Bio CP5 Investigating the effect of antibiotics

Your teacher may watch to see if you can:

- work safely and aseptically with microorganisms.

Aim

To investigate the effect of antibiotics on bacteria.

Method

Apparatus

- Petri dish with lid
- screw-top bottle of sterile liquid nutrient agar (keep in water bath until needed) sterilised in an autoclave
- bacterial culture in screw-top bottle
- sterile pipette in wrapper
- sterile spreader in wrapper
- beaker of disinfectant
- two small filter paper discs of different antibiotic concentration or type
- small disc of sterile filter paper
- sticky tape
- marker pen
- forceps
- ethanol (IDA)
- ruler
- Bunsen burner and heat-resistant mat

⚠ Safety

Plates must be taped closed as in the diagram. This allows air in and does not encourage the growth of pathogenic bacteria.

Dispose of all cultures and equipment safely, as instructed by your teacher.

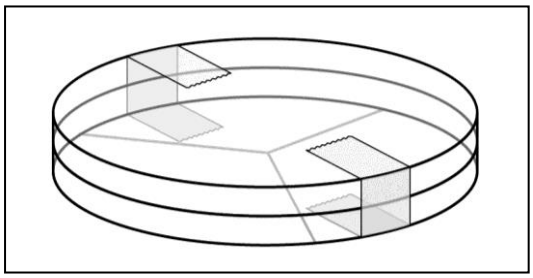
Take care to avoid burning fingers when flaming the neck of an agar bottle.

Avoid touching eyes or skin after handling bacterial cultures. Wash splashes immediately with water.

Wash hands thoroughly before the practical and again before leaving the laboratory.

Ethanol (IDA) is hazardous and highly flammable. Keep away from open flames, except as described in the method.

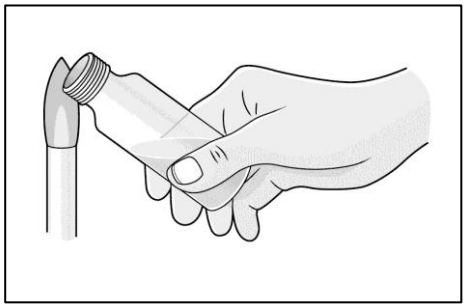
Pouring an agar plate



A Keeping the lid on the Petri dish, turn the dish upside down. Use the pen to draw three sections on the base, as shown in the diagram. Label one section ‘control’ and the other two sections with the name or concentration of each antibiotic. Add your initials and the date near the edge of the dish. Turn the dish the right way up.

B Light the Bunsen burner and set it to a roaring flame.

C Work in a pair to pour the plate. One student should unscrew the cap of the nutrient agar bottle and quickly pass the glass neck of the bottle through the Bunsen flame, as shown in the diagram. The other student should lift the lid of the Petri dish just enough for the agar to be poured carefully into the dish. Pour in enough agar to half fill the depth of the Petri dish base. Replace the dish lid immediately. Flame the open neck of the agar bottle again before screwing the cap back on.



D Leave the agar to solidify.

Making a bacterial plate

E Remove the pipette from its wrapper and do not put it down.

F Unscrew the cap of the bottle of bacterial culture and quickly pass the neck of the bottle through the Bunsen flame.

G Draw a small amount of culture into the pipette then flame the neck of the bottle again and replace the lid.

H Lifting the lid of the Petri dish as little as possible, gently add two drops of culture to the agar. Replace the dish lid and place the pipette in disinfectant.

I Unwrap the spreader and, lifting the dish lid as little as possible, gently spread the culture drops across the agar using a back-and-forwards motion with the spreader.

J Replace the dish lid and place the spreader in disinfectant.

Adding antibiotic discs

K Sterilise the forceps by dipping them into ethanol then passing them through the Bunsen flame to ignite the ethanol.

L Use the forceps to pick up the sterile filter paper disc. Lift the dish lid just enough so that the forceps do not touch the bacterial layer on the agar in the dish, and carefully place the disc on the section labelled 'control'. Replace the dish lid as quickly as possible.

M Sterilise the forceps as before (step **K**). Then repeat step **L** with one of the antibiotic discs, placing it on the appropriate section of agar. Remember to replace the dish lid as quickly as possible.

N Repeat step **M** with the other antibiotic disc.

O Tape the lid onto your Petri dish with two pieces of tape, as shown in the top diagram on the previous last page, and invert the dish. Leave it at 20–25 °C for two to three days.

P Look carefully at your dish. **Do not open it**.

Recording your results

1 Measure the diameter of the circle around each disc where there is no bacterial growth.

2 Divide each diameter by 2 to calculate the radius (r) for each circle, and then calculate the area of no bacterial growth using the formula area = πr^2

3 Draw up and complete a table to record the area of no bacterial growth for each disc.

Considering your results/conclusions

4 Explain what your results show about the effect of antibiotics on bacteria.

5 Compare your results with those of other groups.

a Did other groups get the same results? If not, suggest why not.

b Explain which of the discs was the most effective at killing bacteria.

Evaluation

6 Suggest a way in which the method could be improved, and give a reason for your suggestion.

7 **a** Identify steps in the method that contributed to working aseptically.

b Explain the importance of working aseptically in this practical.