ACTIVITY BRIEF

Researching and planning bioassays

The science at work

Dictionary.com defines a drug in the pharmacological sense as “a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being.” Employed as medicines, drugs can be used for a wide range of therapeutic purposes. Yet, because they are bioactive chemicals, they don’t just have good effects in controlling disease but also have the potential to cause harm. Sometimes this is a question of concentration: many drugs taken in excess can have a damaging effect even though, at the correct dose, they help us to feel and or to get better. For example, non-steroidal anti-inflammatory drugs (NSAIDs), like aspirin and paracetamol, are drugs that have analgesic, antipyretic and anti-inflammatory properties. Taken in the correct quantities they reduce pain, fever and inflammation. Millions of people benefit from taking them, yet an overdose can kill you.

Scientists and technicians employed in the pharmaceutical industry use their knowledge and understanding to develop and trial different medicines. In the development of new drugs, scientists not only need to establish their active and safe doses, but also whether they can cause harm, even in very low concentrations. Ultimately this must be done using human volunteers, but before this can happen, every step must be taken to ensure that a potential new drug will be as safe as possible before it comes to clinical trial. Bioassays are techniques used to test the effects of chemicals on living tissues. This may be in vivo using whole organisms, but for ethical and economical reasons, these tests commence in vitro, using tissue cultures or lower organisms.

Your brief

Your task is to research and use bioassay techniques to test and measure the biological activity of two medicines.

You will need to find out about the available techniques and procedures. You will then use or adapt suitable methods to carry out bioassay procedures to test your two chosen medicines.

Note that you will need to choose and match both methods and medicines. Your choices will depend on:

- the medicines which are available for your use
- the bioassay techniques which can be conducted safely, using the resources available to you.
Task 1 Research bioassay methods
A variety of techniques and procedures are available which can be used or adapted for use in the school laboratory. Your first task is to find out about and select from these.
Use Study sheet: Choosing bioassay methods.

Task 2 Carry out bioassay methods on two medicines
Your second task is to carry out and report on bioassays of two medicines.
Use Study sheet: Using bioassay methods.
STUDY SHEET

Choosing bioassay methods

Introduction
You will need to carry out bioassays of two medicines. You will need to look at the methods available and decide which are most suitable to use on your two medicines. Because of constraints placed on you by your centre (available resources, safety issues, ethical issues, time, cost and so on), you may need to think carefully to come up with a good match between medicines and methods! So ‘most suitable’ includes the practical considerations of what you will actually be able to carry out yourself.

You will need to:
- decide on a clear definition of bioassay
- research potential methods for carrying out bioassays of medicines
- choose a medicine and a method and have them approved by your teacher
- construct a detailed plan to suit your chosen medicine and have it approved
- obtain/organise all the resources that you need for your bioassay
- carry out a bioassay of your medicine
- write a report, including an evaluation, of what you have done
- repeat the process for a second, contrasting medicine.

What is a bioassay?
‘Bioassay’ has come to mean slightly different things. Your first task is to decide on the meaning that you will use in designing your own investigations. For example, the following definition has been taken from an on-line scientific dictionary:

“An assay for the activity or potency of a substance that involves testing its activity on living material.”


Defining ‘bioassay’
With two or three other students, spend about 20 minutes using library resources and/or the Internet to find some contrasting definitions of ‘bioassay’.

1 Record your definitions in a suitable way to be able to communicate them to the rest of your class (using flip charts, overhead projector acetate or data projectable word processing etc.).

2 Share and discuss the definitions found by your class.

3 Decide on those aspects of the definitions which are most important for designing and carrying out a bioassay.

4 Agree on and record a final working definition which will be used by everyone.
After you have decided the best definition for ‘bioassay’, your next task is to research and choose a bioassay method to apply to a medicine of your choice.

Choosing bioassay methods

You need to bioassay two medicines. Ideally, you should investigate those you use for other parts of this unit. It is hard to find methods that can be used in school for chemical analysis and bioassay of the same chemical, so keep an open mind and be prepared to have to reconsider the method or the medicine you choose.

When you carry out your research, you will find that bioassay methods are often used in the assessment of environmental pollution. Because these methods are assessing the effect of chemicals on living material, they can often be successfully adapted for bioassay of medicines. You may be able to take a procedure more or less ‘off the peg’, but more likely you will need to find a method that you can adapt to use the resources that are available to you. If you have the right contacts, you may be able to gain outside help from a scientific institution or research establishment.

Finding out about bioassay methods

Your teacher will give you time limits for this and the following task.

With one or two other students, use some of the URLs below or other sources, to research ideas for different bioassay methods. Some techniques you might look at include:

- Turbidimetric analyses of broth cultures to test antimicrobials
- Bacterial lawns in Petri dishes to test antimicrobials
- Testing yeast cultures with anti-fungals by measuring the rate of fermentation
- Testing anti-fungals by measuring hyphal growth of filamentous fungi

Note that you will need to be selective and may need to consider adapting some of the methods to suit your purposes: the sources listed do not necessarily include appropriate ideas.

https://kb.osu.edu/dspace/bitstream/1811/3443/1/V44N06_278.pdf
http://ei.cornell.edu/toxicology/bioassays/daphnia/culture.asp
http://ei.cornell.edu/toxicology/bioassays/lettuce/RefTest.asp
http://ei.cornell.edu/toxicology/bioassays/lettuce/
http://ei.cornell.edu/toxicology/bioassays/Duckweed/method.asp
http://www-saps.plantsci.cam.ac.uk/records/rec154.htm
http://www-saps.plantsci.cam.ac.uk/docs/antimicrobials/pb.doc
http://www.fortunecity.com/greenfield/clearstreets/84/brine.htm
1 Skim through to get an overview of each method.
2 Make summary notes (no details yet).
3 Choose some to consider in more detail. For each of these:
   - make notes to describe it
   - suggest some medicines that might be assayed using it
   - list some advantages of using the method
   - list some disadvantages of using the method
   - rate it on a scale of 1 to 5 (useless to highly suitable) to indicate how valuable it is to follow up in more detail.
4 Discuss your ideas with the rest of your class. In particular:
   - suggest and explain which methods seem to be the most promising
   - describe and explain some of the problems that you have found.

**Planning a bioassay of a medicine**

Working on your own, choose one medicine and one method to carry out the bioassay. (Remember that you will need to do the same for a second medicine, so save any other useful ideas you find.)

1 When you have selected a promising method, carry out an Internet and literature search to find out more about it. You may need to design your own method by adapting and modifying those that you have found to suit your own specific requirements.
2 Check that your ideas match the definition of bioassay agreed by your class.
3 Sketch out a plan for your bioassay. You should include an outline of the method and a list of resources and materials.
4 Carry out an initial risk assessment.
5 You may need to change your mind at some stage! Have ideas checked regularly, so that you do not waste time going down a dead end. Pay special attention to:

- safety
- expense
- ethical considerations
- availability of materials or equipment
- operating skills required (Will you need to learn how to/be able to carry out the procedure? Remember that you should be able to work more-or-less unaided and not need directing how to proceed or how to modify procedures.)
- time constraints.

6 Discuss your draft plan with your teacher or technician. If they approve, go ahead and develop a detailed plan. This should include:

- full practical instructions
- full risk assessment (using the standard procedure for your centre)
- a complete list of equipment and materials
- laboratory and other specialist requirements, with times and dates
- a diary of all events, with detailed timing for the completion of the assignment so that you can meet the deadlines and allowing
  - time for each aspect of the bioassay, including setting up and testing apparatus, preparation of reagents etc.
  - contingency time for unseens
  - time to write-up and check your report
  - time for other work you have to do
- have your plan checked and approved.

If, at any stage, your ideas are not approved you will need to act on the reasons for this. You may be able to modify and resubmit your plan or you may need to choose and plan another method or use a different medicine.

Once you have your detailed plan accepted, your next task is to organise and carry out your bioassay.

Use *Study sheet: Using bioassay methods*
FACT SHEET

Bioassay of antimicrobials

The potency of antimicrobials can be tested by using the measurement of inhibition zones on microbial lawns grown in nutrient agar. For example, pathology service microbiologists routinely test microbes, obtained from patient samples, for antibiotic sensitivity, so that treatment can be recommended. The techniques given use sterile filter paper discs which are impregnated with different concentrations of the antimicrobial under test.

References

- MISAC The Microbiology in Schools Advisory Committee http://www.microbiologyonline.org.uk/misac.html

Health and safety

A risk assessment must be carried out before starting work. It is important you work as aseptically as possible. Wear eye protection and gloves. When the Bunsen burner is not being used, close the air hole so you can see a yellow flame and turn it off when you have finished with it. All micro-organisms present in a sample multiply during incubation, including any harmful ones, so do not open incubated Petri dishes. Dispose of any waste that has come into contact with micro-organisms as directed by your teacher.

Media preparation

Prepare nutrient broth and nutrient agar. Both nutrient agar and nutrient broth are general purpose media used in the cultivation of micro-organisms. Some more sensitive micro-organisms require more specialist media. The main ingredients of nutrient agar and broth are beef extract and peptone which supply a quantity of carbon, nitrogen and vitamins for the growth of most non-fastidious micro-organisms (organisms without special nutrient requirements).

Nutrient agar and broth can be purchased from a number of suppliers including Philip Harris and Scientific and Chemical. You should follow the instructions that come with the media but a general method for preparing 1.5% nutrient agar or broth from powder is as follows.

1. Suspend 1.5 g of powder in 100 cm³ of distilled/deionised water. Dissolve the agar medium by heating to a boil and stirring. **WARNING**: Agar can easily boil over. Heat in a water bath, NOT over a Bunsen flame. Never add agar to boiling water.

2. Dispense about 25 cm³ of the medium at a time into sterile Universal/McCartney bottles ready to dispense onto plates (agar) or to use directly (broth). Screw down the lids.
3 Unscrew the lids one quarter turn and sterilize the medium in an autoclave at 121 °C for 15 minutes. Do not sterilise agar for longer than recommended by the supplier as it will adversely affect the agar. Screw down the lids when the bottles have cooled.

4 STORAGE
   - Store dry medium at room temperature (15-30 °C). The dehydrated powder should be a light tan colour, homogeneous and free-flowing. Deterioration of powdered medium may be recognized by: (a) granulation, clumping, or particulate matter throughout the powder, (b) pH change, (c) inability to promote growth when properly used.
   - Prepared plates: 1 month at 2-8 °C.
   - Prepared tubes and flasks: 3 months at 2-8 °C.

Preparation of agar plates
1 To prepare nutrient agar plates you need to work using aseptic technique.
2 All culture plates and containers must be labelled to identify the micro-organism, antimicrobial, date and experimenter.
3 If agar is to be used directly from the autoclave, allow to cool for 20 minutes before placing in a water bath at 50 °C.
4 Arrange your workspace so you have a Bunsen burner with a yellow flame and a sterile Petri dish in easy reach (do not open the Petri dish yet).
5 Collect a bottle of sterile liquid agar from the water bath. Dry the outside as you remove it.
6 Open the air hole of the Bunsen burner to obtain a quiet blue flame. Remove the lid of the bottle and immediately flame the glass rim of the bottle before pouring the molten agar into the Petri dish. While pouring the agar, open the Petri dish lid as little as possible, hold it at an angle, and make sure the lid is kept directly over the Petri dish. Immediately replace the lid. You should obtain a depth of about 4 mm (about 25 cm³ agar in a 90 mm Petri dish).
7 Gently rotate the dish on the work top to spread the agar and allow to set on a level surface. Thin or uneven plates will affect your experiments.
8 Allow the plates to dry until the surface of the agar is free of visible surface moisture but do not over dry. This will probably take about 10 minutes. If not required immediately, store plates upside down in a refrigerator, in sealed sterile bags to prevent over drying.

Inoculation for susceptibility testing
To carry out your experiment, you need to grow the bacteria evenly over the agar plate. Bacteria which grow so that they form a continuous layer over the plate will stop growing and are called confluent. You do not want this to happen, they need to be semi-confluent - evenly distributed but still with space to grow.
1 Dip a dry sterile cotton swab in the broth suspension and remove excess fluid by turning the swab against the inside of the tube.
2 Spread the inoculum evenly over the surface of the dry agar plate by swabbing in three different directions, rotating the dish through 45° between each streaking operation.
Make sure you go close to the edges, but do not touch the Petri dish with the swab. You need to make sure that you will get even growth without streaks on the plate.

**Antimicrobial application**

1. Use cooled flamed forceps to place antimicrobial discs on plates. Apply to plates within 15 minutes of inoculation, before organisms have begun to grow too much. Use the forceps to press down discs gently if necessary.

   NB: The antimicrobials will begin to diffuse from the disc into the agar as soon as contact is made, so do not move the discs once they have touched the agar.

2. Incubate plates at 25 ºC for 24-48 hours until distinct zones of inhibition have formed.

**Reading results**

It is not necessary to remove the lid of plates to read results. If the bacteria were susceptible to an antimicrobial, there will be a circular zone of inhibited growth around the disc.

Measure zones of inhibition from the underside of the plate using a ruler.

If zones of inhibition are not circular, it is possible adjacent antimicrobials interfered with each other. The test will need to be repeated with larger distances between the discs.
STUDY SHEET

Using bioassay methods

Introduction
Once it is approved, you need to carry out your plan for the bioassay. Do not attempt any work that has not been checked by your teacher, especially for health and safety considerations. Be sure to keep all the records that you need, including notes on any modifications or problems, so you can make a full report (see below). Remember that you also need to carry out a second bioassay of another contrasting medicine. Use information and ideas that you gain from your first investigation to help you to plan and carry this out more efficiently and effectively, to optimise your chances of gaining top marks.

Procedure
1. Carry out a ‘dry run’. Work through your plan and check that you have all the necessary resources that you need.
2. Make the necessary arrangements to carry out your bioassay (order equipment and materials including statements of quantities and concentrations, book laboratory and computer time etc.) and construct a timetable of events. Allow time to check the reliability of your data and contingency time for unseens.
3. Carry out your bioassay and collect all the essential data. Make notes on:
   - observations of events, especially if unexpected
   - any problems, modifications or improvements.
4. If possible, chart and/or average your results as you obtain them to:
   - spot anomalies or errors
   - decide if
     - the range needs to be extended
     - more repeats are needed to improve the accuracy of the results
     - more precise measurement is needed.
5. When you have all the data that you need, to be able to form valid conclusions, carry out a complete analysis of your data. Do not leave this until you write up your report, as you may find that you need additional information or data.
6. Write up your report using the format expected by your teacher. Break formal reporting into clear sections with sub-headings. You might be asked to include an abstract, introduction, method, results, conclusions and discussion.
7. To obtain top marks, the evidence that you provide should include:
   - evidence of research into methods of analysis
   - plans of the approach to the investigations
   - justification of the choice of methods
   - full details of practical procedures followed - including choice and description of apparatus/equipment and materials used.
• appropriate risk assessments with explanations of their significance based on scientific reasoning
• full and comprehensive observations for two bioassays
• results and observations which allow evaluation
• full appropriate evaluations of methodology, observations and results
• application of detailed and correct scientific knowledge throughout.

Remember: complete your report *in good time* before the deadline, so that you can carry out any necessary remedial work.
Teacher notes

AQA Unit 12 The actions and development of medicines

This activity relates to the following parts of the specification:

21.2 How you will be assessed

You need to produce a portfolio of evidence which considers the actions and development of two different medicines. You need to conduct a quantitative analysis and a bioassay for each medicine.

C. a report on the bioassay of each of the two chosen medicines including:

- researching a suitable method(s) for the bioassay including appropriate background scientific principles
- a list of information sources
- describing the practical procedures to be used
- selecting and using safely, appropriate equipment
- detailing carrying out the bioassay
- carrying out risk assessment(s)
- evaluating the method(s) used.

21.3 You need to know, understand and be able to demonstrate

In order to complete the requirements of this unit you will need to know, understand and be able to demonstrate relevant knowledge of the following:

- the different categories of medicines and their action
- the development of a medicine
- methods of analysing medicines.

Methods of analysing medicines

You will need to:

- research and provide details of a suitable method(s) to be used in the quantitative analysis and a suitable method(s) to be used in the bioassay. This will include details of the underlying scientific principles to be used.
- describe in detail the practical procedures in the quantitative analysis and the practical procedures used in the bioassay including an explanation of the scientific principles involved
- select appropriate apparatus for both the quantitative analysis and bioassay
- carry out a risk assessment for the quantitative analysis and bioassay and complete the procedures in a safe manner
- evaluate the method used in the quantitative analysis and bioassay.
In the AQA *Teachers’ Guide*:

- state that the different categories of medicines available are: antibiotics, antivirals, analgesics, anti-inflammatory agents, antihistamines, antihypertensive agents, vaccines, antiseptics, and vitamins
- suggest testing of antimicrobial agents which either inhibit or kill microorganisms such as toothpaste, mouthwash, skin antiseptic, throat lozenges and TCP
- suggest use of antibiotic assays to test the activity of various antibiotics against recommended bacteria.

**Aims and teaching strategies**

The two tasks for this activity allow students to carry out research to find practical procedures and techniques, to carry out bioassays on two medicines. In *Task 1* they are asked to choose a method or methods and medicines to assay, then in *Task 2* they use those methods.

The specifications for the unit also require the chemical analysis of two medicines. At the time of writing (March 2008) the AQA no longer required that the same two medicines be investigated for both bioassay and chemical analysis. Please refer to the latest guidance from AQA.

There is likely to be considerable differences in the extent to which students will be able to work autonomously on this unit. Consequently, it is likely that students will be at significantly different stages as time spent on the unit elapses. Some students will need considerable help in both the research and the practical phases of the unit.

**Task 1 using Study sheet: Choosing bioassay methods**

This is intended to help students to get started on the assignment of finding, developing and using bioassay methods. As such, it can be started as a task for small groups who can report their findings back in a class discussion. This should help everyone to become aware of where good information is to be found and the principles to be applied in choosing appropriate techniques and medicines to use.

The task is set in two steps:

- comparing definitions of bioassay to obtain a working definition
- researching techniques and procedures to choose an appropriate method to apply to a chosen medicine.

Two single sessions or a double period can be used to start the process, which can then be continued in each student’s own time, or individually in dedicated class time.

The term ‘bioassay’ can be used in different ways, so it is important to establish what is required at an early stage. *Study sheet: Choosing bioassay methods* starts with an exercise to find and compare definitions of ‘bioassay’, so it is possible at this point to establish what you expect. The specification requirements allow different interpretations. Please check any guidelines from the board. Otherwise, it is useful to establish your own criteria to facilitate consistent marking for your centre.
Students are provided with the following definition which has been taken from an on-line scientific dictionary: “An assay for the activity or potency of a substance that involves testing its activity on living material” (JM Lackie & JAT Dow 1999 The Dictionary of Cell & Molecular Biology Third edition, Academic Press, London) http://on.to/cellbiology (accessed 14 April 2008)

This definition has the advantage of being vague about the nature of the assay. It is possible to use any appropriate measurement of the effect of the chemical on the activity of a selected living material. For example, changes in growth or metabolic rates could be measured either to assess the desired effect or an unwanted harmful effect.

Students should have no problems finding contrasting definitions for class discussion. The outcome of the discussion should be directed to a definition which allows the students to design bioassays which meet the criteria that you prefer for the purposes of being able to design satisfactory bioassays. Some examples of other definitions which may be used for discussion are given below:

“A procedure for determining the concentration, purity, and/or biological activity of a substance (e.g., vitamin, hormone, plant growth factor, antibiotic, enzyme) by measuring its effect on an organism, tissue, cell, enzyme or receptor preparation compared to a standard preparation.”
Chemicool Internet Chemical Dictionary http://www.chemicool.com/definition/bioassay.html

“Determination of the active power of a drug sample by comparing its effects on a live animal or an isolated organ preparation with those of a reference standard.”
Dorland’s Medical Dictionary for Health Consumers, Saunders (Elsevier, Inc.)

“1. Determination of the strength or biological activity of a substance, such as a drug or hormone, by comparing its effects with those of a standard preparation on a culture of living cells or a test organism.
2. A test used to determine such strength or activity.”

“1. Determination of the relative purity of a substance, such as a drug or hormone, by comparing its effects with those of a standard preparation on a culture of living cells or a test organism.
2. A test used to determine such purity.”

The Answers.com health website contains a good list of definitions and also a helpful discussion of the issues. http://www.answers.com/topic/bioassay?cat=health
After establishing the requirements for a bioassay, students are asked to research potential methods and choose one to develop in outline for a chosen medicine. The URLs for a variety of websites are provided as a starting point. It would be helpful if students were provided with this list in a Word document file so that they can click on the hotlinks rather than have to type in the URLs.

Some useful techniques and procedures can be found, but also a variety of ideas which can be modified as bioassays of medicines. Turbimetric analyses of broth cultures offer alternatives to antimicrobial investigations using bacterial lawns in Petri dishes. As fungi are important in causing disease (10 million cases in Europe and the USA every year) an investigation of an antifungal agent could be applied to yeast cultures, which opens up the possibility of measuring the rate of fermentation by collecting carbon dioxide gas as an indicator of inhibition. Hyphal growth of filamentous fungi can be measured using stage micrometers or rate tubes can be made from glass tubes or plastic pipettes filled with agar (the distance that the fungus grows up the tube is measured against time).

The outline plan is to be submitted for checking and approval before they go on to Study sheet: Using bioassay methods. They should be encouraged to check ideas, even if tentative, at regular intervals with teachers or technicians during the early stages. This is to avoid wasting too much time on dead-end routes, particularly those for which materials may be too expensive, unsafe, unethical or not available. One approach would be to raise awareness of problems by having feedback sessions when students report their progress in class. Also, carrying out the first bioassay before attempting the second will allow pitfalls to be avoided the second time round.

Students will need at least two sessions to carry out this activity and before going on to plan and execute their bioassays for their chosen medicines. In the first section they can be introduced to the task and review the procedures to be found in the listed websites. They can be set the task of carrying out further library and Internet research before a follow up discussion in the second session. This can be used as an opportunity to troubleshoot difficulties, vet decisions and give extra help to any who are experiencing difficulties. Such assistance can be reflected in the grades awarded according to the assessment criteria.

This is something of a chicken and egg situation. Students can start from the perspective of a medicine they wish to test or a technique that they wish to apply. In either case, they may find that they have to make changes before they can make a medicine and a technique fit together successfully to give an investigation that can be carried out in a school laboratory.

Ideally, students should be given a free hand in determining the materials that they will use. However, realistically and in the interests of efficient planning and economy, after initial decisions have been made, you can require them to cooperate for the ordering of materials such as bacteria and nutrient media, or ask them to select from a list of materials which you make available. Original designs can then be modified according to the constraints that you apply.

Both study sheets are designed to get students started on the tasks. They allow students to gain maximum credit for their work. However, many students are likely to need additional help and guidance, which may affect the marks that they can be awarded. Time and opportunities for such guidance should be built into the teaching and learning scheme for the unit. If students can concurrently be working flexibly on other aspects of the unit, time can more easily be made available for individual attention as and when the need arises.
Task 2 using Study sheet: Using bioassay methods

This study sheet summarises what has to be done to provide the evidence for good marks for the bioassay assignments. It should be used only after a student has completed their planning and had their proposals thoroughly checked.

In addition to setting a final deadline for the completion and write-up of the bioassay work, intermediate goals can be set, such as deadlines for:

- choosing the techniques and medicines to be used
- submitting outline plans for approval
- writing the detailed procedures to be adopted
- having the methods checked
- ordering the materials and confirming laboratory availability
- conducting the bioassays and obtaining the data
- completing and handing in the reports.

Fact sheet: Bioassay of antimicrobials

An optional fact sheet is provided which is a modified version of the method to be found in the activity provided in this resource base for Unit 15 The role of the pathology service. This gives practical details of the Bioassay of antimicrobials using a bacterial lawn grown in a Petri dish culture. It may be given to students who are experiencing difficulty in finding and developing a bioassay method on their own. It will need some modification depending on their choice of medicine (see below*).

The procedure is based upon several standard operating procedures (SOP’s) written as national standards for clinical microbiologists from the Health Protection Agency (http://www.hpa-standardmethods.org.uk/pdf_sops.asp):

BSOP 54 Inoculation of culture media
BSOP 9 Investigation of throat swabs
BSOP 45 Susceptibility testing

Health and safety

A risk assessment should be carried out before starting work. Students should wear gloves and clean lab coats. Health and safety guidelines should be followed from the CLEAPSS Laboratory handbook section 15.2 Microbiology. This practical should ONLY be used with POST-16 students who have a good level of skill in aseptic technique. Students should have learned and practised aseptic technique before undertaking this practical.

Safety hints

- Check nutrient media for contamination before giving them to students to use.
- Keep windows and doors shut to avoid drafts.
- Avoid walking around the room: make sure all students have everything at their work stations before anyone begins aseptic work.
- Make sure the workspaces are cleaned with disinfectant before and after the lesson.
Although it is desirable for students to be able to prepare agar and nutrient broth, you might decide that it is more expedient (with regard to expense, safety and time) for a laboratory technician to prepare the nutrient agar and nutrient broth in Universal/McCartney bottles, ready for use. Once plates have been inoculated with swabs they should be left for about 2 days at room temperature (~ 20ºC) until colonies of bacterial growth can be seen. They must then be carefully examined to check for contaminants. If they have been contaminated they should not be opened (although they may be sealed with tape and shown to students). It is advisable to prepare some spare plates so that if a student does contaminate their work they will still be able to continue with the next stage of the practical.

Students can then select a colony to culture in nutrient broth. These should be left for about 12-24 hours at room temperature (~ 20ºC) until bacterial growth can be seen. Again, these should be examined for contaminants before allowing students to open them to plate out the bacteria.

*The method suggested tests the bacteria against a number of antibiotics using a mast ring. 
For the purpose of carrying out a bioassay of a given antimicrobial medicine, this can be modified by using sterile filter paper discs which have been soaked in solutions of different concentrations of the chemical under test. In this way, students have the opportunity to select and justify their choice of procedure.

An approximate timetable would be:

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Prepare nutrient agar and nutrient broth</td>
</tr>
<tr>
<td></td>
<td>Pour agar plates</td>
</tr>
<tr>
<td></td>
<td>Inoculate plates with patient swab</td>
</tr>
<tr>
<td>Day 2</td>
<td>Subculture a colony grown on agar into nutrient broth.</td>
</tr>
<tr>
<td>Day 3</td>
<td>Inoculate agar plates with bacteria grown in nutrient broth.</td>
</tr>
<tr>
<td></td>
<td>Apply test discs</td>
</tr>
<tr>
<td>Day 4/5</td>
<td>Read results</td>
</tr>
</tbody>
</table>

Materials may be stored in a refrigerator over weekends if necessary.

**Requirements**

*Study sheet: Choosing bioassay methods* assumes that students will be able to use a computer with Internet access.

Follow-up work involving *Study sheet: Using bioassay methods* will require a variety of resources and materials depending on the choices made by each student. It is important that they make decisions early so that they can make their requests and have time to adapt their methods to meet any limitations set by your centre.
The method outlined in *Bioassay of antimicrobials* requires the following materials. This gives some idea of the kind of requirements that are likely to be necessary when students determine their own methods for antimicrobial medicines such as antibiotics.

- refrigerator
- autoclave or pressure cooker
- balance (accurate to 0.1g)
- thermostatically controlled water bath
- incubator (or suitable area for incubation)
- Universal or McCartney bottles (glass)
- weighing boats
- Petri dishes
- Bunsen burner
- sterilised cotton wool swabs (Sterile swabs can be bought. Cosmetic cotton buds are not suitable as they often contain antimicrobial agents.)
- gloves
- tissues
- disinfectant spray for cleaning surfaces
- disinfectant (VirKon)
- beakers
- inoculation loops
- conical flasks
- forceps/tweezers
- ruler
- autoclave tape
- bags for collecting contaminated waste
- nutrient agar powder
- nutrient broth powder
- distilled/deionised water
- antibiotic mast rings or filter paper discs with other antimicrobials

* Bacillus subtilis, Micrococcus luteus, Staphylococcus albus * (e.g. available from Beecroft & partners [http://www.beecroft-science.co.uk/home/](http://www.beecroft-science.co.uk/home/))

The suggested bacteria are inhibited by most easily available antibiotics. Other bacteria usable in schools and colleges, such as *E. coli* strains K12 or B, may be resistant to some of the antibiotics available from the educational suppliers (Philip Harris or Scientific & Chemical).
Further reading

*A viable alternative* Richard van Noorden

*Practical Microbiology and Biotechnology for Schools*, The Society for General Microbiology (SGM), MacDonald Educational

Microbiology On-line The Microbiology in Schools Advisory Committee (MISAC)
http://www.microbiologyonline.org.uk/misac.html

Micro-organisms for investigations in schools and colleges MISAC / SGM
http://www.microbiologyonline.org.uk/forms/BPMlist.pdf