Teaching A2 Biology Practical Skills

UNIVERSITY of CAMBRIDGE
International Examinations

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.

Contents

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 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 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. 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 ay 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 mol dm^{-3} hydrochloric acid will cause much less severe eye injuries than the same accident with a glass bottle containing 5.0 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 mol dm^{-3}	corrosive if $\geq$ 6 mol dm^{-3}
sodium hydroxide	irritant if $\geq$ 0.05 mol dm^{-3}	corrosive if $\geq$ 0.5 mol dm^{-3}
hydrochloric acid	irritant if $\geq$ 2 mol dm^{-3}	corrosive if $\geq$ 6.5 mol dm^{-3}
nitric acid	irritant if $\geq$ 0.1 mol dm^{-3}	corrosive if $\geq$ 0.5 mol dm^{-3}
sulphuric acid	irritant if $\geq$ 0.5 mol dm^{-3}	corrosive if $\geq$ 1.5 mol dm^{-3}
barium chloride	harmful if $\geq$ 0.02 mol dm^{-3}	toxic if $\geq$ 0.2 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 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 may 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 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
L	**Practical 11 - Respirometer**		
(k),(l), (m)	• Identify the independent and dependent variables • Test a hypothesis • Experience relevant methods, analysis, conclusions and evaluation • Explain RQ values in terms of substrate use • Suggest modifications for use with photosynthesising organisms	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. Students should be asked to how the apparatus can be modified to test a green plant.	Biology Resource Pack A2 Lea, Lowrie and McGuigan
M	**Practical 12 - Immobilised Algae – effect of limiting factors on the rate of photosynthesis**		
(f), (g)	• Evaluation • *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;	Use sodium alginate mixed with algae to produce beads by transferring the alginate/algae mixture drop wise into calcium chloride solution 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). Students could be asked to plan to test the effect of either carbon dioxide or temperature	Advanced Biology Study Guide Clegg and Mackean

Syllabus section	Skills/Learning Outcomes	Notes	Sources
N	**Practical 13 - Factors that affect the opening and closing of stomata**		
(f), (g)	• Identify the independent and dependent variables • Formulate a hypothesis and express this in words and graphically • Experience relevant methods and analysis, conclusions and evaluation • Plan an investigation	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.	Advanced Biology Study Guide Clegg and Mackean

Syllabus section	Skills/Learning Outcomes	Notes	Sources
N	**Practical 14 - Reflexes**		
(e), (f), (g)	• Identify the independent and dependent variables • Formulate a hypothesis and express this in words and graphically • Experience relevant methods, analysis, conclusions and evaluation • Describe and explain the reflex arc that occurs during a knee-jerk reflex, swallowing reflex, iris reflex and blinking reflex • Evaluate the procedure, discussing why it is difficult to quantify this investigation	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. 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. 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. 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.	

Syllabus section	Skills/Learning Outcomes	Notes	Sources
N	**Practical 15 - Reaction time**		
(e),(f), (g)	• Identify the independent and dependent variables • Make a hypothesis and express this in words and graphically • Experience relevant methods, analysis, conclusions and evaluation • Describe and explain the reflex arc that occurs during reaction timing • Explain the ability to improve reaction time with practice	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. Students should be asked to record their reaction time and repeat several times, observing any improvement with practice.	Biology 9700 University of Cambridge International Examinations

Syllabus section	Skills/Learning Outcomes	Notes	Sources
(c)	• Recognise the gross structure of the mammalian kidney • Interpret the microscopic image of the kidney via viewing sections under a light microscope • Experience relevant methods • Produce a biological drawing of kidney histology, as viewed under a light microscope	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. 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. Students should produce a drawing of their observations.	An Atlas of Histology Freeman and Bracegirdle Bioscope

Syllabus section	Skills/Learning Outcomes	Notes	Sources
N	**Practical 16 - Model kidney tubule**		
(c)	• Identify the independent and dependent variables • Make a hypothesis and express this in words and graphically • Experience relevant methods, analysis, conclusions and evaluation • Describe and explain the function of the kidney tubule	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.	
N	**Practical 17 - Structure and histology of an Islet of Langerhans in the mammalian pancreas**		
(m)	• Interpret the microscopic image of the pancreas by viewing sections under a light microscope • Experience relevant methods • Produce a biological drawing of pancreas histology, as viewed under a light microscope	Students should observe a prepared section of a pancreas under the microscope. Students should be asked to identify the two different types of secretary tissue present. Students should draw a high power drawing showing the arrangement of the two different types of secretary material. These diagrams should be annotated with the main features that distinguish exocrine gland (enzyme) from the endocrine (islet of Langerhans) tissue.	An Atlas of Histology Freeman and Bracegirdle Bioscope

Syllabus section	Skills/Learning Outcomes	Notes	Sources
O	**Practical 18 - Meiosis**		
(a)	• Use of microscope at medium power to observe the nuclei of cells undergoing meiosis • Use appropriate apparatus and techniques	Use anthers from a flowering plant of *Tradescantia virginiana* or a dormant bulb of *Hyacinthus*. 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.	Advanced Biology Study Guide by Clegg and Mackean Philip Harris-meiosis sets Bioscope *Lilium* anther
O	**Practical 19 - Chi-Squared Test**		
(f)	• Application of Chi-squared test • Use of Chi-squared test to evaluate results of breeding experiments • Use of Chi-squared test to evaluate Mendelian ratios • Data handling	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 use to evaluate the results of ecological sampling in the context of Q (d).	Advanced Biology Study Guide by Clegg and Mackean A2 Biology AQA B by Lea, Lowrie and McGuigan
O	**Practical 20 - Mutation in a Fungus**		
(b) (g) (f)	• Use the fungus *Sordaria fimicola* to obtain Mendelian ratios. • Use of microscope to visualise and score Asci • Data analysis to determine crossing over frequencies	It is possible to culture *Sordaria fimicola*. 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
P	**Practical 21 - Variation – Using statistics**		
(b)	• Application of statistics in Biology to test for the significance of differences between samples. • Use standard deviation to estimate the spread of data • Use t-test to compare two sets of normally distributed data • Data handling	Calculate mean and standard deviation for a set of grouped data. Calculate the value of t and determine if there is a significant difference. Samples of leaves from the same species growing in different areas, height of seedlings grown in different pH	Advanced Biology Study Guide by Clegg and Mackean Advanced Level Practical Work for Biology Hodder and Stoughton
P	**Practical 22 - Simulation of selection and evolution**		
(g)	• Analysis of results • Express results graphically • Draw conclusions	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. Can modify to show isolation by splitting beads into 2 populations an applying different selection pressure.	Biology 9700 University of Cambridge International Examinations Scheme of work

Syllabus section	Skills/Learning Outcomes	Notes	Sources
P	**Practical 23 - Population growth**		
(c)	• Analyse results • Draw conclusions • Express results graphically • Use a complex technique	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
Q	**Practical 24 - Comparing diversity in a managed and unmanaged environment**		
(a) (g)	• Analysis of results • Express results graphically • Statistical analysis • Draw conclusions	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
R(g)	**Practical 25 - Electrophoresis**		
	• Identify the independent and dependent variables • Make a hypothesis and express this in words and graphically • Experience relevant methods, analysis, conclusions and evaluation • Describe the processes of electrophoresis as used in DNA fingerprinting and DNA sequencing • Describe and explain an electrophoresis gel after running and staining	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. Students should analyse the results of the electrophoresis and compare to other result or DNA fingerprints to try and identify 'matches' *Can be followed up by discussion on genetic screening and counselling.	NCBE DNA technology kit Bio-Rad DNA fingerprint kits *NCBE DICE work pack
S	**Practical 26 - ELISA - Using antibodies to detect disease**		
(h)	• Describe antigen-antibody interactions • Understand how HIV is detected • Learn how disease agents are transmitted, diagnosed and tracked • Understand how antibodies are produced in the laboratory for use in diagnostic tests • Study enzyme-substrate mechanics.	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. 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.	Bio-Rad Laboratories Immuno Explorer Kit

Syllabus section	Skills/Learning Outcomes	Notes	Sources
T	**Practical 27 - Adaptations in crop plants**		
(h)	• Recognise the structure and • make accurate drawings of a maize leaf • Understand the difference in organisation of a C4 and C3 leaf • Recognise sorghum and rice • Be able to describe and explain the adaptations of rice and sorghum • Plan an investigation to test the effect of ethanol concentration on the growth of germinated rice.	Microscope slides of maize leaves to look at distribution of chloroplasts. Revisit dicot leaf from Syllabus section M. Draw up comparisons. Actual or museum specimens of sorghum and rice - observations of general morphology. Germinated rice seedlings – grow in different concentrations of ethanol	Bio-Rad Laboratories Immuno Explorer Kit
U	**Practical 28 - Microscopic examination of reproductive organs**		
	• Identify a section of an ovary and testis • Recognise different stages in the gametogenesis in a testis by appearance and position of cell • Recognise the appearance of immature and mature follicles in an ovary • Recognise the appearance of a corpus luteum in an ovary • Estimate the number of ovulations by counting corpora albicans	Can be a circus activity if slides are limited. Student work sheet with labelled diagrams and photomicrographs. Students identify and draw the specified structures. Demonstration dissection or museum specimens to show location of reproductive structures. These can also be used to simulate the techniques of ivf. Slides of uterus wall can be used to demonstrate layers.	An Atlas of Histology Freeman and Bracegirdle Human Systems Griffin and Redmore

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^3 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/time taken for blue colour to disappear. If >10 minutes, then record 1/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

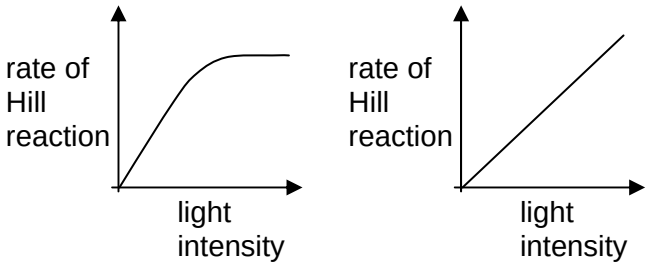
Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	**Teacher / Student Activities**
end of previous lesson	**Preparation** - 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - 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	**Introduction to method** - 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 9o green in a minute or two in normal daylight - add some more DCPIP if needed).
12-20	**Identification of variables, writing and graphing hypotheses and listing control variables** - 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	**Carrying out the practical** - 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	**Drawing together the threads** - 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).



- 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 bight 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^3 or 2 cm^3

13 Make up 500 cm^3 of phosphate buffer solution to use as below, using 4.48 g $Na_2HPO_4.12H_2O$ and 1.7g KH_2PO_4 made up to 500 cm^3 with distilled water. Store in a fridge. Students do **not** need to be given any of this solution.

14 2 cm^3 of very cold extraction medium, labelled **extraction medium**. To make up 250 cm^3 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^3 with the phosphate buffer solution. Store in a fridge for no more than 48 hours and supply very cold for use.

15 2 cm^3 DCPIP solution labelled **DCPIP solution**. To make up 250 cm^3 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^3 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 **oxidising agent**.
	Calcium chloride is an **irritant**.
	Iron (II) sulphate is **harmful**.
 	Zinc sulphate is **harmful** and **dangerous to the environment**.
	Manganese chloride is **harmful**.
	Molybdenum trioxide is **harmful**.
 	Copper sulphate is **harmful** and **dangerous to the environment**.
	Cobalt nitrate is **harmful**.
Bleach may have been used for sterilising materials and may be **harmful**, **oxidising** or **corrosive**.	

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^{-3}
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^3 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^3 of boiled pond water.

Preparation of culture and making observations

1 Pour 200 cm^3 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^3 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^3 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^3 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^3 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^3	Volume of water / cm^3	Concentration of albumin solution / mg dm^{-3}
10	0	
8	2	
6	4	
4	6	
2	8	
0	1	

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	**Preparation** – 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation

4-9	**Context** - 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.
10-14	**Introduction to method** - 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. 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.
15 – 45 46 - 50	**Carrying out the practical** - 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. **Optional measurement of protein content**. 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
51-60	**Drawing together the threads** - 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.
Lesson 2 0-5	**End of previous lesson** - students reminded about the practical and reminded to bring the student work sheets to the lesson.
0-4	**Introduction** – 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.
5 – 25	**Carrying out the practical** – 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.
26 – 40	**Optional preparation of standard protein solutions.** – 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 if a refrigerator if necessary.
26 – 60 or 41 – 60	**Drawing together the threads** –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

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 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 others 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^3 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^3 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^3 syringe or Pasteur pipette.

14 Sterile 250 cm^3 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^{-3}
Potassium nitrate(KNO_3)	1.25
Potassium hydrogen phosphate (KH_2PO_4)	1.25
Magnesium sulphate ($MgSO_4.7H_2O$)	1.00
Calcium chloride ($CaCl_2$)	0.084
Boric acid (H_3BO_3)	0.014
Iron (II) sulphate ($FeSO_4 7H_2O$)	0.050
Zinc sulphate ($ZnSO_4$)	0.088
Manganese chloride ($MnCl_2.4H_2O$)	0.014
Molybdenum trioxide (MoO_3)	0.007
Copper sulphate ($CuSO_4.5H_2O$)	0.016
Cobalt nitrate ($Co(NO_3)_2.6H_2O$)	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

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 **irritant**.
 	Ammonia is **corrosive** and **dangerous to the environment**.
 	Benedict's solution is **harmful** and **dangerous to the environment**.

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^3		
Glucose	Negative	Negative	
Ketones	Negative	Negative	
Microscopic examination RBC WBC casts	 3-4 Occasional	 Rare 0-4 Rare	

Method

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

Initial examination of urine

1 Obtain approximately 10 cm^3 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^3 of the urine sample into a clean test tube.

2 Put 1 cm^3 of Biuret A (sodium hydroxide) into the test tube.

3 Put 1 cm^3 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^3 of the urine sample into a clean boiling tube.

2 Add 2 cm^3 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	**Preparation –** 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** – briefly discuss urine production and potential problems with it – teacher led discussion / question and answer session.
8-12	**Introduction to method** – 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	**Student preparation exercises** – students to work through second box on page 1.

20-50	**Carrying out the practical** – 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	**Drawing together the threads** - 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:

Colour	colourless or pale	diabetes or chronic kidney / renal disease
Appearance	hazy or cloudy	bacterial infection or blood cells present
Odour	ammonia	urea breakdown by bacteria
	foul or putrid	bacterial infection
	sweet or fruity	diabetes mellitus (sugar diabetes)
pH	<4.5	respiratory acidosis or starvation
	>8.0	urinary tract infection
Protein	present	protein urea or renal disease
Glucose	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^3 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^3 distilled water
	1 g sodium chloride
	1 g potassium phosphate
	1 g albumen powder)
	3 cm^3 1M ammonia (pH10)
Sample 3 (patient suffering from renal disease)-	250 cm^3 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 1cm^3 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^3 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^3 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^3 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 7different 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.

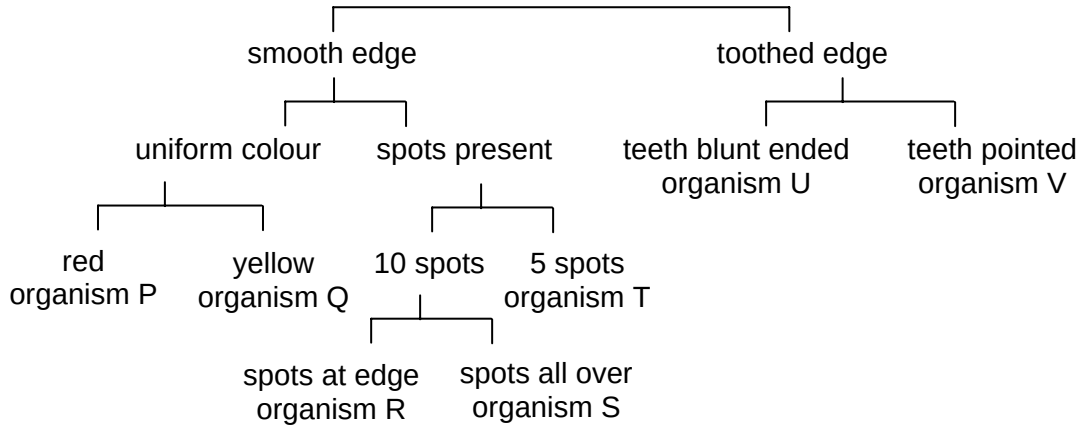
1	has smooth edge	2
	has toothed edge	3
2	uniform colour	4
	spots present	5
3	teeth blunt ended	organism U
	teeth pointed	organism V
4	colour red	organism P
	colour yellow	organism Q
5	ten spots	6
	five spots	organism T
6	spots at edges	organism R
	spots all over	organism S

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	**Preparation** - 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - 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	**Introduction to method** - teacher demonstration using a selected organism to indicate suitable features for assigning an organism to a kingdom.
12-22	**Assigning organisms to kingdoms.** 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 31 – 50 51 – 55	**Using and constructing a key** 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. Students construct a key to identify the organisms provided. 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	**Drawing together the threads** – 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.

- 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 ($CaCl_2$) 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	**Preliminary study** - The goal of genetic transformation is to change an organism's phonotype. Before any change in the phenotype can be detected, an examination of the organism's natural (pre-transformed) phenotype must be made. Students will need to look at the colonies of *E. coli* on the starter plate and list all observable traits or characteristics that can be described.
Day one 0-4	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - review of bacterial transformation. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-12	**Introduction to method** - teacher demonstration of transformation procedure.
12-50	**Carrying out the practical** - 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	**Drawing together the threads** - 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation – review of day one.
4-8	**Day One Review Questions** – teacher led discussion of answers to review questions.
8-12	**Introduction to day two** - teacher to introduce analysis of results session.
12-25	**Observations** – students make and record observations and compare these with those made in the preliminary study.
25-35	**Drawing together the observations** - teacher-led class discussion on the observations.
35-50	**Analysis** – students complete an analysis of the results and draw conclusions.
50-60	**Drawing together the threads** - 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 **highly flammable**. There should be no flames in the same room.
 	Methylated spirit (IMS) is **highly flammable**. There should be no flames in the same room.
	Protease enzymes such as Bromelain and Papain are all **harmful**.
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 3 g of table salt with 10 cm^3 of washing up liquid.

2 Add 90 cm^3 of water so you have 100 cm^3 all together.

3 Add 50 g 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^3 of filtrate.

2 Pour the 10 cm^3 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^3 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)

Timings/ minutes	**Teacher / Student Activities**
End of previous lesson	**Preparation** – students to review the structure of a plant cell and the action of enzymes
0-5	**Introduction** to the aims, intended outcomes and background information - teacher led oral presentation
5-10	**Context** - review plant structure, action of enzymes and location of DNA – teacher led questioning, student responses/discussion
10-15	**Introduction to method** - teacher to go through method
15-50	**Carrying out the practical** - 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	**Drawing together the threads** – 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^3 measuring cylinder (this will need to be washed before being reused)

4 1 100 cm^3 measuring cylinder

5 2 500 cm^3 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 3 g table salt or 3 g sodium chloride

12 Washing up liquid

13 2 cm^3 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/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 **harmful**.
	Fluorescein is an **irritant**. It will stain skin or clothes.
	Methylene Blue is **harmful**. It will stain skin or clothes.
 	Crystal violet is **harmful** and **dangerous to the environment**. 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 '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^3 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^3 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

Timings/ minutes	**Teacher / Student Activities**
end of previous lesson	**Preparation –** students given some theory on DNA fingerprinting or instructed to research on topic for homework.
0-4	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** – class discussion on application of DNA fingerprinting. Teacher to summarise overall process of DNA fingerprinting on board (possibly in flow-chart format).
8-12	**Introduction to method** – teacher demonstration of setting up electrophoresis tank. Teacher demonstration of using micropipette, pouring gel and connecting power supply.

12 -50	**Carrying out the practical** – 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	**Drawing together the threads** – 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^3 per student

  3 Electrophoresis tank, including casting gates, comb and battery pack – 1 per student

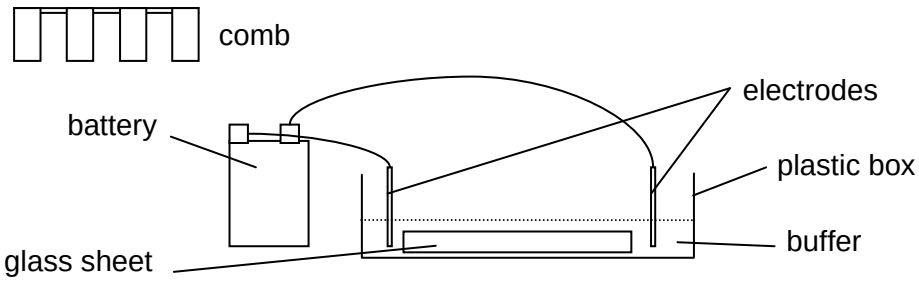
  4 Micropipette – 1 per student (with a clean tip for each dye to be used)

  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 – 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/ and click on the Life Science education tab

     http://wardsci.com/product.asp?pn=365164

     http://www.sdr.com.au/electrophoresis_horizontal.html

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^3 pipette to place 0.5 cm^3 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^3 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)

Timings/ minutes	**Teacher / Student Activities**
Previous lesson	**Preparation** – 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	**Introduction** to the aims, intended outcomes and background information - teacher led oral presentation.
5-10	**Context** - review disc diffusion test, bacterial lawn and aseptic technique - teacher led class discussion / question and answer session.

10-15	**Introduction to method** - teacher to go through method step by step, emphasising the importance of aseptic technique throughout.
15-50	**Carrying out the practical** - students carry out the practical work.
50-60	**Drawing together the threads** – 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	**Gathering results -**
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 my 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^3 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^3 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^3 bacterial culture in nutrient broth

3 10 cm^3 molten nutrient agar*

4 10 cm^3 sterile water*

5 Sterile 90 mm Petri dish

6 1 cm^3 pipette*

7 Filter paper discs* *or* prepared penicillin discs

8 Forceps

9 10 cm^3 1% penicillin solution (students may carry out dilution of this to achieve several concentrations for testing)

10 20 cm^3 absolute ethanol, to enable sterilisation of forceps

11 50 cm^3 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 (3rd 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 **irritant**.
	All enzymes including sucrase enzyme should be assumed to be **harmful**.

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^3 of 1% sucrase solution into a beaker.

2 Add 6 cm^3 of sodium alginate to the sucrase. 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^3 of calcium chloride into a clean beaker.

5 Using a dropping pipette, transfer the sucrase / 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.

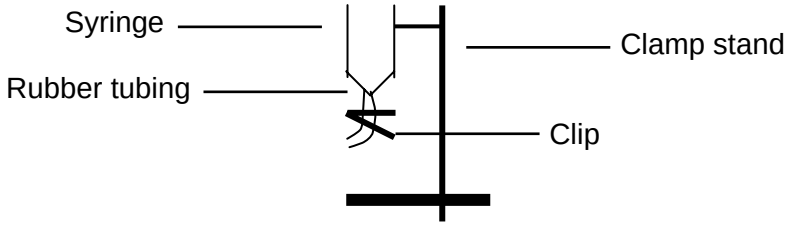
6 Fix a 15 cm^3 syringe to a clamp stand and place a small piece of muslin in the bottom to prevent the nozzle from becoming blocked.

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	**Preparation** – 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	**Introduction** to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	**Context** - review industrial uses of enzymes, reasons for use of immobilised enzymes and methods of immobilisation. Also, review Benedict's test.
8-12	**Introduction to method** - teacher demonstration of alginate bead production. Teacher may have a column already set up to enable students to visualise it.

12-20	**Student preparation exercises** Students list the variables that should be controlled, using their knowledge of enzymes and describe ways in which each variable may be controlled. Suggest a suitable control experiment to prove the model is working correctly.
20-50	**Carrying out the practical** - students carry out the practical work and tidy away apparatus when they have finished.
50-60	**Drawing together the threads** - 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.

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^3 of 1% Sucrase solution provide
- 6 cm^3 of 2% Sodium alginate solution
- 1 mol dm^{-3} Calcium chloride solution (the molecular weight of sucrose = 342g) – provide enough for 50 cm^3 per student
- 10 cm^3 graduated pipettes, syringes / measuring cylinders – 2 per student
- Beakers (at least 50cm^3 volume) – 2 per student
- Dropping / teat / Pasteur pipettes – 1 per student
- 15 cm^3 syringe – 1 per student
- 2 cm^2 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 shows 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 monoecius – their flowers have either an androecium or a gynoecium. Some plants are dioecius – 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.
- Monoecius plants cannot be self pollinated. Dioecius plants and hermaphrodite plants can be self pollinated.
- Outbreeders are plants that do not normally self pollinate. If they are dioecius or hermaphrodite there are structural and physiological methods of preventing self pollination.
- Inbreeders are plants that normally self pollinate. If they are dioecius 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	**Introduction** 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	**Context** – review of flower structure. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-20	**Introduction to method** – 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. 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.
20-45	**Carrying out the practical** - 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	**Drawing together the threads** - 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) Optional t-test – teacher produced guide sheet or access to a computer programme.

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^3 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.

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