Before you read this manual,
have you put your
Bacterial Slopes
in a cool dark room?

Please refer to page 8
for more information

CONTENTS

Welcome to The X-Bacteria! 2

The X-Bacteria Teacher Notes 3
Equipment and materials required 3
Detailed lesson-by-lesson instructions 4
Introduction 5
Outline of the practical activity 7
Who is this practical work suitable for? 7
Planning and classroom organisation 8
Lesson overview 8
Lesson 1: Mating the bacteria 10
Lesson 2: Plating out the cultures 11
Lesson 3: Interpreting the results 14
Microbiology guidelines 15
Additional information 19

Curriculum links 20
Post-2009 legacy 25

Technician Notes 27
Equipment and materials required 27
Detailed lesson-by-lesson instructions 28
Introduction 29
Outline of the practical activity 29
Who is this practical work suitable for? 29
Planning and classroom organisation 30
Lesson overview 30
Lesson 1: Mating the bacteria 32
Lesson 2: Plating out the cultures 33
Lesson 3: Interpreting the results 36
Microbiology guidelines 36
Additional information 40

Student Notes 43
Outline of the practical activity 43
Lesson 1: Mating the bacteria 43
Lesson 2: Plating out the cultures 44
Lesson 3: Interpreting the results 45
Microbiology safety guidelines 45
Additional information 48

A Question of Taste 50
Background information 50
PTC strips – Instructions for use 50

Acknowledgements 53

Wellcome Trust Education Activities 55
Other Links 56

WELCOME TO THE X-BACTERIA!

This kit contains everything you need to carry out the 'The X-Bacteria' protocol with your class, as well as this manual which will help you to get the most out of this resource. Within this there are separate sections for students and for technicians, which you may wish to photocopy and distribute. The 99% Ape book will provide you with a comprehensive guide to evolution and the latest thinking on the subject. Plus the taste test strips included will allow you to take part in a mass participation experiment about taste perception and discover if you are a taster or non-taster of a particular chemical.

The X-Bacteria is part of the Survival Rivals series of experiments. There are three kits in this series:

I'm a Worm, Get Me Out of Here for 11-14 year olds
Brine Date for 14-16 year olds
The X-Bacteria for 16-19 year olds

Every secondary state school within the UK is entitled to one of each of these kits for free. You can find out more and order at **www.survivalrivals.org**.

Survival Rivals is designed to allow secondary schools to celebrate the 200th anniversary of Charles Darwin and the 150th anniversary of the publication of On the Origin of Species by Means of Natural Selection. The three kits enable young people of different ages to carry out practical investigations and to explore Darwin's ideas of evolution and selection.

In The X-Bacteria the spread of antibiotic resistance between bacteria is investigated. Conjugation takes place between two strains of bacteria which are resistant to different antibiotics, resulting in some cells which are resistant to both antibiotics. Students will practice using aseptic techniques and will investigate this method of horizontal gene transfer.

The Survival Rivals website (www.survivalrivals.org) is packed with additional resources and information to support you in delivering these experiments, including online games and videos about carrying out the protocols.

Also on the website are details of A Question of Taste, an experiment about taste perception. On page 51 of this manual you can find details of how to run this with your class and you can then feed your results into the UK wide experiment online.

For further support about the Survival Rivals experiments and delivering evolution in the classroom please see the Science Learning Centres website at www.slcs.ac.uk/darwin200 for details of their courses.

Survival Rivals is funded by the Wellcome Trust, who are also running a number of other Darwin-related initiatives. Find out more about the Great Plant Hunt for primary schools, an animated version of the Tree of Life and various other projects at **www.wellcome.ac.uk/darwin200**.

The Teachers notes (pages 3 to 25) contain all the information. The Technicians notes (pages 27 to 40) and Students notes (pages 43 to 48) contain extracts of the Teachers information for their use. Please photocopy the Technicians notes and Students notes and distribute accordingly.

NOW GET STARTED WITH THE X-BACTERIA...

The X-Bacteria Teacher Notes

Equipment and materials required

The following items are provided in the Survival Rivals: The X-Bacteria kit:

Item No	✓	Description	Quantity
1	☐	Bacterial Slope *E. coli* HT-99	1
2	☐	Bacterial Slope *E. coli* J-53R	1
3	☐	Chloramphenicol 25mg	1
4	☐	Rifampicin 100mg	1
5	☐	Nutrient Agar (Powder) 28g	1
6	☐	Nutrient Broth (Powder) 13g	1
7	☐	Methanol 40mL	1
8	☐	Petri Dishes - sterile	40
9	☐	Loops Plastic sterile -10microlitre	54
10	☐	Syringe 1mL sterile	20
11	☐	Sterile Water - 100mL pack	2
12	☐	Universal containers Non-sterile	40
13	☐	Syringe 30mL sterile	2
14	☐	Syringe 5ml sterile	5
15	☐	Syringe Needles Sterile	5
16	☐	Virkon Disinfectant 50g sachet	1

If you have any items missing from your kit, please call **0845 120 4529**

Extra items unrelated to this protocol

- 99% Ape: How evolution adds up by Jonathan Silvertown [Ed] (2008) London: Natural History Museum/Open University. ISBN: 978 0 565 09231 3. This book will provide you with a comprehensive guide to evolution and the latest thinking on the subject.
- PTC tasting strips, vial of 100. These will allow you to take part in a mass participation experiment. See page X for futher information.

You will also need:

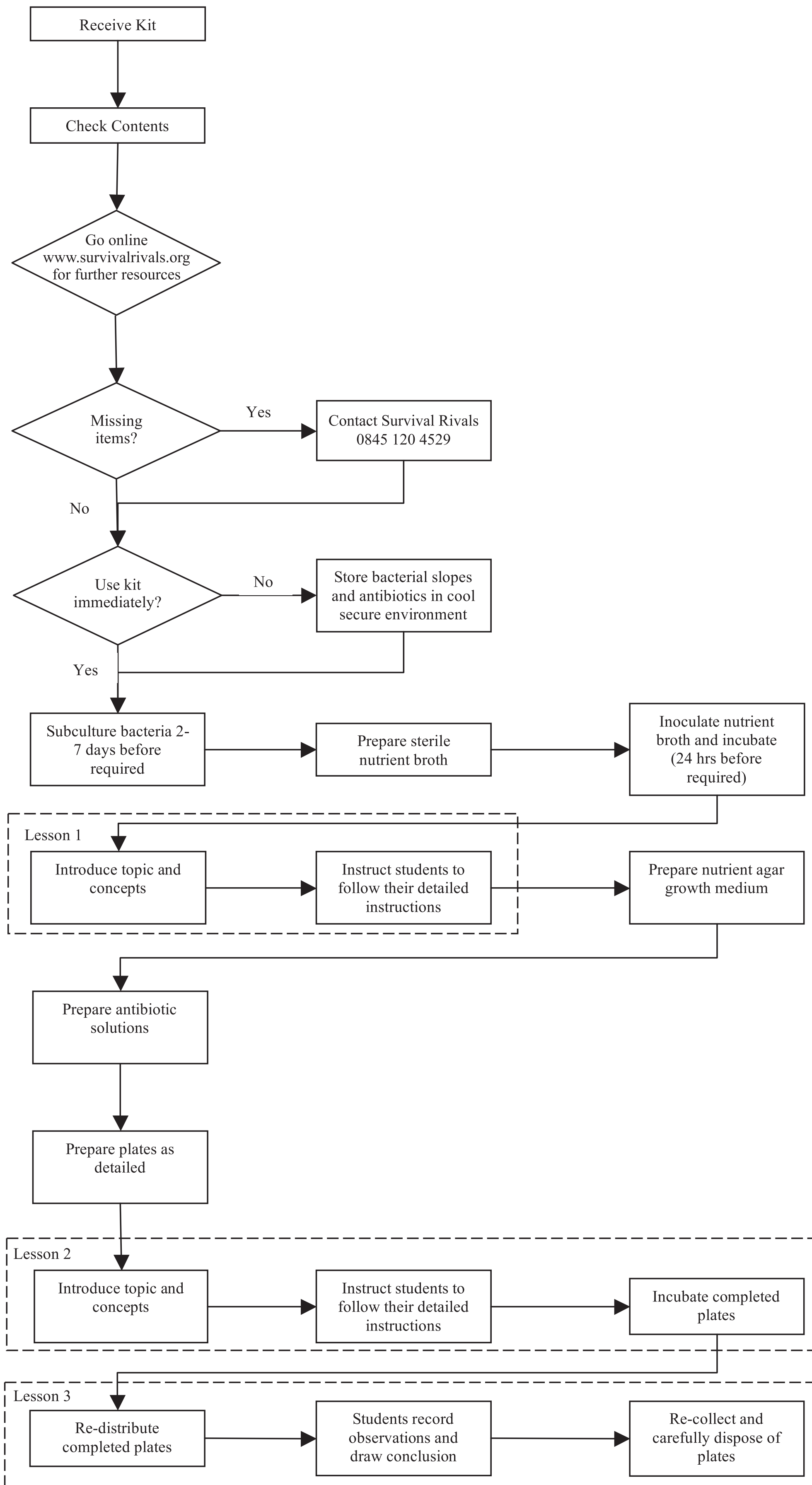
For the class

- An autoclave or pressure cooker
- An incubator, maintained at 30°C
- Aluminum foil
- One or more thermostatically-controlled water baths, set at 50°C
- 500mL flasks, 3 (for preparing growth media)
- Distilled or demineralised water
- Autoclavable disposal bags (at least four, for contaminated paper towels, cultures, plates etc.)
- A gas lighter or matches (for the Bunsens)
- Access to a hand wash basin with soap and paper towels
- OPTIONAL: Adhesive tape for securing the lids to the Petri dishes

For each working group (e.g. for each pair of students)

- A Bunsen burner (Note: heat-resistant mats should not be used, as these can make it difficult to clean up any spills effectively)
- A waste container for holding the VirKon® solution, such a a plastic measuring cylinder
- A waterproof marker pen for labelling the plates
- Tissue paper or paper towels for swabbing the benches

Detailed lesson-by-lesson instructions



Introduction

Shortly after the use of antibiotics became widespread in the 1940s, strains of bacteria began to emerge that were resistant to them. Selection pressures caused by the inappropriate or careless use of antibiotics tend to increase the prevalence of resistant populations of micro-organisms; hence current concerns about hospital-acquired infections, a heightened awareness of the need for cleanliness and attempts to more strictly control the use of antibiotics. Antibiotic resistance is one of the few modern examples of evolution to be found in current school textbooks. The usual examples cited are methicillin-resistant Staphylococcus aureus (MRSA) and strains of Mycobacterium tuberculosis (the usual cause of tuberculosis) that are resistant to antibiotic treatment (the latter is often treated with rifampicin).

Charles Darwin, of course, knew nothing about this: the nature of disease was a mystery to him for most of his life. He worried that his own marriage to his cousin, Emma Wedgwood, might be the cause of a 'hereditary weakness' that had contributed to the death of his beloved daughter Annie. So concerned was he that he tried to have a question on the topic of first cousin marriages included in the National Census of 1871, but the idea was turned down by parliament as an unwarranted breach of privacy. Darwin made his anger clear in the Descent of Man, where he fumed that 'ignorant members of our legislature' had prevented an important, potentially life-saving scientific enquiry.

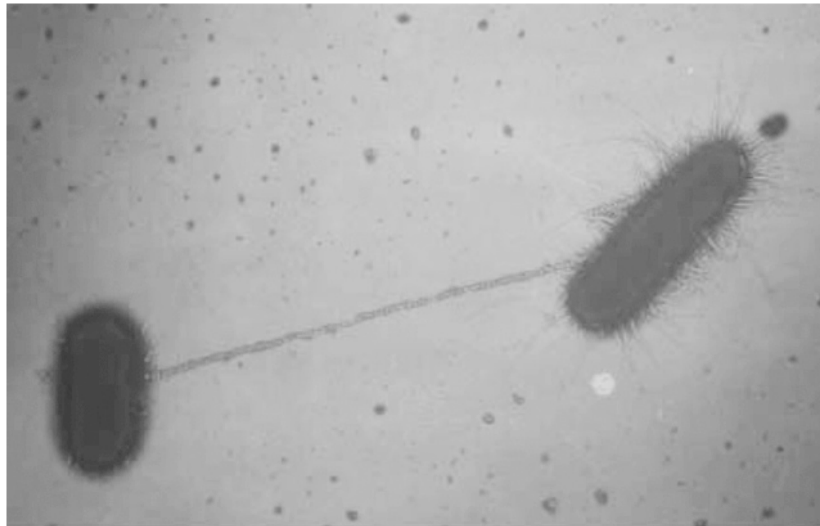
In 1877, as the germ theory of disease was being developed, Ferdinand Cohn, a friend at the University of Breslau (now Worcław in Poland) sent him one of the first photographs of a micro-organism. Charles replied: "I well remember saying to myself between twenty and thirty years ago, that if ever the origin of any infectious disease could be proved, it would be the greatest triumph to Science; and now I rejoice to have seen that triumph". It was twenty-six years since Annie had died. The photograph had been taken by Robert Koch, who was to identify the cause of tuberculosis five years later, less than a month before Darwin died. The evidence suggests that Annie had died from tuberculosis. [1]

Darwin assumed that hereditary characteristics were always passed from one generation to the next, 'vertically'. He also thought that evolutionary change occurred only slowly and by the gradual accumulation of mutations. This is often the case with micro-organisms — genes conferring resistance to antibiotics are sometimes located on the bacterial chromosome as in, for example, the antibiotic resistance in MRSA. Recent research has cast light upon how such resistance can arise and be maintained through the selective pressure of antibiotic treatment in a medical context [2]. Genes that remain in the bacterial chromosome are transferred vertically ('down the generations') and not between bacterial strains or species. Often, however, microbial genes are transferred 'horizontally', that is, from one bacterial strain or species to another.

There are three principal modes of horizontal gene transfer: transformation, in which 'naked' DNA is picked up by cells; transduction, in which the transfer is mediated by a virus and conjugation, in which DNA is transferred between cells via a special 'sex pilus' or tube.

Please refer to The X-Bacteria interactive on the Survival Rivals website which shows conjugation www.survivalrivals.org/the-x-bacteria/animation.

Transformation is probably the most common mechanism of horizontal gene transfer in nature; many bacteria are naturally 'competent', that is, able to pick up DNA from their environment. However, the fate of this DNA once it has entered the cell varies — often it is degraded by enzymes or is not functional. In contrast, transduction is a central, if not the most important, gene transfer mechanism in the generation of genomic diversity and bacterial evolution. Conjugation is also relatively common. Here the DNA is transferred in the form of a plasmid. Plasmids are small independently-replicating rings of DNA, typically carrying a handful of non-essential genes — these are often genes which impart the ability to withstand antibiotic action. Conjugation is therefore an important means by which antibiotic resistance is spread between bacteria. The transferred genes may subsequently be incorporated into the bacterial chromosome, as has happened for instance with resistance to antibiotics such as tetracycline and streptomycin.



E. coli in the process of conjugation

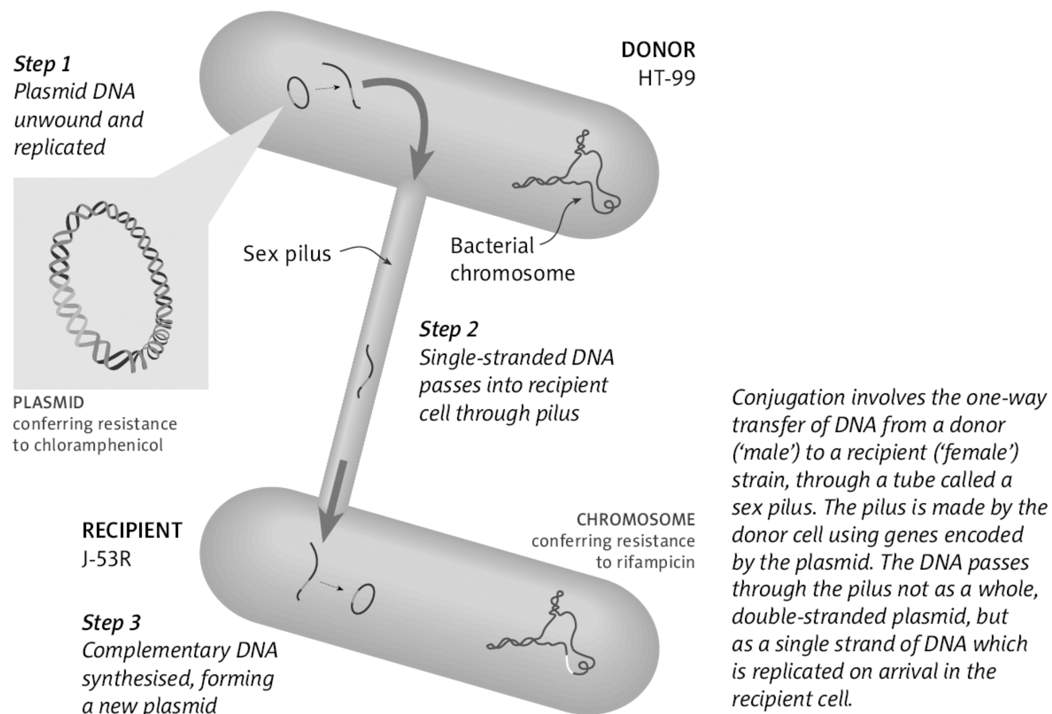
Plasmid-mediated evolution is fast because whole functional 'modules' are lost and gained, rather than the gradual process of mutation and selection that is more commonly encountered in the chromosomal DNA [3]. These are mechanisms of evolution that Charles Darwin did not anticipate.
Microbial evolution is now known to be very different from the tree-like, branching phylogeny that was proposed by Darwin [4]. Studies of horizontal gene transfer provide evidence for the exchange of genetic information within bacterial species and also between distant taxonomic groups and even across kingdom borders [5].

References

[1] *Annie's box: Charles Darwin, his daughter and human evolution* by Randal Keynes (2001) London: Fourth Estate. ISBN: 978 1841150604.
[2] Mwangi, M. et al (2007) Tracking the *in vitro* evolution of multidrug resistance in Staphylococcus aureus by whole-genome sequencing. *PNAS* 104: 9451–9456.
[3] *The biology of plasmids* by David Summers (1996) Oxford: Blackwell Science. ISBN: 0 632 03436 X.
[4] Kunin, V. *et al* (2005) The net of life: Reconstructing the microbial phylogenetic network. Genome Research 15: 954–959.
[5] *A review of gene transfer from genetically-modified micro-organisms* by T.M. Timms-Wilson, A. K. Lilley and M.J. Bailey (1999) Health and Safety Executive Contract Research Report 221/1999. Norwich: HSE Books. ISBN: 0 1776 2445 5.

Outline of the practical activity

In this practical protocol students investigate one way in which bacteria acquire antibiotic resistance through conjugation. The recipient strain *Escherichia coli* J-53R carries on its chromosome a gene conferring resistance to the antibiotic rifampicin. The donor strain, *E. coli* HT-99, harbours a plasmid that includes a gene conferring resistance to a second antibiotic, chloramphenicol. Liquid cultures of the two strains of bacteria are 'mated'. The recipient, donor and 'mated' cells are then plated on three different types of media: one containing rifampicin, one containing chloramphenicol and a one containing both antibiotics. After incubation, students interpret the results and they should find that antibiotic resistance has been transferred from one strain of *E. coli* to the other.



Conjugation involves the one-way transfer of DNA from a donor ('male') to a recipient ('female') strain, through a tube called a sex pilus. The pilus is made by the donor cell using genes encoded by the plasmid. The DNA passes through the pilus not as a whole, double-stranded plasmid, but as a single strand of DNA which is replicated on arrival in the recipient cell.

Who is this practical work suitable for?

Because of the manipulative skills required and the scientific concepts involved, this practical work is in general best-suited to students over the age of 16 years.

Good microbiology laboratory practice is essential to the success and safe execution of this practical activity. Teachers carrying out the work should therefore be trained in the use of aseptic techniques, and should teach these to the students. Non-specialist teachers (e.g. those without a biological background who may be teaching science) should not carry out or supervise this work. Also bear in mind that without specialist knowledge, non-biology teachers may not be able to adequately evaluate or explain the hazards associated with the use of *E. coli* and antibiotics to students and others who may be concerned about these aspects of the work.

Laboratory technicians must also have appropriate microbiology training to prepare the microbiological media and cultures and to dispose of them safely.

Protective clothing

Students, teachers and technicians undertaking this work must wear laboratory coats. Protective glasses should be worn if appropriate e.g. when diluting disinfectants such as VirKon®. Disposable gloves should not generally be worn as they are unnecessary and can be hazardous e.g. if they catch fire or interfere with manipulation (an exception might be if the person involved has cuts or scratches on their hands which cannot be adequately covered by plasters).

Planning and classroom organisation

When you receive the kit

When you receive the kit, check that everything is in the box. If you find that items are missing, please contact Survival Rivals on **0845 120 4529** immediately.

The two slope cultures of *E. coli* will have been prepared some weeks before you received them, so it is a wise precaution to subculture these into fresh nutrient agar up to a week before you plan to carry out the practical lessons. This will ensure that the cells are actively-growing and if you inoculate plates from the slopes, it will enable you to check for contamination (see Microbiology guidelines on page 15).

Slopes and plates of *E. coli* should not be refrigerated but kept at a cool room temperature (10–15°C), preferably in the dark. If you do not plan to carry out the work immediately, you may wish to leave the slope cultures in the box **in a secure place** until you are ready to subculture them.

The dried antibiotic powders can be kept in this form at room temperature. Once you dissolve them, however, they have a limited shelf life and must be stored correctly and used promptly (see below). **Because the antibiotics are potentially harmful, both the powders and solutions must be stored in a secure place**.

It is also a good idea to check at this stage that you have all of the other equipment and materials listed above that you need for the practical work.

The lessons: an overview

This investigation takes place over three lessons and it also presents opportunities for preparatory and follow-up tasks (for example, as homework). Careful planning and advance preparation of the growth media and cultures is essential for success in this practical work; there are many materials to prepare and good technical support and understanding of basic microbiological techniques is vital. Here is a brief overview which may help you to plan the work.

Lesson 1: Mating the bacteria

The first lesson introduces the concept of horizontal gene flow between bacteria by conjugation and some basic aseptic techniques. Students 'mate' two cultures of bacteria and incubate the donor, recipient and 'mated' strains overnight at 30°C. Students can be asked to prepare for this lesson by researching bacterial cell structure and mechanisms of microbial gene transfer. Aseptic techniques are probably best learnt first hand however, by watching a demonstration then having a go. The practical activity for students is relatively short, but this means that there should be time to introduce the topic well and prepare for the following lesson which is more challenging, both intellectually and practically. Technicians will need to prepare fresh bacterial cultures and liquid media in advance of this lesson. **It is *essential* that these overnight cultures are plated out the following day. It may therefore be necessary to ask students to return in their lunch break to do this.**

Lesson 2: Plating out the cultures

In the second lesson students plate out the bacteria on selective (antibiotic-containing) growth media and learn more advanced aseptic techniques. This also introduces the concept of 'controls' in scientific investigations. Depending upon the ability of the students, they can be asked in advance to plan the 'controls' that might be required in the investigation. They can also be asked to find information about the two antibiotics involved (rifampicin and chloramphenicol) and the topic of antibiotic resistance in general. There are several video recordings showing standard aseptic techniques available on-line (see Additional resources), but once again such techniques are best taught practically. Technicians will need to prepare antibiotic-containing plates for this lesson and have the students' previously-incubated liquid cultures to hand. The plates should be incubated for 24 hours at 30°C, but after that they can be refrigerated and kept for up to a fortnight. This gives greater flexibility in lesson timing should this be required.

Lesson 3: Interpreting the results

In the third lesson, students examine and interpret the results. This should not take very long. Students may wish to photograph the plates so that they have a permanent record of their work. Here there is an opportunity to discuss the emergence of antibiotic resistance in natural bacterial populations, the heath care and social consequences of such resistance, and the ways in which microbial evolution, where genes are passed both 'vertically' and 'horizontally', differs from that of multicellular organisms. It is also a good time to emphasise the fact that all of the cultures will be destroyed after use by autoclaving, to ensure that no contribution is made to the spread of antibiotic resistance.

Group size

The kit has been designed with students working in groups of three in mind. This is because there are three treatments and consequently three cultures to be prepared in the second lesson. The work has also been carried out successfully by students working in pairs. We do not recommend that students work individually (which could be very time-consuming) or in larger groups (in which at least one student would just be an observer).

Scope for open-ended investigations

The kit has been designed for use as a whole-class activity and for safety and other practical reasons the investigation is relatively prescriptive. If, however, you have a suitable small class and sufficient time is available, students may also wish to investigate the effects of varying factors such as incubation time, temperature, the proportions of 'donor' and 'host' strains, agitation etc on the efficiency of conjugation. Students may be asked to consider how the investigation could be made quantitative rather than qualitative. You may wish to challenge the students to devise the appropriate treatments and 'controls' for Lesson 2.

Lesson 1: Mating the bacteria

Preparation of cultures (this information is also included in the Technician's notes)

For successful conjugation, the bacteria must be in an active stage of growth. If cultures are taken straight from the slopes provided in the kit, they are highly unlikely to be at the correct stage of growth. Therefore bacteria from the slopes in the kit should first be streaked onto nutrient agar plates (without any antibiotics) and incubated at 30°C for 24 hours. Individual colonies should then be picked off the plates and used to inoculate the nutrient broth cultures that are needed by the students. The broth cultures should be incubated overnight, starting the day before the cultures are needed by the students. You will therefore need to start preparing the cultures for this work at least two days before the first lesson. A wise precaution is to prepare two plates of each culture from the slopes so that if one does not grow well, or becomes contaminated, you will have a second in reserve. There are sufficient Petri dishes and nutrient agar in the kit for you do this.

Each working group of students (we suggest that they work in groups of three) will need about 10mL of each of the two bacterial cultures in nutrient broth (in fact, the students will need less than 10mL of each culture, but smaller volumes are hard to autoclave and dispense).

If you have ten working groups (that is, 30 students) it will therefore be necessary to prepare 20 x 10mL bottles of sterile nutrient broth and to inoculate half of them with *E. coli* J-53R and the rest with *E. coli* HT-99. The inoculated broth should then be incubated overnight at 30°C.
Note that each group of students will also need 10mL of uninoculated sterile nutrient broth — this can be prepared at the same time as the two cultures.

The lesson

The teacher should explain the process of conjugation and its significance. Students can prepare for this in advance of the lesson (e.g. for homework) then the teacher need only provide a reminder at the start of the lesson if time is limited. The necessity of aseptic techniques should be explained and these techniques must be demonstrated before the students prepare the mixed cultures. Preparing the cultures should take at most 30 minutes, so the entire lesson should fit comfortably within a 50–60-minute period.

Equipment and materials

Required by each student or working group

- Overnight culture of *E. coli* J-53R, 10mL
- Overnight culture of *E. coli* HT-99, 10mL
- Bottle containing 10mL of sterile nutrient broth
- Sterile 1mL syringes (without needles), 2
- Waste container of disinfectant (VirKon® solution) (Note: A measuring cylinder may be used as a waste container.)
- Bunsen burner
- Waterproof marker pen

Additional equipment required by the class

- Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

Students' procedure (these instructions are also included in the Students' notes)

1. Wash your hands with soap and water.
2. Wipe down the bench surface with disinfectant (VirKon® solution).
3. There should be a lit Bunsen burner on the bench near where you are working to create an upward flow of warm air, to carry away potentially contaminating micro-organisms.
4. Label the bottle containing sterile nutrient broth: 'Mating'.
5. Open a sterile syringe at the end of the packet furthest from the tip, taking care not to touch the barrel of the syringe or the tip. Use the syringe to aseptically remove 1.8mL of an overnight culture of *E. coli* J-53R and transfer it to the nutrient broth in the bottle you have just labelled. (Since you have a 1mL syringe, you will need to transfer 2 x 0.9mL.)
6. Discard the used syringe into the waste container of disinfectant (VirKon® solution). (Note: Discarded syringes should be completely immersed in the disinfectant and left for 24 hours before disposal in the normal waste. The disinfectant solution should be discarded after use.)
7. Taking care as before, use a new sterile syringe to aseptically transfer 0.2mL of the *E. coli* HT-99 culture to the nutrient broth.
8. Discard the second syringe into the waste container of disinfectant.
9. Place the mixed culture and the other two cultures in an incubator at 30°C (where they should be left for 4–16 hours).
10. Wipe down the bench with disinfectant.
11. Wash your hands with soap and water.

Lesson 2: Plating out the cultures

Preparation of antibiotic plates (this information is also included in the Technician's notes)
These plates should be prepared in advance by the teacher or technician for the whole class. Each student or working group will require three plates:

- one with nutrient agar + rifampicin;
- one with nutrient agar + chloramphenicol; and
- one with nutrient agar + both antibiotics.

The quantities given here are sufficient for 12 sets of plates (36 plates), allowing ~17–20mL of medium per plate. This number provides two extra plates of each medium in case of accidents, etc.

If you have a smaller class or wish students to work in smaller or larger teams, you will need to adjust the number of plates you prepare accordingly.

Preparing the nutrient agar growth media

1. Prepare three separate batches of nutrient agar by adding 7 g of nutrient agar powder to each of three 500mL conical flasks While stirring, to one flask, add 200mL of distilled or deionised water; two each of the others, add 225mL of distilled or deionised water.
2. Cover the tops of the flasks with aluminium foil, then autoclave them at 121°C for 15–20 minutes.
3. While the liquid is being autoclaved, the nutrient agar may sink to the bottom of the flasks. Therefore you should swirl the flasks to mix the nutrient agar after autoclaving. Let the flasks cool to 55–60°C (that is, until the flasks can be held comfortably in your hands). While the medium is cooling, label the bases of the sterile Petri dishes: one third with 'NA + R', third with 'NA + C' and a third with 'NA + RC'.Keep the nutrient agar molten by standing the flasks in a water bath at ~50°C. *Note: if the agar begins to solidify, it can be remelted by autoclaving again for no more than 5 minutes.*

Handling the antibiotics

- Wear eye protection and protective clothing when handling the antibiotics.
- Do not allow the antibiotic powder or solutions to come into contact with your skin or eyes.
- Do not inhale the antibiotic powder.

For additional precautions and first aid instructions, please refer to the material safety data sheets provided in the kit.

The chloramphenicol and rifampicin powders will need to be dissolved before they are used. The final concentrations required in the growth media are:

Chloramphenicol: 25 µg per mL
Rifampicin: 100 µg per mL

Because the antibiotic solutions are heat-labile, powders rather than solutions have been provided the kit. Most schools do not have facilities for weighing out very small quantities of these powders, so small pre-weighed amounts to which the solvent can be added have been supplied.
Although when they are dissolved in methanol and protected from the light (by wrapping the bottles in aluminium foil) the antibiotics can be stored in a fridge for up to a week, once dissolved in water they will deteriorate rapidly. *You should therefore plan this preparation carefully and only make up the antibiotic solutions when you need them.*
The amounts provided in the kit make up far more antibiotic solution than is required. Unused antibiotics should be disposed of with care. *Any unused antibiotic solution must be autoclaved to denature it before disposal down the sink with plenty of tap water.*

Preparation of Antibiotic solutions

The enclosed vials contain :- 100mg Rifampicin & 25mg Chloramphenicol respectively.

Do not allow the contents into contact with your skin or eyes. Do not inhale dust. Wear appropriate protective equipment and clothing!

- Tap each vial gently to move the contents into the base of the vial before opening.

- Remove lids and add 5ml of Methanol to each vial using a syringe. Replace lids and agitate vials until contents dissolve to give a clear solution. (partial immersion in a water bath at 40-45˚C may aid this procedure).

- You have been provided with Sterile water in 100ml sealed bottles. In the centre of the aluminium bottle cap is a 10mm removable disc. Carefully remove this using a fingernail or screwdriver blade, to expose the grey rubber septum beneath it.

For each Antibiotic in turn:-
- Take a 5ml sterile syringe, and carefully attach a sterile needle (take care not to touch the needle directly with your fingers!). Depress the syringe plunger to expel all air, and then fill the syringe with the contents of the vial holding the Antibiotic solution in Methanol.

- Plunge the syringe needle through the grey rubber septum cap of the Sterile Water, and slowly depress the syringe plunger to inject the contents into the bottle. This is best done with the bottle in an upright position, and the syringe vertically above it.

- When all the liquid has transferred, carefully remove the syringe and swirl the bottle to mix the contents.

- Label the bottles ‘Rifampicin Solution’ & ‘Chloramphenicol Solution’.

- These solutions are best used within a few hours of preparation. It is, however, good practice to warm them, by partial immersion of the bottles in a 50˚C water bath for 5mins (maximum!), before adding to your Nutrient Agar.

Preparing the Nutrient Agar plates

Wash your hands and wipe down the bench with disinfectant. There should be a lit Bunsen burner on the bench near to where you are working to create an upward flow of warm air, to carry away potentially contaminating microorganisms. Ensure that the Methanol / Antibiotic solutions are not placed near the flame!

- Take a 30ml sterile syringe, and carefully attach a sterile needle (take care not to touch the needle with your fingers!). Depress the syringe plunger to expel all air, and then insert into the Sterile Water bottle (now holding Antibiotic solution). With the bottle upside down, and the needle tip within the contents – slowly pull back the syringe plunger to transfer 25ml of solution to the syringe.

- Restore the bottle to an upright position and remove the syringe. (you may choose to retain the needle in the septum, as the process will need to be repeated!)

Flask 1, labelled 'NA + R' (containing 225 mL of Agar medium)
Transfer 25 mL of Rifampicin solution from the syringe to the nutrient agar

Flask 2, labelled 'NA + C' (containing 225 mL of Agar medium)
Transfer 25 mL of Chloramphenicol solution to the nutrient agar

Flask 3, labelled 'NA + RC' (containing 200 mL of Agar medium)
Add 25 mL of rifampicin solution and 25mL of Chloramphenicol solution to the nutrient agar

- After you have added the antibiotic solutions to the agar, swirl them to mix. *Important! Do not allow the bottles to cool excessively,as the agar will set. Return the media to the waterbath as necessary to ensure that it remains molten. (If the agar sets, it can only be melted again by boiling it, which will destroy the antibiotics.)*
- Arrange the Petri dishes on the bench in three groups, ready to pour the medium into them.
- Lift the lid of each Petri dish in turn, just enough to pour the nutrient agar in. Do not put the lid down on the bench. Quickly add enough agar medium to cover the bottom of the plate to a depth of about 3 mm (17–20mL). Replace the lid then tilt the plate to spread the medium evenly over the Petri dish.
- Continue pouring the plates, using the appropriate medium for each. As you do so, flame the mouth of the flask occasionally to maintain sterility.
- To remove any bubbles from the surface of the poured nutrient agar, *very briefly* touch the surface with a Bunsen burner flame while the medium is still molten.
- Leave the medium to set, undisturbed, for about 15 minutes. If time permits, invert the plates and incubate them for several hours or overnight at 30°C. This dries the medium, limiting condensation; it also allows any contaminated plates to be detected.
- Stack the plates in their original plastic sleeves and store them at room temperature.
- Wipe down the bench with disinfectant (*VirKon*® solution).
- Wash your hands with soap and water.

Although the prepared plates can be kept for up to a week in a refrigerator at 3–5°C, we advise that you do not do this. Plates stored for any length of time in a fridge will accumulate condensation and be difficult to use. It is better to try to make the plates the day before you need them.

The lesson

The aseptic techniques required in this lesson are slightly different to and build upon those used in the previous one. These techniques will need to be explained and demonstrated by the teacher (perhaps by now, however, the students will be able to offer explanations for the procedures as they are shown).

The teacher may need to briefly recapitulate on the process of conjugation and its role in the spread of antibiotic resistance and discuss with students what they might expect to see on the various plates after incubation. Again this work should fit comfortably within a 50–60 minute period.

An important constraint here is that the broth cultures must be prepared no more than 24 hours before the lesson. If it proves

difficult to arrange for students to do this, it could be done for them by a teacher or technician, but ideally students should experience setting this up too.

Equipment and materials
Required by each student or working group

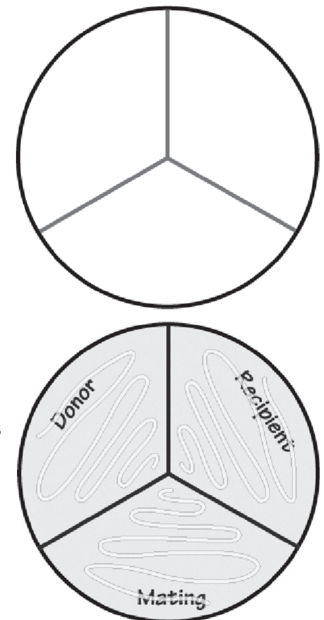
- Plate of nutrient agar containing rifampicin
- Plate of nutrient agar containing chloramphenicol
- Plate of nutrient agar containing rifampicin and chloramphenicol
- The three microbial cultures from the previous lesson
- Waste container of disinfectant (VirKon® solution)
- Sterile disposable loops, 3
- Bunsen burner
- Waterproof marker pen

Additional equipment required by the class

- Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

Students' procedure (these instructions are also included in the Students' notes)

1. Wash your hands with soap and water.
2. Wipe down the bench with disinfectant (VirKon® solution).
3. Turn over each plate so that the base is uppermost. With the marker pen, divide each plate into three segments as shown in the diagram. Label the top two segments 'Donor' [HT-99] and 'Recipient' [J-53R] and the third segment as 'Mating'.
4. Arrange the three plates on the bench in front of you. Use a new sterile loop to aseptically streak each segment of each of the plates with culture from the appropriate bottle. Use a new loop for each of the different cultures, and dispose of the loops into the disinfectant solution as you use them.
5. When the agar has absorbed any excess liquid, invert the plates and incubate them for 24 hours at 30°C.
6. Wipe down the bench with disinfectant (VirKon® solution).
7. Wash your hands with soap and water.



Lesson 3: Interpreting the results

After incubation, the plates can be stored in a fridge and examined at any time up to a fortnight later. This should make timetabling more convenient for schools. A suitable worksheet (e.g. with a chart) could be prepared for students to use when interpreting their results.

Specimen results

- Since the donor strain *E. coli* HT-99 is resistant to chloramphenicol and sensitive to rifampicin, it should be able to grow in the presence of the former antibiotic but not in the presence of the latter.
- Conversely, the recipient strain *E. coli* J-53R is resistant to rifampicin and sensitive to chloramphenicol, so it should be able to grow in the presence of the former but not the latter antibiotic.
- The 'mated' cultures should be able to grow in the presence of both antibiotics, indicating that gene transfer has occurred by conjugation.

Disposal of cultures (this information is also included in the Technician's notes; see the Microbiology guidelines below also)

It is very important to dispose of all the materials used in a practical class properly, especially cultures of antibiotic-resistant bacteria. All containers used for storing and growing cultures must be autoclaved after use to ensure that all micro-organisms are killed.

Two autoclave bags should be available in the laboratory: one for reusable glassware and another for disposable materials. There should be a waste discard jar with disinfectant solution (*VirKon*®) near each work area for items such as disposable loops and waste liquid cultures. A bucket should be available in the laboratory or prep room for disposal of any broken glassware.

After use, disinfectant in the waste jars should be poured away and the items in it should be disposed of. Note that contaminated items should be left fully immersed in the disinfectant for at least 24 hours. Contaminated paper towels, cloths and plastic Petri dishes should be put into the autoclave bag reserved for disposable items. Any contaminated glassware should be put into the autoclave bag for glassware.

Glassware that is not contaminated can be washed normally. Broken glassware should be put in a waste bin reserved exclusively for that purpose. If the glassware is contaminated it must be autoclaved before disposal. Uncontaminated broken glassware can, of course, be disposed of immediately.

Microbiology guidelines

Use of *Escherichia coli*

All micro-organisms should be regarded as potentially harmful. However, the strains of the bacterium *E. coli* used in the current investigation present minimum risk given good practice. These laboratory strains should not be confused with the highly-pathogenic *E. coli* Ø157:H7, other pathogenic strains or the normal 'wild type' *E. coli* that is found in the human gut.

Maintenance and storage of cultures

In schools, microbial cultures are often maintained on agar slopes. These should be transferred onto fresh medium every 8–12 weeks or so, and incubated until the organisms have grown. They should then be stored in a cool (10–15°C), dark place, *not in a refrigerator*. New cultures should be obtained regularly, as repeated sub-culturing can lead to contamination or genetic changes in the cells.

Good microbiological practice

General precautions

- Any exposed cuts or abrasions should be protected with waterproof dressings before the practical work starts.
- Everyone involved — teachers, technicians and students — should always wash their hands before and after practical work.
- The laboratory door and windows should be closed when work is in progress. This will reduce air movements and consequently the risk of accidental contamination of plates, etc.
- Laboratory coats must be worn, and where necessary (e.g. when heating liquids), eye protection.
- High standards of cleanliness must be maintained. Non-porous work surfaces should be used and they should be swabbed with an appropriate laboratory disinfectant before and after each practical session.
- To reduce the risk of ingesting microbes, no hand-to-mouth operations should occur (e.g. chewing pencils, licking labels, mouth pipetting). For the same reason, eating, drinking and smoking must not be allowed in the laboratory.

Spills and breakages

Accidents involving cultures should be dealt with as follows:

- Disposable gloves should be worn.
- The broken container and/or spilt culture should be covered with a cloth soaked in disinfectant.
- After not less than 10 minutes, it must be cleared away using paper towels and a dustpan.
- The contaminated material must be placed in an infected waste container or disposal bag. This must be autoclaved before disposal.
- The dustpan should also be autoclaved or placed in a suitable solution of disinfectant for 24 hours.

Contamination of skin or clothing

As soon as possible, anyone who has been splashed should wash. Severely contaminated clothing should be placed in disinfectant before it is laundered. Contaminated cleaning cloths should be autoclaved or soaked in disinfectant.

Aseptic techniques

The aims of aseptic techniques are:

- To obtain and maintain pure cultures of micro-organisms;
- To make working with micro-organisms safer.

A ‘pure culture’ contains only one species of micro-organism, whereas a ‘mixed culture’ contains two or more species.

Contamination of cultures is always a threat because microbes are found everywhere; on the skin, in the air and on work surfaces and equipment. To obtain a pure culture, sterile growth media and equipment must therefore be used and contaminants must be excluded. These are the main principles of aseptic techniques.

Growth media must be sterilised before use — usually by autoclaving. Sterile containers (flasks, Petri dishes, etc.) should be used. Lids must be kept on these containers to prevent contamination.

It is essential to prepare the work area carefully before you start. The bench surface should be swabbed thoroughly with a suitable disinfectant (e.g. *VirKon®*) and allowed to dry. All necessary equipment and materials should be arranged so that they are readily-at-hand. Work should be done near a lighted Bunsen burner. Rising air currents from the flame will help to carry away any microbes that could contaminate growth media and pure cultures.

When cultures are transferred between containers, tops and lids should not be removed for any longer than necessary. After a lid has been taken from a bottle, it should be kept in your hand until it is put back on the bottle. This stops contamination of the bench and the culture.

A blue flame about 5 cm high should be used for sterilising wire loops and flaming the necks of bottles. After removal of the top, the neck of the culture bottle should be flamed briefly. This will kill any microbes present there and cause convection currents which will help to prevent accidental contamination of the culture from the atmosphere. Bottles should not be heated until they become hot and dangerous to handle.

Sterile, plastic disposable loops are provided in this kit. There is no need to flame these, but they should be opened carefully (at the handle end) immediately before use, used, then discarded immediately into a suitable disinfectant (such as VirKon®). A similar procedure should be adopted with the sterile, plastic disposable syringes.

If you use wire loops, however, these must be heated until they glow red hot along the entire length of the wire part. This should be done both before and after cultures are transferred. Heat the stem of the loop first as it is brought into the Bunsen burner flame, to reduce sputtering and aerosol formation. Allow the loop to cool before you use it to transfer a culture (some people like to cool the loop after flaming it, by touching it briefly onto the agar at the edge of a culture plate).

When the Bunsen burner is not in use, it should be kept on a visible yellow flame.

Incubation

Label the Petri dish around the edge of the base before inoculation. Your name, date and the name and/or source of the organism used will allow the plate and its contents to be identified.

Where appropriate, use self-adhesive tape to seal Petri dishes as shown here:

The seal will ensure that the plates are not accidentally opened or tampered with. Do not seal plates completely round their edges as this could create anaerobic growth conditions within the dish.

Bacterial cultures in Petri dishes should usually be incubated with the base uppermost, so that any condensation that forms falls into the lid and not on the colonies. (If there is heavy condensation in the sterile Petri dish before inoculation, it should be allowed to dry before use.)

The strains of *E. coli* used in this investigation should be incubated at 30°C — this is the maximum incubation temperature usually recommended for use in schools.

Disposal and sterilisation

It is very important to dispose of all the materials used in a practical class properly. All containers used for storing and growing cultures must be autoclaved, then washed and rinsed as necessary, before re-use.

At least two autoclave bags should be available in the laboratory: one for reusable glassware and another for disposable materials. There should be a discard jar near each work area for materials such as waste liquid cultures and pipettes. A bucket should be available for disposal of any uncontaminated broken glassware.

After use, disinfectant in the discard jar should be poured away and the items in it should be autoclaved and disposed of. Contaminated paper towels, cloths and plastic Petri dishes should be put into the autoclave bag reserved for disposable items. Any contaminated glassware should be put into the autoclave bag for glassware.

Glassware that is not contaminated can be washed normally. Broken glassware should be put in a waste bin reserved exclusively for that purpose. If the glassware is contaminated it must be autoclaved before disposal. Uncontaminated broken glassware can, of course, be disposed of immediately.

Autoclaving

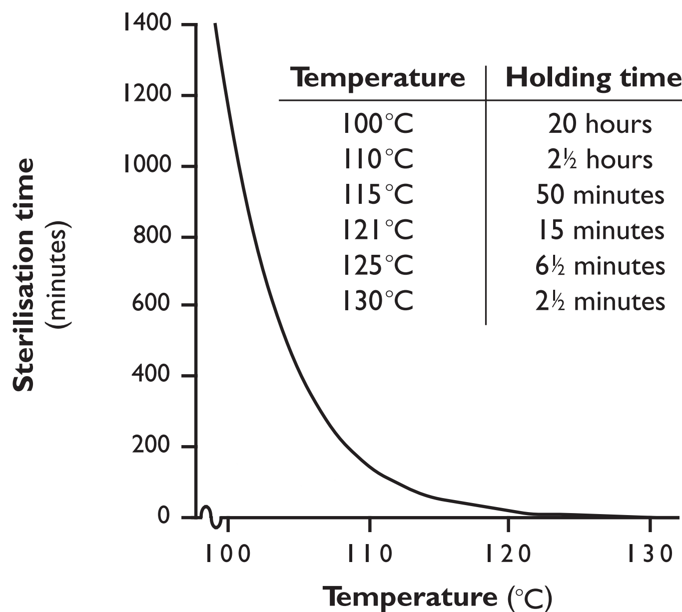
Sterilisation is the complete destruction of all micro-organisms, including their spores. All non-sterile equipment and media should be sterilised before starting practical work so that there are no contaminants. Cultures and any contaminated material should also be sterilised after use for safe disposal.

Autoclaving is the preferred method of sterilisation for culture media, aqueous solutions and discarded cultures. The process uses high pressure steam, usually at 121°C. Microbes are more readily killed by moist heat than dry heat as the steam denatures their proteins. A domestic pressure cooker or a purpose-built autoclave can be used. Domestic pressure cookers can be used in school laboratories but their small capacity can be a disadvantage when dealing with class sets of material.

Principles of autoclaving

Two factors are critical to the effectiveness of the process. Firstly, all air must be driven from the autoclave. This ensures that high temperature steam comes into contact with the surfaces to be sterilised: if air is present the temperature at the same steam pressure is lower. The materials to be sterilised should be packed loosely so that the air can be driven off. Screw-capped bottles and jars must have their lids loosened slightly to allow air to escape and to prevent a dangerous build-up of pressure inside them.

Secondly, sufficient time must be given for heat to penetrate (by conduction) to the centre of media in flasks or other containers. The times for which media or apparatus must be held at various temperatures for sterilisation are shown below:



Temperature	Holding time
100°C	20 hours
110°C	2½ hours
115°C	50 minutes
121°C	15 minutes
125°C	6½ minutes
130°C	2½ minutes

Notice that just a small difference in temperature can result in a great difference in the time required for sterilisation. It is also important that these temperatures are reached by all materials to be sterilised for the specified time e.g. the broth in the very centre of a flask. Three factors determine the duration of the autoclaving process:

- penetration time: the time taken for the innermost part of the autoclave's contents to reach the required temperature (say, 5 minutes);
- holding time: the minimum time in which, at a given temperature, all living organisms will be killed (say, 15 minutes);
- safety margin: roughly half the holding time (about 5 minutes).

Most domestic pressure cookers and autoclaves operate at 121°C. This gives a total autoclaving period of around 25 minutes. For larger volumes of liquid, such as 1 litre of growth medium in a flask, the holding time should be increased to 20–25 minutes.

The effectiveness of an autoclave can be checked by using autoclave test strips which change colour if the process has worked properly (autoclave tape, which also changes colour after heating, does not show this).

Use and routine care of autoclaves

Different autoclaves and pressure cookers will have different operating instructions and it is important that the manufacturer's instructions are always followed. Care should be taken to ensure that there is enough water in the autoclave so that it does not boil dry during operation. A domestic pressure cooker requires at least 250mL of water — larger autoclaves may need much greater volumes. The use of distilled or deionised water in the autoclave will prevent the build-up of limescale or corrosion of the metal pressure vessel. Autoclaves should be dried carefully before storage to prevent damage to the vessel.

When the autoclave is used, steam should be allowed to flow freely from it for about one minute to drive off all the air inside. Only then should the exit valve be tightened. After the autoclave cycle is complete, sufficient time must be allowed for the contents to cool and return to normal atmospheric pressure. Premature release of the lid and the subsequent reduction in pressure will cause any liquid inside the autoclave to boil. Therefore the vessel or valve must not be opened whilst under pressure as this may cause scalding and the agar or broth will froth up and may boil over the outside of the containers within.

Chemical sterilisation

WARNING! Eye protection should be worn when dispensing concentrated disinfectant solutions.

Many different chemicals are used for sterilisation of used equipment and work surfaces. Some disinfectants, such as

MicroSol 3+ and VirKon® can be safely used for most laboratory purposes and are recommended for use in schools. Others have specialist uses. The manufacturer's and supplier's instructions should always be followed with care.

Disposable plastic items

Modern microbiology makes extensive use of disposable plastic items, such as Petri dishes, single-use pipettes and loops. Although they can appear wasteful, we strongly recommend their use as they are inexpensive, save on preparation time and enhance safety by reducing the risk of contamination. After use, such items can be sterilised and disposed of in the normal waste.

Additional information

Antibiotic resistance

There are numerous web sites with high-quality information on the topic of antibiotic resistance. Here are two typical examples:

- PBS (USA) Evolution of antibiotic resistance (TV programme) www.pbs.org/wgbh/evolution/library/10/4/1_104_03.html
- UK Department of Health www.dh.gov.uk/en/Publichealth/Patientsafely/Antibioticresistance/index.html

Microbiology safety

- *Safety in science education* Department for Education and Employment (1996) Her Majesty's Stationery Office. ISBN: 011 270915 X.
- *Topics in safety* Association for Science Education (2001) [Third Edition] Association for Science Education ISBN: 0 86357 316 9.
- *Basic practical microbiology:* A manual by Dariel Burdass (2003) Society for General Microbiology ISBN: 978 0953683833. [Available from the SGM — see address below.]
- Videos about aseptic technique are available at www.sci-eng.mmu.ac.uk/intheloop

Health and Safety advice

- Teachers and technicians of member schools may contact CLEAPSS (for England, Wales and Northern Ireland) and SSERC (for Scotland).
- CLEAPSS, The Gardiner Building, Brunel Science Park, Kingston Lane, Uxbridge UB8 3PQ
  T: 01895 251496 I F: 01895 814372 I W: www.cleapss.org.uk
- SSERC, 2 Pitreavie Court, South Pitreavie Business Park, Dunfermline KY11 8UB
  T: 01383 626070 I F: 01383 842793 I W: www.sserc.org.uk
- *The Society for General Microbiology (SGM) and the Microbiology in Schools Advisory Committee (*MISAC) (which can be contacted via the SGM) are also useful sources of advice and information.
  SGM, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG
  T: 0118 988 1800 I F: 0118 988 5656 I W: www.sgm.ac.uk
  The SGM also has a web site dedicated to school microbiology: www.microbiologyonline.org.uk

Curriculum Links

The threat to health and the economic costs posed by antibiotic-resistant pathogens are great and education has the potential to contribute towards the reduction of this threat. Because of the rapidly-emerging nature of biomedical science, most adults will find it necessary to learn about new concepts through informal learning, but many are not well equipped by their school experience to do so. The topic of antibiotic resistance features in the pre-16 science curriculum, often in the context of evolution: in fact, it is the only modern example of evolution mentioned in current GCSE textbooks. The links between evolution and genetics are seldom made explicit, however. For example, two current (recently revised) GCSE textbooks mention the spread of antibiotic resistance in their chapters on evolution, although the mechanism is not explained and only one book refers to the involvement of genes in this phenomenon. Post-16, both the specifications and the textbooks are more up-to-date and all the foundations are there: prokaryotic cell structure, the rudiments of bacterial genetics, the role of selection in evolution and even the topic of antibiotic resistance itself. This practical protocol should help students to bring together these disparate elements of the biology curriculum in a modern and important context. It provides opportunities for understanding 'how science works' and for discussing the wider health and social impact of medical science.

This practical work therefore covers and augments much of the knowledge, concepts and skills required in post-16 biology. Key elements of the relevant specifications from awarding bodies in England, Wales and Northern Ireland are listed below. Although only one specification mentions bacterial conjugation explicitly, almost all include antibiotic resistance and all cover genetic modification, which this work could link to (bearing in mind, however, the difference between conjugation and artificial transformation).

England

AQA BIOLOGY AS Level

Knowledge and understanding

Unit 1
- Pathogens include bacteria, viruses and fungi.
- The structure of prokaryotic cells to include cell wall, plasma membrane, capsule, circular DNA, flagella and plasmid.

Unit 2
- Variation exists between members of a species.
- Similarities and differences between individuals within a species may be the result of genetic factors, differences in environmental factors, or a combination of both.
- Genes are sections of DNA that contain coded information as a specific sequence of bases. Genes code for polypeptides that determine the nature and development of organisms.
- In prokaryotes, DNA molecules are smaller [than they are in eukaryotes], circular and are not associated with proteins.
- Similarities and differences between organisms may be defined in terms of variation in DNA. Differences in DNA lead to genetic diversity.
- Antibiotics may be used to treat bacterial disease.
- DNA is the genetic material in bacteria as well as in most other organisms.
- Mutations are changes in DNA and result in different characteristics.
- Mutations in bacteria may result in resistance to antibiotics.
- Resistance to antibiotics may be passed to subsequent generations by vertical gene transmission.
- Resistance may also be passed from one species to another when DNA is transferred during conjugation. This is horizontal gene transmission.
- Antibiotic resistance in terms of the difficulty of treating tuberculosis and MRSA.

  Candidates should be able to:
- analyse, interpret and evaluate data concerning early experimental work relating to the role and importance of DNA.

- apply the concepts of adaptation and selection to other examples [that is, examples in addition to antibiotic resistance in bacteria].
- evaluate methodology, evidence and data relating to antibiotic resistance.
- discuss ethical issues associated with the use of antibiotics.
- discuss the ways in which society uses scientific knowledge relating to antibiotic resistance to inform decision-making.

Investigative and practical skills

Unit 2
Candidates are required to:
- use their knowledge and understanding to pose scientific questions and define scientific problems.
- carry out investigative activities, including appropriate risk management, in a range of contexts.
- analyse and interpret data they have collected to provide evidence.
- evaluate their methodology, evidence and data, resolving conflicting evidence.

Unit 3
Candidates should be able to:
- show full regard for safety and the ethical issues involved with the well-being of living organisms and the environment.
- carry out an investigation in a methodical and organised way demonstrating competence in the required manipulative skills and efficiency in managing time.

AQA HUMAN BIOLOGY AS/A2 Level

Knowledge and understanding

Unit 1
- Bacteria may cause infectious diseases.
- The structure of a prokaryotic cell, and the functions of its organelles: restricted to cell wall, plasma membrane, genetic material, plasmid, capsule, ribosome and flagellum.
- Antibiotics can be used to treat bacterial disease by interfering with bacterial metabolism: limited to prevention of cell wall synthesis and protein production.
- When provided with appropriate information, candidates should be able to evaluate the evidence for the links between use of antibiotics and the development of MRSA and other antibiotic resistant bacteria.

Unit 2
- DNA as genetic material.
- Individuals within a population may show a wide range of variation.
- Predation, disease and competition result in differential survival and reproduction.
- Those organisms with a selective advantage are more likely to survive, reproduce and pass on their alleles to the next generation.
- Candidates should be able to analyse and interpret experimental evidence that DNA is the genetic material.
- Candidates should be able to interpret data and use unfamiliar information to explain how natural selection may produce change within a population.

Unit 5
- Humans have introduced large amounts of antibacterial agents into the environment of bacteria.
- Evolution of resistance to antibacterial agents.
- When provided with appropriate information, candidates should be able to evaluate evidence relating to the impact of the widespread use of antibacterial agents.

Investigative and practical skills

Unit 1

Candidates will be expected to have carried out practical investigations in the following areas:

- investigations involving sterile technique and bacterial growth on agar plates.

Unit 3

Candidates should be able to:

- where necessary, describe how and explain why appropriate control experiments should be established.
- show full regard for safety and the ethical issues involved with the well-being of living organisms and the environment.
- carry out an investigation in a methodical and organised way demonstrating competence in the required manipulative skills and efficiency in managing time.

Unit 5

Opportunities to carry out practical work should be provided in the context of this module:

- the effects of antibacterial agents on the growth of bacterial culture.

Unit 6

Candidates should be able to:

- where necessary, describe how and explain why appropriate control experiments should be established.
- show full regard for safety and the ethical issues involved with the well-being of living organisms and the environment.
- carry out an investigation in a methodical and organised way demonstrating competence in the required manipulative skills and efficiency in managing time.

OCR BIOLOGY AS/A2 Level

Knowledge

AS Unit F211 (Module 1)

- Compare and contrast, with the aid of diagrams and electron micrographs, the structure of prokaryotic cells and eukaryotic cells.

AS Unit F212 (Module 1)

- State that a gene is a sequence of DNA.

AS Unit F212 (Module 3)

- Define the term variation.
- Discuss the fact that variation occurs within as well as between species.
- Outline how variation, adaptation and selection are major components of evolution.
- Discuss why the evolution of … drug resistance in micro-organisms has implications for humans.

A2 Unit F215 (Module 2)

- Explain the importance of asepsis in the manipulation of micro-organisms.
- Explain how plasmids may be taken up by bacterial cells... [although this is in the context of bacterial transformation, not conjugation].

Investigative and practical skills

AS Unit F213 and A2 Unit F216

- Carry out experimental and investigative activities, including appropriate risk management, in a range of contexts.
- Analyse and interpret data to provide evidence, recognising correlations and causal relationships.
- Evaluate methodology, evidence and data, and resolve conflicting evidence.

OCR HUMAN BIOLOGY AS/A2 Level

Knowledge and understanding

AS Unit F222 (Module 3)
- Outline the use of antibiotics in the treatment of infectious disease.

AS Unit F222 (Module 3)
- Explain how the use of antibiotics leads to the evolution of resistant strains, with reference to TB and MRSA.
- Outline the precautions that should be used to reduce the spread of resistant bacteria in hospitals.

Investigative and practical skills

AS Unit F222 (Module 3)
Evaluation of data collection strategies:
- assess the problems involved in investigating antibacterial activity.

AS Unit F223 and A2 Unit F226
- Carry out experimental and investigative activities, including appropriate risk management, in a range of contexts.
- Analyse and interpret data to provide evidence, recognising correlations and causal relationships.
- Evaluate methodology, evidence and data, and resolve conflicting evidence.

EDEXCEL SALTERS-NUFFIELD ADVANCED BIOLOGY AS/A2 Level

Knowledge and understanding

Unit 4
- Distinguish between bacteriostatic and bacteriocidal antibiotics.
- Describe how an understanding of the contributory causes of hospital acquired infections have led to codes of practice relating to antibiotic prescription and hospital practice relating to infection prevention and control.

Investigative and practical skills

Note that the 'How science works' section includes numerous statements that can be mapped onto this practical activity, especially if the teacher structures the work in a more open-ended fashion, permitting the students to design parts of the investigation themselves.

Unit 3
- Use apparatus skilfully and safely to carry out manipulative tasks in an appropriate manner.
- Produce and record valid and reliable measurements and observations with precision.
- Present and analyse data using appropriate methods and identify trends, patterns and/or observations.

Unit 4
- Describe how to investigate the effect of different antibiotics on bacteria [a recommended core practical].

Wales

WJEC BIOLOGY AS/A2 Level

Knowledge
AS Unit BY1
Structure of prokaryotic cells and viruses.
A2 Unit BY4
Culture of micro-organisms in the laboratory.
Conditions necessary for [microbial] growth.
Principles of aseptic technique.

A2 Unit BY5
- Genetic and environmental factors produce variation between individuals.
- Selective agencies (e.g. supply of food, breeding sites, climate).
- Selection can change the frequency of alleles in a population.

Investigative and practical skills

Practical work related to the content of A2 Unit BY4 (above).

Northern Ireland

CCEA BIOLOGY AS/A2 Level

Knowledge and understanding

Unit AS 1
- Prokaryotic cells (e.g. bacteria) as those without nuclei, mitochondria or endoplasmic reticulum and possessing naked, circular DNA, small ribosomes, possibly plasmids, and a cell wall.

Unit A2 2
- Understand that a gene is a sequence of bases on the DNA molecule which codes for a sequence of amino acids in a polypeptide chain.

In the context of genetic modification (transformation, not conjugation):
- incorporation of donor genes into a 'vector', e.g. bacteriophages and bacterial plasmids.
- transformation of recipient cells, e.g. *Escherichia coli*, Saccharomyces cerevisiae.
- method used to check that the recipient cell contains the recombinant DNA e.g. use of marker (antibiotic resistance) genes.
- selection and cloning of transformed cells.

Scotland

CURRICULUM FOR EXCELLENCE

The entire Scottish 3-18 curriculum is being reviewed at time of print.
Please see http://www.ltscotland.org.uk/curriculumforexcellence/outcomes/science/index.asp for information. The Survival Rivals website will be updated to show the specific links once the new Scottish curriculum is published. The cover paper from the new draft states that "The new draft experiences and outcomes are designed to allow teachers to 'raise the bar', permitting greater depth and challenging young people to be ambitious in their learning, whilst ensuring that learning is enjoyable. They are designed to encourage a range of learning and teaching styles, whilst at the same time actively encouraging participation and the development of a range of skills - particularly important given the recognition given to schools' contribution to skills development in the Scottish Government's recently published Skills Strategy." (http://www.ltscotland.org.uk/Images/overarching_cover_paper_tcm4-442673.pdf). Survival Rivals will provide teachers with resources to enable them to achieve this.

Investigative and practical skills
The two practical modules present opportunities for the incorporation of this protocol, especially if an open-ended approach is adopted and students are encouraged to devised elements of the investigation themselves.

Post-2009 legacy
This experiment has the potential to be repeated in schools following the Darwin anniversary year. The legacy will be a novel protocol covering antibiotic resistance in bacteria. You can buy many of the items supplied in this kit from Philip Harris at www.philipharris.co.uk.

Technician Notes

Equipment and materials required

The following items are provided in the Survival rivals: The X-Bacteria kit:

Item No	✓	Description	Quantity
1	☐	Bacterial Slope *E. coli* HT-99	1
2	☐	Bacterial Slope *E. coli* J-53R	1
3	☐	Chloramphenicol 25mg	1
4	☐	Rifampicin 100mg	1
5	☐	Nutrient Agar (Powder) 28g	1
6	☐	Nutrient Broth (Powder) 13g	1
7	☐	Methanol 40mL	1
8	☐	Petri Dishes - sterile	40
9	☐	Loops Plastic sterile -10microlitre	54
10	☐	Syringe 1mL sterile	20
11	☐	Sterile Water - 100mL pack	2
12	☐	Universal containers Non-sterile	40
13	☐	Syringe 30mL sterile	2
14	☐	Syringe 5ml sterile	5
15	☐	Syringe Needles Sterile	5
16	☐	Virkon Disinfectant 50g sachet	1

If you have any items missing from your kit, please call **0845 120 4529**

Extra items unrelated to this protocol

- 99% Ape: How evolution adds up by Jonathan Silvertown [Ed] (2008) London: Natural History Museum/Open University. ISBN: 978 0 565 09231 3. This book will provide you with a comprehensive guide to evolution and the latest thinking on the subject.
- PTC tasting strips, vial of 100. These will allow you to take part in a mass participation experiment. See page 50 for futher information.

You will also need:

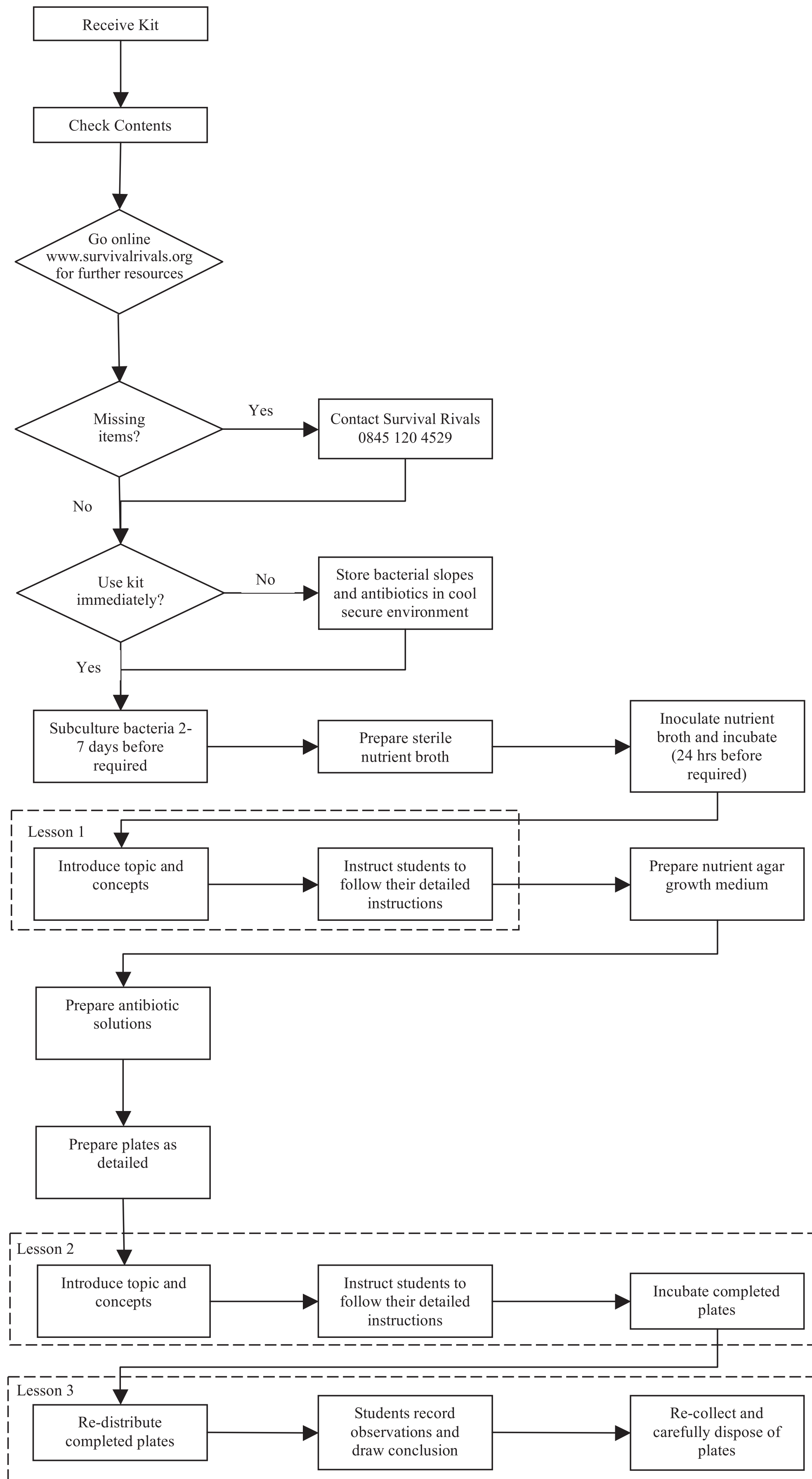
<u>For the class</u>

- An autoclave or pressure cooker
- An incubator, maintained at 30°C
- Aluminum foil
- One or more thermostatically-controlled water baths, set at 50°C
- 500mL flasks, 3 (for preparing growth media)
- Distilled or demineralised water
- Autoclavable disposal bags (at least four, for contaminated paper towels, cultures, plates etc.)
- A gas lighter or matches (for the Bunsens)
- Access to a hand wash basin with soap and paper towels
- OPTIONAL: Adhesive tape for securing the lids to the Petri dishes

<u>For each working group</u> (e.g. for each pair of students)

- A Bunsen burner (Note: heat-resistant mats should not be used, as these can make it difficult to clean up any spills effectively)
- A waste container for holding the *VirKon*® solution, such a a plastic measuring cylinder
- A waterproof marker pen for labelling the plates
- Tissue paper or paper towels for swabbing the benches

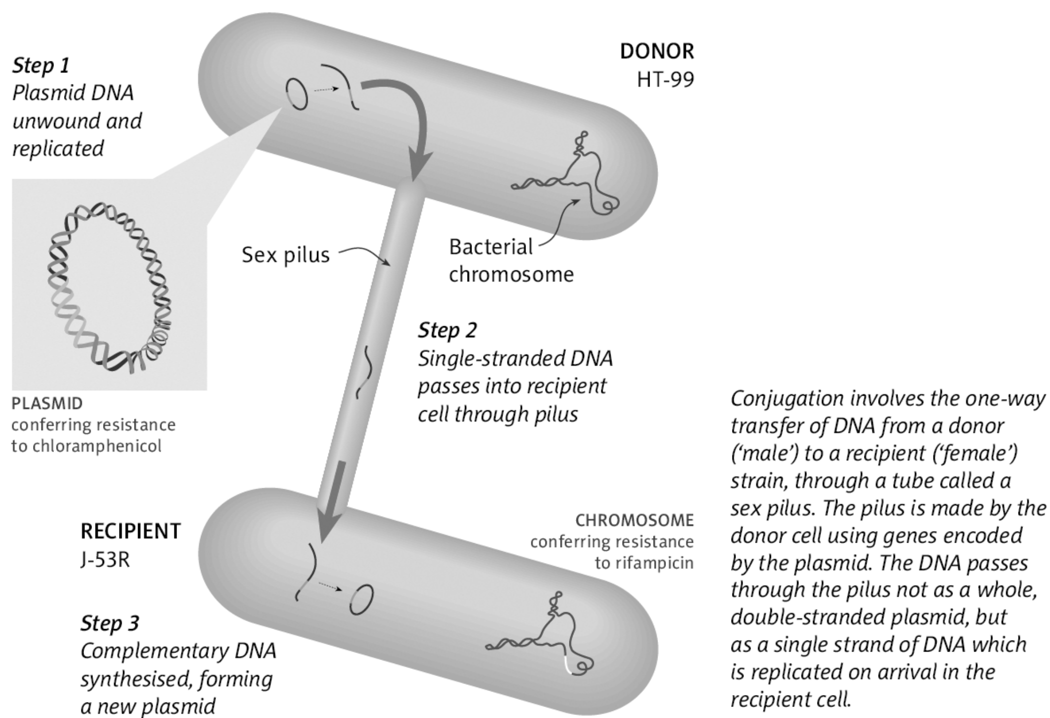
Detailed lesson-by-lesson instructions



Introduction

Outline of the practical activity

In this practical protocol students investigate one way in which bacteria acquire antibiotic resistance through conjugation. The recipient strain *Escherichia coli* J-53R carries on its chromosome a gene conferring resistance to the antibiotic rifampicin. The donor strain, *E. coli* HT-99, harbours a plasmid that includes a gene conferring resistance to a second antibiotic, chloramphenicol. Liquid cultures of the two strains of bacteria are 'mated'. The recipient, donor and 'mated' cells are then plated on three different types of media: one containing rifampicin, one containing chloramphenicol and a one containing both antibiotics. After incubation, students interpret the results and they should find that antibiotic resistance has been transferred from one strain of *E. coli* to the other.



Conjugation involves the one-way transfer of DNA from a donor ('male') to a recipient ('female') strain, through a tube called a sex pilus. The pilus is made by the donor cell using genes encoded by the plasmid. The DNA passes through the pilus not as a whole, double-stranded plasmid, but as a single strand of DNA which is replicated on arrival in the recipient cell.

Who is this practical work suitable for?

Because of the manipulative skills required and the scientific concepts involved, this practical work is in general best-suited to students over the age of 16 years.

Good microbiology laboratory practice is essential to the success and safe execution of this practical activity. Teachers carrying out the work should therefore be trained in the use of aseptic techniques, and should teach these to the students. Non-specialist teachers (e.g. those without a biological background who may be teaching science) should not carry out or supervise this work. Also bear in mind that without specialist knowledge, non-biology teachers may not be able to adequately evaluate or explain the hazards associated with the use of *E. coli* and antibiotics to students and others who may be concerned about these aspects of the work.

Laboratory technicians must also have appropriate microbiology training to prepare the microbiological media and cultures and to dispose of them safely.

Protective clothing

Students, teachers and technicians undertaking this work must wear laboratory coats. Protective glasses should be worn if appropriate e.g. when diluting disinfectants such as *VirKon®*. Disposable gloves should not generally be worn, however, as these are unnecessary and can be hazardous e.g. if they catch fire or interfere with manipulation (an exception might be if the person involved has cuts or scratches on their hands which cannot be adequately covered by plasters).

Planning and classroom organisation

When you receive the kit

When you receive the kit, check that everything is in the box. If you find that items are missing, please contact Survival Rivals on **0845 120 4529** immediately.

The two slope cultures of *E. coli* will have been prepared some weeks before you received them, so it is a wise precaution to subculture these into fresh nutrient agar up to a week before you plan to carry out the practical lessons. This will ensure that the cells are actively-growing and if you inoculate plates from the slopes, it will enable you to check for contamination (see Microbiology guidelines on page 36).

Slopes and plates of *E. coli* should not be refrigerated but kept at a cool room temperature (10–15°C), preferably in the dark. If you do not plan to carry out the work immediately, you may wish to leave the slope cultures in the box in a secure place until you are ready to subculture them.

The dried antibiotic powders can be kept in this form at room temperature. Once you dissolve them, however, they have a limited shelf life and must be stored correctly and used promptly (see below). Because the antibiotics are potentially harmful, both the powders and solutions must be stored in a secure place.

It is also a good idea to check at this stage that you have all of the other equipment and materials listed above that you need for the practical work.

The lessons: an overview

This investigation takes place over three lessons and it also presents opportunities for preparatory and follow-up tasks (for example, as homework). Careful planning and advance preparation of the growth media and cultures is essential for success in this practical work; there are many materials to prepare and good technical support and understanding of basic microbiological techniques is vital. Here is a brief overview which may help you to plan the work.

Mating the bacteria

The first lesson introduces the concept of horizontal gene flow between bacteria by conjugation and some basic aseptic techniques. Students 'mate' two cultures of bacteria and incubate the donor, recipient and 'mated' strains overnight at 30°C. Technicians will need to prepare fresh bacterial cultures and liquid media in advance of this lesson. **It is essential that these overnight cultures are plated out the following day. It may therefore be necessary to ask students to return in their lunch break to do this.**

Plating out the cultures

In the second lesson students plate out the bacteria on selective (antibiotic-containing) growth media and learn more advanced aseptic techniques. Technicians will need to prepare antibiotic-containing plates for this lesson and have the students' previously-incubated liquid cultures to hand. The plates should be incubated for 24 hours at 30°C, but after that they can be refrigerated and kept for up to a fortnight. This gives greater flexibility in lesson timing should this be required.

Interpreting the results

In the third lesson, students examine and interpret the results. This should not take very long. Students may wish to photograph the plates so that they have a permanent record of their work. After this lesson, all of the cultures must be destroyed after use by autoclaving, to ensure that no contribution is made to the spread of antibiotic resistance.

Group size

The kit has been designed with students working in groups of three in mind. This is because there are three treatments and consequently three cultures to be prepared in the second lesson. The work has also been carried out successfully by students working in pairs. We do not recommend that students work individually (which could be very time-consuming) or in larger groups (in which at least one student would just be an observer).

Lesson 1: Mating the bacteria

Preparation of cultures

For successful conjugation, the bacteria must be in an active stage of growth. If cultures are taken straight from the slopes provided in the kit, they are highly unlikely to be at the correct stage of growth. Therefore bacteria from the slopes in the kit should first be streaked onto nutrient agar plates (without any antibiotics) and incubated at 30°C for 24 hours. Individual colonies should then be picked off the plates and used to inoculate the nutrient broth cultures that are needed by the students. The broth cultures should be incubated overnight, starting the day before the cultures are needed by the students. You will therefore need to start preparing the cultures for this work at least two days before the first lesson. A wise precaution is to prepare two plates of each culture from the slopes so that if one does not grow well, or becomes contaminated, you will have a second in reserve. There are sufficient Petri dishes and nutrient agar in the kit for you do this.

Each working group of students (we suggest that they work in groups of three) will need about 10mL of each of the two bacterial cultures in nutrient broth (in fact, the students will need less than 10mL of each culture, but smaller volumes are hard to autoclave and dispense).

If you have ten working groups (that is, 30 students) it will therefore be necessary to prepare 20 x 10mL bottles of sterile nutrient broth and to inoculate half of them with *E. coli* J-53R and the rest with *E. coli* HT-99. The inoculated broth should then be incubated overnight at 30°C.
Note that each group of students will also need 10mL of uninoculated sterile nutrient broth — this can be prepared at the same time as the two cultures.

The lesson

The teacher should explain the process of conjugation and its significance. Students can prepare for this in advance of the lesson (e.g. for homework) then the teacher need only provide a reminder at the start of the lesson if time is limited. The necessity of aseptic techniques should be explained and these techniques must be demonstrated before the students prepare the mixed cultures. Preparing the cultures should take at most 30 minutes, so the entire lesson should fit comfortably within a 50–60-minute period.

Equipment and materials
Required by each student or working group

- Overnight culture of *E. coli* J-53R, 10mL
- Overnight culture of *E. coli* HT-99, 10mL
- Bottle containing 10mL of sterile nutrient broth
- Sterile 1mL syringes (without needles), 2
- Waste container of disinfectant (VirKon® solution) (Note: A measuring cylinder may be used as a waste container.)
- Bunsen burner
- Waterproof marker pen

Additional equipment required by the class

- Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

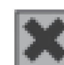
Students' procedure (these instructions are also included in the Students' notes)

1. Wash your hands with soap and water.
2. Wipe down the bench surface with disinfectant (VirKon® solution).
3. There should be a lit Bunsen burner on the bench near where you are working to create an upward flow of warm air, to carry away potentially contaminating micro-organisms.
4. Label the bottle containing sterile nutrient broth: 'Mating'.
5. Open a sterile syringe at the end of the packet furthest from the tip, taking care not to touch the barrel of the syringe or the tip Use the syringe to aseptically remove 1.8mL of an overnight culture of *E. coli* J-53R and transfer

it to the nutrient broth in the bottle you have just labelled. (Since you have a 1mL syringe, you will need to transfer 2 x 0.9mL.)
6. Discard the used syringe into the waste container of disinfectant (VirKon® solution). (Note: Discarded syringes should be completely immersed in the disinfectant and left for 24 hours before disposal in the normal waste. The disinfectant solution should be discarded after use.)
7. Taking care as before, use a new sterile syringe to aseptically transfer 0.2mL of the *E. coli* HT-99 culture to the nutrient broth.
8. Discard the second syringe into the waste container of disinfectant.
9. Place the mixed culture and the other two cultures in an incubator at 30°C (where they should be left for 4–16 hours).
10. Wipe down the bench with disinfectant.
11. Wash your hands with soap and water.

Lesson 2: Plating out the cultures

Preparation of antibiotic plates



These plates should be prepared in advance by the teacher or technician for the whole class. Each student or working group will require three plates:

- **one with nutrient agar + rifampicin;**
- **one with nutrient agar + chloramphenicol; and**
- **one with nutrient agar + both antibiotics.**

The quantities given here are sufficient for 12 sets of plates (36 plates), allowing ~17–20mL of medium per plate. This number provides two extra plates of each medium in case of accidents, etc.

If you have a smaller class or wish students to work in smaller or larger teams, you will need to adjust the number of plates you prepare accordingly.

Preparing the nutrient agar growth media

1. Prepare three separate batches of nutrient agar by adding 7 g of nutrient agar powder to each of three 500mL conical flasks While stirring, to one flask, add 200mL of distilled or deionised water; two each of the others, add 225mL of distilled or deionised water.
2. Cover the tops of the flasks with aluminium foil, then autoclave them at 121°C for 15–20 minutes. While the liquid is being autoclaved, the nutrient agar may sink to the bottom of the flasks. Therefore you should swirl the flasks to mix the nutrient agar after autoclaving.
3. Let the flasks cool to 55–60°C (that is, until the flasks can be held comfortably in your hands). While the medium is cooling, label the bases of the sterile Petri dishes: one third with 'NA + R', a third with 'NA + C' and a third with 'NA + RC'.
   Keep the nutrient agar molten by standing the flasks in a water bath at ~50°C. Note: if the agar begins to solidify, it can be re melted by autoclaving again for no more than 5 minutes.

Handling the antibiotics

Wear eye protection and protective clothing when handling the antibiotics.

- Do not allow the antibiotic powder or solutions to come into contact with your skin or eyes.
- Do not inhale the antibiotic powder.

For additional precautions and first aid instructions, please refer to the safety sheets provided in the kit.

The chloramphenicol and rifampicin powders will need to be dissolved before they are used. The final concentrations required in the growth media are:

Chloramphenicol: 25 µg per mL
Rifampicin: 100 µg per mL

Because the antibiotic solutions are heat-labile, powders rather than solutions have been provided the kit. Most schools do not have facilities for weighing out very small quantities of these powders, so small pre-weighed amounts to which the solvent can

be added have been supplied.
Although when they are dissolved in methanol and protected from the light (by wrapping the bottles in aluminium foil) the antibiotics can be stored in a fridge for up to a week, once dissolved in water they will deteriorate rapidly. *You should therefore plan this preparation carefully and only make up the antibiotic solutions when you need them.*
The amounts provided in the kit make up far more antibiotic solution than is required. Unused antibiotics should be disposed of with care. *Any unused antibiotic solution must be autoclaved to denature it before disposal down the sink with plenty of tap water.*

Preparation of Antibiotic solutions

The enclosed vials contain :- 100mg Rifampicin & 25mg Chloramphenicol respectively.

Do not allow the contents into contact with your skin or eyes. Do not inhale dust. Wear appropriate protective equipment and clothing!

- Tap each vial gently to move the contents into the base of the vial before opening.

- Remove lids and add 5ml of Methanol to each vial using a syringe. Replace lids and agitate vials until contents dissolve to give a clear solution. (partial immersion in a water bath at 40-45°C may aid this procedure).

- You have been provided with Sterile water in 100ml sealed bottles. In the centre of the aluminium bottle cap is a 10mm removable disc. Carefully remove this using a fingernail or screwdriver blade, to expose the grey rubber septum beneath it.

For each Antibiotic in turn:-
- Take a 5ml sterile syringe, and carefully attach a sterile needle (take care not to touch the needle directly with your fingers!). Depress the syringe plunger to expel all air, and then fill the syringe with the contents of the vial holding the Antibiotic solution in Methanol.

- Plunge the syringe needle through the grey rubber septum cap of the Sterile Water, and slowly depress the syringe plunger to inject the contents into the bottle. This is best done with the bottle in an upright position, and the syringe vertically above it.

- When all the liquid has transferred, carefully remove the syringe and swirl the bottle to mix the contents.

- Label the bottles 'Rifampicin Solution' & 'Chloramphenicol Solution'.

- These solutions are best used within a few hours of preparation. It is, however, good practice to warm them, by partial immersion of the bottles in a 50°C water bath for 5mins (maximum!), before adding to your Nutrient Agar.

Preparing the Nutrient Agar plates

Wash your hands and wipe down the bench with disinfectant. There should be a lit Bunsen burner on the bench near to where you are working to create an upward flow of warm air, to carry away potentially contaminating microorganisms. Ensure that the Methanol / Antibiotic solutions are not placed near the flame!

- Take a 30ml sterile syringe, and carefully attach a sterile needle (take care not to touch the needle with your fingers!). Depress the syringe plunger to expel all air, and then insert into the Sterile Water bottle (now holding Antibiotic solution). With the bottle upside down, and the needle tip within the contents – slowly pull back the syringe plunger to transfer 25ml of solution to the syringe.

- Restore the bottle to an upright position and remove the syringe. (you may choose to retain the needle in the septum, as the process will need to be repeated!)

Flask 1, labelled 'NA + R' (containing 225 mL of Agar medium)
Transfer 25 mL of Rifampicin solution from the syringe to the nutrient agar

Flask 2, labelled 'NA + C' (containing 225 mL of Agar medium)
Transfer 25 mL of Chloramphenicol solution to the nutrient agar

Flask 3, labelled 'NA + RC' (containing 200 mL of medium)
Add 25 mL of rifampicin solution and 25mL of chloramphenicol solution to the nutrient agar

- After you have added the antibiotic solutions to the agar, swirl them to mix. *Important! Do not allow the bottles to cool excessively, as the agar will set. Return the media to the waterbath as necessary to ensure that it remains molten. (If the agar sets, it can only be melted again by boiling it, which will destroy the antibiotics.)*
- Arrange the Petri dishes on the bench in three groups, ready to pour the medium into them.
- Lift the lid of each Petri dish in turn, just enough to pour the nutrient agar in. Do not put the lid down on the bench. Quickly add enough agar medium to cover the bottom of the plate to a depth of about 3 mm (17–20mL). Replace the lid then tilt the plate to spread the medium evenly over the Petri dish.
- Continue pouring the plates, using the appropriate medium for each. As you do so, flame the mouth of the flask occasionally to maintain sterility.
- To remove any bubbles from the surface of the poured nutrient agar, *very briefly* touch the surface with a Bunsen burner flame while the medium is still molten.
- Leave the medium to set, undisturbed, for about 15 minutes.
  If time permits, invert the plates and incubate them for several hours or overnight at 30°C. This dries the medium, limiting condensation; it also allows any contaminated plates to be detected.
- Stack the plates in their original plastic sleeves and store them at room temperature.
- Wipe down the bench with disinfectant (*VirKon*® solution).
- Wash your hands with soap and water.

Although the prepared plates can be kept for up to a week in a refrigerator at 3–5°C, we advise that you do not do this. Plates stored for any length of time in a fridge will accumulate condensation and be difficult to use. It is better to try to make the plates the day before you need them.

The lesson
An important constraint here is that the broth cultures must be prepared no more than 24 hours before the lesson. If it proves difficult to arrange for students to do this, it could be done for them by a teacher or technician, but ideally students should experience setting this up too.

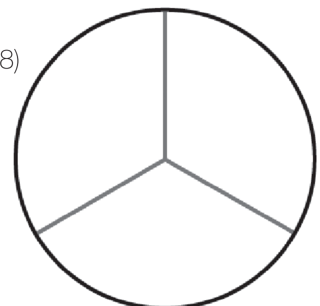
Equipment and materials
Required by each student or working group

- Plate of nutrient agar containing rifampicin
- Plate of nutrient agar containing chloramphenicol
- Plate of nutrient agar containing rifampicin and chloramphenicol
- The three microbial cultures from the previous lesson
- Waste container of disinfectant (VirKon® solution)
- Sterile disposable loops, 3
- Bunsen burner
- Waterproof marker pen

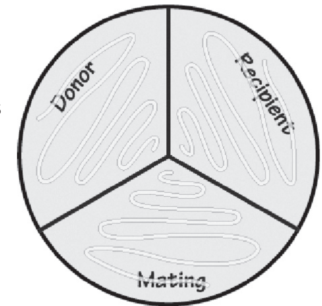
Additional equipment required by the class
Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

Students' procedure (these instructions are also included in the Students' notes on pages 43 to 48)

1. Wash your hands with soap and water.
2. Wipe down the bench with disinfectant (VirKon® solution).
3. Turn over each plate so that the base is uppermost. With the marker pen, divide each plate into three segments as shown in the diagram. Label the top two segments 'Donor' [HT-99] and 'Recipient' [J-53R] and the third segment as 'Mating'.
4. Arrange the three plates on the bench in front of you. Use a new sterile loop to aseptically streak each segment of each of the plates with culture from the appropriate bottle.



Use a new loop for each of the different cultures, and dispose of the loops into the disinfectant solution as you use them.
5. When the agar has absorbed any excess liquid, invert the plates and incubate them for 24 hours at 30°C.
6. Wipe down the bench with disinfectant (VirKon® solution).
7. Wash your hands with soap and water.



Lesson 3: Interpreting the results

After incubation, the plates can be stored in a fridge and examined at any time up to a fortnight later. This should make timetabling more convenient.

Specimen results

Since the donor strain *E. coli* HT-99 is resistant to chloramphenicol and sensitive to rifampicin, it should be able to grow in the presence of the former antibiotic but not in the presence of the latter.

- Conversely, the recipient strain *E. coli* J-53R is resistant to rifampicin and sensitive to chloramphenicol, so it should be able to grow in the presence of the former but not the latter antibiotic.
- The 'mated' cultures should be able to grow in the presence of both antibiotics, indicating that gene transfer has occurred by conjugation.

Disposal of cultures (see the Microbiology guidelines below also)

It is very important to dispose of all the materials used in a practical class properly, especially cultures of antibiotic-resistant bacteria. All containers used for storing and growing cultures *must* be autoclaved after use to ensure that all micro-organisms are killed.

Two autoclave bags should be available in the laboratory: one for reusable glassware and another for disposable materials. There should be a waste discard jar with disinfectant solution (*VirKon*®) near each work area for items such as disposable loops and waste liquid cultures. A bucket should be available in the laboratory or prep room for disposal of any broken glassware.

After use, disinfectant in the waste jars should be poured away and the items in it should be disposed of. Note that contaminated items should be left fully immersed in the disinfectant for at least 24 hours. Contaminated paper towels, cloths and plastic Petri dishes should be put into the autoclave bag reserved for disposable items. Any contaminated glassware should be put into the autoclave bag for glassware.

Glassware that is not contaminated can be washed normally. Broken glassware should be put in a waste bin reserved exclusively for that purpose. If the glassware is contaminated it must be autoclaved before disposal. Uncontaminated broken glassware can, of course, be disposed of immediately.

Microbiology guidelines

Use of *Escherichia coli*

All micro-organisms should be regarded as potentially harmful. However, the strains of the bacterium *E. coli* used in the current investigation present minimum risk given good practice. These laboratory strains should not be confused with the highly-pathogenic *E. coli* Ø157:H7, other pathogenic strains or the normal 'wild type' *E. coli* that is found in the human gut.

Maintenance and storage of cultures

In schools, microbial cultures are often maintained on agar slopes. These should be transferred onto fresh medium every 8–12 weeks or so, and incubated until the organisms have grown. They should then be stored in a cool (10–15°C), dark place, *not in a refrigerator*. New cultures should be obtained regularly, as repeated sub-culturing can lead to contamination or genetic changes in the cells.

Good microbiological practice
General precautions
Any exposed cuts or abrasions should be protected with waterproof dressings before the practical work starts.
Everyone involved — teachers, technicians and students — should always wash their hands before and after practical work.
The laboratory door and windows should be closed when work is in progress. This will reduce air movements and consequently the risk of accidental contamination of plates, etc.
Laboratory coats must be worn, and where necessary (e.g. when heating liquids), eye protection.
High standards of cleanliness must be maintained. Non-porous work surfaces should be used and they should be swabbed with an appropriate laboratory disinfectant before and after each practical session.
To reduce the risk of ingesting microbes, no hand-to-mouth operations should occur (e.g. chewing pencils, licking labels, mouth pipetting). For the same reason, eating, drinking and smoking must not be allowed in the laboratory.

Spills and breakages
Accidents involving cultures should be dealt with as follows:

- Disposable gloves should be worn.
- The broken container and/or spilt culture should be covered with a cloth soaked in disinfectant.
- After not less than 10 minutes, it must be cleared away using paper towels and a dustpan.
- The contaminated material must be placed in an infected waste container or disposal bag. This must be autoclaved before disposal.
- The dustpan should also be autoclaved or placed in a suitable solution of disinfectant for 24 hours.

Contamination of skin or clothing
As soon as possible, anyone who has been splashed should wash. Severely contaminated clothing should be placed in disinfectant before it is laundered. Contaminated cleaning cloths should be autoclaved or soaked in disinfectant.

Aseptic techniques

The aims of aseptic techniques are:

- To obtain and maintain pure cultures of micro-organisms;
- To make working with micro-organisms safer.

A 'pure culture' contains only one species of micro-organism, whereas a 'mixed culture' contains two or more species.

Contamination of cultures is always a threat because microbes are found everywhere; on the skin, in the air and on work surfaces and equipment. To obtain a pure culture, sterile growth media and equipment must therefore be used and contaminants must be excluded. These are the main principles of aseptic techniques.

Growth media must be sterilised before use — usually by autoclaving. Sterile containers (flasks, Petri dishes, etc.) should be used. Lids must be kept on these containers to prevent contamination.

It is essential to prepare the work area carefully before you start. The bench surface should be swabbed thoroughly with a suitable disinfectant (e.g. *VirKon*®) and allowed to dry. All necessary equipment and materials should be arranged so that they are readily-at-hand. Work should be done near a lighted Bunsen burner. Rising air currents from the flame will help to carry away any microbes that could contaminate growth media and pure cultures.

When cultures are transferred between containers, tops and lids should not be removed for any longer than necessary. After a lid has been taken from a bottle, it should be kept in your hand until it is put back on the bottle. This stops contamination of the bench and the culture.

A blue flame about 5 cm high should be used for sterilising wire loops and flaming the necks of bottles. After removal of the top, the neck of the culture bottle should be flamed *briefly*. This will kill any microbes present there and cause convection currents which will help to prevent accidental contamination of the culture from the atmosphere. Bottles should not be heated until they become hot and dangerous to handle.

Sterile, plastic disposable loops are provided in this kit. There is no need to flame these, but they should be opened carefully (at the handle end) immediately before use, used, then discarded immediately into a suitable disinfectant (such as *VirKon*®). A similar procedure should be adopted with the sterile, plastic disposable syringes.

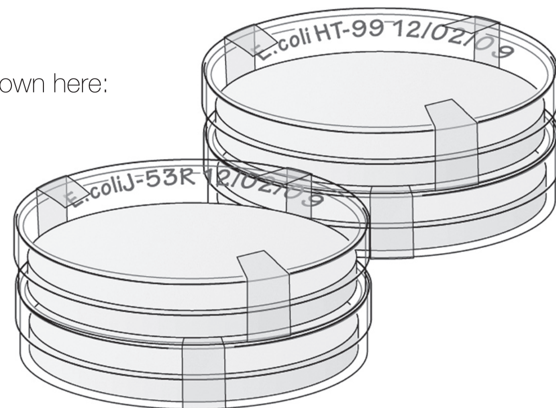
If you use wire loops, however, these must be heated until they glow red hot along the entire length of the wire part. This should be done both before and after cultures are transferred. Heat the stem of the loop first as it is brought into the Bunsen burner flame, to reduce sputtering and aerosol formation. Allow the loop to cool before you use it to transfer a culture (some people like to cool the loop after flaming it, by touching it briefly onto the agar at the edge of a culture plate).

When the Bunsen burner is not in use, it should be kept on a visible yellow flame.

Incubation

Label the Petri dish around the edge of the base before inoculation. Your name, date and the name and/or source of the organism used will allow the plate and its contents to be identified.

Where appropriate, use self-adhesive tape to seal Petri dishes as shown here:



The seal will ensure that the plates are not accidentally opened or tampered with. Do not seal plates completely round their edges as this could create anaerobic growth conditions within the dish.

Bacterial cultures in Petri dishes should usually be incubated with the base uppermost, so that any condensation that forms falls into the lid and not on the colonies. (If there is heavy condensation in the sterile Petri dish before inoculation, it should be allowed to dry before use.)

The strains of *E. coli* used in this investigation should be incubated at 30°C — this is the maximum incubation temperature usually recommended for use in schools.

Disposal and sterilisation

It is very important to dispose of all the materials used in a practical class properly. All containers used for storing and growing cultures must be autoclaved, then washed and rinsed as necessary, before re-use.

At least two autoclave bags should be available in the laboratory: one for reusable glassware and another for disposable materials. There should be a discard jar near each work area for materials such as waste liquid cultures and pipettes. A bucket should be available for disposal of any uncontaminated broken glassware.

After use, disinfectant in the discard jar should be poured away and the items in it should be autoclaved and disposed of. Contaminated paper towels, cloths and plastic Petri dishes should be put into the autoclave bag reserved for disposable items. Any contaminated glassware should be put into the autoclave bag for glassware.

Glassware that is not contaminated can be washed normally. Broken glassware should be put in a waste bin reserved exclusively for that purpose. If the glassware is contaminated it must be autoclaved before disposal. Uncontaminated broken glassware can, of course, be disposed of immediately.

Autoclaving

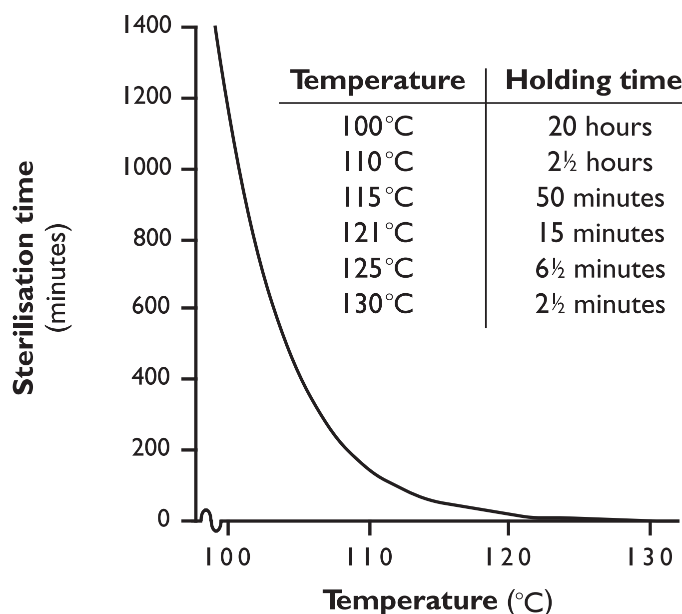
Sterilisation is the complete destruction of all micro-organisms, including their spores. All non-sterile equipment and media should be sterilised before starting practical work so that there are no contaminants. Cultures and any contaminated material should also be sterilised after use for safe disposal.

Autoclaving is the preferred method of sterilisation for culture media, aqueous solutions and discarded cultures. The process uses high pressure steam, usually at 121°C. Microbes are more readily killed by moist heat than dry heat as the steam denatures their proteins. A domestic pressure cooker or a purpose-built autoclave can be used. Domestic pressure cookers can be used in school laboratories but their small capacity can be a disadvantage when dealing with class sets of material.

Principles of autoclaving

Two factors are critical to the effectiveness of the process. Firstly, all air must be driven from the autoclave. This ensures that high temperature steam comes into contact with the surfaces to be sterilised: if air is present the temperature at the same steam pressure is lower. The materials to be sterilised should be packed loosely so that the air can be driven off. Screw-capped bottles and jars must have their lids loosened slightly to allow air to escape and to prevent a dangerous build-up of pressure inside them.

Secondly, sufficient time must be given for heat to penetrate (by conduction) to the centre of media in flasks or other containers. The times for which media or apparatus must be held at various temperatures for sterilisation are shown below:



Temperature	Holding time
100°C	20 hours
110°C	2½ hours
115°C	50 minutes
121°C	15 minutes
125°C	6½ minutes
130°C	2½ minutes

Notice that just a small difference in temperature can result in a great difference in the time required for sterilisation. It is also important that these temperatures are reached by all materials to be sterilised for the specified time e.g. the broth in the very centre of a flask. Three factors determine the duration of the autoclaving process:

- penetration time: the time taken for the innermost part of the autoclave's contents to reach the required temperature (say, 5 minutes);

- holding time: the minimum time in which, at a given temperature, all living organisms will be killed (say, 15 minutes);
- safety margin: roughly half the holding time (about 5 minutes).

Most domestic pressure cookers and autoclaves operate at 121°C. This gives a total autoclaving period of around 25 minutes. For larger volumes of liquid, such as 1 litre of growth medium in a flask, the holding time should be increased to 20–25 minutes.

The effectiveness of an autoclave can be checked by using autoclave test strips which change colour if the process has worked properly (autoclave tape, which also changes colour after heating, does not show this).

Use and routine care of autoclaves
Different autoclaves and pressure cookers will have different operating instructions and it is important that the manufacturer's instructions are always followed. Care should be taken to ensure that there is enough water in the autoclave so that it does not boil dry during operation. A domestic pressure cooker requires at least 250mL of water — larger autoclaves may need much greater volumes. The use of distilled or deionised water in the autoclave will prevent the build-up of limescale or corrosion of the metal pressure vessel. Autoclaves should be dried carefully before storage to prevent damage to the vessel.

When the autoclave is used, steam should be allowed to flow freely from it for about one minute to drive off all the air inside. Only then should the exit valve be tightened. After the autoclave cycle is complete, sufficient time must be allowed for the contents to cool and return to normal atmospheric pressure. Premature release of the lid and the subsequent reduction in pressure will cause any liquid inside the autoclave to boil. Therefore the vessel or valve must not be opened whilst under pressure as this may cause scalding and the agar or broth will froth up and may boil over the outside of the containers within.

Chemical sterilisation
WARNING! *Eye protection should be worn when dispensing concentrated disinfectant solutions.*
Many different chemicals are used for sterilisation of used equipment and work surfaces. Some disinfectants, such as MicroSol 3+ and VirKon® can be safely used for most laboratory purposes and are recommended for use in schools. Others have specialist uses. The manufacturer's and supplier's instructions should always be followed with care.

Disposable plastic items
Modern microbiology makes extensive use of disposable plastic items, such as Petri dishes, single-use pipettes and loops. Although they can appear wasteful, we strongly recommend their use as they are inexpensive, save on preparation time and enhance safety by reducing the risk of contamination. After use, such items can be sterilised and disposed of in the normal waste.

Additional information

Microbiology safety
- *Safety in science education* Department for Education and Employment (1996) Her Majesty's Stationery Office. ISBN: 011 270915 X.
- *Topics in safety* Association for Science Education (2001) [Third Edition] Association for Science Education ISBN: 0 86357 316 9.
- *Basic practical microbiology:* A manual by Dariel Burdass (2003) Society for General Microbiology ISBN: 978 0953683833. [Available from the SGM — see address below.]
- Videos about aseptic technique are available at www.sci-eng.mmu.ac.uk/intheloop

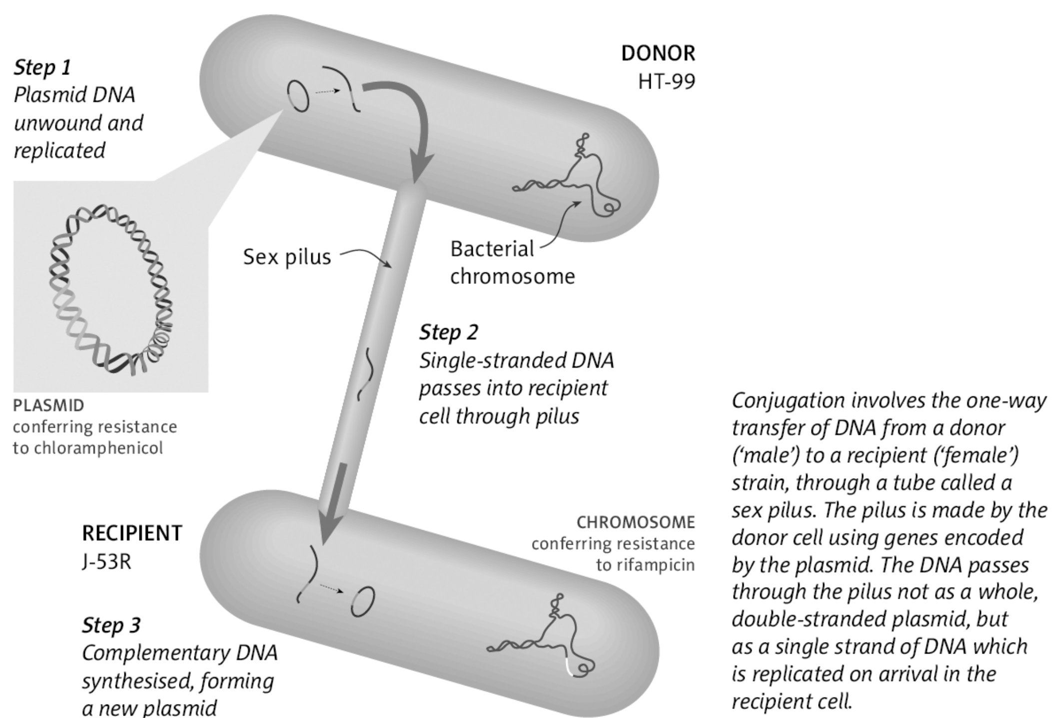
Health and Safety advice
- Teachers and technicians of member schools may contact CLEAPSS (for England, Wales and Northern Ireland) and SSERC (for Scotland).

- CLEAPSS, The Gardiner Building, Brunel Science Park, Kingston Lane, Uxbridge UB8 3PQ
  T: 01895 251496 I F: 01895 814372 I W: www.cleapss.org.uk
- SSERC, 2 Pitreavie Court, South Pitreavie Business Park, Dunfermline KY11 8UB
  T: 01383 626070 I F: 01383 842793 I W: www.sserc.org.uk
- *The Society for General Microbiology (SGM) and the Microbiology in Schools Advisory Committee (*MISAC) (which can be contacted via the SGM) are also useful sources of advice and information.
  SGM, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG
  T: 0118 988 1800 I F: 0118 988 5656 I W: www.sgm.ac.uk
  The SGM also has a web site dedicated to school microbiology: www.microbiologyonline.org.uk

Student Notes

Outline of the practical activity

In this practical protocol you will investigate one way in which bacteria acquire antibiotic resistance through conjugation. The recipient strain *Escherichia coli* J-53R carries on its chromosome a gene conferring resistance to the antibiotic rifampicin. The donor strain, *E. coli* HT-99, harbours a plasmid that includes a gene conferring resistance to a second antibiotic, chloramphenicol. Liquid cultures of the two strains of bacteria are 'mated'. The recipient, donor and 'mated' cells are then plated on three different types of media: one containing rifampicin, one containing chloramphenicol and a one containing both antibiotics. After incubation, you will interpret the results and see whether and how antibiotic resistance has been transferred from one strain of *E. coli* to the other.



Conjugation involves the one-way transfer of DNA from a donor ('male') to a recipient ('female') strain, through a tube called a sex pilus. The pilus is made by the donor cell using genes encoded by the plasmid. The DNA passes through the pilus not as a whole, double-stranded plasmid, but as a single strand of DNA which is replicated on arrival in the recipient cell.

Good microbiology laboratory practice

When you carry out this practical work, you will need to follow good microbiology laboratory practice. Your teacher will explain the techniques and special precautions that must be used.

Everyone undertaking this work must wear a laboratory coat. Protective glasses should be worn if appropriate e.g. when diluting disinfectants such as *VirKon*®. Disposable gloves should not generally be worn, however, as these are unnecessary and can be hazardous e.g. if they catch fire or interfere with manipulation (an exception might be if the person involved has cuts or scratches on their hands which cannot be adequately covered by plasters).

Lesson 1: Mating the bacteria

Equipment and materials

Required by each student or working group

- Overnight culture of *E. coli* J-53R, 10mL
- Overnight culture of *E. coli* HT-99, 10mL
- Bottle containing 10mL of sterile nutrient broth
- Sterile 1mL syringes (without needles), 2

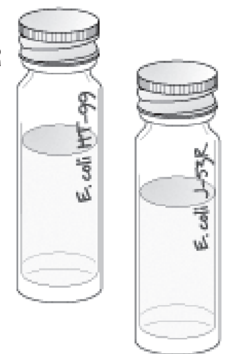
- Waste container of disinfectant (VirKon® solution) (Note: A measuring cylinder may be used as a waste container.)
- Bunsen burner
- Waterproof marker pen

Additional equipment required by the class

Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

Procedure

1. Wash your hands with soap and water.
2. Wipe down the bench surface with disinfectant (VirKon® solution).
3. There should be a lit Bunsen burner on the bench near where you are working to create an upward flow of warm air, to carry away potentially contaminating micro-organisms.
4. Label the bottle containing sterile nutrient broth: 'Mating'.
5. Open a sterile syringe at the end of the packet furthest from the tip, taking care not to touch the barrel of the syringe or the tip. Use the syringe to aseptically remove 1.8mL of an overnight culture of *E. coli* J-53R and transfer it to the nutrient broth in the bottle you have just labelled. (Since you have a 1mL syringe, you will need to transfer 2 x 0.9mL.)
6. Discard the used syringe into the waste container of disinfectant (VirKon® solution). (Note: Discarded syringes should be completely immersed in the disinfectant and left for 24 hours before disposal in the normal waste. The disinfectant solution should be discarded after use.)
7. Taking care as before, use a new sterile syringe to aseptically transfer 0.2mL of the *E. coli* HT-99 culture to the nutrient broth.
8. Discard the second syringe into the waste container of disinfectant.
9. Place the mixed culture and the other two cultures in an incubator at 30°C (where they should be left for 4–16 hours).
10. Wipe down the bench with disinfectant.
11. Wash your hands with soap and water.



Lesson 2: Plating out the cultures

Equipment and materials
Required by each student or working group

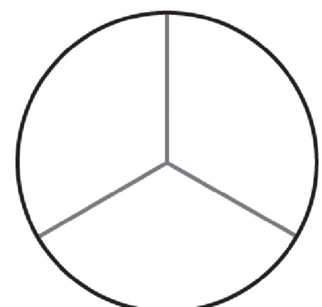
- Plate of nutrient agar containing rifampicin
- Plate of nutrient agar containing chloramphenicol
- Plate of nutrient agar containing rifampicin and chloramphenicol
- The three microbial cultures from the previous lesson
- Waste container of disinfectant (VirKon® solution)
- Sterile disposable loops, 3
- Bunsen burner
- Waterproof marker pen

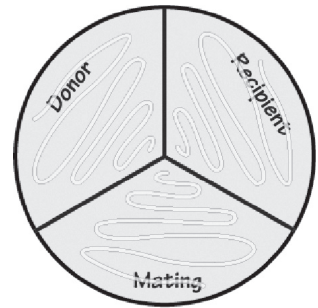
Additional equipment required by the class

Access to an incubator set at 30°C (Note: For safety reasons, 30°C is the preferred incubation temperature for *E. coli* in schools.)

Procedure

1. Wash your hands with soap and water
2. Wipe down the bench with disinfectant (VirKon® solution).
3. Turn over each plate so that the base is uppermost. With the marker pen, divide each plate into three segments as shown in the diagram.





Label the top two segments 'Donor' [HT-99] and 'Recipient' [J-53R] and the third segment as 'Mating'.

4. Arrange the three plates on the bench in front of you. Use a new sterile loop to aseptically streak each segment of each of the plates with culture from the appropriate bottle. Use a new loop for each of the different cultures, and dispose of the loops into the disinfectant solution as you use them.
5. When the agar has absorbed any excess liquid, invert the plates and incubate them for 24 hours at 30°C.
6. Wipe down the bench with disinfectant (VirKon® solution).
7. Wash your hands with soap and water.

Explain why each treatment has been used.
What 'controls' have been used in this investigation, and why?
What would you expect to grow on each are of each plate, and why?

Lesson 3: Interpreting the results

You should record your results by noting what is growing on the three zones on each plate.
What is your interpretation of the results that you have obtained?
How do your results help to explain how antibiotic resistance spreads?
How is this form of selection and evolution different to that which Charles Darwin proposed?

Microbiology safety guidelines

Use of *Escherichia coli*

All micro-organisms should be regarded as potentially harmful. However, the strains of the bacterium *E. coli* used in the current investigation present minimum risk given good practice. These laboratory strains should not be confused with the highly-pathogenic *E. coli* Ø157:H7, other pathogenic strains or the normal 'wild type' *E. coli* that is found in the human gut.

Good microbiological practice

General precautions

- Any exposed cuts or abrasions should be protected with waterproof dressings before the practical work starts.
- Everyone involved — teachers, technicians and students — should always wash their hands before and after practical work.
- The laboratory door and windows should be closed when work is in progress. This will reduce air movements and consequently the risk of accidental contamination of plates, etc.
- Laboratory coats must be worn, and where necessary (e.g. when heating liquids or diluting concentrated disinfectants), eye protection.
- High standards of cleanliness must be maintained. Non-porous work surfaces should be used and they should be swabbed with an appropriate laboratory disinfectant before and after each practical session.
- To reduce the risk of ingesting microbes, no hand-to-mouth operations should occur (e.g. chewing pencils, licking labels, mouth pipetting). For the same reason, eating, drinking and smoking must not be allowed in the laboratory.

Aseptic techniques

The aims of aseptic techniques are:

- To obtain and maintain pure cultures of micro-organisms;
- To make working with micro-organisms safer.

A 'pure culture' contains only one species of micro-organism, whereas a 'mixed culture' contains two or more species. Contamination of cultures is always a threat because microbes are found everywhere; on the skin, in the air and on work surfaces and equipment. To obtain a pure culture, sterile growth media and equipment must therefore be used and contaminants must be excluded. These are the main principles of aseptic techniques.

Growth media must be sterilised before use — usually by autoclaving. Sterile containers (flasks, Petri dishes, etc.) should be used. Lids must be kept on these containers to prevent contamination.

It is essential to prepare the work area carefully before you start. The bench surface should be swabbed thoroughly with a suitable disinfectant (e.g. *VirKon®*) and allowed to dry. All necessary equipment and materials should be arranged so that they are readily-at-hand. Work should be done near a lighted Bunsen burner. Rising air currents from the flame will help to carry away any microbes that could contaminate growth media and pure cultures.

When cultures are transferred between containers, tops and lids should not be removed for any longer than necessary. After a lid has been taken from a bottle, it should be kept in your hand until it is put back on the bottle. This stops contamination of the bench and the culture.

A blue flame about 5 cm high should be used for sterilising wire loops and flaming the necks of bottles. After removal of the top, the neck of the culture bottle should be flamed briefly. This will kill any microbes present there and cause convection currents which will help to prevent accidental contamination of the culture from the atmosphere. Bottles should not be heated until they become hot and dangerous to handle.

Sterile, plastic disposable loops are often used for inoculation. There is no need to flame these, but they should be opened carefully (at the handle end) immediately before use, used, then discarded immediately into a suitable disinfectant (such as VirKon®). A similar procedure should be adopted with the sterile, plastic disposable syringes.

If you use wire loops, however, these *must* be heated until they glow red hot along the entire length of the wire part. This should be done both before and after cultures are transferred. Heat the stem of the loop first as it is brought into the Bunsen burner flame, to reduce sputtering and aerosol formation. Allow the loop to cool before you use it to transfer a culture (some people like to cool the loop after flaming it, by touching it briefly onto the agar at the edge of a culture plate).
When the Bunsen burner is not in use, it should be kept on a visible yellow flame.

Incubation
Label the Petri dish around the edge of the base before inoculation. Your name, date and the name and/or source of the organism used will allow the plate and its contents to be identified.

Where appropriate, use self-adhesive tape to seal Petri dishes as shown here:

The seal will ensure that the plates are not accidentally opened or tampered with. Do not seal plates completely round their edges as this could create anaerobic growth conditions within the dish.

Bacterial cultures in Petri dishes should usually be incubated with the base uppermost, so that any condensation that forms falls into the lid and not on the colonies. (If there is heavy condensation in the sterile Petri dish before inoculation, it should be allowed to dry before use.)

The strains of *E. coli* used in this investigation should be incubated at 30°C — this is the maximum incubation temperature usually recommended for use in schools.

Disposal and sterilisation

It is very important to dispose of all the materials used in a practical class properly. All containers used for storing and growing cultures must be autoclaved, then washed and rinsed as necessary, before re-use.

At least two autoclave bags should be available in the laboratory: one for reusable glassware and another for disposable materials. There should be a discard jar near each work area for materials such as waste liquid cultures and pipettes. A bucket should be available for disposal of any uncontaminated broken glassware.

After use, disinfectant in the discard jar should be poured away and the items in it should be autoclaved and disposed of. Contaminated paper towels, cloths and plastic Petri dishes should be put into the autoclave bag reserved for disposable items. Any contaminated glassware should be put into the autoclave bag for glassware.

Glassware that is not contaminated can be washed normally. Broken glassware should be put in a waste bin reserved exclusively for that purpose. If the glassware is contaminated it must be autoclaved before disposal. Uncontaminated broken glassware can, of course, be disposed of immediately.

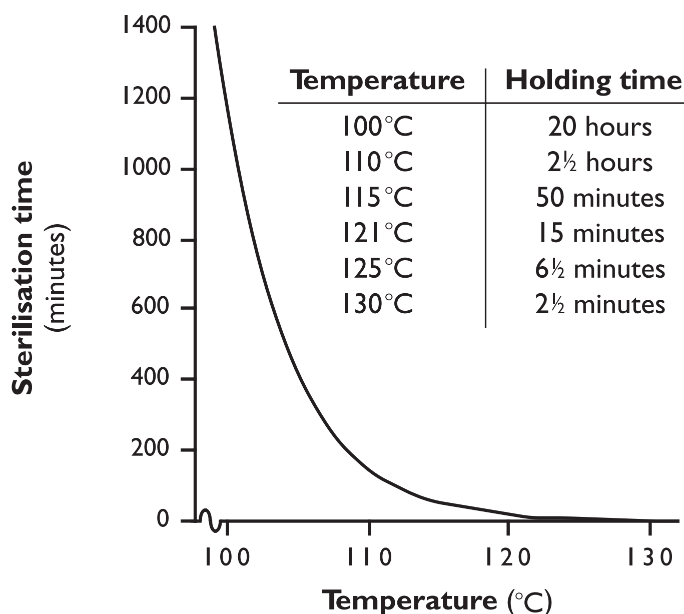
Autoclaving

Sterilisation is the complete destruction of all micro-organisms, including their spores. All non-sterile equipment and media should be sterilised before starting practical work so that there are no contaminants. Cultures and any contaminated material should also be sterilised after use for safe disposal.
Autoclaving is the preferred method of sterilisation for culture media, aqueous solutions and discarded cultures. The process uses high pressure steam, usually at 121°C. Microbes are more readily killed by moist heat than dry heat as the steam denatures their proteins. A domestic pressure cooker or a purpose-built autoclave can be used. Domestic pressure cookers can be used in school laboratories but their small capacity can be a disadvantage when dealing with class sets of material.

Principles of autoclaving

Two factors are critical to the effectiveness of the process. Firstly, all air must be driven from the autoclave. This ensures that high temperature steam comes into contact with the surfaces to be sterilised: if air is present the temperature at the same steam pressure is lower. The materials to be sterilised should be packed loosely so that the air can be driven off. Screw-capped bottles and jars must have their lids loosened slightly to allow air to escape and to prevent a dangerous build-up of pressure inside them.

Secondly, sufficient time must be given for heat to penetrate (by conduction) to the centre of media in flasks or other containers. The times for which media or apparatus must be held at various temperatures for sterilisation are shown below:



Temperature	Holding time
100°C	20 hours
110°C	2½ hours
115°C	50 minutes
121°C	15 minutes
125°C	6½ minutes
130°C	2½ minutes

Notice that just a small difference in temperature can result in a great difference in the time required for sterilisation. It is also important that these temperatures are reached by all materials to be sterilised for the specified time e.g. the broth in the very centre of a flask. Three factors determine the duration of the autoclaving process:

- penetration time: the time taken for the innermost part of the autoclave's contents to reach the required temperature (say, 5 minutes);
- holding time: the minimum time in which, at a given temperature, all living organisms will be killed (say, 15 minutes);
- safety margin: roughly half the holding time (about 5 minutes).

Most domestic pressure cookers and autoclaves operate at 121°C. This gives a total autoclaving period of around 25 minutes. For larger volumes of liquid, such as 1 litre of growth medium in a flask, the holding time should be increased to 20–25 minutes.

The effectiveness of an autoclave can be checked by using autoclave test strips which change colour if the process has worked properly (autoclave tape, which also changes colour after heating, does not show this).

Use and routine care of autoclaves
Different autoclaves and pressure cookers will have different operating instructions and it is important that the manufacturer's instructions are always followed. Care should be taken to ensure that there is enough water in the autoclave so that it does not boil dry during operation. A domestic pressure cooker requires at least 250mL of water — larger autoclaves may need much greater volumes. The use of distilled or deionised water in the autoclave will prevent the build-up of limescale or corrosion of the metal pressure vessel. Autoclaves should be dried carefully before storage to prevent damage to the vessel. When the autoclave is used, steam should be allowed to flow freely from it for about one minute to drive off all the air inside. Only then should the exit valve be tightened. After the autoclave cycle is complete, sufficient time must be allowed for the contents to cool and return to normal atmospheric pressure. Premature release of the lid and the subsequent reduction in pressure will cause any liquid inside the autoclave to boil. Therefore the vessel or valve must not be opened whilst under pressure as this may cause scalding and the agar or broth will froth up and may boil over the outside of the containers within.

Chemical sterilisation
WARNING! *Eye protection should be worn when dispensing concentrated disinfectant solutions.*

Many different chemicals are used for sterilisation of used equipment and work surfaces. Some disinfectants, such as MicroSol 3+ and VirKon® can be safely used for most laboratory purposes and are recommended for use in schools. Others have specialist uses. The manufacturer's and supplier's instructions should always be followed with care.

Disposable plastic items
Modern microbiology makes extensive use of disposable plastic items, such as Petri dishes, single-use pipettes and loops. Although they can appear wasteful, we strongly recommend their use as they are inexpensive, save on preparation time and enhance safety by reducing the risk of contamination. After use, such items can be sterilised and disposed of in the normal waste.

Additional information

Antibiotic resistance
There are numerous web sites with high-quality information on the topic of antibiotic resistance. Here are two typical examples:
- PBS (USA) Evolution of antibiotic resistance (TV programme) www.pbs.org/wgbh/evolution/library/10/4/1_104_03.html
- UK Department of Health www.dh.gov.uk/en/Publichealth/Patientsafely/Antibioticresistance/index.html

Microbiology safety

- *Safety in science education* Department for Education and Employment (1996) Her Majesty's Stationery Office. ISBN: 011 270915 X.
- *Topics in safety* Association for Science Education (2001) [Third Edition] Association for Science Education ISBN: 0 86357 316 9.
- *Basic practical microbiology:* A manual by Dariel Burdass (2003) Society for General Microbiology ISBN: 978 0953683833. [Available from SGM www.sgm.ac.uk]
- Videos about aseptic technique are available at www.sci-eng.mmu.ac.uk/intheloop

A Question of Taste

Within The X-Bacteria kit you may also have noticed some PTC taste test strips. These can be used to carry out another short exercise with your students, as detailed below.

Background information

People differ in their ability to taste phenylthiocarbamide (PTC). To people who can taste it, PTC is very bitter, however, to some people it is completely tasteless. The ability to taste PTC depends largely on differences in a gene called TAS2R38. This gene encodes a taste receptor protein which is found in the taste buds on the tongue. The ability to taste PTC can be influenced by other factors including: the density of taste buds, smoking, age, other genes that are involved in taste, and a person's perception of what they can taste.

This short practical involves tasting paper strips that have been soaked in PTC, to identify the participants' ability to taste this bitter chemical. It forms part of a larger practical workshop which uses molecular biology techniques to investigate differences in the TAS2R38 gene of the student participants. For more information please see: www.survivalrivals.org.uk/taste

PTC strips – Instructions for use

This document provides instructions for tasting the control (normal paper) and PTC paper strips provided in your Survival Rivals kit.

Advance preparation

It is advisable to make one set of the strips distinguishable from the other. One easy way to do this is to cut the corners off of one set of strips using a clean pair of scissors. As the strips can be tricky to separate, it's a good idea to distribute one of each paper strip on a paper towel to the student's benches before the activity.

How to do the taste test

a) Preparing the students

It is beneficial if students are not made aware of the taste they are expecting, as perception of taste may be affected by prior expectations. If possible, ask the students to keep their reactions to themselves until all of the participants have recorded their results. This may be difficult as PTC tastes extremely unpleasant to tasters.

b) Tasting the strips

1. Ensure students have washed their hands.
2. Provide each student with one control strip and one PTC strip.
3. Ask students to taste the first paper strip (the control) which has the corner removed from the bottom. To do this, ask students to put the paper on the middle of the tongue and close their mouths. Students should note down any taste and its intensity from a scale of 0 - 2, (0 = no taste, 1 = weak taste, 2 = strong taste).
4. Repeat step 3 with the PTC strips.

c) Recording the results

It may be interesting to gather the class data to identify how many people are:

- Non-tasters – those who recorded 0 after tasting the PTC strip
- Weak tasters – those who recorded 1 after tasting the PTC strip
- Tasters – those who recorded 2 after tasting the PTC strip

These data can be entered onto the Survival Rivals website www.survivalrivals.org.uk/taste. We hope to build a national picture of bitter tasting ability to quantify the percentage of tasters and identify any regional differences that may exist.

Expected results

The ability to taste PTC is genetically dominant over the inability to taste. Therefore, the expected results should be approximately 25% tasters, 50% weak tasters, 25% non-tasters. However, as PTC tastes very unpleasant, the results might be skewed to show that students have reported themselves as 75% tasters and 25% non-tasters.

Health and Safety Information

CLEAPSS has advised that students should not taste more than two PTC strips within this activity. Teachers should ensure that students wash their hands before putting paper strips into their mouths. Paper strips should be disposed of immediately into a normal bin to avoid the spread of germs.

Ethical Information

It is strongly recommended that children and their parents do not participate together in this activity.
The ability to taste PTC is mainly due to differences at various locations within the TAS2R38 gene. Therefore, it is a trait which is inherited from parents. Because of this, there are some combinations of results which could cause anxiety to parents and children who participate together in this activity. There are several explanations for unexpected results. These include the number of differences within the TAS2R38 gene that can affect ability to taste PTC, differences in other genes involved in taste, age, smoking, and the density of a person's taste buds. These complex factors that contribute to the ability to taste PTC suggest that the taste test cannot definitively reveal any sensitive information between family members. However, it is strongly advised that this situation is avoided by preventing parents and children participating in this activity together and by ensuring that students do not have access to PTC strips that they could take home.
This taste test protocol has been considered by a leading UK geneticist who has stated that differences in the ability to taste PTC are highly unlikely to be associated with any other health conditions for the participants.

Acknowledgements

Wellcome Trust

The Wellcome Trust is the most diverse biomedical research charity in the world. It spends £600 million every year in the UK and around the world to support and promote research that will improve the health of humans and animals. The Trust's funding has supported the work of scientists that has helped to expand our understanding of evolution in humans and other species. For example, research on the human genome has revealed patterns of human migration and ancestry. As part of Darwin 200, the Wellcome Trust is engaging with the public in educational, broadcast and cultural activities.

The Wellcome Trust have funded and co-ordinated the development and delivery of Survival Rivals.
Thanks to Clare Matterson, Daniel Glaser, Amy Sanders and Stephanie Forman.

Website: www.wellcome.ac.uk

Philip Harris

Philip Harris has an outstanding heritage in education, dating back to 1817. They are the science education market leader both in the UK and internationally, with an unbeatable range. Their science resources cover Early Years right through to Further Education and are for teachers and technicians who want good value innovative resources, relevant to the changing demands of the science curriculum.

Philip Harris are producing and distributing the three kits for Survival Rivals.
Thanks to Emma Markey, Sharon Hawksworth, John Cotton and John Hurst.

Website: www.philipharris.co.uk

National Centre for Biotechnology Education, University of Reading

National Centre for Biotechnology Education, University of Reading was the first school biotechnology centre in the world. Since its establishment in 1984-5, the NCBE has gained an international reputation for the development of innovative educational resources.

The NCBE has produced detailed protocols for Survival Rivals activities for secondary students, advised in the pilot stage of the project and written resources to accompany the experiments.
Thanks to Dean Madden and John Schollar.

Website:www.ncbe.reading.ac.uk

Guardian Professional

Guardian Professional is a commercial division of Guardian News and Media Ltd (GNM). We provide products and services to professionals across education, media and the public sector. These include marketing, digital content and web build, publications, advertising, consultancy, research and conferences and events. We have extensive experience of developing highly interactive, user-friendly websites, with years of experience in devising educational content for clients such as Directgov, the Department for Children, Families and Schools, Kew, EDF Energy and LOCOG.

Guardian Professional have designed and delivered the marketing and communications for Survival Rivals.
Thanks to Emma Whitehead, Tom Jackson, Helen Warner and Alex Williams.

Website: www.guardianprofessional.co.uk

guardianprofessional

Other Contributors

Special thanks to Leighton Dann and Jenny Edrich for carrying out the Survival Rivals pilot experiments and to the following schools for taking part in these:

Swavesey Village College

Oundle School, Peterborough

King Ecgbert School, Sheffield

Comberton Village College

King Edward VI Grammar School, Chelmsford

St Clement Danes School, Chorleywood

Samuel Ward School, Haverhill

JFK Roman Catholic School, Hemel Hempstead

Watford Grammar School for Girls, Watford

Huge thanks to Stephen Tomkins at Homerton College, Cambridge and Mark Boyd at RSPB for your advice.

And to the many people who have helped in the development and delivery of Survival Rivals, many thanks.

Finally, thank you to all the teachers for ordering Survival Rivals kits and running the experiments with young people.

Wellcome Trust Education Activities

The Wellcome Trust is committed to engaging with the public on science and has a strong interest in supporting science teachers through initiatives such as the National Science Learning Centre (http://www.sciencelearningcentres.org.uk). The Trust also produces a twice-yearly publication, the Big Picture, for science teachers and there is an edition of this all about evolution which is free to download or order at www.wellcome.ac.uk/bigpicture/evolution.

To celebrate 200 years since the birth of Charles Darwin, the Wellcome Trust is commissioning, developing and funding a unique and ambitious set of projects to engage the widest UK public with Darwin, his ideas, and his influence on contemporary science and culture.

The Trust is providing Darwin-inspired practical activity kits for every state school in the UK. Survival Rivals is the Wellcome Trust's offering for secondary schools. The Great Plant Hunt is the equivalent programme for primary schools. See www.greatplanthunt.org for more information.

The Trust has developed the Tree of Life – an interactive fly-through of evolution on Earth, narrated by Sir David Attenborough- as well as Darwin-inspired visual arts, poetry and short film projects, placing Darwin and evolution into contemporary culture. See www.wellcometreeoflife.org for more information.

Working with the BBC, the Wellcome Trust is highlighting a series of short visual clips, that help teachers to teach evolution related topics, available on BBC Learning Zone Broadband www.bbc.co.uk/learningzone.

DNA to Darwin is an education project funded by the Wellcome Trust and run by the NCBE (National Centre for Biotechnology Education). DNA to Darwin will allow 16-19 year old biology students to explore the molecular evidence for evolution by using computers to analyse DNA and protein sequence data. Each of the student activities centre around an engaging story from recent research in molecular genetics encompassing microbiology, plant and animal biology and human evolution. See www.dnadarwin.org.

Find out more about all the Wellcome Trust's Darwin projects at www.wellcome.ac.uk/darwin200.

Other Links

Great Plant Hunt, Survival Rivals equivalent offering for primary schools, in partnership with Kew Gardens www.greatplanthunt.org

BBC Learning Zone includes clips on evolution www.bbc.co.uk/learningzone

Tree of Life An interactive fly-through of evolution on Earth, narrated by Sir David Attenborough. www.wellcometreeoflife.org

DNA to Darwin free resources for 16-19 year olds around molecular evidence of evolution www.dnadarwin.org

Wellcome Trust's Darwin projects www.wellcome.ac.uk/darwin200

Darwin200 is a national programme of events celebrating Charles Darwin's scientific ideas and their impact around his two hundredth birthday on 12 February 2009 www.darwin200.org

Evolution Megalab Did you know that thanks to a common little snail that you can find in your garden, in the park or under a hedge, you can see evolution in your own back yard? www.evolutionmegalab.org

CREST Awards to accredit young people's work in science and technology www.britishscienceassociation.org/crest

Royal Society for the Protection of Birds Information about birds, including education pages www.rspb.org.uk

Society for General Microbiology Information about microbiology, including educational resources www.sgm.ac.uk

Singtastic Featuring the Mr Darwin song www.singtastic.com

Teachers TV have produced a range of programmes for Darwin200 www.teachers.tv/evolution

Practical Biology Find lots of other investigations to carry out in school www.practicalbiology.org

Association for Science Education helping teachers teach science www.ase.org.uk

Your Genome Educational information from the Sanger Centre www.yourgenome.org

Inside DNA A genomic revolution www.insidedna.org.uk