minutes</b>	<b>Teacher/ Student Activities</b>
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. Students to look at examples of drawings of cells and tissues in their textbooks.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review the protocols for setting up and using a microscope to observe slides
5 - 10	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns regarding breaking slide by focussing down with the objective lens.
10 - 25	<b>Carrying out the practical</b> – students carry out the practical work.

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25 - 50	<b>Obtain results</b> – Students observe the plant tissue and produce clear labelled diagrams as requested, then clear away apparatus as soon as they have finished
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the manipulation and observational skills that have been developed as well as discussion on results obtained. Teacher to go through the mark scheme with the students and students compare their marks and understand why marks were not awarded.



**Practical 2 - Technical information****Microscopic observation of cells and tissues**

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 1 x prepared slide TS *Lamium* stem
2. 1 x microscope with suitable illumination and;
  - high-power objective lens e.g. x40 (equal to 4mm or 1/6")
  - low-power objective lens e.g. x10 (equal to 16mm or 2/3")
3. suitable white paper, HB (Medium hard) pencil and rubber

**Safety Precautions.**

No specific hazards have been identified in this practical, however a risk assessment should be carried out as a matter of course.

### Practical 3 - The identification of biological chemicals present in solutions






This practical focuses on making decisions about measurements and observations, recording and presenting data and observations, interpretation, drawing conclusions and suggesting improvements. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Decide what tests to carry out and what observations to make
- Use an appropriate means to record your observations, constructing any tables before you make the observations
- Describe and summarise the key points of your observations
- Draw conclusions in terms of the presence or absence of different chemicals in the solutions
- Suggest alternative strategies for identifying some of the materials

#### Safety information

	You should wear eye protection throughout this practical.
	Amylase is <b>harmful</b> , avoid contact with eyes and skin.
 	Benedict's solution is <b>harmful</b> and <b>dangerous to the environment</b> .
	1 mol dm <sup>-3</sup> hydrochloric acid is <b>harmful</b> .

#### Background information

- Make sure that you know how to carry out Benedict's test, what it is used for and what the positive and negative results should be.
- Make sure that you know how to carry out biuret test, what it is used for and what the positive and negative results should be.
- Think about how Benedict's test and the enzyme amylase can be used to confirm the presence of a polysaccharide such as starch.
- Think about how acid hydrolysis, neutralisation and Benedict's test can be used to confirm the presence of the non-reducing disaccharide, sucrose.

You will use the materials provided to identify the unknown materials in the solutions A, B, C and D

- Read and think about the information above.
- The solutions A, B, C, D and E each contain only one of the following materials, but not necessarily in this order
  - A reducing sugar
  - A non-reducing sugar
  - A polysaccharide that can be hydrolysed by amylase
  - Proteins including amylase
  - No dissolved material
- You are also provided with materials for biuret test and for Benedict's test, as well as dilute hydrochloric acid, calcium hydrogen carbonate powder and a waterbath at 35°C

### Method

#### Preparations and making observations

1. You need to decide what tests to do and in what order so that it is possible to use the amylase to test some of the other solutions.
2. Decide how you are going to record your observations so that it will be absolutely clear what you did to which solutions, what you observed and your interpretation of the observations.
3. Prepare a piece or pieces of paper in accordance with your decisions.
4. Make a risk assessment of your proposed methods and decide what precautions to take to reduce the likelihood of an accident and to reduce the damage any accidents might cause – ask your teacher to confirm that you may go ahead with the tests.
5. Carry out the tests with full regard to safety, recording your observations and interpretations.
6. Record the identity of the unknown solutions.

#### Write-up

- hand in your original laboratory records, including your methods, observations and interpretations.
- suggest improvements to the method including some of the following:
  - a simpler way of testing for the presence of starch,
  - starch would also be hydrolysed by acid. Suggest a better order to do your tests if this caused you difficulties, or a way of using amylase to confirm that it is non-reducing sugar rather than starch that is present,
  - if a solution contained a small amount of reducing sugar and also non-reducing sugar, suggest how it might be possible to use repeated benedict's tests, filtering the precipitate out after each, to remove the reducing sugar before testing for non-reducing sugar.

### Practical 3 - Lesson Plan

#### The identification of biological chemicals present in solutions

##### Context

A practical investigation set in the context of 9700 syllabus – The identification of biological chemicals present in solutions

##### Key aims of the lesson

This practical is designed to develop the skills of decision-making, observation, interpretation and evaluation.

##### Intended learning outcomes

By the end of this practical the student should be able to:

- Decide what tests to carry out and what observations to make
- Use an appropriate means to record your observations, constructing any tables before you make the observations
- Describe and summarise the key points of your observations
- Draw conclusions in terms of the presence or absence of different chemicals in the solutions
- Suggest alternative strategies for identifying some of the materials

##### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

##### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – student worksheet given out for students to read in preparation for the practical lesson. To ensure understanding of methods and their use as well as to consider decisions about which observations to make and in what order, and the means of recording
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of biochemicals and biochemical tests - key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly reminds students of methods of tests, demonstrating as necessary. Teacher answers any student questions on procedure. Teacher emphasises safety concerns with acidic and harmful solutions and heating of solutions
8 - 45	<b>Carrying out the practical</b> – students carry out the practical work and record their observations and interpretations.
45 - 50	<b>Clear up</b> – Students clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Teacher to emphasise importance of handing in original laboratory records – (these can be collected in at the end of the lesson, photocopied and given back to students if they are thought likely to ‘copy them up’). Practical write up to be completed in following lesson or as homework activity.
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### Useful information

The most effective methodology is likely to be

- Test a small sample of each of the solutions with biuret test to identify the protein including amylase – set aside solution B for later use (identified with this test)
- Carry out Benedict’s test, heating the tubes gently to 90°C until one gives a positive result, on small samples of each of solutions A, C, D and E to identify which contains reducing sugar – set aside solution A (identified with this test)
- Mix small samples from each of solutions C, D and E with equal volumes of solution B and incubate at 35°C for a few minutes. Test sample from each of the incubated tubes with benedict’s test to identify in which the polysaccharide has been hydrolysed to give a reducing sugar – set aside tube E (identified with this test)
- Mix small samples from tubes C and D with an equal volume of hydrochloric acid and boil carefully for two minutes. Add a small excess of calcium hydrogen carbonate (until it just stops effervescing) to neutralise the acid and test with Benedict’s test to identify which contains non-reducing sugar (C) and therefore which contains only water (D).

### Summary of contents of the five solutions

solution	material present in solution
A	reducing sugar
B	proteins including amylase
C	non-reducing sugar
D	no dissolved material
E	polysaccharide that can be hydrolysed by amylase

- To improve the methodology, iodine solution would be a more elegant way of confirming the presence of the polysaccharide.
- To detect non-reducing sugar in the presence of reducing sugar – react the latter with excess Benedict’s and filter out the resulting precipitate. Re-test to confirm the absence of reducing sugar. Now hydrolyse with acid, neutralise and re-test.

### Practical 3 - Technical information

#### The identification of biological chemicals present in solutions

The apparatus and materials required for this practical are listed below.


The amount of apparatus listed is for one student or one group of students if they are to work in groups.



- 5 small beakers or other containers as follows


label on beaker	20 cm <sup>3</sup> of solution in beaker
A	1 mol dm <sup>-3</sup> glucose solution made by dissolving 18 g of glucose in 80 cm <sup>3</sup> of distilled water and making up to 100 cm <sup>3</sup>
B	10% egg white and 1% amylase solution made up by dissolving 10 cm <sup>3</sup> of fresh egg white (or 1 g of dried egg white) and 1 g of amylase in 80 cm <sup>3</sup> of cold distilled water, mixing until dissolved and making up to 100 cm <sup>3</sup>
C	0.5 mol dm <sup>-3</sup> sucrose solution made by dissolving 17 g of sucrose in 80 cm <sup>3</sup> of distilled water and making up to 100 cm <sup>3</sup>
D	no dissolved material
E	polysaccharide that can be hydrolysed by amylase

- 5 test tubes in a rack and a means of washing the tubes such as a sink and running water
- a glass marker, such as a wax pencil or a permanent OHP pen or small labels and pencil
- the usual materials that the students are used to using for biuret test, labelled appropriately
- the usual materials and heating arrangements that the students are used to using for Benedict's test, labelled appropriately
- 1 mol dm<sup>-3</sup> hydrochloric acid in a small dropper bottle, labelled hydrochloric acid
- Sodium hydrogen carbonate powder in a small specimen tube with a stopper and a spatula that fits in the tube to dispense it
- A thermostatic waterbath or plastic trough containing water at about 35°C for use as a waterbath

#### Safety Precautions/Risks

Amylase = H 

Benedict's solution = H, N  

Hydrochloric acid (1 mol dm<sup>-3</sup>) = H 

A risk assessment should be carried out as a matter of course.

### Practical 4 - Investigation of the carbohydrates metabolised by yeast



This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between temperature and membrane permeability.
- Evaluate procedures

#### Safety Information

	You should wear eye protection throughout this practical.
	Methylene Blue is <b>harmful</b> . Avoid contact with eyes and skin. It will stain skin or clothes.

#### Background information

- Yeast can metabolise carbohydrates under two different conditions. When oxygen is present aerobic respiration occurs yielding a large amount of energy for the organism and producing carbon dioxide & water as waste products.
- However when oxygen is in short supply (anaerobic conditions) the yeast will break down the carbohydrate into ethanol & carbon dioxide with a much reduced energy output (alcoholic fermentation).
- Both of these forms of respiration in addition to most metabolic processes are catalysed by specific enzymes.
- The process of how efficient the yeast is in metabolising different carbohydrates can be monitored by observing the time taken for Methylene Blue to be discoloured

In this experiment you will investigate the relative efficiency with which different carbohydrates can be metabolised by yeast.

- Read the information above
- Identify and write down the dependent and independent variables
- Consider which type of carbohydrate (monosaccharide, disaccharide, polysaccharide) will be metabolised by the yeast and why. Explain your reasoning.
- Write down what you expect to happen as a hypothesis in which you make specific predictions about which carbohydrates you might expect yeast to metabolise.

- Identify any variables that should be controlled and outline how this should be done

### Method

#### Preparations and making observations

1. Label seven boiling tubes A - G.
2. In tube A place 5cm<sup>3</sup> distilled water, in tube B 5cm<sup>3</sup> glucose, in tube C 5cm<sup>3</sup> fructose and continue with as many carbohydrates provided placing each tube in a rack.
3. Into each tube add 3 drops of Methylene blue.
4. Add 5cm<sup>3</sup> yeast solution to each tube noting the time.
5. Shake each tube to mix the contents and place back into the rack.
6. Do not disturb the tubes again but note the time taken for the blue colour to disappear from each tube.

#### Write-up

- Record your results in a clear table ensuring units are put in headers where possible.
- Represent the results of the experiment in a suitable chart to show type of carbohydrate against the time taken for the blue colour to disappear.
- Explain your findings in terms of enzymes activity and carbohydrate structure.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment



## Practical 4 - Lesson Plan

### Investigation of the different carbohydrates metabolised by yeast.

#### Context

A practical investigation set in the context of 9700 syllabus – Enzymes, biological molecules, respiration.

#### Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between temperature and the permeability of cell membranes.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on cell membranes
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of enzyme controlled reactions, biological molecules & respiration. Key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with methylene blue
8 - 40	<b>Carrying out the practical</b> – students carry out the practical work. Whilst they are waiting for the colour change to occur they can write up the first part, identifying variables, hypothesis, results table.

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40 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity

### Useful information

#### Safety precautions:

- **Methylene blue is harmful and can be a skin irritant. Safety glasses should be worn. Additionally as it is a protein stain it will stain any natural material. Please emphasise to students the importance of safety when pipetting the methylene blue.**

Discussion / evaluation points should include:

- Why should the tubes remain still after the initial mixing?
- What is being measured by the methylene blue discolouration (i.e. removal of oxygen from the system by the aerobically respiring yeast) ?
- Suggest why some sugars are metabolised and others are not.
- Why was the yeast incubated for about 30 minutes before the experiment started?
- What was the purpose of the tube with distilled water and yeast solution?
- Ensure that the students are aware of what type of organism that yeast belongs to.
- Yeasts live in many different environments. Suggest why the following are suitable places for yeast growth
  - a) fruit skin
  - b) Human body
- What precautions could be undertaken to ensure that all the tubes remained at a constant temperature?
- for students unable to obtain a full set of results the following could be used for analysis. Please note that other students results may not agree with these ones.

Type of carbohydrate	Time taken for blue colour to disappear (minute:secs)
Glucose	6:15
Fructose	24:45
Galactose	No change
Lactose	No change
Maltose	25:30
Sucrose	8:40
Starch	42:00

## Practical 4 - Technical information

### The metabolism of different carbohydrates by yeast

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 5cm<sup>3</sup> of as many of the following carbohydrates as available. Each made up to 5% concentration – Fructose, Galactose, Glucose, Lactose, Maltose, Starch, Sucrose
2. 5cm<sup>3</sup> distilled water
3. 7 boiling tubes (or as many as the number of carbohydrates available plus control)
4. Methylene blue, 3 drops per sample
5. 5cm<sup>3</sup> yeast solution (prepared in advance) per sugar used
6. test tube rack
7. Timer
8. Safety glasses

Additionally each student will require access to a sink & running water.

The yeast should be prepared according to local conditions so that it is activated and ready for use.

#### Safety Precautions/Risks.

Methylene blue = H



A risk assessment should be carried out as a matter of course.

### Practical 5 - The effect of pH on enzymes




This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Identify dependent and independent variables
- Make a hypothesis and express this in words and graphically
- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between pH and enzyme activity
- Evaluate procedures

#### Safety Information

	You should wear eye protection throughout this practical.
	Hydrogen peroxide is <b>corrosive</b> . Avoid contact with eyes or skin. It will bleach skin or clothes.
	Citric acid is <b>harmful</b> .

#### Background information

- Most enzymes have an optimum pH near to 7 (the pH found inside most cells)
- pH is the measurement of the concentration of Hydrogen ions ( $H^+$ )
- Hydrogen ions will affect the hydrogen and ionic bonds within the enzyme
- If these bonds are changed the three dimensional shape is changed altering the shape of the active site
- When an enzymes shape is altered it becomes denatured
- Potatoes are a good source of catalase

You will investigate the effect of pH on the enzyme catalase as it breaks down toxic Hydrogen peroxide, a by-product of some biochemical reactions, into water and oxygen.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen

- Identify any variables that should be controlled and outline how this should be done
- What would be the best method for collecting the oxygen produced?
- A graph of pH against rate of activity will be produced after the practical. Make sure you know how to calculate rate.
- Know what a buffer solution is and what it does.

### Method

#### Preparations and making observations

1. Use a cork borer to cut cylinders of fresh potato tissue. You will require a piece (pieces) between 6 – 7cm in length. Place on a tile and cut into at least 60 discs, each 1mm wide.
2. Place all the discs in a small beaker of water.
3. Set up the equipment as follows. Clamp a boiling tube to a stand and carefully insert the manometer (with fluid) into a rubber bung.
4. Using a syringe or small measuring cylinder place 5cm<sup>3</sup> of buffer solution pH3 into the boiling tube.
5. Carefully add 10 of the potato discs followed by 5cm<sup>3</sup> of hydrogen peroxide solution.
6. Place the bung back into the boiling tube as quickly as possible.
7. Start the stop watch and time how long it takes for the manometer fluid to rise by 5cm. (Mark start point on tube and measure 5cm)
8. Carefully agitate the tube to make sure that the potato discs do not stick together.
9. Wash out the boiling tube and repeat the experiment using a different pH buffer and ten new potato discs each time.
10. Make sure you use a clean syringe / measuring cylinder with each different buffer solution.
11. If time allows repeat the procedure for increased reliability.

#### Write-up

- Record your results in a clear table ensuring units are put in headers.
- If replicate results not done obtain a set of mean readings by using other class members results.
- Calculate the rate of reaction.
- Plot a graph of pH against rate.
- Explain your findings using your knowledge of enzymes.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment
- How could you measure the volume of gas produced by this method and by altering the method

## Practical 5 - Lesson Plan

### The effect of pH on enzyme activity

#### Context

A practical investigation set in the context of 9700 syllabus – The effect of pH on enzyme activity

#### Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between pH and enzyme activity.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – 2 page student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on cell membranes
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of enzymes, key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with cork borer (or sharp knife) and hydrogen peroxide
8 - 45	<b>Carrying out the practical</b> – students carry out the practical work..
45 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity
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### Useful information

Discussion / evaluation points should include:

- explanation of the shape of the graph
- consistency of the enzyme within the potato, age of potato
- possible problems with the method e.g. Lack of temperature control in reaction tube, loss of gas before bung inserted
- for students unable to obtain a full set of results the following could be used for analysis

pH	Time taken for manometer to move 5cm (minutes)
3	10
4	8
5	6
6	4
7	2
8	5
9	8
10	10



## Practical 5 - Technical information

### The effect of pH on enzymes

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. fresh potato (each student requires a core (cores) approximately 10cm in length). (More if experiment is to be repeated)
2. 1 boiling tube
3. Single bore rubber bung
4. cork borer
5. white tile
6. scalpel
7. small beaker
8. 2 x 10cm<sup>3</sup> graduated pipette or measuring cylinder or syringe
9. Manometer tube (3mm diameter)
10. Stop watch
11. Forceps
12. 20 volume Hydrogen peroxide
13. Range of buffers (pH 3 – 8).

Additionally each student will require access to a sink and running water.


Commercial buffer tablets are available from most chemical wholesalers, however it is possible to make up buffer solutions in the laboratory. (Details from, Laboratory Manual for Schools. Heinemann. 1977)


Sodium hydrogen phosphate/citric acid buffer – range pH 3.0 – 8.0.

To make up 100cm<sup>3</sup> of buffer use 0.1M Citric Acid & 0.2M Sodium hydrogen phosphate in the following proportions:

pH	Citric acid / cm <sup>3</sup>	Sodium hydrogen phosphate / cm <sup>3</sup>
3.0	79.45	20.55
4.0	61.45	38.55
5.0	48.50	51.50
6.0	36.85	63.15
7.0	17.65	82.35
8.0	2.75	97.25

Safety Precautions/Risks.

Hydrogen peroxide = C 

Citric acid = H 

A risk assessment should be carried out as a matter of course.

### Practical 6 - The effect of inhibitors on enzyme activity




This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience relevant methods, analysis and conclusion.
- Describe and explain the effect of a non competitive inhibitor on enzyme activity.
- Evaluate procedures.

#### Safety Information

	You should wear eye protection throughout this practical.
	Amylase is <b>harmful</b> . Avoid contact with eyes or skin.
	Iodine solution is <b>harmful</b> . Avoid contact with eyes or skin. It will stain skin or clothes.

#### Background information

- A non competitive inhibitor binds to a part of the enzyme away from the active site
- The shape of the enzyme is changed, thus changing the shape of the active site
- No enzyme-substrate complexes can be formed, hence no product produced
- Increasing the amount of substrate does not overcome the effect of this type of inhibitor

You will investigate the effect of increasing the amount of Lead nitrate on the hydrolysis of starch by the enzyme amylase.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen
- Identify any variables that should be controlled and outline how this should be done

A colorimeter should be used to compare the colours of the solutions obtained after a given time.

### Method

#### Preparations and making observations

1. Set up a thermostatically controlled water bath set at 40°C
2. Label six boiling tubes A – F and place in a test tube rack
3. Add 10cm<sup>3</sup> starch solution to each tube
4. Add the following quantities of Lead nitrate to 5cm<sup>3</sup> distilled water in six test tubes labelled 1 – 6 : 0g, 0.1g, 0.2g, 0.3g, 0.4g, 0.5g and shake to ensure it dissolves
5. Pour contents of tube 1 into boiling tube A, tube 2 into boiling tube B etc.
6. Add 5cm<sup>3</sup> amylase solution to each of the boiling tubes, agitate well and start the timer after placing the tubes into the water bath
7. Allow the reaction to proceed for 20 minutes
8. During this time adjust the colorimeter using a solution of 1cm<sup>3</sup> iodine solution in a boiling tube containing 10cm<sup>3</sup> starch solution and 5cm<sup>3</sup> distilled water. Set the colorimeter to 0% transmission with this solution
9. After 20 minutes add 1cm<sup>3</sup> iodine solution to each tube
10. Test each of the tubes A – F in the colorimeter noting down the absorbance for each tube and record in a table. (Note for centres without access to a colorimeter the method could be adapted to using a spotting tile with one drop of iodine in each well. At intervals of one minute a drop of the reaction mixture is placed on the tile and the time taken for the black colour to disappear noted.)

#### Write-up

- Record your results in a clear table ensuring units are put in headers where possible.
- Plot a graph of transmission against mass of lead nitrate added. (If experiment done using spotting tile method a graph of rate of reaction against mass of lead nitrate added should be drawn)
- Explain your findings using your knowledge of enzymes and inhibitors.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment.
- Why was it necessary to control the temperature of the reaction?
- What further experiments could be done to investigate non-competitive inhibitors?
- What are the advantages of repeating an experiment?
- Suggest why the iodine solution was not added at the same time as the other solutions

## Practical 6 - Lesson Plan

### The effect of inhibitors on enzyme activity

#### Context

A practical investigation set in the context of 9700 syllabus – Enzymes and enzyme inhibitors

#### Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the effect of a non competitive inhibitor

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on enzymes.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of enzyme action,
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with the use of chemicals.
8 - 40	<b>Carrying out the practical</b> – students carry out the practical work. Whilst they are waiting for the 20 minute period they can write up the first part, identifying variables, hypothesis, results table. Teacher to demonstrate the use of colorimeter to those students unfamiliar with this piece of equipment.

40 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in flowing lesson or as homework activity

**Useful information**

- For centres without access to a colorimeter the practical results will not be as accurate but will still be of an objective nature. The pupils could evaluate to suggest improvements to include a more objective measurement.
- If the centre does not have access to thermostatically controlled water baths, manually controlled ones could be substituted or left out altogether but the need for temperature control needs to be discussed in the evaluation.

Other Discussion / evaluation points should include:

- explanation of the shape of the graph
- the differences between competitive and non competitive inhibitors needs to be emphasised
- for students unable to obtain a full set of results the following could be used for analysis

Mass of Lead nitrate / g	Transmission / arbitrary units
0.0	85
0.1	52
0.2	37
0.3	27
0.4	23
0.5	21

## Practical 6 - Technical information

### The effect of inhibitors on enzyme activity

The apparatus and materials required for this practical are listed below.


The amount of apparatus listed is for one student or one group of students if they are to work in groups.


1. 6 boiling tubes.
2. 6 test tubes
3. test tube rack
4. labels/marker pen
5. 1% starch solution – allow 100cm<sup>3</sup> per repeat
6. Amylase solution 1% - allow 50cm<sup>3</sup> per repeat
7. Iodine solution – allow 10cm<sup>3</sup> per repeat
8. Distilled water – 50cm<sup>3</sup> per repeat
9. 10cm<sup>3</sup> graduated pipette / measuring cylinder / syringe
10. Stopclock
11. colorimeter cuvettes

Additionally each student will require access to a colorimeter, thermostatically controlled water bath, sink & running water.

If using spotting tile method also add 1 x spotting tile and glass rods.

#### Safety Precautions/Risks.

Amylase = H 

Iodine solution = H 

A risk assessment should be carried out as a matter of course.

### Practical 7 - The effect of temperature on membrane permeability in beetroot

This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between temperature and membrane permeability.
- Evaluate procedures

#### Safety Information



You should wear eye protection throughout this practical.

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- The colour of beetroot is due to the presence of a red pigment called anthocyanin
- The cell membrane is mainly made up of two types of molecules, phospholipids and proteins scattered around in the membrane.
- The membrane is partially permeable
- Protein structure is denatured at high temperatures

You will investigate the effect of temperature on the permeability of the cell membrane in beetroot.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen
- Identify any variables that should be controlled and outline how this should be done

A colorimeter should be used to compare the colours of anthocyanin solutions obtained.



## Method

### Preparations and making observations

1. Use a cork borer to cut cylinders of fresh beetroot tissue. Place on a tile and cut into 30 discs, each 3mm wide.
2. Place all the discs in a small beaker and wash under a running tap for at least five minutes.
3. Label six test tubes – 30°C, 40°C, 50°C, 60°C, 70°C, 80°C.
4. Add 10cm<sup>3</sup> cold distilled / de-ionised water to each tube.
5. Set up a water bath using a large beaker, tripod, gauze and Bunsen burner.
6. Heat the water gently until a temperature of 80°C is reached then remove heat source.
7. Take five of the beetroot discs and impale on a mounted needle with space between each disc.
8. Immerse the discs in the water bath for exactly one minute, then remove and carefully push the discs into the test tube labelled 80°C and set aside.
9. Reduce the temperature of the water bath to 70°C and take a second set of five discs and repeat the process of immersion for one minute followed by putting them into the next tube.
10. Continue the process for each of the temperatures.
11. After the discs have stood for thirty minutes shake the tubes and pour this liquid into a cuvette.
12. Fill a second cuvette with distilled water.
13. Place a blue filter into the colorimeter and use the distilled water to zero the machine adjusting the pointer to zero absorbance.
14. Measure the colour density of the 70°C solution.
15. Wash out the cuvette and repeat the procedure to record the light absorbance for each of the temperatures.

### Write-up

- Record your results in a clear table ensuring units are put in headers where possible.
- Plot a graph of relative concentration of pigment against temperature.
- Explain your findings using your knowledge of cell membranes.
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment

## Practical 7 - Lesson Plan

### The effect of temperature on the permeability of cell membranes

#### Context

A practical investigation set in the context of 9700 syllabus – cell membranes and the effect of temperature.

#### Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between temperature and the permeability of cell membranes.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – 2 page student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on cell membranes
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of cell membranes, key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with cork borer (or sharp knife) and water baths
8 - 40	<b>Carrying out the practical</b> – students carry out the practical work. Whilst they are waiting for the 30 minute period they can write up the first part, identifying variables, hypothesis, results table. Teacher to demonstrate the use of colorimeter to those students unfamiliar with this piece of equipment.

40 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in flowing lesson or as homework activity

**Useful information**

- For centres without access to a colorimeter the practical results will only be of a subjective nature. However the principles of denaturation of the proteins in the membrane can still be discussed and the pupils could evaluate to suggest improvements to include a more objective measurement.
- If the centre has access to a number of thermostatically controlled water baths these could be substituted for the individual ones suggested in the method.

Discussion / evaluation points should include:

- explanation of the shape of the graph
- consistency of the pigment within the beetroot
- why the discs were washed before heating
- possible problems with the method e.g. impaling onto a mounted needle
- for students unable to obtain a full set of results the following could be used for analysis

Temperature / °C	Absorbance / arbitrary units
30	0.12
40	0.16
50	0.29
60	0.83
70	1.62
80	1.41

**Practical 7 - Technical information****The permeability of beetroot cell membrane**

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. fresh beetroot (each student requires a core approximately 10cm in length).
2. 6 test tubes
3. test tube rack
4. cork borer
5. white tile
6. scalpel
7. small beaker
8. 10cm<sup>3</sup> graduated pipette or measuring cylinder
9. mounted needle
10. large beaker
11. thermometer
12. Bunsen burner
13. tripod
14. heat proof mat
15. gauze
16. colorimeter cuvettes (2)

Additionally each student will require access to a sink, running water and a colorimeter.

**Safety Precautions/Risks.**

No specific hazards identified.

A risk assessment should be carried out as a matter of course.

### Practical 8 - Broad bean root tip squash



This practical focuses on setting up and manipulating apparatus and making and recording observations. Further skills can be developed using additional information after the practical has been completed.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience simple techniques to observe mitosis in cells in the root tip of broad beans
- Further your knowledge about mitosis
- Interpret and record observations

#### Safety Information

	You should wear eye protection throughout this practical.
	Acetic orcein is <b>corrosive</b> . Avoid contact with eyes or skin. It will stain skin and clothes.

#### Background information

- The role of chromosomes is to store information in the DNA coding and be able to replicate by cell division.
- Mitosis is a type of cell division found in somatic cells, that produces diploid cells.
- Chromosomes are only visible when the cell is dividing. They can be made more visible using appropriate staining techniques.
- During mitosis each chromosome separates into two chromatids.
- Each chromatid is pulled to opposite ends of the spindle prior to the cell dividing into two.
- The different stages of cell division are called interphase, prophase, metaphase, anaphase, telophase and back to interphase and are characterised by the position of the chromatids.
- During interphase the DNA of the chromatids replicates so that at prophase, whole chromosomes are visible once more.
- Mitosis can be observed in the meristematic tissues found in the apical meristem of actively growing roots of plants such as broad bean.

You will investigate how root tip tissue can be prepared and stained so that chromosomes in cells undergoing mitosis can be viewed through a microscope. You will then identify the various stages of cell division.

- Read the information above.
- Read your textbook and look carefully at the diagrams and photographs on the section concerning mitosis.
- Write down the key features that will enable you to identify root tip cells at each of the different stages of mitotic cell division.

### Method

#### Preparation

1. You have been provided with a germinated seedling of a broad bean or similar.
2. Using a sharp knife or scalpel, carefully cut off 5mm from the apical tip of five lateral roots.
3. Place the root tips into a test-tube containing acetic orcein stain.

Warning! Acetic orcein is an acidic stain that turns protein purple. Any stain that gets on skin or natural clothing will stain the material purple.

4. Warm the acetic orcein and root tips in a hot water bath but do NOT allow the solution to boil. The stain should be kept so that it is gently steaming for at least five minutes.
5. Using a mounted needle or similar, carefully remove one of the root tips. The root tips will now be very soft and easily damaged so do not remove them with forceps.
6. Leave the remaining four root tips in the hot stain.
7. Place the root tip onto a clean microscope slide.
8. Using a sharp knife cut it in half transversely so that each length is approximately 2.5mm.
9. Discard the half that is furthest away from the root tip.
10. Using a pipette, carefully add two or three drops of acetic orcein stain to the root tip on the slide.
11. Using a mounted needle or similar gently break the tissue of the root tip apart.
12. Carefully lower a cover slip over the root tip and using the blunt end of your mounted needle or a pencil, gently push the cover slip down onto the slide so that the root tip is squashed. Care is needed to ensure that as few air bubbles are present as possible.
13. Gently blot any excess stain from the slide.
14. Examine the slide using a microscope for the different stages in mitosis.
15. If insufficient staining has taken place, the process may be repeated with the other four root tips that have been left in the hot stain.

#### Making observations

1. Initially observe the tissue using the low power of your microscope. This will enable you to find areas of cells where the nuclear material is clearly visible and undergoing mitosis.

2. Using the high power of your microscope examine individual cells at various stages of division.
3. Using the high power of your microscope make large, labelled drawings of each of the stages of cell division.

Write-up

- Write up the method and answering the following questions
1. Suggest why the tissue was heated when placed in the acetic orcein stain.
  2. Explain how you placed the coverslip on the slide in order to reduce the number of air bubbles.
  3. What was the diploid number of chromosomes in the cells of the root tip that you examined?
  4. Explain why the haploid number is always even.

## Practical 8 - Lesson Plan

### Broad bean root tip squash

#### Context

A practical investigation set in the context of 9700 syllabus – Cell and nuclear division

#### Key aims of the lesson

This practical is designed to develop the skills of manipulation of apparatus and observation, and the recording and interpretation of observations.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods.
- Describe and explain the reasons behind the methods.
- Extend knowledge on the structure and function of mitosis

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. Students to read and look at drawings and photographs of mitosis in their textbooks.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review the sequence of mitosis and determine the key features of each of the stages with key points written on board
5 - 10	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns when using hot acetic orcein stain
10 - 25	<b>Carrying out the practical</b> – students carry out the practical work.
25 - 50	<b>Obtain results</b> – Students observe mitosis and produce clear labelled diagrams of each stage, then clear away apparatus as soon as they have finished



50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the manipulation and observational skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity
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**Useful information**

- Other plant root tips can be substituted for broad bean, such as other types of bean, onions or sunflowers.
- The intensity of the stain depends upon how long the root tips are left in the hot acetic orcein stain.

Discussion / evaluation points should include:

- explanation of the methods used and how to successfully produce a mounted slide
- possible problems with the method such as the degree of staining, sufficient squashing of tissue and microscope technique

## Practical 8 - Technical information

### Broad bean root tip squash

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 1 x actively growing broad bean seedling such that at least five lateral roots have developed. Broad bean may be substituted with any other bean, or onion or sunflower.
2. sharp knife or scalpel
3. 1 x test-tube and hot water bath that can be maintained at approximately 90°C
4. 1 x microscope slide and cover slip
5. 1 x mounted needle or similar
6. 5cm<sup>3</sup> of acetic orcein stain. If not previously prepared, the acetic orcein should be mixed with 1M HCl in the proportions of ten parts stain to one part acid.
7. Access to microscope with both low and high power objective lenses

#### Safety Precautions/Risks.

Acetic orcein = C



A risk assessment should be carried out as a matter of course.

#### **NOTE**

**Acetic orcein is an acidic stain that turns protein purple. Any stain that gets on skin or natural clothing will stain the material purple.**

### Practical 9 - The extraction of DNA from onions




This practical focuses on setting up and manipulating apparatus and making observations. Further skills can be developed using additional information after the practical has been completed.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Experience simple techniques to extract DNA from living material.
- Further your knowledge about the structure of DNA

#### Safety Information

	You should wear eye protection throughout this practical.
	Protease is <b>harmful</b> . Avoid contact with eyes or skin.
	Ethanol is <b>highly flammable</b> . There should be no flames in the same room.

#### Background information

- DNA is a polymer made up of monomers called nucleotides
- A gene is a set of coded instructions made up of a particular order of nucleotides
- A nucleotide consists of three parts:
  - i) a pentose sugar
  - ii) a nitrogen containing base
  - iii) a phosphate group
- DNA molecule is a double helix held together by hydrogen bonds between the complementary base pairs

You will investigate how DNA can be extracted from living material such as onion.

- Read the information above
- Read your text book and notes on DNA

#### Method

##### Preparations and making observations

1. Prepare the degrading mixture by adding 3g of sodium chloride (table salt) to 10cm<sup>3</sup> liquid detergent (washing up liquid). Make this up to 100cm<sup>3</sup> with distilled water and stir well to ensure the salt has dissolved.
2. The onion should be chopped into small pieces and added to the detergent

solution and stir.

3. Place the beaker into a water bath maintained at 60°C for fifteen minutes.
4. Immediately cool down the onion mixture in an ice cold water bath for five minutes again stirring frequently.
5. Blend using a food blender for no more than five seconds.
6. Using coffee filter paper (laboratory filter paper not coarse enough) filter the mixture into a new beaker.
7. Once you have obtained enough liquid pour 10cm<sup>3</sup> into a boiling tube and add 2 – 3 drops of a protease enzyme and shake the tube to mix the contents well.
8. Pour 10cm<sup>3</sup> ice cold ethanol slowly into the boiling tube and place the tube into rack for about five minutes.
9. DNA should appear where the two liquids meet.

#### Write-up

- Write up the method and answering the following questions
1. What effect would the washing up liquid (detergent) have on the cell membranes?
  2. Why was the beaker placed in a hot water bath for 15 minutes and then immediately cooled?
  3. Why was the mixture blended, but only for 5 seconds?
  4. What type of enzyme would now be needed to separate the DNA into smaller pieces?

## Practical 9 - Lesson Plan

### The extraction of DNA from onions

#### Context

A practical investigation set in the context of 9700 syllabus –

#### Key aims of the lesson

This practical is designed to develop the skills of observation and manipulation of apparatus.

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods.
- Describe and explain the reasons behind the methods.
- Extend knowledge on the structure and function of DNA.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – Student worksheet given out for students to read in preparation for the practical lesson. To consider the structure of DNA and reinforce previous learning
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of DNA structure, key points written on board
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns with sharp knives and use of ethanol
8 - 45	<b>Carrying out the practical</b> – students carry out the practical work. Whilst they are waiting for the 15 minute period they can write up the first part of the method and consider the questions.
40 - 50	<b>Obtain results</b> – Students observe DNA produced then clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity
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**Useful information**

- Other vegetables/fruit can be substituted for onions, however mixed results are often obtained.

Discussion / evaluation points should include:

- explanation of the methods used
- possible problems with the method

## Practical 9 - Technical information

### Extraction of DNA from onions

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. fresh onion, approximately tennis ball sized
2. sharp knife
3. chopping board
4. 2 x 250cm<sup>3</sup> beakers
5. 1 x 400cm<sup>3</sup> beaker or jug (for the ice)
6. 3g salt
7. 10cm<sup>3</sup> washing up liquid
8. 90cm<sup>3</sup> distilled water
9. (Thermostatically controlled) water bath at 60°C.
10. Supply of ice
11. Food blender (household domestic one is ideal)
12. Coffee filter paper
13. Funnel
14. Boiling tube
15. 2-3 drops of protease enzyme, such as neutrase ®
16. 10cm<sup>3</sup> ice cold ethanol

### SAFETY NOTE

The ethanol must be ice cold, this involves leaving it overnight in a freezer. It is essential that it is placed in a sealed, vapour tight plastic bottle. If this is not possible put the ethanol in a sealed container in an ice bath for several hours before the practical is due to start.

#### Safety Precautions/Risks.

Protease = H



Ethanol = F



A risk assessment should be carried out as a matter of course.

### Practical 10 - The effect of wind speed on the rate of transpiration in a leafy shoot

This practical focuses on making measurements and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. You will also develop other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Identify dependent and independent variables
- Make a hypothesis and express this in words and graphically
- Identify the variables that should be controlled
- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between transpiration and wind speed
- Evaluate procedures

#### Safety Information



You should wear eye protection throughout this practical.

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- Transpiration is the movement of water through plants, from the roots where it is absorbed by osmosis, to the leaves where it is lost by evaporation.
- Water leaves a plant's leaves through stomata, the aperture of which is controlled by guard cells.
- Most plants open their stomata during the day and close them at night.
- Plants may close their stomata when stressed by losing too much water.
- The real purpose of stomata is to absorb carbon dioxide and release oxygen from photosynthesis without losing too much water.
- The evaporation of water is affected by wind speed, temperature, humidity and atmospheric pressure.

You will investigate how **wind speed** affects the rate of transpiration from a leafy shoot by using a **potometer**.

- Read the information above
- Identify and write down the dependent and independent variables
- Write down a hypothesis
- Draw a sketch graph to show what you think will happen



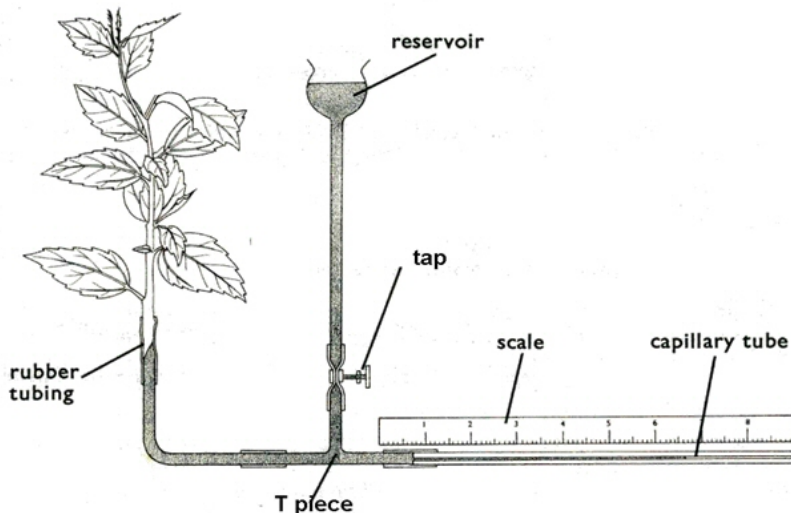
- Identify any variables that should be controlled and outline how this should be done
- What would be the best method for setting up the potometer?
- Plot a graph of the distance of the fan from the shoot, against the rate of water movement in the potometer, after the practical. Make sure you know how to calculate rate.

Wind speed in this case is varied by moving the fan to fixed distances from the leafy shoot. Although the wind speed from the fan will not accurately follow the inverse - square law, you would be well advised to understand how increasing the distance of the fan from the shoot, may affect the wind speed.

## Method

### Preparations

The apparatus should be assembled as shown in the following diagram.



1. Attach the rubber tubing, the capillary tube and the water reservoir to the T piece.
2. Fill the reservoir, capillary tube and rubber tubing with water. This can be done by placing them under water and gently squeezing the rubber tubing until all the air has been removed.
3. Leave the apparatus under water.
4. Cut a fresh leafy shoot with a sharp knife and immediately place the cut end under water.
5. Carefully attach the cut end of the shoot to the rubber tubing. This should be done with the cut end only under water.
6. Close the tap on the water reservoir.
7. Remove the apparatus from the water and attached to a clamp stand or support.
8. Place a mm scale behind the capillary tube.
9. Place a fan at a set distance from the leafy shoot. Do NOT switch on.

### Making observations

1. Note the position of the air bubble in the capillary tube. It may be very close to then end of the tube.
2. Record the time taken for the air bubble to move a set distance along the tube. You will have to determine this distance base on the speed of the bubble. If the bubble is moving quickly the distance will need to be larger than if it is moving slowly.
3. Reset the air bubble to then end of the capillary tube by carefully opening

the tap on the water reservoir.

4. Turn on the fan and repeat the procedure.
5. Reset the apparatus and move the fan to another distance.
6. Repeat the procedure with the fan at at least five different distances.

You are advised to start with the fan at the furthest distance and gradually move it towards the leafy shoot.

#### Calculations

1. Calculate the rate of movement using  $1/\text{time}$  taken for the air bubble to travel a set distance.
2. Record the rate of travel for each distance in the class result table on the board or flip chart.
3. When all of the results have been recorded in the class results table, calculate the mean rate of movement for each distance.
4. (Optional) – calculate the standard error for each distance.

#### Write up

- Record your results in a clear table ensuring units are put in headers.
- Plot a graph to show the mean rate of movement for each distance.
- (Optional – add error bars to your graph)
- Make an evaluation considering:
  - the limitations of the method used,
  - anomalous values if any,
  - replication and range of values of independent variable,
  - the confidence with which the conclusions should be drawn.
- Draw conclusions considering:
  - detailed description of the features of the results,
  - whether your results agree or contradict your hypothesis,
  - a scientific explanation of your results and conclusions,
  - any modifications you could make to improve the experiment.

## **Practical 10 - Lesson Plan**

### **The effect of wind speed on the rate of transpiration in a leafy shoot.**

#### **Context**

A practical investigation set in the context of 9700 syllabus – Investigate experimentally the factors that affect transpiration rate.

#### **Key aims of the lesson**

This practical is designed to develop the skills of planning, observation, analysis and evaluation.

#### **Intended learning outcomes**

By the end of the practical and the write-up the student should be able to:

- make a hypothesis and express this in words and graphically
- identify the dependant and independent variables
- identify the variables that should be controlled
- experience relevant methods, analysis, conclusions and evaluation
- describe and explain the relationship between wind speed and the rate of transpiration in a leafy shoot.

### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

**Planned activities** (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – 2 page student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on water movement through plants.
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review transpiration in plants. Teacher led questioning, student responses / discussion regarding the factors that can affect the rate of transpiration and how they might be measured.
5 - 10	<b>Introduction to method</b> – Teacher briefly demonstrates the procedure for setting up the potometer and explains the importance of not having any air in the apparatus until the introduction of the air bubble. Teacher emphasises safety concerns with the sharp knife.
10 - 40	<b>Carrying out the practical</b> – students carry out the practical work..
40 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished
50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity to include identification of variables, the hypothesis, results and graphs along with a full detailed write of the experiment and an explanation of how the experiment could be extended.

### Useful information

- The evaporation of water from a leaf is affected by wind speed, temperature, humidity and air pressure, the first three having the most significant affect.
- Increased temperature increases the kinetic energy of the water molecules thus increasing the rate of evaporation.
- Increased wind speed blows away evaporated molecules form around the opening of the stomata thus maintaining a greater diffusion gradient for the water molecules.

- Increased humidity lowers the concentration gradient and thus slows down the rate of evaporation.
- Reduced air pressure increases the rate of evaporation.
- Factors such as light are affected by the inverse square law where doubling the distance reduces the light intensity by a factor of four. Although this does not hold true for wind speed, you should be aware that doubling the distance of the fan from the leafy shoot, will not necessarily mean that the wind speed is reduced by half.
- Possible variables to control include temperature and humidity.

For students unable to obtain accurate data, the following table of results may be used.

rate of movement / 1/time in seconds	distance of fan from shoot / cm
0.016	200
0.018	175
0.023	150
0.034	125
0.055	100
0.071	75
0.092	50
0.11	25

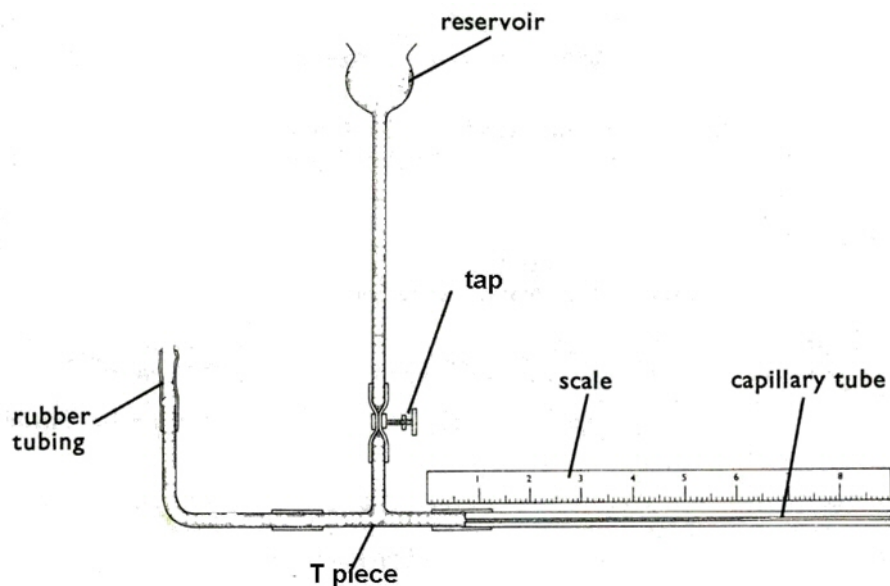
### Practical 10 - Technical information

#### The effect of wind speed on transpiration in a leafy shoot

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

1. 1 freshly cut leafy shoot that has been put immediately into fresh water
2. 1 potometer set up as shown in the diagram below.



3. electric fan
4. meter rule
5. sight of a stop watch or clock

Additionally each student will require access to a sink and running water.

#### Safety Precautions/Risks.

No specific hazards identified.

A risk assessment should be carried out as a matter of course.

### Practical 11 - Investigating the role of carbon dioxide in living organisms.




This practical focuses on manipulation and observations, recording and presenting data, analysis, drawing conclusions and evaluating methods. The practical also develops skills of using material in new and unfamiliar situations.

#### Intended learning outcomes

By the end of this practical you should be able to:

- Identify dependent and independent variables
- Make a hypothesis and express this in words
- Experience relevant methods, analysis and conclusion.
- Describe and explain the relationship between different living organisms and the production of carbon dioxide
- Evaluate procedures

#### Safety Information

	You should wear eye protection throughout this practical.
	Ethanol is <b>highly flammable</b> . There should be no flames in the same room.
	Bicarbonate indicator solution is <b>flammable</b> .

#### Background information

- Carbon dioxide is a gas found in the air at 0.04%
- Carbon dioxide dissolves in water to form carbonic acid thus reducing the pH
- When bicarbonate indicator solution is equilibrated with air it turns red/orange
- Bicarbonate indicator changes colour in different levels of pH
- You will remember from biology learnt in earlier courses that plants both respire and photosynthesise.
- Respiration    glucose + oxygen     $\longrightarrow$     carbon dioxide + water + energy
- Photosynthesis    carbon dioxide + water     $\longrightarrow$     glucose + oxygen
- The point at which the carbon dioxide released by plants from respiration, equals the carbon dioxide absorbed by plants for photosynthesis is called the plant's compensation point.

You will investigate the effect of different living organisms on bicarbonate indicator and use this information to devise an experiment to determine the compensation point in plants.

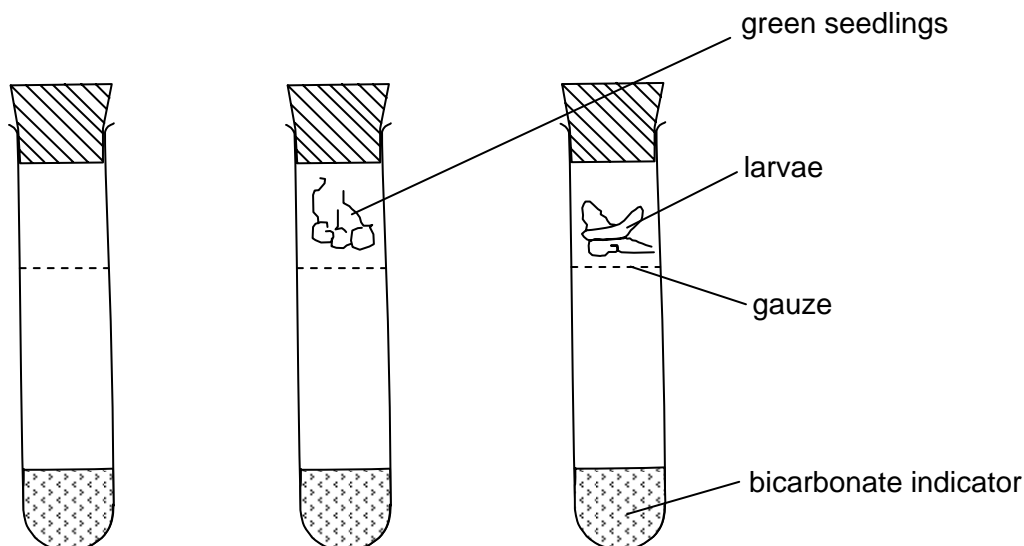


- Read the information above
- Identify and write down the dependent and independent variables
- Write down what you think will happen (do not worry about what the colour the indicator will be – you will discover that by doing the experiment)
- Identify any variables that should be controlled and outline how this should be done
- Write down a hypothesis to explain what will happen to the colour of the bicarbonate indicator when a plant is at its compensation point.

### Method

#### Preparations and making observations

1. Rinse out three large test-tubes with distilled water and then with bicarbonate indicator solution.
2. Using a syringe or small measuring cylinder place 3 – 5 cm<sup>3</sup> of bicarbonate indicator solution into each test-tube.
3. Carefully place a piece of perforated gauze in each test-tube so that it is just above the indicator solution.
4. Place a rubber bung or cork into the first test-tube.
5. Carefully place three green seedlings onto the gauze in the second test-tube and seal with a rubber bung or cork.
6. Carefully place three fly larvae onto the gauze in the third test-tube and seal with a rubber bung or cork.
7. Place the three test-tubes near a bright light source such as a lamp or window



8. Check that the colour of the bicarbonate indicator solution in each test-tube is red/orange at the start of the experiment.
9. Leave the tubes for at least 30 minutes, comparing the colour of each indicator solution every ten minutes.
10. When the colours look different in all three test-tubes, note the final colour of the indicator in each of the three test-tubes.

Write-up

- Record your results in a clear table.
- Explain why one of the test-tubes contained no living material
- Explain your findings using your knowledge of respiration and photosynthesis
- Assess the reliability of the results obtained and suggest any modifications you could make to improve the experiment
- Plan and describe, but do not carry out an experiment using the same technique, to determine the compensation point in plants.

## Practical 11 - Lesson Plan

### Investigating the role of carbon dioxide in living organisms.

#### Context

A practical investigation set in the context of 9700 syllabus – Gaseous exchange

#### Key aims of the lesson

This practical is designed to develop the skills of observation, analysis and evaluation and using knowledge gained in a new and different context

#### Intended learning outcomes

By the end of the practical and the write-up the student should be able to

- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain how an experimental method can be adapted to discover when a plant is at its compensation point.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalk

Practical materials specified on the Technical Information Sheet.

Copies of the student worksheets.

#### Planned activities

Timings/ minutes	Teacher/ Student Activities
End of previous lesson	<b>Preparation</b> – 2 page student worksheet given out for students to read in preparation for the practical lesson. To consider identification of the variables, formulate a hypothesis and review previous learning on cell membranes
0 - 3	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson – teacher led oral presentation
3 - 5	<b>Context</b> – review of pH indicators such as litmus and universal indicator and that carbon dioxide dissolves to form an acidic solution.
5 - 8	<b>Introduction to method</b> – Teacher briefly outlines method and answers any student questions on procedure. Teacher emphasises safety concerns and ethics when handling living material such as fly larvae which must not suffer undue stress.
8 - 40	<b>Carrying out the practical</b> – students carry out the practical work..
40 - 50	<b>Obtain results</b> – Students enter results into table and clear away apparatus as soon as they have finished

50 - 60	<b>Drawing together the threads</b> – Teacher led discussion on the skills that have been developed as well as discussion on results obtained. Practical write up to be completed in following lesson or as homework activity
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**Useful information**

Discussion / evaluation points should include:

- what colour bicarbonate indicator turned in different situation
- the cause of the colour change in the bicarbonate indicator
- what other variables could have affect the results and which variables should be controlled
- how the procedure could be improved to increase reliability
- how the procedure could be modified to determine the compensation point in plants

A numerical value of the compensation point can be determined by using a light metre. The light reading should be taken as close to the plant as possible at the time when the plant is at its compensation point.

For students unable to obtain accurate data, the following table of results may be used.

		no living material	seedlings	fly larvae
colour of bicarbonate indicator	start	red/orange	red/orange	red/orange
	end	red/orange	purple	yellow

## Practical 11 - Technical information

### Investigating the role of carbon dioxide in living organisms.

The apparatus and materials required for this practical are listed below.

The amount of apparatus listed is for one student or one group of students if they are to work in groups.

1. 3 large test-tubes each fitted with a rubber bung or cork
2. gauze or similar to support the specimens in the test-tubes whilst at the same time allowing the transfer of gases
3. supply of distilled water to rinse each test-tube.
4. 40 cm<sup>3</sup> of bicarbonate indicator solution, sufficient to rinse each test-tube and have sufficient remaining to place 5cm<sup>3</sup> into each test-tube.

The stock solution of indicator can be prepared by dissolving 0.2g of thymol blue and 0.1g of cresol red in 20cm<sup>3</sup> of ethanol. Also prepare a solution by adding 0.84g of pure sodium bicarbonate to 900 cm<sup>3</sup> of distilled water. Add the dyes to this solution and make up to 1 dm<sup>3</sup>. To prepare the indicator for use, pipette 25cm<sup>3</sup> of stock solution into a graduated flask and make up to 250 cm<sup>3</sup> with distilled water.


The solution should be equilibrated with air by aspirating atmospheric air through the solution until it is orange/red in colour.


5. 3 germinated seeds such that they have developed green leaves and are photosynthesising. Cress seeds that have been placed on moist cotton wool in a Petri dish will germinate and develop leaves in only a few days. Times will vary depending upon local conditions.
6. 3 large fly larvae that are active and not approaching pupation
7. 10cm<sup>3</sup> graduated pipette or measuring cylinder or syringe

Additionally each student will require access to a sink and running water.

Commercial bicarbonate indicator solution is available from most chemical wholesalers, however it is possible to make up the solution in the laboratory as described above.

#### Safety Precautions/Risks.

Ethanol = F 

Bicarbonate indicator solution = F 

A risk assessment should be carried out as a matter of course.

# Teaching A Level Biology Practical Skills

in preparation for 9700 paper 5

**Your attention is drawn to the section on Risk Assessment on page 15 of the Introduction to this booklet, and to the hazards indicated in Appendices 1 and 2. While all effort has been made to ensure that appropriate safety indications are given, CIE accepts no responsibility for the safety of these experiments and it is the responsibility of the teacher to carry out a full risk assessment for each experiment undertaken, in accordance with local rules and regulations. Hazard data sheets should be available from your suppliers.**

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## Introduction

You may have been teaching AS and A level biology for many years or perhaps you are new to the game. Whatever the case may be, you will be keen to ensure that you prepare your students as effectively as possible for their examinations. The use of a well-structured scheme of practical work will certainly help in this ambition. However it can do so much more. Scientists who are thoroughly trained and experienced in practical skills, will have a 'feel' for the subject and a confidence in their own abilities that is far greater above those with a purely theoretical background. It is true that there are branches of biology that might be described as purely theoretical but they are in the minority. Essentially, biology is a practical subject and we owe it to our students to ensure that those who pursue science further have the necessary basic practical skills to take forward into their future careers. Furthermore, the basic skills of planning, analysis and evaluation will be of great value to those who pursue non-science careers.

### Why should I read this booklet?

Some of you may be wondering why you should need a booklet like this. If your practical skills are of a high order and you feel confident teaching these skills to others, you probably don't need it; but you might find some of the exercises described in the appendices useful. However, if you are like the majority of us, a little help and support is likely to be appreciated. This booklet aims to provide at least some of this support.

It is designed for the teacher rather than for the student. Its objective is to provide a framework within which the practical skills of teachers can develop and grow. Experience shows that as a teacher's practical skills grow, so too do the confidence to teach such skills and the time that you will be prepared to spend on teaching practical work.

### How much teaching time should I allocate to practical work?

The syllabus stipulates that at least 20% of teaching time should be allocated to practical work. This is in addition to any time the teacher chooses to use for practical demonstrations to illustrate the theory syllabus. This emphasis on practical work is not misplaced. Consider the weighting given to assessment objectives in the syllabus: 24% is allocated to experimental skills and investigations and 30% is allocated to handling, applying and evaluating information. Taken together, 55% of the total award is related to a students' ability to interpret data, understand how this has been obtained, recognise limitations and suggest explanations; all of which lend themselves to investigative work involving practical experience. If the specific practical papers are considered in isolation, they still represent 23% of the AS and 24% of the A Level award.

In planning a curriculum, teachers should therefore expect to build in time for developing practical skills. If, for example, the time allowed is 5 hours per week over 35 weeks, then a minimum of 1 hour per week should be built into the plan, so that over the year, a minimum of 35 hours is made available. Bearing in mind the emphasis on assessment objectives that related to information handling and problem solving, a minimum of 2 hours per week might be more appropriate, which at 40% of the time is still less than the overall weighting for these assessment objectives.



### **Can I use the practicals in these booklets in a different order?**

It is assumed in these booklets that for A level candidates, the AS work will be taught in the first year of the course, with the A2 work being covered in the second year. If the linear A Level assessment route is used, care should be taken with regard to in the order in which practical exercises are used, as the skills practiced in these booklet are hierarchical in nature, i.e. the basic skills established in the AS booklet are extended and developed in the A2 Level booklet. Thus, students will need to have practiced basic skills using AS exercises before using these skills to tackle more demanding A Level exercises.

The exercises in these booklets are given in syllabus order. A teacher may well decide to use a different teaching sequence, but the point made above, regarding AS/A2 exercises, still applies.

### **What resources will I need?**

For a practical course in A-level Biology to be successful, it is not necessary to provide sophisticated equipment. Some of the more advanced practicals in these booklets may require less easily obtainable equipment, but the vast majority can be performed using the basic equipment and materials in the lab. Alternative 'low-tech' exercises are also provided where possible.

A list of the basic resources required for assessment may be found in the syllabus. A more detailed list may be found in the booklet '*CIE Planning For Practical Science in Secondary Schools*', Appendix B.

### **Is there a limit to the class size?**

It is true that there is a limit to the class size that is manageable in a laboratory situation, particularly when students may be moving about. The actual size may be determined by the size of the room, but as a general guide, 15 - 20 students is the maximum that one person can reasonably manage, both for safety reasons and so that adequate support can be given to each student. Larger numbers can more easily and safely be accommodated with input from another person with appropriate qualifications / experience or splitting the class into two groups for practical lessons.

### **Why should I teach my students practical skills?**

Although this section is likely to be read once only, it is arguably the most important; for, if it convinces readers that practical work is an essential part of biology as a science and underpins the whole teaching programme, the aim of publishing this booklet will have been achieved.

### **Points to consider**

- It's fun! The majority of students thoroughly enjoy practical work. The passion that many scientists have for their subject grew out of their experiences in the practical classes. Students who enjoy what they are doing are likely to carry this enthusiasm with them and so be better motivated.
- Learning is enhanced by participation as students tend to remember activities they have performed more easily, thus benefiting their long-term understanding of the subject. Students who simply memorise and recall facts find it difficult to apply their knowledge to an unfamiliar context. Experiencing and using practical skills helps develop the ability to use information in a variety of ways, thus enabling students to apply their knowledge and understanding more readily.

- The integration of practical work into the teaching programme quite simply brings the theory to life. Teachers often hear comments from students such as “I’m glad we did that practical because I can see what the book means now.” and “It’s much better doing it than talking about it.”
- Chemistry, physics and biology are by their very nature, practical subjects – both historically and in the modern world. The majority of students who enter careers in science need to employ at least basic practical skills at some time in their career. For all students, whether they regard themselves as scientists or non-scientists, the skills that they develop by doing practical work, hand-eye coordination skills, communication, numeracy and problem solving skills, will prove to be useful transferable skills throughout their future life.
- A practical course develops many cross-curricular skills including literacy, numeracy, ICT and communication skills. It develops the ability to work both in groups and independently and with confidence. It enhances critical thinking skills and it requires students to make judgements and decisions based on evidence, some of which may well be incomplete or flawed. It helps to make students more self-reliant and less dependent on information provided by the teacher.
- The skills developed are of continued use in a changing scientific world. While technological advances have changed the nature of practical procedures, the investigative nature of practical science is unchanged. The processes of observation, hypothesis formation, testing, analysis of results and drawing conclusions will always be the processes of investigative science. The ability to keep an open mind in the interpretation of data and develop an appreciation of scientific integrity is of great value both in science and non-science careers.
- Practical work is not always easy and persistence is required for skills and confidence to grow. Students often relish this challenge and develop a certain pride in a job well done.
- The more experience students have of a variety of practical skills, the better equipped they will be to perform well in the practical exams, both in terms of skills and confidence. While it could be argued that the required skills could be developed for papers 31 and 32 simply by practising past-papers, the all-round confidence in practical ability will be greatly enhanced by a wider experience. Similarly for paper 5, while it might be argued that planning, analysis and evaluation could be taught theoretically, without hands-on experience of manipulating their own data, putting their plans into action and evaluating their own procedures and results, students will find this section difficult and will be at a distinct disadvantage in the examination. Those students who can draw on personal experience, and so are able to picture themselves performing the procedure they are describing, or recall analysing their own results from a similar experiment are much more likely to perform well than those with limited practical skills.

### **What are the practical skills required by this course?**

This course addresses seven practical skills that contribute to the overall understanding of scientific methodology. In a scientific investigation these would be applied in the following sequence.

- 1 Planning the experiment**
- 2 Setting up / manipulating apparatus**
- 3 Making measurements and observations**

- 4 Recording and presenting observations and data**
- 5 Analysing data and drawing conclusions**
- 6 Evaluating procedures**
- 7 Evaluating conclusions**

The syllabus shows how these seven skills are assessed and the structure is common to all three sciences. The emphasis of the AS syllabus is on developing an understanding and practice of scientific procedures, the collection of data, analysis and drawing conclusions. It also starts to develop critical evaluation of procedures by suggesting improvements to experimental procedures. In general students find the performance of practical procedures and the collection of data more accessible than analysis, whilst evaluation is least readily accessed. To enable access to these more demanding skills, students need to understand why an experimental procedure is carried out in a particular way so that they can recognise sources of error or limitations which could affect the reliability of their results. Students will not be able to evaluate until they can critically review a practical procedure.

The A2 syllabus builds upon the skills developed in AS and its emphasis is on the higher level skills of planning, analysis and evaluating. In order to plan effectively, students need to be able to evaluate procedures and critically assess results. This is best achieved by the performance of practical exercises starting in AS with relatively straightforward and familiar contexts and developed in A2 by the use of more complex procedures and less familiar contexts. Data analysis again develops from AS into more complex treatments so that students need to be given opportunities to gather suitable data and perform the appropriate manipulations. The evaluation of conclusions and assessing procedures are very high order skills. Students who have not had sufficient opportunity to plan and trial their own investigations will find these skills difficult. Students are not expected to be able to plan perfectly, but to recognise weaknesses and make reasonable suggestions for improvement. The best learning tool to develop these skills is to devise a plan, carry out the investigation and then assess how well the planned procedure worked. The syllabus gives detailed guidance on the expected skills and learning outcomes.

In summary, as the syllabus clearly shows, skills 2-6 listed above will be assessed at AS level in papers 31 and 32. Skills 1 and 7 will only be assessed at A level in paper 5, which will also take skills 5 and 6 to a higher level.

The above list shows the seven skills in the order in which they would be used in an extended investigation. It is not suggested, nor would it be wise, to teach these skills in this order. Students who are new to practical work will initially lack the basic manipulative skills, and the confidence to use them. It would seem sensible, therefore, to start practical training with skill 2, initially with very simple tasks and paying attention to the establishment of safe working practices.

Once a measure of confidence in their manual dexterity has been established, AS students can move on to exercises that require skills 3 and 4 to be included. Extensive experience in carrying out practical procedures allows students to gain awareness of appropriate quantities and become more organised in time management and the recording of data as it is collected.

It is likely that skill 6, Evaluating Procedures, will be the most difficult to learn at AS level. Critical self-analysis does not come easily to many people. 'My experiment worked well' is a frequent and inappropriate response. If students are to master this skill, they need to develop an appreciation of reliability and accuracy inherent in the equipment and procedure they are using. Only then will they be able to identify anomalous results, or results which fall outside of the 'range of uncertainty' intrinsic in

the choice of apparatus used and so are considered to be inaccurate. Exercises with less reliable/accurate outcomes can be used to provide more scope for the evaluation of procedural, technique or apparatus errors.

Planning is arguably the most demanding of the seven skills. For it to be effective, students need to be very well grounded in skills 2-6, so that they can anticipate the different stages involved in the task, and can provide the level of detail required. It is for this reason that planning skills are not assessed at AS level but form part of the A2 assessment in Paper 5. Unless students use apparatus they do not develop an understanding of how it works and the sort of measurements that can be made using particular sorts of apparatus. Candidates cannot be taught to plan experiments effectively unless, on a number of occasions, they are required:

- to plan an experiment;
- to perform the experiment according to their plan;
- to evaluate what they have done.

The evaluation of conclusions, skill 7, is done by comparison of the outcome of an exercise with the predicted outcome, and so is also an A2 skill. It should be taught and practised as part of the planning exercises.

### Summary of each of the 7 skills

Full details of the requirements for each of these skills may be found on pages 34 to 41 of the syllabus. What follows below is a brief summary of the skills involved.

#### 1 Planning

- **Defining the problem**

Students should be able to use information provided about the aims of the investigation, or experiment, to identify the key variables. They should use their knowledge and understanding of the topic under consideration to make a quantitative, testable, prediction of the likely outcome of the experiment.

- **Methods**

The proposed experimental procedure should be workable. It should, given that the apparatus is assembled appropriately, allow data to be collected without undue difficulty. There should be a description, including diagrams, of how the experiment should be performed and how the key variables are to be controlled. Equipment, of a level of precision appropriate for the measurements to be made, and quantities to be used should be specified. The use of control experiments should be considered.

- **Risk assessment**

Candidates should be able to carry out a simple risk assessment of their plan, identifying areas of risk and suggesting suitable safety precautions to be taken.

- **Planning for analysis, conclusions and evaluation**

Students should be able to describe the main steps by which their results would be analysed in order that that valid conclusions might be drawn. This may well include the generation of a results table and the proposal of graphical methods to analyse data. Also, they should propose a scheme for the interpretation and evaluation of the results themselves, and of the experimental procedure employed in obtaining those results. There should

be an indication of how the outcomes of the experiment would be compared with the original hypothesis.

## **2 Setting up / manipulating apparatus**

It is important that students are allowed sufficient time and opportunity to develop their manipulative skills to the point where they are confident in their approach to experimental science. They must be able to follow instructions, whether given verbally, in writing or diagrammatically, and so be able to set up and use the apparatus for experiments correctly.

## **3 Making measurements and observations**

- **Measuring/observing**

Whilst successfully manipulating the experimental apparatus, it is crucial that students are able to make measurements with accuracy and/or to make observations with clarity and discrimination. Accurate readings of meters or burettes and precise descriptions of colour changes and precipitates will make it much easier for students to draw valid conclusions, as well as scoring more highly in the test.

- **Deciding on what measurements/observations to make**

Time management is important, and so students should be able to make simple decisions on the number and the range of tests, measurements and observations that can be made in the time available. For example, if the results of the first two titrations are in good agreement, there is no need to carry out a third.

Students need to be able to make informed decisions regarding the appropriate distribution of measurements within the selected range, which may not always be uniform, and the timing of measurements made within the experimental cycle. They should also be able to identify when repeated measurements or observations are appropriate.

The strategies required for identifying and dealing with results which appear anomalous should be practised.

## **4 Recording and presenting observations and data**

An essential, but frequently undervalued, aspect of any experimental procedure is the communicating of the results of the procedure to others in a manner that is clear, complete and unambiguous. It is vital that students are well practised in this area.

- **The contents of the results table**

The layout and contents of a results table, whether it is for recording numerical data or observations, should be decided before the experiment is performed. 'Making it up as you go along' often results in tables that are difficult to follow and don't make the best use of space. Space should be allocated within the table for any manipulation of the data that will be required.

- **The column headings in a results table**

The heading of each column must be clear and unambiguous. In columns which are to contain numerical data, the heading must include both the quantity being measured and the units in which the measurement is made. The manner in which this information is given should conform to 'accepted practice'.

- **The level of precision of recorded data**

It is important that all data in a given column is recorded to the same level of precision, and that this level of precision is appropriate for the measuring instrument being used.

- **Display of calculations and reasoning**

Where calculations are done as part of the analysis, all steps of the calculations must be displayed so that thought processes involved in reaching the conclusion are clear to a reader. Similarly, where conclusions are drawn from observational data, the key steps in reaching the conclusions should be reported and should be clear, sequential and easy to follow.

- **Significant figures**

Students should be aware that the number of significant figures to which the answer is expressed shows the precision of a measured quantity. Therefore, great care should be taken with regard to the number of significant figures quoted in a calculated value. The general rule is to use the same number of significant figures as (or at most one more than) that of the least precisely measured quantity.

- **Data layout**

Students should be able to make simple decisions concerning how best to present the data they have obtained, whether this is in the form of tabulated data or as a graph. When plotting graphs they should be able to follow best practice guidelines for choosing suitable axis scales, plotting points and drawing curves or lines of best fit. In drawing tables they should be able to construct a table to give adequate space for recording data or observations.

## 5 Analysing data and drawing conclusions

This skill requires students to apply their understanding of underlying theory to an experimental situation. It is a higher-level skill and so makes a greater demand on a student's basic understanding of the biology involved. Even when that understanding is present, however, many students still struggle. The presentation of a clear, lucid, watertight argument does not come naturally to most people and so much practice in this area is recommended.

- **Interpretation of data or observations**

Once data has been presented in the best form for analysis of the results of the experiment, the student should be able to describe and summarise any patterns or trends shown and the key points of a set of observations. Further values such as the gradient of a graph may be calculated or an unknown value found, for example from the intercept of a graph.

- **Errors**

Students should be used to looking at an experiment, assessing the relative importance of errors and where appropriate, expressing these numerically. Students should be aware of two kinds of error.

- i The 'error' that is intrinsic in the use of a particular piece of equipment. Although we refer to this as an equipment error, we really mean that there is a 'range of uncertainty' associated with measurements made with that piece of equipment. This uncertainty will be present no matter how skilled the operator might be.

- ii Experimental error, which is a direct consequence of the level of competence of the operator or of the effectiveness of the experimental procedure.

- **Conclusions**

Students should learn to use evidence to support a given hypothesis, to draw conclusions from the interpretation of observations, data or calculated values and to make scientific explanations of their data, observations and conclusions. Whatever conclusions are drawn, they must be based firmly on the evidence obtained from the experiment. At the highest level, students should be able to make further predictions and ask appropriate questions based on their conclusions.

## **6 Evaluating procedures**

Arguably, this is one of the most important, and probably one of the most difficult skills for a student to develop. In order for the evaluation to be effective, students must have a clear understanding of the aims and objectives of the exercise, otherwise they will not be able to judge the effectiveness of the procedures used. They must be able to evaluate whether the errors in the data obtained exceed those expected due to the equipment used. If this is the case, they then need to identify those parts of the procedure which have generated these excess errors, and suggest realistic changes to the procedure which will result in a more accurate outcome. Students should also be able to suggest modifications to a procedure to answer a new question.

The evaluation procedure may include:

- i the identification of anomalous values, deducing possible causes of these anomalies and suggesting appropriate means of avoiding them,
- ii an assessment of the adequacy of the range of data obtained,
- iii an assessment of the effectiveness of the measures taken to control variables,
- iv taking an informed judgement on the confidence with which conclusions may be drawn.

## **7 Evaluating conclusions**

This is also a higher-level skill, which will demand of the student a thorough understanding of the basic theory that underpins the science involved.

The conclusions drawn from a set of data may be judged on the basis of the strength or weakness of any support for or against the original hypothesis.

Students should be able to use the detailed scientific knowledge and understanding they have gained in theory classes in order to make judgements about the reliability of the investigation and the validity of the conclusions they have drawn.

Without practice in this area, students are likely to struggle. In order to increase the confidence in drawing conclusions, it is recommended that practical exercises, set within familiar contexts, be used to allow students the opportunity to draw conclusions, make evaluations of procedure and assess the validity of their conclusions.

In the examination, students may be required to demonstrate their scientific knowledge and understanding by using it to justify their conclusions.

## Ways of doing practical work

Science teachers should expect to use practical experiences as a way to enhancing learning. Practical activities should form the basis on which to build knowledge and understanding. They should be integrated with the related theory, offering opportunities for concrete, hands-on, learning rather than as stand-alone experiences. In planning a scheme of work it is important to consider a mosaic of approaches that include those that allow students to participate in their own learning.

Some practical activities should follow the well established structure that includes a detailed protocol to follow. Such well-structured learning opportunities have a vital role to play in introducing new techniques, particularly in rapidly developing fields such as biotechnology. In these new areas of science, teachers will often find themselves leading practical work that they have not had the chance experience themselves as students.

Other practical activities should offer the students the opportunity to devise their own methods or to apply to solving a problem the methods that they have been taught. The excitement generated by exposure to “new” and unfamiliar techniques provides a stimulus to engage a student’s interest and challenge their thinking.

Practical activities may be used as a tool to introduce new concepts – for example, introducing catalysis by experimentation, followed up by theoretical consideration of the reasons for the unexpected results obtained. On other occasions, practical work can be used to support and enhance the required knowledge and understanding – for example in building upon a theoretical consideration of the limiting factors of photosynthesis with a series of practicals investigating the effect of light intensity and hydrogen carbonate concentration on photosynthesis in water weed. In all cases, learning will be enhanced most effectively by practical work that encourages students to be involved, to think, to apply and use their knowledge, understanding and skills.

Practical work does not always have to be laboratory based. In classrooms, the use of models, role play and paper cut outs to simulate processes can be equally valuable. Field studies also contribute greatly to a students’ appreciation of Biology. No amount of reading or viewing videos can substitute for being exposed to an environment and the organisms living there. Even a carefully managed environment like a school lawn represents a challenge to recognise the species and to understand how they can survive.

There are a variety of strategies by which practical work can be integrated into a scheme of work. Teachers should use a variety of methods, enhancing a variety of subject specific skills and simultaneously developing a variety of transferable skills that will be useful throughout their future professional lives. Some of the ways of delivering practical work also enable the teacher to interact on a one-to-one basis with individual students. This allows a teacher to offer support at a more personal level and develop a greater awareness of an individual students needs.

Your choice of the specific strategy to use will depend on such issues as class size, laboratory availability, the availability of apparatus, the level of competence of your students, availability and expertise of technical support, the time available, your intended learning outcomes for the activity and safety considerations. The following are some possible strategies for delivery of practical work:

- **Teacher demonstrations**

These require less time than a full class practical, but give little opportunity for students to develop manipulative skills or gain familiarity with equipment. Careful planning can give opportunity for limited student participation. Teacher



demonstrations are a valuable way of showing an unfamiliar procedure at the start of a practical session, during which students go on to use the method.

**Considerations** in choosing to do a demonstration **might include:**

- i **Safety** – some exercises carry too high a risk factor to be performed in groups.
- ii **Apparatus** – complicated procedures or those using limited resources
- iii **Time** – demonstrations usually take less time
- iv **Outcome** – some results are difficult to achieve and may be beyond the skill level of most of the students. A failed experiment may be seen as a waste of time.
- v **Students' attention** – a danger is that the attention of some students will drift.
- vi **Manipulative experience** – the teacher gets experience, the students' don't.

There are many good reasons for the teacher performing a demonstration but do be aware that most students have a strong preference for hands-on experimentation. So, where possible, do let them do it!

- **Group work**

**Whole class practical sessions.** These have an advantage in terms of management as all the students are doing the same thing. Students may be working individually, in pairs or in small groups. Integrating this type of practical is straightforward as lessons beforehand can be used to introduce the context and following lessons can be used to draw any conclusions and develop evaluation. Where specialised equipment or expensive materials are in short supply this approach may not be feasible.

**Small group work.** This can provide a means of utilising limited resources or managing investigations that test a range of variables and collect a lot of measurements. Although the same procedure may be performed, each student group collects only one or a few sets of data which are then pooled. For example, if five concentrations of the independent variable are being tested, each of which need to be measured at two minute intervals for thirty minutes, then a group of five students can each test one concentration. Field studies also lend themselves to group activities as a lot of data has to be collected in a short period of time. The individual student has the opportunity to develop their subject specific skills. Part of the role of the teacher is to monitor and maintain safety and also to enable and persuade reluctant learners to take part. Group work aids personal development as students must interact and work co-operatively.

**Considerations might include:**

- i **Learning** – successful hands-on work will reinforce understanding; also, students will learn from each other.
- ii **Confidence** – this will grow with experience
- iii **Awareness/insight** – should grow with experience
- iv **Team building** – a most desirable outcome.
- v **Setting out** – all students doing the same thing is easier for the technicians

- vi **Confusion** – incomplete, ambiguous or confusing instruction by the teacher will waste time while the instructions are clarified but may also compromise safety and restrict learning.
- vii **Opting out** – some students will leave it for others to do and so learn very little.
- viii **Safety** – this could be a serious issue and constant vigilance is essential.
- ix **DIY** – the urge to adapt their experiments, to ‘see what would happen if’, must be strictly dealt with.
- x **Discipline** – practical time must not be allowed to become ‘play time’.

Working in groups, whether as part of a whole-class situation or where groups are working on parts of a whole, is probably the preferred option for many students. At A level, it is highly desirable to include opportunities for students to work on their own, developing their own skills and independence. In Papers 31 and 32, a student’s practical skills will be assessed on an individual basis, so an individual’s experience, competence and confidence are of considerable importance.

- **Circus of experiments**

A circus comprises of a number of different exercises that run alongside each other. Individual or groups of students work on the different exercises and, as each exercise is completed, move on to the next one. These are a means by which limited resources can be used effectively.

There are two basic approaches. Most commonly, during a lesson a number of short activities are targeted at a specific skill. Alternatively, over a series of lessons, a number of longer practical activities are used, addressing a variety of skills. The circus arrangement may be more difficult to manage as the students are not all doing the same activity. This puts more pressure on the teacher as they have to cope with advising and answering questions from a variety of investigations. With circuses spread over a number of sessions, careful planning is needed to enable the teacher to engage each group of students, to maintain a safe environment. In these situations it is useful to have at least two of the circus activities that involve no hands-on practical work - using data response based simulations or other activities. In this way the teacher can interact with groups that need a verbal introduction or short demonstration and can monitor their activities more effectively.

- i **Apparatus** – if the amount apparatus used in an exercise is limited, students are able to use it in rota.
- ii **Awareness** – students by observing their peers will become more aware of the pitfalls of the exercise and so will learn from the experience of others.
- iii **Safety** – different exercises may well carry different safety risks, all of which would need to be covered.
- iv **Setting out** –students doing different exercises will make it more difficult for the technicians
- v **Opting out** – some students may be tempted to ‘borrow’ the results of earlier groups.

- **Within theory lessons**

This option should be considered whenever it is viable. It is likely that the practical work would be by demonstration, as this would take less time. Given

the power of visual images, the inclusion of a short practical to illustrate a theoretical point will reinforce that point and so aid the learning process. It is critical, however, that the practical works correctly, otherwise the flow of the lesson is disrupted and confidence in the theory may be undermined. The exercise should therefore be practiced beforehand.

- **Project work**

Projects are a means by which a student's interest in a particular topic, which is not always directly on the syllabus, can be used to develop investigative skills. It can also be used to access parts of the syllabus that have little laboratory based investigation. For example, in gene technology students might use internet based research to find examples of genetic modification and present a poster display showing the implications. Another might be in aspects of human reproduction, where research into the control of human reproduction and look at trends in access to contraception or IVF together with ethical considerations. This sort of investigative work can be individual, or a group activity. Once the project is underway, much of the work can be student based outside the class room. Care is needed in selecting the topics and setting a time scale, so that the relevance is maintained to the syllabus context. The work can be directed at the production of posters, presentations to give to the group or reports from the group or individual.

- **Extra-curricular clubs**

The role that these can play is in stimulating scientific enquiry methods. There are a number of ways of using clubs. One way is to hold the club session during the teaching day so that all students can attend. In effect this becomes additional lesson time in which students can practice investigative skills, including laboratory work. Such lab work involves materials that have a cost, which must be planned for beforehand. If however the club is held outside the teaching day it may be voluntary. Syllabus specific activities should be limited and the most made of the opportunities for exciting work unrelated to syllabuses. After school clubs could be vehicle for project work that is related to science and of social or economic importance, for example, endangered species. Students who do attend the club could be used as a teacher resource by bringing back their finding to a class room session.

## **Keeping records**

Students often find it a problem to integrate the practical work to the theory. This is particularly true when a series of experiments or a long term investigation or project is undertaken. Some potential issues include:

- Some students use odd scraps of paper in the laboratory, which are lost or become illegible as chemicals are spilled on them. One important criterion is that students are trained to immediately and accurately record results.
- Practical procedures may be provided, or students write their own notes from a teacher demonstration. These may be lost, so students end up with results but no procedure or context.
- When results take a period of time to collect, analysis becomes isolated from the context of the investigation and may not be completed.

The key to minimising these issues is to train students into good work practices. This is particularly important in colleges where students join at the start of their A levels from a variety of feeder schools. It is also vital for students with specific learning

difficulties that affect their ability to organise their work such as dyslexia and Asperger's syndrome.

Students may be encouraged to integrate the practical in the same file as the theory. Alternatively, students may be encouraged to keep an entirely separate practical book or file. Loose leaf files make it easy to add to the file, but may make it easier to lose items. Exercise books can be used but students should be encouraged to glue provided protocols and their laboratory records into the book so that they are not lost. Depending on how they learn, individuals may vary in their preferred method. Whichever option is chosen, students need to be encouraged to relate their investigations to the appropriate theory and to regard it as something that needs to be thoroughly assimilated.

- Integrating the materials generated by practical work with the note and other items from learning of theory can be achieved by interspersing the records of investigations with the relevant section of theory. This may still require cross-referencing where several learning outcomes and assessment objectives are targeted by work.
- Keeping a separate practical book enables records of all the practical investigations to be kept in one place. Students need training to manage practical files effectively, particularly in keeping the contexts and cross referencing to the theory. If care is not taken to develop and keep up these skills, students may perceive practical as something different from theory.
- An intermediate between these two extremes is having a separate section for practical investigations in each student's file with each syllabus section and cross referenced to the relevant theory.

## How is a practical activity organised?

Preparing for practical work needs thought and organisation. The practical work may be an activity that forms part of a lesson, it may comprise an entire lesson, or it may be an investigation designed to last for several lessons, but in every case, thorough preparation is a key prerequisite to success.

Practical and investigative work should be integrated into the programme of study. The scheme of work should identify appropriate practical investigative experiences for use at the most suitable time. In designing the scheme of work,

- the resource implications should be considered in terms of equipment and materials in stock,
- thought should be given to the seasonal availability of materials such as organisms or specific stages of organisms, and the sometimes short shelf-life of thermo-sensitive substances such as enzymes or hygroscopic substances such as some salts
- the time taken from order to delivery, potential for damage during despatch and cost of materials to be obtained from local, national or international suppliers should be considered
- careful scheduling may be needed in Centres with a large number of students. It may be possible to permit several groups to do the work simultaneously or in quick succession, or it may be essential to re-order the scheme of work for different groups so that scarce resources can be used effectively.
- note must be taken of national or local health and safety regulations relating to chemicals, electricity, growing microorganisms etc. There may also be

regulations controlling use of controversial materials such as genetically modified organisms.

Once the scheme of work has been established, the next stage is to consider each practical activity or investigation. In an ideal course, each of the following stages would be gone through in developing each practical exercise in a course. This is not always realistically possible the first time through a course, which is one reason for the existence of this booklet. It is better to get going and to get some practical work done with students than to hold out for perfection before attempting anything. Obviously, all practical work should be subject to careful and rigorous risk assessment no matter how provisional the rest of the supporting thinking and documentation.

- Decide on the aims of the work – the broad educational goals, in terms of the broad skill areas involved (e.g. planning) and the key topic areas (e.g. animal transport systems or unfamiliar material)
- Consider the investigative skills being developed. Reference should be made to the syllabus, which in the practical skills section, includes learning outcomes relating to practical skill. In the 2007 syllabus these are identified by bullet points, but from 2008 onwards, alpha-numeric identifiers will be used. For instance, if the practical work intended is to be a planning exercise, which of the specific skills identified in the learning outcomes will be developed?
- With reference to the topics included, decide on the intended learning outcomes of the practical activity or investigation, again referring to the syllabus. For instance, which of the transport learning outcomes will be achieved? In a few cases during the course, the material on which the practical is to be based may be unfamiliar, in which case there may be no topic-related intended learning outcomes. Thus, A2 contexts may be used for AS practicals, and topic areas not on the 9700 syllabus at all may be used for AS or A2 practicals.
- In addition, it may be useful to assess any other context of the practical work investigation. For instance, is it intended as part of the introduction of a concept, or to support a theory, or to demonstrate a process?
- Produce a provisional lesson plan, allocating approximate times to introduction, student activities and summarising.
- Produce and trial a student work sheet. Published procedures or those produced by other teachers can be used. Alternatively produce your own. As a rule schedules produced by others need modifying to suit individual groups of students or the equipment available. It helpful to ask students or another teacher to read work sheets before they are finalised as they can identify instructions that are ambiguous or use inaccessible terminology.
- Refine the lesson plan in relation to the number of students for which the investigation is intended (whole class or a small group), the available equipment (does some have to be shared?) and materials. There are examples of lesson plans and student work sheets in appendix 2.
  - Carry out a detailed and careful risk assessment (see below) before any preparatory practical work is done, and certainly well before students do any of the practical work. You should consider
    - the likelihood that any foreseeable accident might occur – for example, pupils putting glass tube through bungs are quite likely to break the tube and push it though their hand

- the potential severity of the consequences of any such accident – for example dropping onto a desk a plastic dropper bottle of  $0.01 \text{ mol dm}^{-3}$  hydrochloric acid will cause much less severe eye injuries than the same accident with a glass bottle containing  $5.0 \text{ mol dm}^{-3}$  hydrochloric acid.
- the means that can be taken to reduce the severity of the effect of any accident – for example, the teacher or technician preparing bungs with glass tubes before the lesson, or using eye protection such as safety spectacles during all practical work.
- Make an equipment and materials list. This may need to be in sections;
  - materials and apparatus per student or per group (chemicals and glassware)
  - shared equipment per laboratory (water baths, microscopes, pH meters)
  - any chemicals should include concentrations and quantities needed
  - any equipment should include number required
  - any hazard associated with specific chemicals or equipment should also be noted and cross referenced to the risk assessment. Sources of information about safety may be listed in the syllabus (and are reproduced below).
  - The location of storage areas for equipment and chemicals may be cross referenced to this equipment and materials list.
- Set up and maintain a filing system where master copies of the work sheets, lesson plans and equipment lists can be stored. It is helpful to have these organised, or at least indexed, by both their syllabus context and skills developed.
- Once an investigation has been used by a group of students it should be evaluated in relation to intended outcomes and the lesson plan. It is important to obtain feedback from the students about their perception of the work. For example,
  - was the time allocation appropriate,
  - were the outcomes as expected,
  - did the students enjoy the work,
  - did the students understand the instructions,
  - was the point of the work clear to the students?

If necessary the work sheet and lesson plan should be revised.

## Risk assessment

All practical work should be carried out in accordance with the health and safety legislation of the country in which it is done. No activities should be attempted if they conflict with such legislation.

Hands-on practical work can be carried out safely in schools. If it is to be safe, then the hazards need to be identified and any risks from them reduced to insignificant levels by the adoption of suitable control measures. These risk assessments should be done for all the activities involved in running practical science classes including storage of materials, preparatory work by the teacher and by any technical support staff and the practical activities that are carried on in the classroom, whether

demonstrations by the teacher or practical activities for the students. Such risk assessments should be carried out in accordance with the health and safety legislation of the country in which they are done.

Risk assessment involves answering two basic questions:

- 1 how likely is it that something will go wrong?** For example, pupils using a double sided razor blade to cut up carrots are quite likely to cut themselves.
- 2 how serious would it be if it did go wrong?** For example the consequences of a spark from an experiment landing in an open bottle of magnesium powder are likely to be serious, including spraying burning magnesium all over the laboratory, burning many pupils and setting the laboratory ceiling on fire (based on a real accident).

With the answers to these questions it is now possible to plan the practical activity to minimise the risk of an accident and to minimise how severe any accident might be. In our examples, this might include cutting up the carrot before giving to young pupils, or providing older pupils with an appropriate sharp knife, it might include bringing in to the laboratory only the amount of magnesium powder required for the activity.

How likely it is that something will go wrong depends on who is doing it and what sort of training and experience they have had. You would obviously not ask 11 year old students to heat concentrated sulphuric acid with sodium bromide, or to transfer *Bacillus subtilis* cultures from one Petri dish to another, because their inexperience and lack of practical skills makes a serious accident all too likely. By the time they reach post-16 they should have acquired the skills and maturity to carry such activities out safely.

Decisions need to be made as to whether an activity should be a teacher demonstration only, or could be done by students of various ages. This means that some experiments should normally only be done as a teacher demonstration or by older students. Perhaps with well-motivated and able students it might be done earlier, but any deviation from the model risk assessment needs discussion and a written justification beforehand.

There are some activities that are intrinsically dangerous, and, if included in the suggested activities, should always be changed to more safe modes of practice, for example, there are **no** circumstances under which mouth pipetting is acceptable – pipette fillers of some sort should **always** be used.

Teachers tend to think of eye protection as the main control measure to prevent injury. In fact, personal protective equipment, such as goggles or safety spectacles, is meant to protect from the unexpected. If you expect a problem, more stringent controls are needed. A range of control measures may be adopted, the following being the most common. Use:

- a less hazardous (substitute) chemical;
- as small a quantity as possible;
- as low a concentration as possible;
- a fume cupboard; and
- safety screens (more than one is usually needed, to protect both teacher and students).

The importance of lower concentrations is not always appreciated, but the following examples, showing the hazard classification of a range of common solutions, should make the point.

ammonia (aqueous)	irritant if $\geq 3 \text{ mol dm}^{-3}$	corrosive if $\geq 6 \text{ mol dm}^{-3}$
sodium hydroxide	irritant if $\geq 0.05 \text{ mol dm}^{-3}$	corrosive if $\geq 0.5 \text{ mol dm}^{-3}$
hydrochloric acid	irritant if $\geq 2 \text{ mol dm}^{-3}$	corrosive if $\geq 6.5 \text{ mol dm}^{-3}$
nitric acid	irritant if $\geq 0.1 \text{ mol dm}^{-3}$	corrosive if $\geq 0.5 \text{ mol dm}^{-3}$
sulphuric acid	irritant if $\geq 0.5 \text{ mol dm}^{-3}$	corrosive if $\geq 1.5 \text{ mol dm}^{-3}$
barium chloride	harmful if $\geq 0.02 \text{ mol dm}^{-3}$	toxic if $\geq 0.2 \text{ mol dm}^{-3}$ (or if solid)

Reference to the above table will show, therefore, that if sodium hydroxide is in common use, it should be more dilute than  $0.5 \text{ mol dm}^{-3}$ . The use of more concentrated solutions requires measures to be taken to reduce the potential risk.

### Material Safety Data Sheets. (MSDS)

Your risk analysis should consider the hazards associated with the materials you propose to use. These risks are best assessed by reference to MSDS's appropriate to the chemical(s) in use. These are generally supplied by the chemical manufacturer and supplied with the chemical. If this is not the case then there are many internet sites that have this information freely available. These sheets also provide useful information on the actions to take following an accident, including first aid measures, and should therefore be considered essential for all practical experiments involving chemicals, as part of the risk assessment process.

#### Hazard key.

The following key applies.

**C = Corrosive substance**

**F = Flammable substance**

**H = Harmful or irritating substance**

**O = Oxidising substance**

**T = Toxic substance**

**N = Harmful to environment**

**B = Biohazard**

#### Eye protection

Clearly students will need to wear eye protection. Undoubtedly, chemical splash goggles give the best protection but students are often reluctant to wear goggles. Safety spectacles give less protection, but may be adequate if nothing which is classed as corrosive or toxic is in use.

Your risk assessment should not restrict itself simply to the materials, procedures and equipment being used, but should have a wider remit, covering the time from when the class enter the room until they leave it.

Practical science can be - and should be - fun. It must also be safe. The two are not incompatible.

*Safeguards in the School Laboratory*, 10th edition, ASE, 1996

*Topics in Safety*, 2nd edition, ASE, 1988

*Hazcards*, CLEAPSS, 1998 (or 1995)

*Laboratory Handbook*, CLEAPSS, 1997

*Safety in Science Education*, DfEE, HMSO, 1996

*Hazardous Chemicals Manual*, SSERC2, 1997.



## Appendix 1

### A2 Skills and Designing a practical course for A2

A2 skills build on the AS skills developed. It cannot be emphasised enough that students will not become competent in these skills without practical experience. The specific investigations to which references are made can be found in appendix 2.

### Extending AS skills for the A2 year

As part of their AS studies students will be expected to develop skills in manipulating and measuring using standard laboratory apparatus. These will form a basis on which more advanced manipulative skills will be developed. During their AS course it is assumed that students will learn how to measure accurately and to manage space and time effectively, so that they are confident in their use of apparatus.

- These practical skills will be extended by more complex investigations and the use more specialised apparatus. For instance an investigation into *The effect of nitrate concentration on biomass* extends over several weeks. There is also an opportunity in this investigation to use a more accurate method of measuring concentration using a bioassay. In the Applications section of the syllabus there will be many unfamiliar techniques and quite complex equipment. Here, it is important that the students gain confidence in the use of the apparatus and understand how it works. A number of the investigations are intended to introduce students to the technique, for example electrophoresis equipment, growing microorganisms and using immobilised enzymes. It is anticipated that teachers will develop these into evaluation or planning exercises. For some biotechnology investigations equipment may be restricted, nevertheless, demonstrations can form the basis for planning and evaluation.
- The analysis and evaluation will also be more extensive. Analysis data will involve calculations and statistical testing. The investigation into the effect of nitrate expects the processing of data and the use of error bars. An investigation into *The effect of penicillin on bacterial growth* makes use of t-test to assess results. The Chi square test can be used to evaluate the results of a breeding experiment.

### Teaching students to evaluate

Evaluation refers to a number of skills concerned with the design of an experiment – in effect “How well did the experiment work”. Students should question the way in which a procedure is carried out, comment on the reliability of the results and understand the limitations of a method. Students need to acquire these skills before they can progress to the high order A2 skill of planning. The more practice the better – ideally every investigation could be evaluated using a simple check list until it becomes an automatic response by a student.

- In AS students will have been taught to evaluate procedures and suggest improvements. These skills be utilised in A2 and developed into the higher order skills recognising the cause of anomalous and contradictory results and determining how a procedure can be modified to remove potential sources of error. The skill of evaluation is further developed by learning how to assess results in relation to the stated aim or hypothesis of the investigation.

- Students need to be able to judge the reliability of their results. Many students confuse reliability – consistent repeatable results, with accuracy – measuring with the appropriate equipment. One strategy is to compare class results or to compare actual results to theoretical results. Once the reliability is known students can then relate to the aim of the experiment. To develop these skills students need to be encouraged to question. Initially a check list of questions such as: Do I have enough results? How much variability is there in my results? How many results are anomalous? How accurate was the equipment used? Have all the variables been controlled – if not, what should I do to improve this? How else could I have measured? Do my results support the aim/hypothesis – if not, which part and how can I change the procedure?
- Many of the investigations in appendix 4 have aspects of evaluation. *Producing a model industrial immobilised enzyme column*, *The effect of nitrate concentration on biomass* and *The effect of light intensity on rate of the Hill reaction* address these skills in a variety of ways. Any other procedure can be evaluated.

### Teaching students to plan experiments

Planning the experiment requires students to formulate a hypothesis, recognise variables and determine how to test a hypothesis. Students cannot access these skills without familiarity with experimental procedures and experience of using apparatus. Skills that are apparently straightforward, such as choosing suitable apparatus and devising an appropriate procedure, become problematical as students are uncertain what to measure or how to measure. Awareness of safety does not really develop unless students are actively involved in activities that involve a potential risk. It is expected that students will be encouraged to use safety information sources, such as Hazcards.

- Evaluation skills are a starting point for planning. At a preliminary level this may be to modify an existing procedure to generate more reliable results. The investigations, *The effect of nitrate concentration on biomass* and *The effect of light intensity on rate of the Hill reaction* can both be used for recognising uncontrolled variables. The investigation *Urine Analysis* could be used for improving reliability by asking students, working in groups, to suggest methods of measuring more accurately the glucose content of sample 1. Part of this activity could include asking the students to identify the potential risks and how they have been addressed by the procedure. The suggested improvement from each group could then be trialled and assessed by another group. This strategy has an additional benefit of training students in writing clear instructions that can be followed by someone else. To develop this skill further, students could be given the task of producing a plan for an investigation using an existing experimental set for a different purpose. The investigation *Producing a model industrial immobilised enzyme column* could be used for this purpose.
- To design their own experiment, students initially need to be in a familiar context. It is helpful to have a check list to prevent critical features of the plan from being omitted. Devising a generic check list by student participation can help to clarify the principles of planning as required by the syllabus learning outcomes. This could be a list of questions or a work sheet to complete. Initially, teachers may choose to give a hypothesis and ask the students to devise a plan. An investigation using *Immobilised algae* could be used in this way by a hypothesis such as "*The greater the concentration of*

*carbon dioxide, the more oxygen is released*” or “*The greater the concentration of carbon dioxide, the more oxygen is released*”.

- Once students have reached the stage of planning their investigations it is essential that they try them out. Often plans do not work as anticipated, so students need to evaluate and refine their plans. It is common for students to make unrealistic choices of apparatus and quantities, but unless they are given the opportunity to try, they remain unconvinced. Students should be encouraged to use the apparatus available, which may limit the syllabus contexts from which planning exercises may be drawn. If resources are limited then many biotechnology contexts are unsuitable, although investigations *The effect of penicillin on bacterial growth* and *Estimating the population growth of yeast* provide contexts from which hypotheses could be devised and tested usually relatively inexpensive equipment. For example, “*Bacteria are killed more effectively by soap than by detergent*” or “*Yeast population increases faster at low pH*”
- The plans produced by students are by their nature different from each other. If the same hypothesis is being tested, then there may be similarities. However, once students devise their own hypotheses then there may well be significant differences. This has implications for both resources and supervision. One strategy mentioned in the section on delivering practical skills is to incorporate planning into a circus of activities, particularly if resources are limited. However, planning and evaluation do not need to be carried out in a laboratory. So these could be carried out in a classroom, planning in a lesson before hand, trialling in a laboratory and evaluating as homework or as a follow up classroom activity. Another issue to consider is the preparation time for student planned activities. As part their plan students should produce an equipment list, with quantities that can be handed in to the person responsible for the preparation. For standard laboratory equipment, students should know where this is stored and be able to get it for themselves, but the person responsible for resources will need to know the overall requirements to ensure that there is sufficient available.

## Outline List of Practical Experiments

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>L</b>	<b>Practical 11 - Respirometer</b>		
<b>(k),(l), (m)</b>	<ul style="list-style-type: none"> <li>Identify the independent and dependent variables</li> <li>Test a hypothesis</li> <li>Experience relevant methods, analysis, conclusions and evaluation</li> <li>Explain RQ values in terms of substrate use</li> <li>Suggest modifications for use with photosynthesising organisms</li> </ul>	<p>Use small invertebrates. Blow fly larvae, woodlice, cockroaches and germinating seeds. Evaluating a single arm respirometer allows the introduction of more complex respirometers with balanced pressure.</p> <p>Students should be asked to how the apparatus can be modified to test a green plant.</p>	<p>Biology Resource Pack A2</p> <p>Lea, Lowrie and McGuigan</p>
<b>M</b>	<b>Practical 12 - Immobilised Algae – effect of limiting factors on the rate of photosynthesis</b>		
<b>(f), (g)</b>	<ul style="list-style-type: none"> <li>Evaluation</li> <li>*discuss limiting factors in photosynthesis and carry out investigations on the effects of light intensity and wavelength, carbon dioxide and temperature on the rate of photosynthesis;</li> </ul>	<p>Use sodium alginate mixed with algae to produce beads by transferring the alginate/algae mixture drop wise into calcium chloride solution</p> <p>Students should be asked to consider the advantages of immobilising the algae in this way and review other methods of immobilisation (in relation to syllabus section S).</p> <p>Students could be asked to plan to test the effect of either carbon dioxide or temperature</p>	<p>Advanced Biology Study Guide Clegg and Mackean</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>N</b>	<b>Practical 13 - Factors that affect the opening and closing of stomata</b>		
<b>(f), (g)</b>	<ul style="list-style-type: none"> <li>• Identify the independent and dependent variables</li> <li>• Formulate a hypothesis and express this in words and graphically</li> <li>• Experience relevant methods and analysis, conclusions and evaluation</li> <li>• Plan an investigation</li> </ul>	<p>Use a number of solutions to bathe whole leaves or leaf discs. Calcium chloride, sodium chloride, potassium chloride, sucrose, glucose can all be used. Concentrations will need to be trialled depending on the leaves used. Measure the size of the stomata using epidermal strips or nail varnish impressions. Find the mean size and present graphically. Test whether any differences are significant. Plan an investigation to determine the effect of abscissic acid.</p>	<p>Advanced Biology Study Guide Clegg and Mackean</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>N</b>	<b>Practical 14 - Reflexes</b>		
<b>(e), (f), (g)</b>	<ul style="list-style-type: none"> <li>• Identify the independent and dependent variables</li> <li>• Formulate a hypothesis and express this in words and graphically</li> <li>• Experience relevant methods, analysis, conclusions and evaluation</li> <li>• Describe and explain the reflex arc that occurs during a knee-jerk reflex, swallowing reflex, iris reflex and blinking reflex</li> <li>• Evaluate the procedure, discussing why it is difficult to quantify this investigation</li> </ul>	<p>Using a tendon hammer and working in pairs, students should test each others knee-jerk reflexes by tapping the joints just below the knee, and then the ankle. Students should compare the intensity of the reactions from the knee and ankle.</p> <p>Students can also observe that it is virtually impossible to swallow twice in rapid succession unless liquid is present in the mouth. Students should try to explain the reflex which the liquid induces.</p> <p>Students can observe one another's eyes to note the change in the iris when their partner looks toward a light then try to explain the reflex arc that occurs.</p> <p>Students should try and explain the reflex arc that occurs when a person claps their hands in front of another's face, causing them to blink.</p>	

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>N</b>	<b>Practical 15 - Reaction time</b>		
<b>(e),(f), (g)</b>	<ul style="list-style-type: none"> <li>• Identify the independent and dependent variables</li> <li>• Make a hypothesis and express this in words and graphically</li> <li>• Experience relevant methods, analysis, conclusions and evaluation</li> <li>• Describe and explain the reflex arc that occurs during reaction timing</li> <li>• Explain the ability to improve reaction time with practice</li> </ul>	<p>Using a reaction timing ruler and working in pairs, students should test each other's reaction time. This is done by one student holding the ruler between their partner's thumb and forefinger, which should be approx 2 cm apart, their elbow resting on a table to keep the hand from moving up or down. The ruler is then dropped and the student catches it as soon as possible, reading the reaction time off the ruler from the position of their thumb.</p> <p>Students should be asked to record their reaction time and repeat several times, observing any improvement with practice.</p>	<p>Biology 9700</p> <p>University of Cambridge International Examinations</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
(c)	<ul style="list-style-type: none"> <li>• Recognise the gross structure of the mammalian kidney</li> <li>• Interpret the microscopic image of the kidney via viewing sections under a light microscope</li> <li>• Experience relevant methods</li> <li>• Produce a biological drawing of kidney histology, as viewed under a light microscope</li> </ul>	<p>Students should dissect a pig's or lamb's kidney along the perimeter of the convex side so it may be opened up and the internal structures observed. Students should be asked to identify the ureter, pelvis, cortex and medulla of the kidney and the glomeruli may be observed using a hand lens.</p> <p>Students should be asked to observe a prepared section of kidney under a light microscope and to identify the glomeruli, renal capsule (Bowman's capsule), the renal tubules, the collecting ducts and the loops of Henle.</p> <p>Students should produce a drawing of their observations.</p>	<p>An Atlas of Histology</p> <p>Freeman and Bracegirdle</p> <p>Bioscope</p>



Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>N</b>	<b>Practical 16 - Model kidney tubule</b>		
<b>(c)</b>	<ul style="list-style-type: none"> <li>• Identify the independent and dependent variables</li> <li>• Make a hypothesis and express this in words and graphically</li> <li>• Experience relevant methods, analysis, conclusions and evaluation</li> <li>• Describe and explain the function of the kidney tubule</li> </ul>	<p>Students should produce a model of the filtration in a kidney tubule using visking tubing and a syringe. Pressure applied by pushing the syringe simulates hydrostatic pressure. Small molecules will pass through the membrane (to be re-absorbed) whilst others will remain inside the tubing. This is possible by using albumin and glucose and placing the model in a beaker of water. The water can then be tested for both protein and glucose using Biuret and Benedict's reagent respectively.</p>	
<b>N</b>	<b>Practical 17 - Structure and histology of an Islet of Langerhans in the mammalian pancreas</b>		
<b>(m)</b>	<ul style="list-style-type: none"> <li>• Interpret the microscopic image of the pancreas by viewing sections under a light microscope</li> <li>• Experience relevant methods</li> <li>• Produce a biological drawing of pancreas histology, as viewed under a light microscope</li> </ul>	<p>Students should observe a prepared section of a pancreas under the microscope. Students should be asked to identify the two different types of secretory tissue present. Students should draw a high power drawing showing the arrangement of the two different types of secretory material. These diagrams should be annotated with the main features that distinguish exocrine gland (enzyme) from the endocrine (islet of Langerhans) tissue.</p>	<p>An Atlas of Histology</p> <p>Freeman and Bracegirdle</p> <p>Bioscope</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
O	<b>Practical 18 - Meiosis</b>		
(a)	<ul style="list-style-type: none"> <li>Use of microscope at medium power to observe the nuclei of cells undergoing meiosis</li> <li>Use appropriate apparatus and techniques</li> </ul>	Use anthers from a flowering plant of <i>Tradescantia virginiana</i> or a dormant bulb of <i>Hyacinthus</i> . Stain anthers with acetic orcein. If pollen grains are visible then meiosis has already occurred. If large nuclei are present the plant is too young.	<p>Advanced Biology Study Guide by Clegg and Mackean</p> <p>Philip Harris-meiosis sets</p> <p>Bioscope <i>Lilium</i> anther</p>
O	<b>Practical 19 - Chi-Squared Test</b>		
(f)	<ul style="list-style-type: none"> <li>Application of Chi-squared test</li> <li>Use of Chi-squared test to evaluate results of breeding experiments</li> <li>Use of Chi-squared test to evaluate Mendelian ratios</li> <li>Data handling</li> </ul>	Calculate Chi-squared value of a set of data obtained from a genetic experiment. Test the significance of differences between observed and expected results. The Chi-squared test can also be used to evaluate the results of ecological sampling in the context of Q (d).	<p>Advanced Biology Study Guide by Clegg and Mackean</p> <p>A2 Biology AQA B by Lea, Lowrie and McGuigan</p>
O	<b>Practical 20 - Mutation in a Fungus</b>		
(b) (g) (f)	<ul style="list-style-type: none"> <li>Use the fungus <i>Sordaria fimicola</i> to obtain Mendelian ratios.</li> <li>Use of microscope to visualise and score Asci</li> <li>Data analysis to determine crossing over frequencies</li> </ul>	It is possible to culture <i>Sordaria fimicola</i> . Using standard corn agar. Use the strain with black ascospores crossed with the strain with off-white ascospores to obtain Mendelian ratios.	Practical Genetics Open University Press

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>P</b>	<b>Practical 21 - Variation – Using statistics</b>		
<b>(b)</b>	<ul style="list-style-type: none"> <li>• Application of statistics in Biology to test for the significance of differences between samples.</li> <li>• Use standard deviation to estimate the spread of data</li> <li>• Use t-test to compare two sets of normally distributed data</li> <li>• Data handling</li> </ul>	<p>Calculate mean and standard deviation for a set of grouped data. Calculate the value of t and determine if there is a significant difference.</p> <p>Samples of leaves from the same species growing in different areas, height of seedlings grown in different pH</p>	<p>Advanced Biology Study Guide by Clegg and Mackean</p> <p>Advanced Level Practical Work for Biology Hodder and Stoughton</p>
<b>P</b>	<b>Practical 22 - Simulation of selection and evolution</b>		
<b>(g)</b>	<ul style="list-style-type: none"> <li>• Analysis of results</li> <li>• Express results graphically</li> <li>• Draw conclusions</li> </ul>	<p>A large number of beads (counters/plastic) of two different colours in a beaker. Start at 50% each colour to represent alleles. One colour represents dominant. Pick at random 2 beads and place together to represent genotypes. Repeat until all beads used – group according to phenotype. Decide on selection pressure e.g. 25% of dominant phenotype and remove. Return all others to beaker and repeat several generations. By changing the selection pressure can show results graphically.</p> <p>Can modify to show isolation by splitting beads into 2 populations an applying different selection pressure.</p>	<p>Biology 9700</p> <p>University of Cambridge International Examinations</p> <p>Scheme of work</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>P</b>	<b>Practical 23 - Population growth</b>		
<b>(c)</b>	<ul style="list-style-type: none"> <li>• Analyse results</li> <li>• Draw conclusions</li> <li>• Express results graphically</li> <li>• Use a complex technique</li> </ul>	Use yeast or bacterial culture. Measure population growth using haemocytometer. Bacteria measure twice daily, 3-4 days. Yeast daily, 5-6 days. Population growth curve, should show slowing down due to limiting factors.	Advanced Biology Study Guide by Clegg and Mackean
<b>Q</b>	<b>Practical 24 - Comparing diversity in a managed and unmanaged environment</b>		
<b>(a) (g)</b>	<ul style="list-style-type: none"> <li>• Analysis of results</li> <li>• Express results graphically</li> <li>• Statistical analysis</li> <li>• Draw conclusions</li> </ul>	Two areas relatively easily accessible, one managed in some way – lawns, parts, farm land, fished water; the other unmanaged – woodland, conservation sites with access, waste land, unfished water. One could be the Centre grounds the other the side of a road. Use suitable sampling equipment to collect data on the number of different species in an area. Use Lincoln index and Chi square	

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>R(g)</b>	<b>Practical 25 - Electrophoresis</b>		
	<ul style="list-style-type: none"> <li>• Identify the independent and dependent variables</li> <li>• Make a hypothesis and express this in words and graphically</li> <li>• Experience relevant methods, analysis, conclusions and evaluation</li> <li>• Describe the processes of electrophoresis as used in DNA fingerprinting and DNA sequencing</li> <li>• Describe and explain an electrophoresis gel after running and staining</li> </ul>	<p>Students should first extract a sample of DNA from fruit or vegetables (see extended practical) and then cut the DNA using restriction enzymes. The cut DNA can then be used in electrophoresis (see extended practical) and stained with an appropriate stain when complete.</p> <p>Students should analyse the results of the electrophoresis and compare to other result or DNA fingerprints to try and identify 'matches'</p> <p>*Can be followed up by discussion on genetic screening and counselling.</p>	<p>NCBE DNA technology kit</p> <p>Bio-Rad DNA fingerprint kits</p> <p>*NCBE</p> <p>DICE work pack</p>
<b>S</b>	<b>Practical 26 - ELISA - Using antibodies to detect disease</b>		
<b>(h)</b>	<ul style="list-style-type: none"> <li>• Describe antigen-antibody interactions</li> <li>• Understand how HIV is detected</li> <li>• Learn how disease agents are transmitted, diagnosed and tracked</li> <li>• Understand how antibodies are produced in the laboratory for use in diagnostic tests</li> <li>• Study enzyme-substrate mechanics.</li> </ul>	<p>The kits come with a selection of investigations and work sheets. These can be used directly or modified to become the basis of planning exercises.</p> <p>The kit gives the flexibility to perform 3 different ELISA-based protocols. Protocols I and II test for the presence of antigen in unknown samples and Protocol III for the presence of antibody. The positive control is either an antigen or an antibody depending on the protocol being followed. Each kit includes a Teacher's Guide, Student Manual and graphic Quick Guide.</p>	<p>Bio-Rad Laboratories Immuno Explorer Kit</p>

Syllabus section	Skills/Learning Outcomes	Notes	Sources
<b>T</b>	<b>Practical 27 - Adaptations in crop plants</b>		
<b>(h)</b>	<ul style="list-style-type: none"> <li>• Recognise the structure and</li> <li>• make accurate drawings of a maize leaf</li> <li>• Understand the difference in organisation of a C4 and C3 leaf</li> <li>• Recognise sorghum and rice</li> <li>• Be able to describe and explain the adaptations of rice and sorghum</li> <li>• Plan an investigation to test the effect of ethanol concentration on the growth of germinated rice.</li> </ul>	<p>Microscope slides of maize leaves to look at distribution of chloroplasts. Revisit dicot leaf from Syllabus section M. Draw up comparisons.</p> <p>Actual or museum specimens of sorghum and rice - observations of general morphology.</p> <p>Germinated rice seedlings – grow in different concentrations of ethanol</p>	<p>Bio-Rad Laboratories Immuno Explorer Kit</p>
<b>U</b>	<b>Practical 28 - Microscopic examination of reproductive organs</b>		
	<ul style="list-style-type: none"> <li>• Identify a section of an ovary and testis</li> <li>• Recognise different stages in the gametogenesis in a testis by appearance and position of cell</li> <li>• Recognise the appearance of immature and mature follicles in an ovary</li> <li>• Recognise the appearance of a corpus luteum in an ovary</li> <li>• Estimate the number of ovulations by counting corpora albicans</li> </ul>	<p>Can be a circus activity if slides are limited. Student work sheet with labelled diagrams and photomicrographs. Students identify and draw the specified structures.</p> <p>Demonstration dissection or museum specimens to show location of reproductive structures. These can also be used to simulate the techniques of ivf.</p> <p>Slides of uterus wall can be used to demonstrate layers.</p>	<p>An Atlas of Histology</p> <p>Freeman and Bracegirdle</p> <p>Human Systems</p> <p>Griffin and Redmore</p>

## Appendix 2 – practicals for which full details are provided

### Practical 1 - M(b)(c) The effect of light intensity on rate of the Hill reaction

#### Defining the Problem

This practical focuses on Planning - Defining the problem. You will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Identify the independent and dependent variables
- Make a hypothesis and express this in words and graphically
- Identify the variables that should be controlled
- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between light intensity and photosynthesis
- Explain the relationship between this experiment and the light dependent reactions of photosynthesis

#### Safety information



You should wear eye protection throughout this practical.

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP, also using hydrogen ions from photolysis of water.
- If chloroplasts are broken, the enzymes that are involved in this process can reduce other oxidised materials in the same way. This is termed the Hill reaction after its discoverer.
- Oxidised DCPIP is bright blue, and when reduced, for example by high-energy electrons and hydrogen ions from the light dependent reaction of photosynthesis, becomes colourless.
- DCPIP provides a way of measuring how fast the light dependent reaction is happening, as well as giving an interesting insight into the light dependent reaction itself.

You will investigate the effect of **light intensity** on the **rate of DCPIP reduction** by the Hill reaction.

- Read the information above.
- Identify and write down the independent and dependent variables.
- Write down the hypothesis.

- Represent the hypothesis as a sketch graph.
- List the variables that should be controlled.
- Outline how each such variable might be controlled.

Light intensity in this case is varied by using filters to absorb some of the available light.

filter	light intensity as percentage of available light
none	100
pale	70
medium	50
dark	25
under foil	0

### Method

#### Preparations

- 1 Put the leaf onto a tile.
- 2 Discard large veins and chop the leaf finely.
- 3 Put it into a plastic tube and add 2 cm<sup>3</sup> of very cold extraction medium.
- 4 Grind with a glass rod for 1 minute to give green juice (the leaf extract).
- 5 Decant (pour) the leaf extract slowly into a Petri dish with one edge resting on the clean white tile.
- 6 Place a loose aluminium foil cover over the Petri dish to keep light out but be easy to remove to take samples.
- 7 Fold the three grey filters along their length to make little tents, and put them on the tile.
- 8 Make up a table to include the colours of the contents of tubes A, B, C, D, E and F every minute from 0 to 10 minutes.

#### Preparation of capillary tubes and making observations - steps 4 and 5 need to be done fast

- 1 Stand one of the capillary tubes in the leaf extract in the Petri dish so that some extract rises up the tube. This is tube A. Lay tube A on the tile.
- 2 Add 5 drops of DCPIP solution to the leaf extract in the Petri dish. Mix, and if not blue colour is visible add another 5 drops, repeating until the green leaf extract is a blue-green colour. Cover.
- 3 Stand another capillary tube in the leaf extract/DCPIP mixture. Lay on the tile under a foil cover as tube B. Cover the Petri dish.
- 4 Stand four more capillary tubes in the dark blue-green leaf extract/DCPIP mixture. On the tile, put tube C under the dark filter, tube D under the mid-grey filter, tube E under the pale filter and tube F without a cover.
- 5 Switch on the lamp so that the light falls evenly all over the tile and start timing. Every minute lift the filters or covers and record the colour of each tube in the table.



### Calculations

- 1 Record the time taken for the blue colour to disappear from each tube. If it is still blue, record '>10 minutes'
- 2 Calculate the rate of reaction using  $1/\text{time}$  taken for blue colour to disappear. If >10 minutes, then record  $1/\text{time}$  taken for blue colour to disappear as 0.
- 3 Record your rate of reaction for each light intensity in the class results table on the board or flipchart.
- 4 When all the results have been recorded in the class results table, calculate the mean rate of reaction for each light intensity.
- 5 (Optional - calculate the standard error for each light intensity.)

### Write-up

- Plot a graph to show the mean rate of reaction for each light intensity
- (Optional - add error bars to your graph.)
- Make an evaluation considering:
  - the limitations of the methods used,
  - anomalous values if any,
  - replication and range of values of independent variable,
  - effectiveness of control of selected variables,
  - the confidence with which conclusions should be drawn
- Draw conclusions considering:
  - detailed description of the features of the results,
  - the meaning of the results in relation to the hypothesis,
  - scientific explanation of the results and conclusions,
  - potential Improvements and further predictions

## Lesson Plan

### The effect of light intensity on rate of the Hill reaction - Defining the Problem

#### Context

A practical investigation set in the context of 9700 Syllabus - aspects of the light dependent reaction and light as a limiting factor in photosynthesis.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify the variables involved and make hypotheses in writing and graphically.

#### Key aims of lesson

This practical is designed to develop the skill of Planning - Defining the problem. Students will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up the student should be able to

- Identify the independent and dependent variables
- Make a hypothesis and express this in words and graphically
- Identify the variables that should be controlled
- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between light intensity and photosynthesis.
- Explain the relationship between this experiment and the light dependent reactions of photosynthesis

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

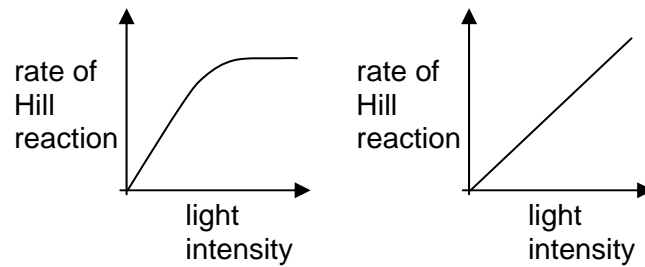
Timings/ minutes	Teacher / Student Activities
end of previous lesson	<b>Preparation</b> - 2 page student worksheet given out for students to read in preparation for the practical lesson, and to consider the identification of variables, hypothesis formulation and listing of control variables, reinforcing previous learning and preparing for this lesson
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> - review of Hill reaction, light independent reactions of photosynthesis, and on light as a limiting factor in photosynthesis. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-12	<b>Introduction to method</b> - teacher demonstration of cutting up leaf, adding extraction medium, grinding, putting extract in Petri dish, loading capillary tube, adding DCPIP to an appropriate green-blue colour, handed round for students to see (this will go green in a minute or two in normal daylight - add some more DCPIP if needed).
12-20	<b>Identification of variables, writing and graphing hypotheses and listing control variables</b> - pupils work through second box on page 1 of the student worksheet - teacher circulates, answering specific queries, praising students who are making a good effort and discussing responses that are not detailed enough or incorrect to help guide students in the right direction. Student activity will be completed for homework
20-50	<b>Carrying out the practical</b> - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.
50-60	<b>Drawing together the threads</b> - teacher-led class discussion on the skills that have been developed, as well as the results and their meaning - teacher led introduction to write-up, which should include class-work, finished off if necessary, (identification of variables, writing and graphing hypotheses and listing control variables), the method sheet to show what was done, annotated to include any modifications that were made, and a full, detailed write-up as described on page 2 of the student worksheet.

### Useful Information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP, also using hydrogen ions from photolysis of water.
- If chloroplasts are broken, the enzymes that are involved in this process can reduce other oxidised materials in the same way. This is termed the Hill reaction after its discoverer.
- Oxidised DCPIP is bright blue, and when reduced, for example by high-energy electrons and hydrogen ions from the light dependent reaction of photosynthesis, becomes colourless.
- DCPIP provides a way of measuring how fast the light dependent reaction is happening, as well as giving an interesting insight into the light dependent reaction itself.
- The Independent variable is light intensity; the dependent variable is rate of the Hill reaction, measured as how fast the blue DCPIP is reduced to colourless.
- The precise hypothesis will vary from student to student, depending what information they use to help guide them. Accept any valid hypothesis, e.g.
  - Students who make use of the curve of light intensity against photosynthesis will come up with something equivalent to: at low light intensities; as light intensity increases, the rate of the Hill reaction also increases (as light is the limiting factor), but at high light intensities; as the light intensity increases, the rate of photosynthesis remains constant (as some other factor is limiting).

## Appendix 2



- Other students may view the process energetically as all the raw materials are present in excess, and will anticipate a straight line in which: as light intensity increases, the rate of the Hill reaction will increase (as the light supplies the energy, and the more energy is supplied, the faster the reaction will go).
- Possible variables to control include:
  - temperature;
  - volumes and concentrations of extraction medium and DCPIP;
  - leaf area / volume of extract used and species / type of leaf;
  - light intensity falling on each filter;
  - spectrum of light falling on each tube ('light temperature');
  - pH;
  - atmospheric pressure;
  - time of exposure of each treatment to bright light during the making of observations;

## Technical Information

### The effect of light intensity on rate of the Hill reaction - Defining the Problem

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

For some of the chemicals, it is convenient to make up more than is required in order to give sufficient quantities for accurate measurement.

- 1 1 fresh green cabbage or spinach leaf per student / group. Any soft, green, non-toxic dicotyledonous leaf would be suitable.
- 2 1 white tile
- 3 1 scalpel or sharp knife
- 4 1 shatterproof plastic specimen tube (minimum 3cm x 1 cm) that will withstand being squeezed and being used as a mortar for grinding the soft green leaves.
- 5 1 glass rod that will fit into the specimen tubes. Very thin rods will break easily, so use reasonably thick glass rod and round off the ends with heat
- 6 1 paper towel
- 7 1 Petri dish base or top (either is suitable)
- 8 3 different plastic neutral density grey filters 1.5 cm by 10 cm cut from a large sheet. There are several manufacturers of such filters, details of which are given below:

description	% of light transmitted	Lee	Cotech	Roscolux
pale grey	70	298	298	397
mid-grey	50	209	209	97
dark grey	25	210	210	98

- 9 1 desk lamp, which could be a proper articulated lamp, or a bulb-holder safely screwed to a small wooden base.
- 10 6 melting point tubes (thin wall capillary tubes 10 cm long) or 6 pieces of capillary tube cut to a length of 4-10 cm each, with any sharp edges removed.
- 11 aluminium foil
- 12 2 syringes, 5 cm<sup>3</sup> or 2 cm<sup>3</sup>
- 13 Make up 500 cm<sup>3</sup> of phosphate buffer solution to use as below, using 4.48 g Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O and 1.7g KH<sub>2</sub>PO<sub>4</sub> made up to 500 cm<sup>3</sup> with distilled water. Store in a fridge. Students do **not** need to be given any of this solution.
- 14 2 cm<sup>3</sup> of very cold extraction medium, labelled **extraction medium**. To make up 250 cm<sup>3</sup> of extraction medium, dissolve 34.23 g sucrose and 0.19 g KCl in phosphate buffer solution (see above) and then make up to 250 cm<sup>3</sup> with the phosphate buffer solution. Store in a fridge for no more than 48 hours and supply very cold for use.
- 15 2 cm<sup>3</sup> DCPIP solution labelled **DCPIP solution**. To make up 250 cm<sup>3</sup> of DCPIP solution, dissolve 0.4 g DCPIP and 0.93 g KCl in phosphate buffer solution **at room temperature** and make up to 250 cm<sup>3</sup> with phosphate buffer solution.

Store in a fridge for no more than 48 hours but **supply for use at room temperature.**

Safety Precautions/Risks.

No specific hazards identified.

A risk assessment should be carried out as a matter of course.

**Practical 2 - M(a)/S(b) The effect of nitrate concentration on the production of biomass by algae.**












**This practical focuses on Analysis** – Evaluation and Conclusions. You will be developing other assessed skills throughout the practical.

**Intended learning outcomes**

By the end of this practical and its write-up you should be able to:

- Draw conclusions based on the key features of the data obtained
- Explain whether the experimental evidence supports the hypothesis
- Describe the relationship between nitrate concentration and biomass production
- Give a scientific explanation of the relationship between photosynthesis, nitrate concentration and the production of biomass.
- Optional describe the relationship between nitrate concentration, photosynthesis and protein production.
- Comment on the experimental design and suggest improvements.

**Safety information**

	You should wear eye protection throughout this practical.
	Potassium nitrate is an <b>oxidising agent</b> .
	Calcium chloride is an <b>irritant</b> .
	Iron (II) sulphate is <b>harmful</b> .
 	Zinc sulphate is <b>harmful</b> and <b>dangerous to the environment</b> .
	Manganese chloride is <b>harmful</b> .
	Molybdenum trioxide is <b>harmful</b> .
 	Copper sulphate is <b>harmful</b> and <b>dangerous to the environment</b> .
	Cobalt nitrate is <b>harmful</b> .
Bleach may have been used for sterilising materials and may be <b>harmful, oxidising or corrosive</b> .	

### Background information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP, also using hydrogen ions from photolysis of water.
- In the light dependent reaction of photosynthesis carbon dioxide is assimilated and used in the synthesis of 3 carbon sugars. Reduced NADPH and ATP from the light dependent reactions are used during these reactions.
- The 3 carbon sugars and mineral ions are used in reactions that enable plants to synthesise all the organic molecules needed to produce new cellular material.
- The new cellular material includes polysaccharide, protein, lipid and nucleic acid that add to the biomass of the plants and thus provides a way of measuring growth in relation to photosynthesis.
- Unicellular algae grow relatively quickly and their biomass can be measured using a balance.

You will investigate the hypothesis that; An increase in **nitrate concentration** increases the **production of biomass** by a unicellular green alga.

Nitrate concentration in this case is varied by different masses of a sodium nitrate.

Experimental set	concentration of sodium nitrate/g dm <sup>-3</sup>
control	0
1	5
2	10
3	15
5	20



## Method

### Preparations

- 1 Wash your hands and then wipe your work area with disinfectant.
- 2 Wash a 250 cm<sup>3</sup> container with sterilising solution.
- 3 Connect a length of clear plastic tubing to a diffusion block. Insert a small piece of cotton wool into the other end of plastic tubing to filter the air supply. The open end of the tubing can be used if a diffusion block is not available.
- 4 Connect the plastic tubing to an aquarium pump.
- 5 If an aquarium pump is not available a water filter pump can be used. In this case two pieces of plastic tubing are needed. One piece of tubing is connected to the diffusion block and is long enough to pass through the cotton wool plug so the diffusion block (or open end) reaches almost to the bottom of the container. The other piece of tubing is connected to the water pump and is long enough for the open end to pass through the cotton wool plug but does not reach the liquid.
- 6 Collect about 250 cm<sup>3</sup> of boiled pond water.

### Preparation of culture and making observations

- 1 Pour 200 cm<sup>3</sup> of boiled pond water into the container. Keep the remaining pond water in a closed, labelled sterile bottle.
- 2 Add a known mass of sodium nitrate to the pond water and plug loosely with cotton wool. Swirl gently until all the sodium nitrate is dissolved.
- 3 Remove the cotton wool plug and add 2 cm<sup>3</sup> of an algal culture to the pond water.
- 4 Mark the level of the liquid in the container and weigh the container and its contents. (Optional – remove 1 cm<sup>3</sup> of mixture and test for protein using Biuret test)
- 5 Place a light source at a known distance from the container at room temperature.
- 6 Place the diffusion block into the liquid in the container and loosely plug in place using fresh cotton wool. Turn on the pump. Alternatively place the plastic tubing connected to the diffuser into the liquid and the tubing connected to the water suction pump into the top of the container and plug loosely with cotton wool. Connect to the water pump and turn on.
- 7 Observe any changes in the culture over several days. If the liquid level falls, top up to the mark with sterile pond water.
- 8 After 2 weeks turn off the air supply, remove the plastic tubing and cotton wool.
- 9 Reweigh the bottle and its contents. (Optional- remove 1 cm<sup>3</sup> of mixture and test for protein using Biuret test)

**Preparing a calibration curve and estimating protein content—Optional.**

- 1 Weigh 3 g of albumin powder or other protein.
- 2 Dissolve in 30 cm<sup>3</sup> of distilled water in a test tube.
- 3 Prepare a series of solutions of known protein concentration using the proportions of albumin solution and water in the table below.

Volume of albumin solution / cm <sup>3</sup>	Volume of water / cm <sup>3</sup>	Concentration of albumin solution / mg dm <sup>-3</sup>
10	0	
8	2	
6	4	
4	6	
2	8	
0	10	

- 4 Carry out Biuret test on each of the solutions.
- 5 Calibrate a colorimeter using the tube without any albumin solution.
- 6 Take a reading of each tube and record the light absorbance.
- 7 Allow the cells to settle in each of the samples tested. Decant the coloured liquid into a suitable tube and read the light absorbance.

**Calculations**

- 1 Record the initial and final mass of the bottle and its contents.
  - 2 Calculate the increase in biomass over time.
  - 3 Record your increase in biomass on a class results table.
  - 4 When all the results have been recorded in the class results table, calculate the mean increase in biomass.
- *Optional calibration curve and protein measurement.*
    - 1 plot a curve of protein concentration against light absorbance.
    - 2 use the calibration curve to find the concentration of protein in your culture at the beginning and end of the investigation.
    - 3 calculate the increase in protein

If a colorimeter is not available the colours obtained from the culture can be compared to a set of standard tubes. These are made in the same way as described for colorimeter measurement, using specimen tube which can be sealed. To improve accuracy in this case, a larger range of concentrations should be made.

### Write-up

- Plot a graph to show the mean increase in mass for each nitrate concentration
- (Optional - add error bars to your graph.)
- Optional estimation of protein content
  - On the same axis as biomass, plot a graph of protein content for each nitrate concentration.
  - Compare the curves for protein content and biomass
- Draw conclusions from the investigation considering:
  - detailed description of relationships between nitrate concentration and biomass,
  - a scientific explanation of the results and conclusions,
  - the extent to which the data supports the hypothesis, commenting on any other factor that may have influenced the biomass.
- Make an evaluation considering:
  - the limitations of the methods used,
  - anomalous values if any,
  - replication and range of values of independent variable,
  - effectiveness of control of variables,
  - the confidence with which conclusions should be drawn,
  - potential improvements and further predictions

## Lesson Plan

### The effect of nitrate concentration on the production of biomass by algae.

#### Analysis – Evaluation and Conclusions.

##### Context

A practical investigation set in the context of 9700 Syllabus - aspects of the transfer of light energy during photosynthesis to produce complex organic molecules. It also has some overlap to Option S with regard to the technique of culturing and producing biomass.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify the variables and to evaluate an experimental design.

##### Key aims of lesson

This practical is designed to develop the skill of Analysis – Evaluation and Conclusions. Students will be developing other assessed skills throughout the practical.

##### Intended learning outcomes

By the end of this practical and its write-up the student should be able to:

- Draw conclusions based on the key features of the data obtained
- Explain whether the experimental evidence supports the hypothesis
- Describe the relationship between nitrate concentration and biomass production
- Give a scientific explanation of the relationship between photosynthesis, nitrate concentration and the production of biomass.
- Optional - describe the relationship between nitrate concentration, photosynthesis and protein production.
- Comment on the experimental design and suggest improvements.

##### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

##### Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
end of previous lesson	<b>Preparation</b> – first two pages of student worksheet given out for students to read in preparation for the practical lesson. Students should identify the control variables and note how they are being standardised in preparation for evaluating the method after the investigation.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation

4-9	<p><b>Context</b> - review light independent reactions of photosynthesis and light independent reactions as a means of producing small organic molecules that can be used to synthesise larger molecules. The requirement for mineral ions for some molecules, emphasising role of nitrate for proteins and nucleic acids. The need for these molecules for growth and reproduction of plants. Teacher-led questioning, student responses / discussion, students building a multi-coloured learning outline on the board.</p>
10-14	<p><b>Introduction to method</b> - teacher demonstration of assembling the diffuser and pump system to be used. If groups are to be working together using different nitrate concentrations – allocation of concentrations.</p> <p>If optional protein measurements are to be carried out - demonstration of colorimeter if necessary. Within a group, each individual can carry out Biuret test on one or more concentrations of protein.</p>
15 – 45	<p><b>Carrying out the practical</b> - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.</p>
46 - 50	<p><b>Optional measurement of protein content.</b> Students carry out Biuret test must be carried out on the initial culture and the coloured liquid decanted into a specimen tube for storage in a refrigerator</p>
51-60	<p><b>Drawing together the threads</b> - teacher-led class discussion on the design of the experiment, identifying independent and dependant variables. These should be and discussed in relation to methods of measurement and any limitations that might affect the results. Variables that might influence the results should also be identified and how these are being controlled discussed. A summary on an OHP or flip chart should be built up for reference at the end of the investigation.</p>
<b>Lesson 2</b> 0-5	<p><b>End of previous lesson</b> - students reminded about the practical and reminded to bring the student work sheets to the lesson.</p>
0-4	<p><b>Introduction</b> – reminder of the aims, intended outcomes and shape of the lesson – teacher led oral presentation using the summary OHP or flip chart from the first session.</p>
5 – 25	<p><b>Carrying out the practical</b> – students carry out the weighing of the sample entering their results into a table on the board and tidying away apparatus as soon as they have finished.</p>
26 – 40	<p><b>Optional preparation of standard protein solutions.</b> – students make a series of protein solutions and carry out Biuret test. Biuret test carried out on sample from culture. Colorimeter readings taken or colour of samples compared to colours of known concentrations. The preparation of the standard series can be carried out at the end of the first session and the tubes stored in a refrigerator if necessary.</p>
26 – 60 or 41 – 60	<p><b>Drawing together the threads</b> –Page 3 of the student work sheet handed out. Teacher-led class discussion on the results and their meaning - teacher led introduction to write-up, which should include class-work, finished off as homework as necessary, (graphing results, conclusions, evaluation of design referring to limitations and any improvements</p>

### Useful Information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons and hydrogen ions from photolysis of water are used to reduce NADP and synthesise ATP from ADP and inorganic phosphate.
- Reduced NADP and ATP are used in the light independent reactions to synthesise glyceraldehyde phosphate (GP), a 3-carbon sugar that feeds into a number of metabolic pathways that lead to the synthesis of simple organic monomers, such as amino acids, hexoses and more complex molecules such as nucleotides and fatty acids.
- Amino acids and nucleic acids require a supply of nitrogen for their synthesis, which is obtained from nitrate. The more protein and nucleic acids synthesised the greater the rate of growth, leading to an increase in biomass.
- Unicellular algae such as *Chlorella sp.* are easy to culture and grow relatively fast. They can be cultured commercially as a source of single cell protein. Some parts of the world grow *Chlorella sp.* in sewage lagoons as a means of reducing pollution from nitrate. The dried cells may be used in animal food. They are also being studied as a means of reducing pollution by toxic metals as they accumulate ions such as aluminium.
- Conclusions – students might be expected to relate the increase in mass to an increase in photosynthesis and the production of more protein/cellular material, leading to more cell division and an increased population. The more nitrate available the greater the production of new biomass. At higher nitrate concentrations may see less increase as other factors are limiting so the results may not fully support the hypothesis. Also students might suggest that sodium influences plant growth.
- The Independent variable is nitrate concentration; the dependent variable is the increase in biomass (or protein content).
- Other variables are:
  - Light – controlled by a constant light source.
  - Carbon dioxide concentration – controlled by the air flow.
  - Other organisms – controlled by using sterile pond water and pure culture. Also the use of semi-sterile conditions during the experimental set up.
  - Volume of pond water – controlled by topping up the container.
  - Quantity of chlorella – controlled by using same volume of pure culture.
  - Exposure of organism to nitrate supply, light carbon dioxide – mixing due to air flow prevents settling of cells and mixes with the required nutrients.
  - Temperature – varies with room temperature
- Possible improvements may include:
  - Temperature; Students may suggest a means of controlling this more effectively, e.g. temperature controlled room, water bath.
  - Concentrations of carbon dioxide- students may suggest this is a limiting factor and use sodium hydrogen carbonate as a source;
  - Light availability; - students may suggest increased light illumination.
  - Volume of chlorella used – students may suggest a greater volume to obtain results faster;

- Range of nitrate concentrations - students may suggest a greater range and/or smaller intervals between the concentrations. Higher nitrate concentrations should be tested to test hypothesis fully.
- Weighing more frequently to find rate of growth/ population increase;
- Source of nitrate - students may suggest another nitrate salt to eliminate the possible effect of sodium on growth or another source of nitrogen such as an ammonium salt;

### Technical Information

#### The effect nitrate concentration on biomass production in algae.

As this investigation takes several days for the results to be obtained it will require a number of students to share results. Ideally at least two students or groups of students should investigate each nitrate concentration and use a means value in their calculations. If resources do not allow for this then one set of values should be sufficient.

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

For some of the chemicals, it is convenient to make up more than is required in order to give sufficient quantities for accurate measurement.

- 1 A supply of pond water. This can be previously sterilised by boiling and kept in a sterile container. If pond water is not available a complete mineral culture solution can be used. A formula for *Chlorella* is supplied
- 2 Sterilising solution. Household peroxide or hypochlorite bleach diluted as indicated by the manufacturer is suitable.
- 3 1 plastic container. Washed, clear plastic drinks containers are suitable.
- 4 1 plastic specimen tube or other container with a lid that can hold 50cm<sup>3</sup> of liquid.
- 5 Plastic tubing – about 50cm
- 6 Diffusion block
- 7 Aquarium air pump or water pump. Most aquarium pumps can drive up to 4 diffusers. If a water pump is used up to 4 can be connected in series. Pumps can be turned off overnight but should be allowed to run for at least 4 hours each day to ensure sufficient air supply.
- 8 2 cm<sup>3</sup> of a pure culture of a unicellular alga e.g. *Chlorella* sp.
- 9 1 desk lamp, which could be a proper articulated lamp, or a bulb-holder safely screwed to a small wooden base.
- 10 Non-absorbent cotton wool
- 11 Marker pen
- 12 Disinfectant for wiping bench
- 13 Sterile 1 cm<sup>3</sup> syringe or Pasteur pipette.
- 14 Sterile 250 cm<sup>3</sup> measuring cylinder or measuring beaker
- 15 Access to a top pan weighing balance
- 16 Optional – Biuret reagents and tubes for testing.

**Chlorella growth medium- pH 6.8**

Component	g/dm <sup>-3</sup>
Potassium nitrate(KNO <sub>3</sub> )	1.25
Potassium hydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.25
Magnesium sulphate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	1.00
Calcium chloride (CaCl <sub>2</sub> )	0.084
Boric acid (H <sub>3</sub> BO <sub>3</sub> )	0.014
Iron (II) sulphate (FeSO <sub>4</sub> 7H <sub>2</sub> O)	0.050
Zinc sulphate (ZnSO <sub>4</sub> )	0.088
Manganese chloride (MnCl <sub>2</sub> .4H <sub>2</sub> O)	0.014
Molybdenum trioxide (MoO <sub>3</sub> )	0.007
Copper sulphate (CuSO <sub>4</sub> .5H <sub>2</sub> O)	0.016
Cobalt nitrate (Co(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O)	0.005
EDTA	0.500











Information about algae and algal cultures can be obtained from;

<http://www.saps.plantsci.cam.ac.uk/docs/algalballs.doc>.

<http://www.botany.ubc.ca/cccm/>

[info@fba.org.uk](mailto:info@fba.org.uk)

**Safety Precautions/Risks.**

Potassium nitrate = O	
Calcium chloride = H (Irritant only)	
Iron (II) sulphate = H	
Zinc Sulphate = H, N	 
Manganese Chloride = H	
Molybdenum trioxide =H	
Copper sulphate = H, N	 
Cobalt Nitrate = H	

If household peroxide or hypochlorite bleach is used for sterilisation, manufacturers instructions should be followed and appropriate safety precautions taken.

A risk assessment should be carried out as a matter of course.



### Practical 3 - N (d)(m)Urine Analysis –Evaluating and reporting on observations



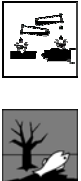
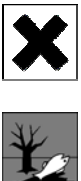
This practical focuses on – **Recording data, drawing conclusions** and **evaluation**.  
You will also be developing other assessed skills throughout the practical

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Use relevant scientific methods to produce a set of data.
- Record data reliably, using appropriate scientific terminology where necessary.
- Diagnose a medical condition by analysis of a sample of ‘urine’.
- Analyse data and produce a reasoned, valid conclusion.
- Support your conclusion with evidence gathered from a reference table.

#### Safety information

	You should wear eye protection throughout this practical.
	Sodium chloride is an <b>irritant</b> .
	Ammonia is <b>corrosive</b> and <b>dangerous to the environment</b> .
	Benedict’s solution is <b>harmful</b> and <b>dangerous to the environment</b> .

#### Background information

- The urine can be used to find out about a person’s health because of the role of the kidney in homeostasis.
- Urine analysis is a particularly useful tool for diagnosis of some metabolic conditions relating as toxins and excess substances are filtered into urine.
- Typical tests that would be performed on a urine sample include odour, clarity, colour, pH and presence or absence of protein or glucose.
- A table of reference values for healthy urine has been provided for comparison. The terms used in the table those appropriate in describing a urine sample.
- The presence of glucose can be tested using Benedict’s reagent.
- The presence of protein can be tested using the Biuret test.

You will carry out an analysis of 3 ‘urine’ samples in order to diagnose medical conditions.

- Read the information above.
- Produce a results table for your investigation.
- Familiarise yourself with the reference values for urine tests.

The 'urine' samples have been obtained from patients who may be suffering from either protein urea, diabetes or renal disease. The 'urine' samples are artificial as actual samples may contain pathogens.

**Table of reference values**

	Normal Reference Values		
	Adult	Child	Newborn
Colour	Light straw / Dark amber	Light straw / Dark yellow	
Appearance	Clear	Clear	Clear
Odour	Aromatic	Aromatic	
pH	4.5 – 8.0	4.5 – 8.0	5.0 – 7.0
Specific gravity	1.005 – 1.030	1.005 – 1.030	1.001 – 1.020
Protein	2-8mg/dm <sup>3</sup>		
Glucose	Negative	Negative	
Ketones	Negative	Negative	
Microscopic examination			
RBC		Rare	
WBC	3-4	0-4	
casts	Occasional	Rare	

**Method**

Use the following procedure to test each of the 'urine' samples.

**Initial examination of urine**

- 1 Obtain approximately 10 cm<sup>3</sup> of one of the urine samples.
- 2 Examine the urine visually for odour and clarity, describe what you see and record this in your results table.
- 3 Comment on the odour of the urine sample by wafting vapours toward you nose with your hand – DO NOT put your nose directly over the sample and inhale.

**Testing for pH**

- 1 Tear off approximately 3 cm of universal indicator paper
- 2 Briefly dip the paper in the urine.
- 3 Remove the paper and compare its colour against a universal indicator chart.
- 4 Record the pH in your results table.

**Testing for protein**

- 1 Put 2 cm<sup>3</sup> of the urine sample into a clean test tube.
- 2 Put 1 cm<sup>3</sup> of Biuret A (sodium hydroxide) into the test tube.
- 3 Put 1 cm<sup>3</sup> of Biuret B (copper II sulphate) into the test tube.
- 4 Observe any colour change and comment on whether protein is present in your results table.

**Testing for glucose**

- 1 Put 2 cm<sup>3</sup> of the urine sample into a clean boiling tube.
- 2 Add 2 cm<sup>3</sup> of Benedict's reagent to the urine.
- 3 Heat the urine sample in a boiling water bath.
- 4 Observe any colour change and comment on whether glucose is present in your results table.

**Diagnosis**

- 1 Using the reference table of normal values, identify any abnormalities of the urine sample.
- 2 Using the clinical information table, make a diagnosis based on your findings.

**Write-up**

- Prepare a report of your findings considering:
  - the condition from which each person may be suffering
  - the scientific explanations of your conclusions that relate to kidney function.
  - an explanation of the changes in metabolism that have caused the changes in the urine.

## Lesson Plan

### Urine Analysis –Evaluating and reporting on observations

#### Context

A practical investigation set in the context of 9700 Syllabus – **section N – excretion, control of water and metabolic wastes**. The investigation will improve understanding of urine production and removal of metabolic wastes and develop a students' evaluating skills. It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills and will be familiar with tests for reducing sugar and protein.

#### Key aims of lesson

This practical is designed to develop the skills of making and recording observations. In addition students will also evaluate observations and experimental data in relation to theoretical knowledge.

#### Intended learning outcomes

By the end of this practical and its write-up a student should be able to:

- Use relevant scientific methods to produce a set of data.
- Record data reliably, using appropriate scientific terminology.
- Diagnose a medical condition via analysis of a sample of 'urine'.
- Analyse data and produce a reasoned, valid conclusion.
- Support conclusions with evidence gathered from a reference table.
- Explain results in relation to theoretical knowledge.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
End of previous lesson	<b>Preparation</b> – students given theory on urine production. Students to consider possible problems that could arise during urine production. This could be presented as class discussion. Students to be reminded of Benedict's test and Biuret test, as experienced in AS syllabus.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> – briefly discuss urine production and potential problems with it – teacher led discussion / question and answer session.
8-12	<b>Introduction to method</b> – teacher to discuss appropriate terms to describe colour, clarity, and odour of urine. Teacher demonstration of correct way to determine odour. Optional teacher demonstration of Benedict's and Biuret tests.
12-20	<b>Student preparation exercises</b> – students to work through second box on page 1.

20-50	<b>Carrying out the practical</b> – students carry out entire investigation on at least 1 urine sample (all three samples, time permitting) and tidy away apparatus when they have finished.
50-60	<b>Drawing together the threads</b> - teacher-led discussion on the diagnoses the students have come to. Discussion on skills used and developed. Introduction to write up, students to complete report as homework.

### Useful information

Students should construct a table that shows the tests carried out, the results obtained and any conclusions based on the results of the tests.

Explanations of the results should be related to metabolic reactions and the role of the kidney, giving reasons why sample 1 is more likely to be from a diabetic than sample 3, why sample 2 is more likely to be from a person with protein urea and why sample 3 is more likely to be from a person with renal failure.

Students should make reference to;

- the role of the liver in converting glucose to glycogen and the role of the pancreas in monitoring and responding to glucose concentration in the blood,
- the role of the kidney is reabsorption of glucose and the effect of high blood glucose on reabsorption,
- the structure of the Bowman's capsule and capillary wall in relation to pore size and the 'normal' filtration of these layers,
- A suitable table of results would incorporate columns indicating the number of the urine sample being tested and rows indicating the test results of each of the different urine tests. A final row could indicate the proposed diagnosis of each particular sample.

The following is a brief guide to the clinical problems indicated by abnormal urine results, in relation to this investigation:

<b>Colour</b>	colourless or pale	diabetes or chronic kidney / renal disease
<b>Appearance</b>	hazy or cloudy	bacterial infection or blood cells present
<b>Odour</b>	ammonia foul or putrid sweet or fruity	urea breakdown by bacteria bacterial infection diabetes mellitus (sugar diabetes)
<b>pH</b>	<4.5 >8.0	respiratory acidosis or starvation urinary tract infection
<b>Protein</b>	present	protein urea or renal disease
<b>Glucose</b>	present	diabetes mellitus or renal disease

### Technical Information

#### Urine Analysis –Evaluating and reporting on observations

- Prepare 3 'urine' samples as follows:

*Sample 1 (patient suffering from diabetes)*

250 cm<sup>3</sup> distilled water  
1 g sodium chloride  
1 g potassium phosphate  
2 g glucose powder  
A few drops of fruit juice  
to give a fruity smell

*Sample 2 (patient suffering from protein urea)-*

250 cm<sup>3</sup> distilled water  
1 g sodium chloride  
1 g potassium phosphate  
1 g albumen powder  
3 cm<sup>3</sup> 1M ammonia  
(pH10)

*Sample 3 (patient suffering from renal disease)-*

250 cm<sup>3</sup> distilled water  
1 g glucose powder  
1 g albumen powder

- All samples can be coloured to appear more realistic by adding tea, yellow ink, food colouring or dye.
- Albumen powder may be substituted by other proteins, for example 1 cm<sup>3</sup> egg white.

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

For some of the chemicals, it is convenient to make up more than is required in order to give sufficient quantities for accurate measurement.

- The 'urine' samples may be prepared up to a week in advance but may require either gently shaking or inverting prior to use as particulate matter will settle.
- It is suggested that students complete a whole investigation on each urine sample but to save time, some students could investigate only sample 1, some only sample 2 and some only sample 3 and then the class may share results.

- 1 10 cm<sup>3</sup> of each 'urine' sample
- 2 Benedict's reagent
- 3 Biuret A solution (Sodium hydroxide)
- 4 Biuret B solution (Copper II sulphate)
- 5 Universal indicator paper and comparison chart
- 6 Test tube
- 7 Boiling tube1

- 8 250 cm<sup>3</sup> beaker
- 9 3 dropping pipettes or other suitable pipette
- 10 Bunsen burner, tripod and gauze – or access to a water bath set at >60°C)
- 11 6 x 10 cm<sup>3</sup> graduated pipettes / syringes / measuring cylinders

Safety Precautions/Risks.

Sodium chloride = H (Irritant only)



Ammonia = C, N



Benedict's = H, N



A risk assessment should be carried out as a matter of course.

### Practical 4 - Q(a) Systematics and classification

This practical focuses on - **Drawing conclusions and evaluating evidence**. You will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Identify the main features used to classify organisms
- Be able to construct and use a simple dichotomous key
- Experience grouping organisms based on biological similarities.

#### Safety Information

There are no particular hazards in this practical, however you must follow your laboratory rules.

#### Background information

- The study of diversity in living organisms(biodiversity) is sytematics
- Living organisms are grouped according to morphological, physiological and biochemical similarities. These groups are called taxons.
- The most commonly used taxons are: Kingdom, Phylum, Class, Order, Family, Genus and Species
- Similarities exist due to evolution from a common ancestral stock.
- The more similarities shown by different organisms, the more closely related and the shorter the time since the organisms became separated by evolution.
- The more similarities, the fewer organisms belong to the taxon. Thus each kingdom contains many different types of organism with a few common features, while a genus contains only a few types of organism with many features in common.
- Nomenclature is the naming of organisms based on the taxons. International agreements exist for most taxa, in particular the binomial system used for genus and species. Rules apply to the correct use of grammar for the Latin names.
- Dichotomous keys are a universal means of classification that allows quick and reliable identification of unknown organisms.
- A key is a series of choices based on observable phenotypic characteristics. In a dichotomous key the choices are given in pairs. Correct choice at each pair gives the specific name of the organism.
- The information used in a key can be qualitative descriptions of physical features, for example, the colour, presence of spots. Alternatively information can be quantitative, for example the number of spots, number of spines, mass.
- There are two ways to set up a dichotomous key.
  - 1 Present the two choices together,
  - 2 Present by relationships, in which case the choices are widely separated.Examples of these types of key are in a student guide sheet.
- Type 1 keys are generally easier to follow, but may give less information about relationships than type 2.



You will investigate the taxonomic relationships between a number of organisms

- Read the information above.
- Group together the organisms in relation to shared features.
- Use the student guide to identify the kingdom to which each of the organisms belongs. List the evidence to support your groupings.
- Use a key to classify at least one organism into its complete taxonomic groupings.
- Use the guide sheet to construct a dichotomous key to identify a group of organisms

### Method

#### Observing features used in classification

- 1 Observe each organism carefully.
- 2 Use the check list to identify the features that link the organism to a particular kingdom.
- 3 Put the organisms that belong to the same kingdom together.
- 4 Make further observations to group each organism into a phylum.
- 5 Using the organism labelled A, work through the key to identify the organism as far as you can.
- 6 Repeat step 5 for the organism labelled B.

#### Preparing to construct a dichotomous key

- 1 Look the organisms provided and sort them into groups based on observable feature.
- 2 There is no rule about which features are chosen, just that they are distinctive. A first grouping could be by size – longer than 2 cm or shorter than 2 cm. Put all the organisms that fit into one or other of the categories together.
- 3 Then look for another distinguishing feature – this might be colour and group the organisms accordingly
- 4 Continue to select a feature that separates the groups of organism until you have each in a separate group.
- 5 Use a piece of paper to lay out the groups like a spider chart and write down what feature you used to separate them. An example of a spider chart is on the guide sheet to constructing a key

### Write-up

- Produce a table that shows the allocation of the organisms to specific kingdoms.
- This should include;
  - the correct names of the kingdoms,
  - One or two distinguishing features of each kingdom,
  - A list of the letters of the organisms provided
- Draw conclusions to complete the table considering:
  - Matching the description of the features of the organisms to the kingdom,
  - Grouping the organisms according to their kingdom,
  - Other features that may not be directly visible in the specimens that might be used to confirm identification
- Produce a table that shows the classification of organisms A and B
- Produce a dichotomous key to identify the organisms provided.
- Evaluate the key in relation to ease of use and accuracy of identification. You could do this your self, but it is more useful to ask another person to see if they can use your key.

**Guide sheet to producing a key**

These keys are based on imaginary flower petals from 7 different organisms:

P, Q, R, S, T, U and V

Whichever key is used organism P should be identified as having red petals with a smooth edge.

**1 Present the two choices together.**

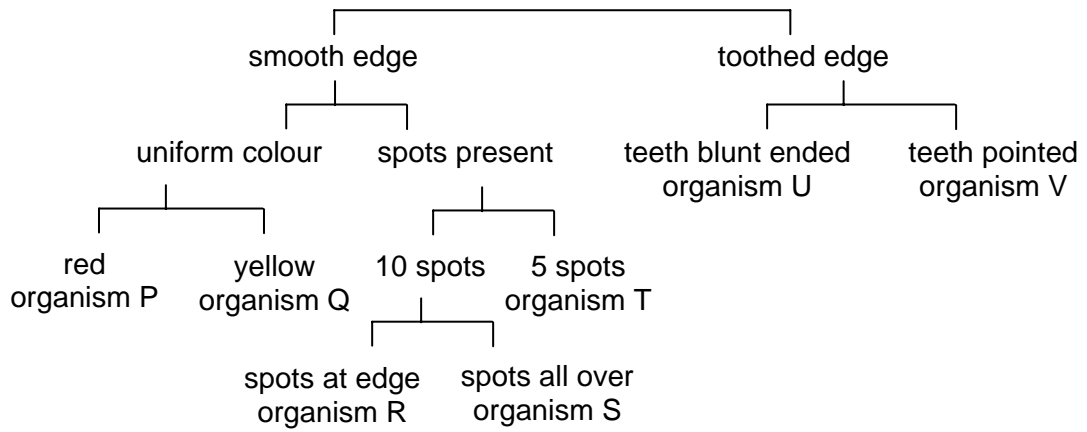
- |   |                         |            |
|---|-------------------------|------------|
| 1 | has smooth edge .....   | 2          |
|   | has toothed edge .....  | 3          |
| 2 | uniform colour .....    | 4          |
|   | spots present .....     | 5          |
| 3 | teeth blunt ended ..... | organism U |
|   | teeth pointed .....     | organism V |
| 4 | colour red .....        | organism P |
|   | colour yellow .....     | organism Q |
| 5 | ten spots .....         | 6          |
|   | five spots .....        | organism T |
| 6 | spots at edges .....    | organism R |
|   | spots all over .....    | organism S |

If your specimen has a smooth edge you would then go to number 2 in the key and look at the next pair of choices. If it has spots then you go to number 5.

**2 Present by relationships.** In this case the choices are widely separated but are still given the same number.

- |   |                    |            |
|---|--------------------|------------|
| 1 | Has a smooth edge  |            |
| 2 | Colour uniform     |            |
| 3 | colour red         | organism P |
| 3 | colour yellow      | organism Q |
| 2 | Spots present      |            |
| 4 | ten spots          |            |
| 5 | spots at edges     | organism R |
| 5 | spots all over     | organism S |
| 4 | five spots         | organism T |
| 1 | Has a toothed edge |            |
| 6 | teeth blunt ended  | organism U |
| 6 | teeth pointed      | organism V |

**Alternative presentation as a spider chart**



## Lesson Plan

### Systematics and classification – Drawing conclusions and evaluating evidence

#### Context

A practical investigation set in the context of 9700 Syllabus Learning outcome (a) – biodiversity and the five kingdom classification.

It is anticipated that students will completed an AS practical course so that they will have good observational skills. It is also anticipated that they will have been given learning opportunities before this so that they will be familiar with the terminology used in classification.

#### Key aims of lesson

This practical is designed to develop the skills of: Drawing conclusions and evaluating evidence. Students will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up the student should be able to:

- Identify the main features by which the five kingdoms are recognised
- Place an organism into the appropriate kingdom based on observable features
- Experience using a simple key to identify an organism
- Construct dichotomous key to identify a group of organisms
- Experience classifying an organism based on observable features

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

#### Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
end of previous lesson	<b>Preparation</b> - 2 page student worksheet and guide to constructing a key given out for students to read in preparation for the practical lesson and to consider appropriate features that might be used in classifying organisms into taxons.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> - review of classification, major taxons used in the five kingdom system. Teacher-led questioning, student responses / discussion, students building a framework for a suitable table to be used in identifying the organisms provided.

8-12	<b>Introduction to method</b> - teacher demonstration using a selected organism to indicate suitable features for assigning an organism to a kingdom.
12-22	<b>Assigning organisms to kingdoms.</b> Students use the table format worked out during context discussion to assign a variety of organisms to a kingdom. There should be at least 10 different organisms, with at least one from each of the major kingdoms. These can be arranged as a circus around the room and students move from one to the other. Every minute students move to the next organism. Teacher circulates, answering specific queries, praising students who are making a good effort and helping to guide students in the right direction.
23-25	Checking the allocations – teacher goes around the class asking each student to give the kingdom and the reasons for their choice. Students check their own answers.
26- 30	<b>Using and constructing a key</b> Optional – teacher / student interactive demonstration on constructing a key from everyday objects e.g. each students puts a writing implement on the table or a shoe. Alternatively the students themselves might be put into an identification key.
31 – 50	Students construct a key to identify the organisms provided.
51 – 55	Students evaluate the key by using another student's key to identify the organism. Comments are made on how easy the key was to use.
56- 60	<b>Drawing together the threads</b> – teacher-led class discussion on the skills that have been developed. Teacher led introduction to write-up, which should include the classification table and the key. Further practice at producing a key or using from teacher generated examples of organisms, using photocopies of actual organisms Students should also select two examples of organisms to generate a complete classification table for each.

### Useful Information

- Taxons are based on similarities and differences that arise due to evolution.
- Some metabolic processes e.g. many of the respiratory reactions are the same in all organisms as they have been highly conserved by evolution. The indicate common ancestry of all organisms. Other processes shows differences due to adaptation to different environments e.g. sequence of digestion from large to small molecule is common but different enzymes may be present, which act in a number of different locations depending on how the organisms feed.
- Physiological and morphological differences due to evolution that can be observed or measured are used in classification. Technological advances have made it possible to measure molecular differences that are now also used in classification.
- Keys used to identify organisms are based on taxonomic groupings.

## Appendix 2

- Keys are usually hierarchical. The first part key is used to assign the organism to a phylum and/or class. Following sub-keys then allow further identification. This allows a faster process of identification
- The most commonly used keys are dichotomous. Simple field identification keys tend to use the paired choice or spider diagram method of presentation. More complex reference keys tend to be based on relationships.
- There is no one correct way of producing a key. The most important feature is that it works and is easy to use.
- There are classification and key construction websites that students could access via a search engine.
- A suitable table for identifying kingdoms might be:

	Feature of Kingdom.				
	Plantae	Animalia	Monera (Prokaryotae)	Protoctista	Fungi

### Technical information

#### Systematics and classification – Drawing conclusions and evaluating evidence

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

- 1** A set of ten organisms, at least one example from of each of the five kingdoms. Photographs, photocopies and museum specimens may be used as well as live specimens
- 2** A set of at least 8 organisms from the same phylum or class. Photographs, photocopies, slides and museum specimens may be used as well as live specimens. Possibilities are: different types of flowering plants-either flowers or leaves or seeds, different types of arthropod, crustacea, arachnida or insecta, different types of mollusc shell, different types of fern – easier to use if spore cases are present, different types of fungi.
- 3** Access to standard keys of local flora and fauna or photocopies of parts of keys. Choice of organisms to use may be influenced by the keys available. Reference text books may also be suitable for the classification exercise.
- 4** websites  
<http://www.iit.edu/bi8611/~smilehtml>  
<http://www.zoo.utoronto.ca/able/volumes/vol-12/7-timme/7-timme.html>  
<http://regentsprep.org/Regents/biology/units/laboratory/dichotomous.cfm>  
<http://www.park.edu/bhoffman/courses/bi225/labs/>

#### Safety Precautions/Risks.

No specific hazards identified.

A risk assessment should be carried out as a matter of course.



### Practical 5 - R(a) Bacterial Transformation

This practical focuses on- **Using complex apparatus, analysis and evaluation**

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Learn and apply the steps involved in the transformation process
- Understand transformation and its applications in biology
- Analyse and interpret experimental results
- Understand regulation of gene expression by the arabinose operon

#### Safety Information

The safety instructions provided with the Bio-Rad Laboratories kit should be followed. You should avoid exposing eyes or skin to UV light. Care should be taken as the UV light may not be visible to human eyes.

#### Background information

- Genetic transformation occurs when a cell takes up and expresses a new piece of genetic information (DNA).
- Genetic transformation has many uses ranging from genetic modification of crops to give them more desirable qualities e.g. frost or drought resistance.
- The desirable gene is cut from human, plant or animal DNA and placed inside bacteria, which then reproduces, replicating the new gene and synthesising the protein coded by the gene.
- You will introduce a gene that codes for green fluorescent protein (GFP) into bacteria.
- The GFP gene is present in jellyfish and codes for the production of GFP, so the jellyfish glows in the dark.
- After the transformation, the bacteria containing the GFP gene should also glow in the dark.

You will introduce the GFP gene into the bacterium *Escherichia coli* using the pGLO Bacterial Transformation Kit from Bio-Rad Laboratories.

## Method

### Preliminary Study

The goal of genetic transformation is to change an organism's phenotype. Before any change in the phenotype can be detected, an examination of the organism's natural (pre-transformed) phenotype must be made.

- 1 Look at the colonies of *E. coli* on the starter plate. List all observable traits or characteristics that can be described.

### Day One – Transformation Procedure

- 1 Label one micro test tube **+pGLO** and a second test tube **-pGLO**.
- 2 Put your initials on the lid of both tubes then place the tubes in a foam test tube rack.
- 3 Open the tubes and using a sterile transfer pipette, transfer 250µl of transformation solution (CaCl<sub>2</sub>) into each tube.
- 4 Put both tubes on ice.
- 5 Using a sterile loop, pick up **one single colony** of bacteria from your starter plate.
- 6 Immerse the loop in the liquid in the tube labelled **+pGLO**. Agitate the loop in the liquid until the colony is dispersed in the solution.
- 7 Place the tube back in the ice and repeat using the other tube labelled **-pGLO**.
- 8 Examine the solution in the pGLO DNA tube provided using a UV lamp and note any observations.
- 9 Immerse a new sterile loop into the pGLO DNA tube, when you remove the loop there should be a film of solution across the ring. Mix this into the contents of the **+pGLO** tube then put it back on ice.
- 10 Leave the tubes on ice for 10 minutes.
- 11 While you wait, label your 4 nutrient agar plates on the **bottom** as follows:  
Label one LB/amp plate **+pGLO**  
Label the LB/amp/ara plate **+pGLO**  
Label the other LB/amp plate **-pGLO**  
Label the LB plate **-pGLO**
- 12 Using the foam rack as a float put the **+pGLO** and **-pGLO** tubes into the water bath (42°C) for 50 seconds.
- 13 After 50 seconds put the tubes back on ice for 2 minutes.
- 14 After 2 minutes remove the tubes from the ice. Using a transfer pipette, add 250µl of LB nutrient broth (provided) to each tube. Allow the tubes to stand at room temperature for 10 minutes.
- 15 After 10 minutes, flick the tubes to ensure the contents are mixed. Using a clean transfer pipette each time, add 100µl of **+pGLO** suspension to the plates you labelled **+pGLO** and 100µl of **-pGLO** suspension to the plates you labelled **-pGLO**.
- 16 Using a new sterile loop for each plate gently spread the liquid across the surface of the agar plates. **DO NOT put the lid down on the bench and DO NOT press into the agar.**
- 17 Stack your plates and tape them together. By tomorrow you should be able to determine whether the bacteria have taken up the GFP gene and expressed the protein.

**Day One - Review considerations**

- 1 Identify the plates you would expect to find bacteria most like the original non-transformed *E. coli* colonies you observed. Explain your predictions.
- 2 Predict which plate(s) are most likely to have any genetically transformed bacterial cells. Explain your predictions.
- 3 Identify which plates should be compared to determine if any genetic transformation has occurred. Explain why.
- 4 What is meant by a control plate? What purpose does a control serve?

**Day Two – Data Collection**

- 1 Observe the results you obtain from the transformation procedure under normal lighting conditions. Then hold the UV lamp over the plates.
- 2 Carefully observe and draw what you see on each of the four plates.
- 3 Write down the following observations for each plate:  
How much bacterial growth do you see on each plate?  
What colour are the bacteria?  
How many bacterial colonies are on each plate (count the spots you see).

*Day Two – Analysis of Results*

- 1 Which of the traits that you originally observed for *E. coli* did not alter?
- 2 Which of the traits did alter?
- 3 If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be concluded about the other genes on the plasmid that you used in your transformation procedure?
- 4 From the results that you obtained, how could you prove that the changes that the changes were due to the procedure that you performed?

## Lesson Plan

### Bacterial Transformation

#### Context

A practical investigation set in the context of 9700 Syllabus – a bacterial transformation procedure.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify transformed bacteria.

The GFP gene will be introduced into the bacterium *Escherichia coli* using the pGLO Bacterial Transformation Kit from Bio-Rad Laboratories

#### Key aims of lesson

This practical is designed to develop the practical, observational, data handling and analysis skills.

#### Intended learning outcomes

By the end of this practical the student should be able to

- Learn and apply the steps involved in the transformation process
- Understand transformation and its applications in biology
- Analyse and interpret experimental results
- Understand regulation of gene expression by the arabinose operon

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
previous lesson	<p><b>Preliminary study</b> - The goal of genetic transformation is to change an organism's phenotype. Before any change in the phenotype can be detected, an examination of the organism's natural (pre-transformed) phenotype must be made.</p> <p>Students will need to look at the colonies of <i>E. coli</i> on the starter plate and list all observable traits or characteristics that can be described.</p>
Day one 0-4	<p><b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation</p>
4-8	<p><b>Context</b> - review of bacterial transformation. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.</p>

8-12	<b>Introduction to method</b> - teacher demonstration of transformation procedure.
12-50	<b>Carrying out the practical</b> - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.
50-60	<b>Drawing together the threads</b> - teacher-led class discussion on the procedure as well as a look at the review questions. Review questions to be completed prior to day two of practical.
Day Two 0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation – review of day one.
4-8	<b>Day One Review Questions</b> – teacher led discussion of answers to review questions.
8-12	<b>Introduction to day two</b> - teacher to introduce analysis of results session.
12-25	<b>Observations</b> – students make and record observations and compare these with those made in the preliminary study.
25-35	<b>Drawing together the observations</b> - teacher-led class discussion on the observations.
35-50	<b>Analysis</b> – students complete an analysis of the results and draw conclusions.
50-60	<b>Drawing together the threads</b> - teacher-led class discussion on the student analysis.

### Useful Information

- The pGLO kit can be used to introduce students to the concept of genes and their basic function of coding for proteins.
- The kit can be used for independent study projects.
- Transformation is commonly used in biotechnology research and industry to study and manufacture proteins so a link to the real world can be made.
- A flow chart can be used to show the transformation procedure.
- Each kit is supplied with a Teacher's Guide, Student Guide and graphic quick guide.
- There is an advanced preparation step which needs to be performed 3 to 7 days before the transformation procedure is undertaken.
- The regulations about investigations using genetic transformation vary between countries. Teachers will need to check the regulations in their countries before embarking on these investigations
- Suppliers may be limited by import regulations and extra taxation in some countries.

## Technical Information

### Bacterial Transformation

The **apparatus and materials** required for this are listed below. The kit provides materials for 32 students or 8 complete student workstations.

Apparatus and materials per group:

- 1 1 pGLO Bacterial Transformation Kit (Bio-Rad Laboratories)
- 2 1 UV lamp
- 3 500 ml distilled water
- 4 1 Beaker of crushed ice

Apparatus to be available in laboratory:

- 1 Water bath at 42 °C
- 2 1 paper towel
- 3 Rubber gloves

### GMO's

- Countries have different regulations with regard to the use of genetically modified organisms.

Teachers will need to be aware of these.

#### Safety Precautions/Risks.

When using the Bio-Rad Laboratories product the manufacturers instructions should be followed.

Due care should be taken with regard to exposure to UV light.

A risk assessment should be carried out as a matter of course.

### Practical 6 - R(a) Extraction of DNA from Fruit and Vegetables






This practical focuses on – **Using complex apparatus and procedures.**

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Describe a method that can be used to extract DNA from plant tissue.
- Explain using theory from A2, the main stages used to extract DNA from plant tissue.

#### Safety Information

	You should wear eye protection throughout this practical.
	Ethanol is <b>highly flammable</b> . There should be no flames in the same room.
 	Methylated spirit (IMS) is <b>highly flammable</b> . There should be no flames in the same room.
	Protease enzymes such as Bromelain and Papain are all <b>harmful</b> .
The general safety precautions for working with DNA, such as those provided by NCBE, should be followed.	

#### Background information

- In forensic science DNA is extracted to obtain DNA for genetic fingerprinting, in genetic engineering it may be used for modifying plants and animals and in medicine it may be used to research inherited diseases and develop cures or gene therapy
- Initially tissue is broken up mechanically. It is important that the tissue is broken down as finely as possible
- Detergent is used to disrupt the cell membranes and nuclear membranes.
- The cell fragments are separated by filtration.
- DNA is separated from the extract.
- A protease enzyme is used to remove soluble proteins.
- DNA is precipitated using ice-cold ethanol.

You will extract DNA from fruit or vegetables using a method which has been adapted from a method that is used in laboratories all over the world.



## Method

### Mechanical break up of plant tissue

- 1 In a large beaker, mix 3g of table salt with 10 cm<sup>3</sup> of washing up liquid.
- 2 Add 90 cm<sup>3</sup> of water so you have 100 cm<sup>3</sup> all together.
- 3 Add 50g of chopped fruit or vegetables.
- 4 Place the beaker in a water bath at 60 °C for **exactly** 15 minutes.
- 5 After 15 minutes, place the beaker into an ice bath for 5 minutes, stirring frequently.
- 6 Filter the mixture through a coffee or large filter paper in a filter funnel; place a clean beaker underneath the funnel to collect the filtrate. Do not over fill the funnel or the filtrate will be contaminated by foam.

**Separation of DNA** – step 5 should be done slowly and carefully so the ethanol forms a layer on top of the filtrate/protease mix.

- 1 Use a measuring cylinder to measure 10 cm<sup>3</sup> of filtrate.
- 2 Pour the 10 cm<sup>3</sup> of filtrate into a boiling tube and add 2-3 drops of protease enzyme using a teat pipette.
- 3 Shake the boiling tube to mix the contents.
- 4 Use a measuring cylinder to measure 6 cm<sup>3</sup> of ice-cold ethanol.
- 5 Slowly and carefully pour the ethanol into the boiling tube containing the filtrate/protease mix.
- 6 Leave the boiling tube in a rack for a few minutes without disturbance.
- 7 After a few minutes you will see a white substance floating out into the ethanol – this is the DNA!

### Review considerations

- 1 The washing-up liquid breaks down the membranes. Why is it necessary to breakdown the nuclear membrane?
- 2 In step 4 a temperature of 60 °C is used to denature DNAases. Why is it important to denature DNAases?
- 3 Why is the mixture filtered?
- 4 Describe the action of the protease enzyme.

**Lesson Plan****Extraction of DNA from Fruit or Vegetables****Context**

A practical set in the context of 9700 Syllabus – a simplified method of extracting DNA from fruit or vegetables.

It is anticipated that students will have completed an AS practical course and so they will have good basic practical skills. It is also assumed that they will have reviewed work completed in AS on the structure of a plant cell and the action of enzymes.

**Key aims of lesson**

This practical is designed to develop practical skills and relate work completed in AS to new situations.

**Intended learning outcomes**

By the end of this practical and by answering the questions the student should be able to:

- Describe a method that can be used to extract DNA from plant tissue.
- Explain the main stages used to extract DNA.

**Resources required**

White board or flipchart and suitable pens or chalkboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

<b>Timings/ minutes</b>	<b>Teacher / Student Activities</b>
End of previous lesson	<b>Preparation</b> – students to review the structure of a plant cell and the action of enzymes
0-5	<b>Introduction</b> to the aims, intended outcomes and background information - teacher led oral presentation
5-10	<b>Context</b> - review plant structure, action of enzymes and location of DNA – teacher led questioning, student responses/discussion
10-15	<b>Introduction to method</b> - teacher to go through method
15-50	<b>Carrying out the practical</b> - students carry out the practical work. At the end the teacher compares the amount of DNA each student or group has extracted. Students tidy away apparatus as soon as they have finished.
50-60	<b>Drawing together the threads</b> – students to complete questions. Teacher-led check of answers through questioning, student response/discussion.

### Useful Information

- It is best to use soft fruit or vegetables. Frozen peas and onion give good results. If hard fruit or vegetables are used the mixture will need to be blended for 5 seconds before filtering. Fruit with a skin will need to be peeled and the skin discarded before weighing. Fish eggs can also be used
- Different types of fruit and vegetables can be compared.
- Students should consider that:
  - 1 Cells and cell membranes have to be broken to release the DNA from the nucleus.
  - 2 DNAase must be denatured as it is an enzyme that breaks down DNA,
  - 3 Filtration separates the cell wall material from the DNA and soluble proteins.
  - 4 Protease enzymes break the peptide bond between the amino acids in the polypeptide chain. The protein binds to the active site of the enzyme lowering the activation energy. The peptide bonds break and peptides are produced.

**Technical Information****Extraction of DNA from Fruit and Vegetables**


The **apparatus and materials** required for this are listed below.



The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.


It is convenient to weigh out the salt so that each student or group has 3g. A measuring cylinder should be used to measure the washing-up liquid.

- 1 Approximately 50g of fruit or vegetable should be used. For example frozen peas or onion.  
The fruit or vegetables should be pre-chopped very finely.
- 2 Top pan balance and spatula
- 3 1 10 cm<sup>3</sup> measuring cylinder (this will need to be washed before being reused)
- 4 1 100 cm<sup>3</sup> measuring cylinder
- 5 2 500 cm<sup>3</sup> beakers
- 6 1 water bath at 60 °C
- 7 1 ice bath
- 8 1 coffee filter or filter paper and funnel
- 9 1 boiling tube and rack
- 10 1 teat pipette
- 11 3g table salt or 3g sodium chloride
- 12 Washing up liquid
- 13 2 cm<sup>3</sup> protease solution (Novo Neutrase available from \*NCBE). Other proteases, such as 2% solutions of papain or bromelain, may be used. These are less effective so the tubes may need to be left longer before DNA separates.
- 14 Ice cold 95% ethanol (place the ethanol in a freezer overnight). Ice cold methylated spirits (IMS) can be used as an alternative.

Safety Precautions/Risks.

Ethanol = F 

Methylated spirits (IMS) = F, H  

Proteases as per individual product, ie Bromelain = H, Papain = H 

General safety precautions for working with DNA can be obtained from NCBE.  
[www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk)

(<http://www.ncbe.reading.ac.uk/NCBE/SAFETY/dnasafety1.html>)

A risk assessment should be carried out as a matter of course.

### Practical 7 - R(g) Electrophoresis as a separation process







This practical focuses on – **Using complex procedures and apparatus, evaluation**

#### Intended learning outcomes

by the end of this practical and its write-up you should be able to:

- Set up and run a gel electrophoresis tank as a model of DNA fingerprinting
- Be able to use new apparatus correctly
- Understand the process of DNA fingerprinting and its usefulness in biotechnology

#### Safety Information

	You should wear eye protection throughout this practical. The coloured dyes used will stain skin or clothes.
	TBE buffer is <b>harmful</b> .
	Fluorescein is an <b>irritant</b> . It will stain skin or clothes.
	Methylene Blue is <b>harmful</b> . It will stain skin or clothes.
 	Crystal violet is <b>harmful</b> and <b>dangerous to the environment</b> . It will stain skin or clothes.

- Electrophoresis is a technique used for separating molecules by charge.
- The molecules to be separated are placed on a supporting surface, usually agarose gel and immersed in a conducting buffer. The buffer ensures a current passes through the gel.
- A potential difference is applied and the molecules move towards the electrodes according to their charge.
- The agarose gel has a structure like a sieve. Small molecules move quickly through it but larger ones are hindered by the gel.
- DNA is a negatively charged molecule so it moves towards the anode (positive electrode).
- All human DNA has the same basic chemical structure.
- However, each human's individual DNA is unique due to the sequence of the base pairs.
- DNA fingerprinting is a way of visualising the sequence of base pairs in a DNA sample, thus identifying whom it belongs to.
- To 'map' the entire sequence of an individual's DNA would be too great an

undertaking; so instead, specific sections of DNA are used, which can be obtained by using restriction enzymes.

- These specific sections (known as variable number tandem repeats – VNTRs, inherited genetically) are known to vary enormously between individuals so when two samples are compared it would be possible to tell the difference
- between the DNA of two different people. It would also be possible to identify individuals who were related to one another.
- The process of DNA fingerprinting consists of 6 stages, of which you will be carrying out stage 3, using dyes to substitute for DNA:
  - 1 Isolation of DNA (DNA extraction), which you may have carried out in another practical
  - 2 Cutting DNA into fragments using restriction enzymes
  - 3 Gel electrophoresis to sort DNA fragments by size
  - 4 DNA denaturation (to make DNA single stranded)
  - 5 Southern blot onto nitrocellulose paper – this transfers the single stranded DNA onto a permanent medium (the gel is not permanent)
  - 6 Hybridisation with a radioactive or fluorescent probes. Radioactive DNA bases are introduced to the southern blot and so bond with the single stranded DNA on it. The places where these specific probes bond can then be visualised.

You will carry out the gel electrophoresis stage of DNA fingerprinting, using coloured dyes to represent DNA samples.

- Read the information above.
- Draw a labelled diagram of a working electrophoresis tank.
- List the variables that should be controlled.
- Outline how each variable might be controlled.

### Method

#### Preparing and pouring the agarose

- 1 Add 0.35 g of agarose powder to 35 cm<sup>3</sup> of TBE buffer in a conical flask.
- 2 Heat the mixture over a Bunsen burner using a tripod and gauze, swirling the flask occasionally to prevent any lumps forming. A microwave can be used, in which case heat for 30 seconds, then in 10 seconds increments until the agarose dissolves.
- 3 Agarose becomes transparent when it boils, when it reaches this stage, remove from the heat and leave to cool for 6-8 minutes or until the temperature of the agarose is between 55°C and 60°C.
- 4 Whilst the gel is cooling prepare the electrophoresis tank by inserting the casting gates and comb.
- 5 When the gel has cooled sufficiently to the desired temperature it can be poured, carefully, into the tank, between the two casting gates.
- 6 Leave the gel to set for at least 15 minutes.

### Loading the gel

- 1 Once the gel is set, carefully remove the casting gates.
- 2 Very gently remove the comb, taking care not to rip the gel. The comb should have introduced 'wells' into the gel, which will be utilised later.
- 3 Pour 40 cm<sup>3</sup> TBE buffer into the tank. This should completely cover the surface of the gel.
- 4 Fill a micropipette or capillary tube with dye A.
- 5 Position the pipette inside the mouth of the first well in the gel and dispense the dye into it.
- 6 Load dye B into the next well in the same way.
- 7 Repeat the loading procedure for each of the six dyes available.
- 8 Connect the battery pack. The positive electrode should be at the same end as the wells loaded with dye.
- 9 After a minimum of 1.5 hours and a maximum of 3 hours, disconnect the battery pack and interpret the results

### Write-up

- Draw a diagram to represent the final positions of the dyes on your gel.
- Draw conclusions, considering the following; the meaning of the results, what the results might be able to tell us if real DNA fragments had been used, scientific explanations of results and conclusions.
- Make an evaluation, considering the following; the limitations of the methods used, the confidence with which conclusions can be drawn, the adaptations that would have to be made for this method to be a reliable tool for forensic investigations or paternity tests.

**Lesson plan****Electrophoresis as a separation process****Context**

A demonstration of electrophoresis set in the context of the 9700 syllabus. It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that students will have been given prior learning opportunities so will be able to handle the tasks required of this practical.

**Key aims of lesson**

This practical is designed to develop the skill with which students use techniques and apparatus, and to boost confidence in learning to use unfamiliar apparatus.

**Intended learning outcomes**

By the end of this practical and its write-up students should be able to:

- Set up and run a gel electrophoresis tank as a model of DNA fingerprinting
- Be able to use new apparatus correctly
- Understand the process of DNA fingerprinting and its usefulness in biotechnology

**Resources required**

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Either gel electrophoresis tanks with battery packs / electrode material, batteries, wire and clips or a commercial Electrophoresis kit.

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons).

It is possible that the time for the gels to run may overlap into another lesson. In this case the student activity can be split and the gels prepared in the previous lesson. The gels can be stored in a container or plastic bag for 24 hours. Alternatively gels can be pre-prepared and used after the technique is demonstrated

<b>Timings/minutes</b>	<b>Teacher / Student Activities</b>
end of previous lesson	<b>Preparation</b> – students given some theory on DNA fingerprinting or instructed to research on topic for homework.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> – class discussion on application of DNA fingerprinting. Teacher to summarise overall process of DNA fingerprinting on board (possibly in flow-chart format).
8-12	<b>Introduction to method</b> – teacher demonstration of setting up electrophoresis tank. Teacher demonstration of using micropipette, pouring gel and connecting power supply.



12 -50	<b>Carrying out the practical</b> – students work through the method in groups and tidy away apparatus whilst waiting for gels to ‘run’. Students can make a sketch of the apparatus and list the variables and how they are controlled whilst the teacher circulates. Students may also begin an evaluation of method before gels have finished ‘running’.
50-60	<b>Drawing together the threads</b> – students observe the completed gels and sketch. Teacher-led class discussion on what has been learned, the results and their meaning. Teacher led introduction to write-up, which may be finished as homework if necessary.

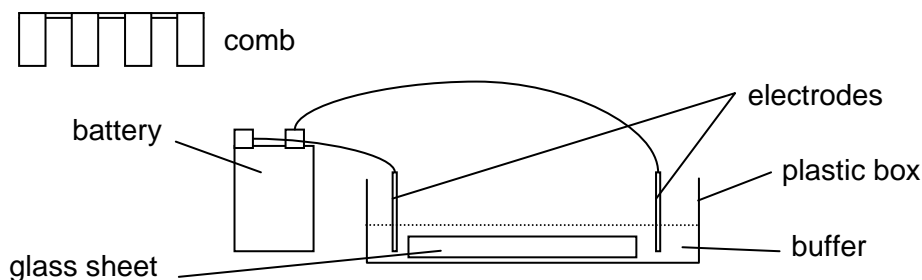
#### Useful information

- Once prepared, agarose gel has a short shelf life and should be discarded after removal from the electrophoresis tank.
- TBE buffer is effective to a minimum of a 10% working concentration and has a shelf life of approximately 12 months.
- Possible variables to control include: temperature, pH, concentration of TBE buffer, volume of TBE buffer, electrical charge through the gel

For a practical that incorporates more aspects of DNA fingerprinting, including cutting DNA with restriction enzymes for example, the purchase of a kit that includes all the necessary materials. A number of companies produce kits with instructions for practical activities.

### Technical Information - Electrophoresis as a separation process

- The list of apparatus is given per student, but this may be adapted if students are to work in pairs or groups.
- It is convenient to make more of the reagents than is required in order to give sufficient quantities for accurate measurements.
  - 1 Agarose powder – 0.35 g per student
  - 2 TBE buffer – 75 cm<sup>3</sup> per student
  - 3 Electrophoresis tank, including casting gates, comb and battery pack – 1 per student
  - 4 Micropipette – 1 per student (with a clean tip for each dye to be used)
  - 5 Bunsen burner, tripod and gauze – 1 per student OR access to a microwave
  - 6 Conical flask, flat-bottomed – 1 per student
  - 7 Coloured dye samples labelled A,B,C,D,E,and F
- Dyes that might be used: bromophenol blue, methylene blue, bromocresol green, crystal violet, fluorescein, Orange G , red food colouring, blue food colouring, green food colouring, yellow food colouring. A mixture of dye may also be used.
  1. It is expected that an electrophoresis kit will be available. Each kit usually has its own individual way of connecting a battery pack but will include instructions.
  2. If electrophoresis kits are not available, they can be made using plastic boxes as buffer tanks, glass sheets for supporting a gel, a pair of electrodes (platinum is preferred although carbon will work) and a 9 volt battery. A thin plastic sheet can be cut to form a comb.





3. Most good electrophoresis kits include dyes which can be used for this practical and also micropipettes.
4. If micropipettes are not available, capillary tubes may be used instead.
5. Suppliers of kits for electrophoresis and DNA fingerprinting:
  - [www.ncbe.reading.ac.uk](http://www.ncbe.reading.ac.uk) – at the time of writing £50 will get you 8 low voltage electrophoresis kits. Price list is a pdf and international order details are on <http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/ordering.html>
  - Bio-Rad –electrophoresis kits and DNA fingerprinting – [www.bio-rad.com/](http://www.bio-rad.com/) and click on the Life Science education tab
  - <http://wardsci.com/product.asp?pn=365164>
  - [http://www.sdr.com.au/electrophoresis\\_horizontal.html](http://www.sdr.com.au/electrophoresis_horizontal.html)

[http://sciencekit.com/category.asp\\_Q\\_c\\_E\\_432358](http://sciencekit.com/category.asp_Q_c_E_432358)



<http://www.bioteach.ubc.ca/TeachingResources/DoingScience/MacgyverProjShirazuEtalMaintext.pdf>

Safety Precautions/Risks.

TBE buffer = H 

Fluorescein = H (irritant) 

Methylene Blue = H 

Crystal violet = H, N  

A risk assessment should be carried out as a matter of course.

### Practical 8 - S(d) The Effect of Penicillin on Bacterial Growth

In this practical focuses on the practical skills of: **Planning – defining the problem**

You will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Pour agar plates using aseptic technique
- Experience growing microorganisms
- Use the disc diffusion technique needed to undertake a microbiological investigation
- Use vernier callipers to measure clear zone diameters.
- Assess the reliability of results

#### Safety Information

See below and the next page.



Culturing microorganisms may be hazardous

#### Background information

- Penicillin is an antibiotic that prevents the synthesis of mucopeptides in bacterial cell walls by preventing the formation of peptide bonds; it is bactericidal.
- *Staphylococcus epidermidis (albus)* is a gram-positive bacterium which forms small white colonies.
- Penicillin is effective against gram-positive bacteria.
- Discs containing penicillin are placed on an agar plate containing *Staphylococcus epidermidis (albus)*.
- If the penicillin has been effective there will be a clear zone around the disc.
- The size of the clear zone is a measure of the effectiveness of the penicillin

All microorganisms should be treated as potential pathogens so it is important to follow safety procedures when working with *Staphylococcus epidermidis (albus)*.

- Read the information above.
- Identify and write down the independent and dependent variables.
- Write down the hypothesis.
- List the variables that should be controlled.
- Outline how each such variable might be controlled.

**Safety in the use of microorganisms****Rules for working with microorganisms:**

- 1 BEFORE STARTING WORK cover all cut or broken skin with a waterproof dressing.
- 2 WEAR tightly fitting disposable gloves when working with all live cultures.
- 3 WEAR a clean laboratory coat with all the fastenings closed.
- 4 BEFORE AND AFTER each working session wash the bench surface with bactericidal disinfectant.
- 5 AFTER each working session dispose of gloves into the sterilin bag or other disposal container provided. WASH your hands with bactericidal soap.
- 6 SWAB any spillages with bactericidal disinfectant. DISPOSE of any contaminated paper in the sterilin bag or other disposal container provided.
- 7 NEVER place anything in your mouth whilst working with microorganisms. This includes foods, liquids, gummed labels, pipettes etc.
- 8 ALL CULTURES should be labelled clearly with the following information:
  - Your name
  - Name of the organism
  - Type of nutrient medium used
  - Date
- 9 NO CULTURE should be left for more than one week. Incubators are checked daily and outdated cultures will be removed for safe disposal.
- 10 WHEN you have completed your work with a culture the petri dish should be placed in a sterilin bag or into a container of bactericidal disinfectant.

**Method****Preliminary study – Safety and Plate Pouring**

After watching the demonstration on plate pouring and aseptic technique prepare a pour plate as follows.

- 1 Read the safety rules for working with microorganisms.
- 2 Use a sterile 1cm<sup>3</sup> pipette to place 0.5cm<sup>3</sup> of *Staphylococcus epidermidis (albus)* into a sterile petri dish aseptically.
- 3 Label the base of the petri dish with your name, name of the organism, type of nutrient agar and the date.
- 4 Add 10cm<sup>3</sup> of nutrient agar to the petri dish using aseptic technique and leave to set.
- 5 Using aseptic technique place an antibiotic disc on the surface of the agar using flamed and cooled forceps. Note the concentration of antibiotic on the disc.
- 6 Seal the petri dish.
- 7 Incubate the petri dish at 25°C for 2 to 3 days.

### **Preliminary Study – Measuring the Clear Zone**

- 1 Watch a demonstration on measuring the size of the clear zone
- 2 Measure the diameter of the clear zone around the disc without opening the lid.

### **Day One – Preparation of the Pour Plate**

Draw lines on the base of the petri dish so that the base is split into 3 equal parts. Label the sections 1 to 3.

- 1 Using aseptic technique prepare a pour plate containing *Staphylococcus epidermis*. (Use the method above.)
- 2 Prepare a control disc by soaking a sterile disc in sterile water.
- 3 Place the control disc in the centre of section 1 using flamed and cooled forceps.
- 4 You have 2 penicillin discs which contain 2 different penicillin concentrations. Using flamed and cooled forceps place the disc containing the lowest concentration in section 2 and the higher concentration disc in section 3.
- 5 Seal the petri dish and incubate the petri dish at 25°C for 2 to 3 days.

### **Follow Up – Data Collection**

- 1 Without opening the lid measure the diameter of the clear zone around each disc using vernier callipers. If vernier callipers are not available, use a pair of dividers and measure against a mm rule
- 2 Tabulate your results to show the effect of penicillin on *Staphylococcus epidermidis (albus)* growth.

### **Write-up**

- Using the class results, plot a bar chart to show the mean diameter of clear zone for each disc.
- (Optional - add error bars to your graph.)
- Do a t-test to see if there is a significant difference between the results obtained at the 2 different concentrations.
- Make an evaluation considering:
  - the limitations of the methods used,
  - anomalous values if any,
  - replication and range of values of independent variable,
  - effectiveness of control of selected variables,
  - the reliability of the results
  - the confidence with which conclusions should be drawn
- Draw conclusions considering:
  - detailed description of the features of the results,
  - the meaning of the results in relation to the hypothesis,
  - scientific explanation of the results and conclusions,
  - potential improvements and further predictions

**Lesson Plan****The Effect of Penicillin on Bacteria****Context**

A practical set in the context of 9700 Syllabus – a timed practical investigation to determine the minimum effective concentration of penicillin against *Staphylococcus epidermidis* (*albus*).

It is anticipated that students will have completed an AS practical course and so they will have good basic practical skills. It is also assumed that they will have undertaken work on the nature of bacteria and the methods by which bacterial growth can be investigated.

**Key aims of lesson**

This practical is designed to develop practical skills and carry out an investigation over a number of lessons.

**Intended learning outcomes**

By the end of this practical and by answering the questions the student should be able to:

- Pour agar plates using aseptic technique
- Experience growing microorganisms
- Use the disc diffusion technique needed to undertake a microbiological investigation
- Use vernier callipers to measure clear zone diameters.
- Assess the reliability of results

**Resources required**

White board or flipchart and suitable pens or chalkboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

<b>Timings/ minutes</b>	<b>Teacher / Student Activities</b>
Previous lesson	<b>Preparation</b> – students to practice / learn aseptic technique by pouring an agar plate and creating a bacterial lawn. It may also be prudent for students to practice the disc diffusion test during this lesson. Student should research the mode of action of penicillin for homework as well as considering suitable controls that should be observed during such an investigation.
0-5	<b>Introduction</b> to the aims, intended outcomes and background information - teacher led oral presentation.
5-10	<b>Context</b> - review disc diffusion test, bacterial lawn and aseptic technique - teacher led class discussion / question and answer session.

10-15	<b>Introduction to method</b> - teacher to go through method step by step, emphasising the importance of aseptic technique throughout.
15-50	<b>Carrying out the practical</b> - students carry out the practical work.
50-60	<b>Drawing together the threads</b> – students to complete questions and begin write up no results will be available at this stage so students should evaluate the method and apparatus used.
Lesson 2	<b>Gathering results -</b>
0 – 10	Teacher demonstration of the correct procedure for measuring 'clear zones' produced by the disc diffusion method either using vernier callipers or other appropriate measuring device.
10 – 30	Students to measure clear zones produced in their investigation and record their results in a suitable table.
30 - 60	Students to complete write up of investigation.

#### Useful Information

- Students may require some practice in mastering aseptic technique. If possible they should attempt various bacterial culturing methods during the practical lessons prior to this investigation.
- A bunsen burner creates a sterile environment in a limited area around the Bunsen. Students should work as close as possible to the flame.
- The gas inlet is a useful prop for sterilised equipment
- Students should be encouraged to treat all organisms being cultured as potential pathogens.



### Technical Information - The Effect of Penicillin on Bacteria

- 1 It is possible to purchase filter paper discs which are already impregnated with penicillin; these are very useful for this investigation. However, if these are not available students may impregnate filter paper discs themselves by soaking them in a solution of antibiotic, using aseptic technique.
- 2 Bacterial cultures should be prepared at least two days in advance by growing bacteria in sterilised nutrient broth.
- 3 Much of the equipment used should be sterilised before student use, these items are marked with \* on the list below (other items either do not need to be sterile or are purchased already sterilized. They may be sterilised by using an autoclave. If an autoclave is not available a chemical antiseptic may be used to clean the equipment although this will not be as effective.
- 4 Students should wear lab coats and latex gloves throughout the procedure, if available.
- 5 It is advisable although not essential to incubate the bacterial lawn plates at 25°C to encourage bacterial growth, suitable growth usually takes 2-3 days at this temperature (longer if not in an incubator).
- 6 Each bacterial lawn will require 10cm<sup>3</sup> molten nutrient agar, the agar should be prepared, sterilised and decanted into individual sterile vessels prior to the practical so that each student has access to their own bottle containing 10cm<sup>3</sup> sterile, molten agar.
- 7 Agar can be prepared well in advance and has a shelf life of years if kept sterile but should be melted down prior to the practical using a boiling water bath.
- 8 Variables that should be controlled include concentration and volume of bacterial culture, concentration and volume of agar used, size of filter paper disc, weight of filter paper and concentration of penicillin solution.
- 9 A suitable control for the experiment would be the presence of a filter paper disc soaked in sterile water.

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

- 1 Bunsen burner, to enable good aseptic conditions
- 2 10 cm<sup>3</sup> bacterial culture in nutrient broth
- 3 10 cm<sup>3</sup> molten nutrient agar\*
- 4 10 cm<sup>3</sup> sterile water\*
- 5 Sterile 90 mm Petri dish
- 6 1 cm<sup>3</sup> pipette\*
- 7 Filter paper discs\* or prepared penicillin discs
- 8 Forceps
- 9 10 cm<sup>3</sup> 1% penicillin solution (students may carry out dilution of this to achieve several concentrations for testing)
- 10 20 cm<sup>3</sup> absolute ethanol, to enable sterilisation of forceps
- 11 50 cm<sup>3</sup> bactericidal disinfectant for containment of used forceps and pipettes, also to clean work surfaces.

Safety Precautions/Risks.

It is recommended that the following sources be used to ensure the safety of all microbiological work by teachers, support staff/technicians (if any) and students.

Topics in Safety (3<sup>rd</sup> Edition, 2001), publisher Association for Safety Education, College Lane, Hatfield, Herts, AL10 9AA. ISBN 0863571042

MISAC Safety guidelines

<http://www.ncbe.reading.ac.uk/NCBE/SAFETY/PDF/Topics15.pdf>

Society for General Microbiology Safety website including GMLP (Good Microbiology laboratory Practice) and Risk Assessment.

<http://www.microbiologyonline.org.uk/safety.html>

**Follow the safety precautions as given below.**

**Safety in the use of microorganisms**



**Rules for working with microorganisms:**

- 1 BEFORE STARTING WORK cover all cut or broken skin with a waterproof dressing.
- 2 WEAR tightly fitting disposable gloves when working with all live cultures.
- 3 WEAR a clean laboratory coat with all the fastenings closed.
- 4 BEFORE AND AFTER each working session wash the bench surface with bactericidal disinfectant.
- 5 AFTER each working session dispose of gloves into the sterilin bag or other disposal container provided. WASH your hands with bactericidal soap.
- 6 SWAB any spillages with bactericidal disinfectant. DISPOSE of any contaminated paper in the sterilin bag or other disposal container provided.
- 7 NEVER place anything in your mouth whilst working with microorganisms. This includes foods, liquids, gummed labels, pipettes etc.
- 8 ALL CULTURES should be labelled clearly with the following information:
  - Your name
  - Name of the organism
  - Type of nutrient medium used
  - Date
- 9 NO CULTURE should be left for more than one week. Incubators are checked daily and outdated cultures will be removed for safe disposal.
- 10 WHEN you have completed your work with a culture the petri dish should be placed in a sterilin bag or into a container of bactericidal disinfectant.

A risk assessment should be carried out as a matter of course.

### Practical 9 - S(e) Producing a model industrial immobilised enzyme column

This practical focuses on: **Defining the problem**




You will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Set up an effective working model of an immobilised enzyme column.
- Understand the usefulness of immobilised enzymes in biotechnology.
- Identify variables that should be controlled.
- Experience relevant methods, including the use of a control.

#### Safety Information

	You should wear eye protection throughout this practical.
	Calcium chloride is an <b>irritant</b> .
	All enzymes including sucrase enzyme should be assumed to be <b>harmful</b> .

#### Background information

- In industry (e.g. the confectionary industry), enzymes are used on a large scale.
- It is very costly to use enzymes only once, but most enzymes are only commercially available in liquid or dehydrated forms and once they have been used in solution it is very difficult and time consuming to separate them from the product.
- To allow their re-use, enzymes may be immobilised. One way of immobilising enzymes is to 'stick' the enzyme molecule to an alginate bead.
- In industry these immobilised enzymes are used in large columns. The substrate enters at the top of the column and the product collected at the bottom.
- Sodium alginate (used to produce alginate beads) will turn from liquid to solid when immersed in calcium chloride.
- Sucrase is an enzyme which breaks down sucrose into glucose and fructose
- The presence of glucose can be tested using Benedict's reagent.

You will produce a model of an immobilised enzyme column.

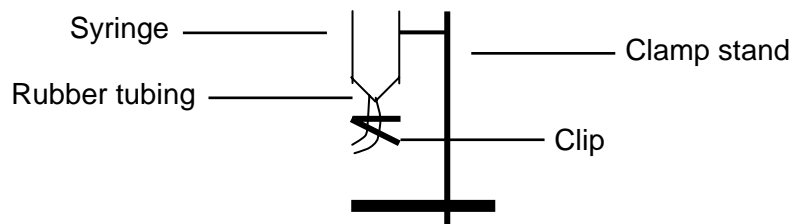
- Read the information above.
- List the variables that should be controlled, using your knowledge of enzymes.
- Describe ways in which each variable may be controlled.
- Suggest a suitable control experiment to prove your model is working correctly.

## Method

### Immobilise enzymes and prepare column

- 1 Put 4 cm<sup>3</sup> of 1% sucrose solution into a beaker.
- 2 Add 6 cm<sup>3</sup> of sodium alginate to the sucrose. Use a measuring cylinder to obtain the correct volume of sodium alginate as it is very viscose and will not be easily expelled from a pipette).
- 3 Stir the sucrose / sodium alginate mixture with a glass rod for at least 2 minutes.
- 4 Put 50 cm<sup>3</sup> of calcium chloride into a clean beaker.
- 5 Using a dropping pipette, transfer the sucrose / sodium alginate mixture *one drop at a time* into the calcium chloride. The drops of alginate mixture will form solid beads when immersed in calcium chloride. These beads have the enzyme (sucrase) on them.
- 6 Fix a 15 cm<sup>3</sup> syringe to a clamp stand and place a small piece of muslin in the bottom to prevent the nozzle from becoming blocked.
- 7 Attach a piece of rubber tubing to the nozzle of the syringe and seal it with a clip.
- 8 Pour the contents of the calcium chloride beaker through the syringe, allowing the liquid to drain away by opening the clip on the rubber tubing. Your column will now be full of immobilised enzyme beads.
- 9 Rinse the beads by passing water through the syringe and allowing it to drain away.

### Diagram



### Test your immobilised enzymes

- 1 Close the clip on the rubber tubing, then pour enough 1M sucrose into the column to fill it.
- 2 After 5 minutes, open the clip on the rubber tubing and collect the liquid in a clean boiling tube. This is your 'product'.
- 3 Test the 'product' you have just collected for the presence of glucose, using Benedict's reagent. If you have clinistix or other glucose testing strips use these to estimate the glucose concentration.
- 4 (Optional) Repeat the whole procedure, omitting the addition of sucrase – this is a *control* experiment.

**Write-up**

- 1** Evaluate the procedure, considering the following:
  - The limitations of the apparatus you used e.g. the size of the syringe (column), the size of the dropping pipette and therefore the size of the beads produced.
  - The effectiveness of the system – was it efficient? Would it be possible to re-use the enzyme? Was a pure product obtained?
- 2** Explain what happened during the reaction in terms of enzymes activity.

Design a further investigation, using this apparatus, to test the rate of flow through the column on the rate of breakdown of sucrose.

## Lesson Plan

### Producing a model industrial immobilised enzyme column

#### Context

To build a working model of an industrial immobilised enzymes column. This will improve understanding of the nature and applications of immobilised enzymes in industry, in the context of the 9700 syllabus – “immobilise an enzyme in alginate and compare the ease of recovering the enzyme and ease of purification of the product compared to the same enzyme that has not been immobilised”.

#### Key aims of lesson

This practical is designed to enable students to visualise an immobilised enzymes column and demonstrate the ability of immobilised enzymes to be re-used.

#### Intended learning outcomes

By the end of this practical and its write-up the student should be able to:

- Set up an effective working model of an immobilised enzyme column.
- Understand the usefulness of immobilised enzymes in biotechnology.
- Identify variables that should be controlled.
- Use relevant methods, including the use of a control.
- Design a method to test the effect of the rate of flow through the column on the hydrolysis of sucrose.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
End of previous lesson	<b>Preparation</b> – student given theory on immobilised enzymes, students to consider industrial applications of enzymes in biotechnology. Students to be reminded of Benedict’s test, as learned in AS syllabus.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	<b>Context</b> - review industrial uses of enzymes, reasons for use of immobilised enzymes and methods of immobilisation. Also, review Benedict’s test.
8-12	<b>Introduction to method</b> - teacher demonstration of alginate bead production. Teacher may have a column already set up to enable students to visualise it.

12-20	<p><b>Student preparation exercises</b></p> <p>Students list the variables that should be controlled, using their knowledge of enzymes and describe ways in which each variable may be controlled.</p> <p>Suggest a suitable control experiment to prove the model is working correctly.</p>
20-50	<p><b>Carrying out the practical</b> - students carry out the practical work and tidy away apparatus when they have finished.</p>
50-60	<p><b>Drawing together the threads</b> - teacher-led class discussion on the skills that have been developed and the knowledge the students have gained. Students begin evaluation and design of a follow up investigation. These are completed as homework.</p>

### Useful Information - Producing a model industrial immobilised enzyme column

- Possible variables to control include: temperature, bead size, column volume / dimensions, time which sucrose remains in column, concentration of sucrose / sucrase / sodium alginate solutions, pH (you may wish to include a buffer e.g. citrate-phosphate buffer (pH 7) into the alginate / sucrase mixture.
- If a positive result is not achieved when doing the Benedict's test, it may be necessary to leave the sucrose in the column for a longer period of time.
- Students should recognise the need to devise a method of passing sucrose solution through the column at a variable rate. Suggestions could be using a burette or a larger syringe and controlling the clip flow. Adding a known volume of sucrose above the beads and recording the time taken for the entire volume to pass through the column. Students should also suggest a means of quantifying the amount of sucrose hydrolysed. This could be using glucose testing strips or making a series of known glucose concentrations and comparing the colour to a Benedict's trust carried out on the collected filtrate.

### Technical Information- Producing a model industrial immobilised enzyme column

The **apparatus and materials** required are listed below. The amount given is **per student or one** group if students are to work in groups.

It is convenient to make up more of the reagents than is required in order to give sufficient quantities for accurate measurements.

- 4 cm<sup>3</sup> of 1% Sucrase solution provide
- 6 cm<sup>3</sup> of 2% Sodium alginate solution
- 1 mol dm<sup>-3</sup> Calcium chloride solution (the molecular weight of sucrose = 342g) – provide enough for 50 cm<sup>3</sup> per student
- 10 cm<sup>3</sup> graduated pipettes, syringes / measuring cylinders – 2 per student
- Beakers (at least 50 cm<sup>3</sup> volume) – 2 per student
- Dropping / teat / Pasteur pipettes – 1 per student
- 15 cm<sup>3</sup> syringe – 1 per student
- 2 cm<sup>2</sup> pieces of muslin or gauze – 1 per student
- Stop clock / watch – 1 per student
- Bunsen burner, tripod and gauze – 1 per student (for Benedict's test, alternatively you may use a water bath set to >60°C)
- Narrow rubber tubing cut to >5cm lengths – 1 per student
- Rubber tubing / Hoffman clips – 1 per student
- Boiling tube – 1 per student
- Clamp stand – 1 per student
- Sodium alginate powder takes a long time to dissolve, so ensure this is prepared well in advance, it has a shelf life of a few months.
- Sucrase and sucrose solutions will keep for up to 1 week in a fridge, calcium chloride solution will keep indefinitely.
- You may wish to prepare the syringes by putting the square of muslin in and attaching the rubber tubing prior to the practical lesson, to save time.

#### Safety Precautions/Risks.

Safety information on the use of enzymes may be found at  
<http://www.ncbe.reading.ac.uk/NCBE/SAFETY/enzymesafety1.html>

Calcium chloride = H (Irritant only)



Sucrase enzyme = H



A risk assessment should be carried out as a matter of course.



**Practical 10 - T(a)(d) The structure of wind pollinated flowers and fruit.**

This practical focuses on recording accurately – Biological drawings. You will be developing other assessed skills throughout the practical.

**Intended learning outcomes**

By the end of this practical and its write-up you should be able to:

- Identify the main features of a wind pollinated flower
- Explain the significance of the adaptations shown by flowers that are wind pollinated
- Identify pollen of a wind pollinated flower
- Observe and measure the rate of germination of pollen grains
- Identify the main features of a maize fruit
- Draw accurately the main structures and organisation of a wind pollinated flower
- Draw accurately the internal structure of a maize fruit
- Experience relevant methods and conclusions.

Safety Precautions/Risks.

No specific hazards identified.

A risk assessment should be carried out as a matter of course.

### Background information

- All flowers are formed from modified leaves and arranged on a specialised stem called the receptacle
- There are four flower components; sepals, petals, stamens and ovules
- The flower components are arranged in rings called whorls, one inside the other on the receptacle. These whorls have collective names: calyx, corolla, androecium, and gynoecium.
- Starting from the outer side the order is: calyx (sepals), corolla (petals), androecium (stamens) and gynoecium (carpels).
- The androecium is the male reproductive component and the gynoecium is the female reproductive component
- Wind pollinated plants generally show only the reproductive component. Sepals and petals are replaced in modified leaves, called bracts, which vary greatly in appearance and are used in taxonomic grouping.
- Wind pollinated flowers are adapted in a variety of ways to increase the chance of pollination. Overall the adaptations result in maximum exposure to the air of the pollen producing anthers and the pollen collecting stigmas.
- Some plants are monoecious – their flowers have either an androecium or a gynoecium. Some plants are dioecious – their flowers have either an androecium or a gynoecium, both types of flower occur on the same plant. Some plants are hermaphrodite – their flowers have both an androecium and a gynoecium.
- Monoecious plants cannot be self pollinated. Dioecious plants and hermaphrodite plants can be self pollinated.
- Outbreeders are plants that do not normally self pollinate. If they are dioecious or hermaphrodite there are structural and physiological methods of preventing self pollination.
- Inbreeders are plants that normally self pollinate. If they are dioecious or hermaphrodite there are structural and physiological methods of encouraging self pollination.

You will investigate the structural organisation of a number of wind pollinated plants. You will also investigate the structure of a maize fruit.

- Read the information above
- Produce a table that you can complete with the number of each flower structure present in the flowers you study.

## Method

### Identification of floral structures

- 1 Observe the appearance of the inflorescence of each of the flowering plants.
- 2 Carefully detach a single flower from each inflorescence and identify the different whorls present.
- 3 Draw a diagram and label the structure of each of the individual flowers.
- 4 Carefully remove each separate structure from the flower and count the number of each structure present.
- 5 Make accurate drawings of a single stamen and ovule. Annotate each diagram with the functions of each of the parts of the stamen and anther, noting those features that adapt the structures to their function.

### Pollen study

- 1 Gently shake each inflorescence onto a sheet of plain paper.
- 2 Place a drop of 0.5% sucrose on a slide. Use a paint brush to transfer some pollen from one of the flowers onto a slide and cover with a cover slip.
- 3 Using x400 magnification count the number of pollen grain visible in the field of view.
- 4 Leave the slide for a minimum of twenty minutes while continuing with 7 and 8.
- 5 Note the time the slide was left and then count the number of pollen grain that have germinated.
- 6 Measure the length of the pollen tubes.
- 7 Using a different slide, transfer some of each type of pollen onto the slide. The pollen may stick better if the slide is slightly damp.
- 8 Observe, measure and draw each type of pollen at x400 magnification. Take care in measuring that you measure the pollen grain only and not any air bladders that may be present.

### Maize grain study

- 1 Observe the outside appearance of a single fresh or soaked maize fruit.
- 2 Draw and label the main features – fruit wall, scars of attachment.
- 3 Cut a vertical section along the widest part of the seed.
- 4 Identify the endosperm and embryo.
- 5 Use a hand lens to identify the main regions of the embryo – radicle, plumule and cotyledon. You may also find the coleoptile and coleorhiza.
- 6 Draw a diagram of a section through the fruit and annotate with the functions of the different structures.

### Calculations

- 1 Calibrate your microscope and calculate the actual size of the pollen grains and pollen tubes.
- 2 Calculate the percentage germination for the pollen grains.
- 3 Calculate the mean size of the pollen grains
- 4 Calculate the growth rate of the pollen tubes.
- 5 Record the percentage germination, mean size of pollen grain and growth rate for each of the germinated pollen grains on a class results table.
- 6 When all the results have been recorded in the class results table, calculate the mean percentage germination and mean growth rate for each type of pollen.
- 7 Optional – use the student T-test to find there is a significant difference in the growth rate of the different types of pollen.
- 8 Work out the magnification of your drawing of a maize fruit.

### Write-up

- Construct a table of the similarities and differences between each of the flowers studied.
- List the features that the pollen grains have in common.
- Explain why the organisation of maize inflorescence favours cross pollination.

## Lesson Plan

### The structure of wind pollinated flowers and fruit

This practical focuses on recording accurately – Biological drawings and measuring using a microscope.

#### Context

A practical investigation set in the context of 9700 Syllabus – the structure of wind pollinated flowers and the structure of maize fruits.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify the components of flowers. These are often easier to recognise initially in an insect pollinated flower.

#### Key aims of lesson

This practical is designed to develop the skill of accurate drawing and measuring. Students will be developing other assessed skills throughout the practical.

#### Intended learning outcomes

By the end of this practical and its write-up the student should be able to

- Identify the structural features of a wind pollinated flower and explain their role
- Identify the structural features of a maize fruit and explain their role
- Measure and calculate size using a microscope graticule
- Experience relevant methods and analysis.
- Calculate the rate of germination of pollen tubes.

#### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

**Planned activities** (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation.  Give out student work sheets and inflorescences to be used.
4-8	<b>Context</b> – review of flower structure. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-20	<p><b>Introduction to method</b> – teacher demonstration of the removal and dissection of a single flower. Each student should have an inflorescence and follow each step along with the teacher. Teacher demonstration of the correct direction for cutting a maize fruit.</p> <p>Optional statistical test – direct students to germinate a particular type of pollen. Depending on the class size two or three types of pollen may be compared.</p>
20-45	<p><b>Carrying out the practical</b> - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.</p>
50-60	<p><b>Drawing together the threads</b> - teacher-led class discussion on the skills that have been developed, as well as the results and their meaning - teacher led introduction to write-up, which should include class-work, finished off if necessary, (production of comparison tables and calculations)</p> <p>Optional t-test – teacher produced guide sheet or access to a computer programme.</p>

### Useful Information

- Most commercially grown wind pollinated flowers are grasses and show a 3 multiple of floral structures. However breeding programmes have caused changes in morphology that obscure these.
- Ripe stamens have anthers are visible outside the protective structures. Most inflorescences will have some ripe anthers.
- Ripe carpels have stigmas outside the protective structures.
- Maize plants have a modified floral structure as all the individual flowers are enclosed by large, protective, modified leaves
- Dry maize fruits need to be soaked at least 24 hours before use. Fruits need to be undamaged
- To gain sufficient data for at T-test at least 20 sets of data are needed. The class size will determine how many students need to be directed to grow a particular type of pollen

### Technical Information - The structure of wind pollinated flowers and fruit

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

- 1 1 example of each of at least three different wind pollinated flowers. For the purposes of the practical the species is immaterial, but it would support the 9700 syllabus to have rice, sorghum and maize.
- 2 2 slides
- 3 5 cm<sup>3</sup> 0.5% sucrose solution
- 4 1 scalpel or sharp knife
- 5 fine forceps
- 6 white paper
- 7 microscope with an eye piece graticule.
- 8 Access to a slide graticule if microscopes have not been previously calibrated
- 9 1 soft bristle paint brush. If these are not available the pollen can be shaken directly onto the slides
- 10 1 hand lens
- 11 Guide sheet to carrying out a student t-test.

#### Safety Precautions/Risks.

No specific hazards identified

A risk assessment should be carried out as a matter of course.