Unit 4 Area of Study 2 – Antibiotics in Action

Advice to teachers

This module is the second in a series of related activities:

a) *Strategies to deal with the emergence of an outbreak of food-borne disease*, where *E. coli* is identified as the pathogen (Unit 4 AOS 2)
b) Antibiotics in Action (practical laboratory activity Unit 4 AOS2), where students perform an experiment to identify an antibiotic that will be effective against *E. coli*
c) AOS3 Practical investigation - a staged investigation to identify the concentration of antibiotic that will be effective against *E. coli*

However, Antibiotics in Action can also be run as a stand-alone practical activity.

This module has been designed to meet key knowledge and key science skills for VCE Biology.

Unit 4 key knowledge:

AOS 2 Biological knowledge and society

- The use of chemical agents against pathogens including the distinction between antibiotics and antiviral drugs with reference to their mode of action and biological effectiveness

VCE Biology key science skills

- ALL key skills

Rationale behind the GTAC module

- This practical activity is designed to teach students the techniques and skills they require to successfully complete the GTAC designed Unit 4 AOS 3 Practical investigation.

- This activity can be easily modified to allow different groups to investigate different species of bacteria.

Resources to support this module

All resources used in this module can be found on the GTAC website at:
Teachers > Teaching resources > Resources for teaching biology Units 3 & 4

Resources include:

Teacher guide (pdf booklet)

- Advice to teachers - Implementation notes
- Teacher notes to accompany AOS2 Student Worksheets
- Laboratory preparation and sourcing materials

Student worksheet

- AOS2 Student Experiment – Antibiotics in Action

Unit 4 AOS2 Practical – Antibiotics in Action

Antibiotics in action: Identifying a suitable treatment for people infected with a food-borne pathogen

'Antibiotics in Action' aims to identify a suitable antibiotic treatment for people infected with the food-borne pathogen. In this practical students learn about the nature of antibiotics and develop the practical microbiology skills for identifying antibiotics that effectively prevent bacterial growth, and approaches to interpreting the results.

This experiment develops all of the key practical and analytical skills for students to conduct the GTAC module designed for the GTAC-designed ***Unit 4 AOS3 Practical Investigation***.

Addressing Key skills

During the investigation students consider the key skills of experimental design, including:

- Plan and conduct investigations safely and ethically
- Examine and analyse collected data to identify patterns, trends or relationships
- Analyse the characteristics of the data
  - Accuracy – Is there a true or accepted value for what is being measured? How much measurement error?
  - Precision – how much spread around the mean value? Have you repeated multiple times/Can you pool results to increase number of samples?
  - Reliability – can someone repeat and generate the same results?
  - Validity – does experimental design allow production of results?
- Draw conclusions consistent with the data

The following notes refer to the Student Worksheet for AOS2 Antibiotics in Action. They provide additional background information and practical tips. See also the Laboratory Preparation notes for specific lab preparation details.

Teacher note 1

Overview for teachers

In 'Antibiotics in Action', students investigate the effects of a range of antibiotics on *E. coli*. The Introduction outlines the experiment. Materials and methods for how to spread a bacterial lawn are described. Results will not be obtained until the next class as the bacteria require a culture period. During this wait period, students answer the Experimental Questions, which include a consideration of controls (which are deliberately omitted from the outline) and come up with a way to measure and record their results. After obtaining the results, class data can be pooled for completion of the Discussion questions. These questions direct students to consider reliability and validity of the experiment. They also conduct background research into the mode of action of different types of antibiotics. Based on this information they predict why some antibiotics are more effective than others against different bacteria.

Antibiotics in Action - Practical set up:

The antibiotic discs are cut from the Mastring rings which contain 6 different antibiotics. Lab technicians can cut the individual discs from the rings prior to class and distribute them in appropriate numbers in a sterile dish for the student work benches. The reasons for cutting off the individual discs include:

a) Students learn and practise handling individual discs with forceps – a skill required for the GTAC designed AOS 3 Practical Investigation.
b) 3 antibiotics can be tested per plate, so one Mastring covers two groups; more students are therefore involved in the practical skills; it is cheaper for you. You choose how many discs to apply to each plate, depending on your class sizes and budget.
c) Compared to the individual antibiotic discs, the colour code can help to distinguish the various different antibiotics.
d) Sometimes, when you have 6 discs on one plate, the zones of inhibition can be too large and overlapping, making interpretation and measurement difficult for beginners. We want students to think about how to measure, and practise measuring, the zone of growth inhibition (a standard method is provided).

The bacteria: *E. coli* is used to follow on from the GTAC designed activity *Strategies to deal with the emergence of an outbreak of food-borne disease*, as *E. coli* is identified as the pathogen. Depending on your class size and budget, you could include other species. Using more than one species provides greater comparison and introduces students to different types of bacteria that may respond to antibiotics in different ways. Comparing other bacteria would be ideal if you are using 'Antibiotics in Action' as a stand-alone practical.

Teacher note 2 – Materials and methods

- If you are not following the GTAC series of classes, where *E.coli* is the pathogen to be tested, then alternative bacteria (*Bacillus subtilis, Staphylococcus epidermidis and Micrococcus luteus)* could be used for, or included in, this practical. Depending on your budget, class size or number of classes, you can use more than one species for comparison within the class.
- Antibiotic discs - cut from Mastring rings prior to class; in a sterile Petri dish

Sterilise the tips of the forceps by dipping the tips into a small volume of ethanol in a beaker, passing the tips through the flame (don't hold the tips in the flame) – as you pass them through the flame, the ethanol alights, burns off, and thus sterilises the tips. Use the forceps to place the paper disc onto the bacterial lawn on the agar surface. Gently press down on each disc to ensure contact with the plate surface.

STUDENTS WORK IN PAIRS. Each pair tests 3 antibiotics from the Mastring. That way one Mastring covers 2 plates and two student pairs. The students are practising the technique of handling individual discs. When the data are pooled all antibiotics are covered.

You may have previously used a whole Mastring antibiotic ring. Reasons why the full Mastring is NOT recommended are:

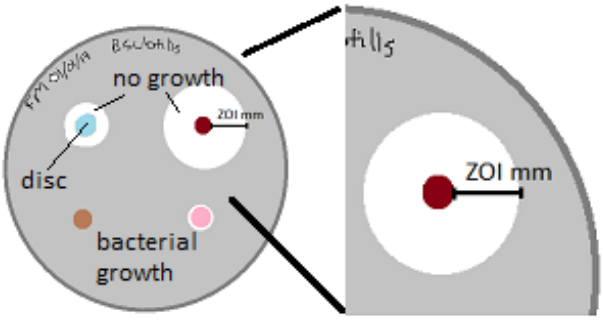
a) Students are learning the technique for handling small individual discs and labelling the plate accordingly.
b) On Nutrient Agar the zones of bacterial death around the discs are often very large and overlapping (see images on the Mastring Product data sheet supplied by Southern Biological for four bacteria).

Large ZOIs can be very difficult to interpret (e.g. you may think the bacteria have not grown at all) and difficult, or impossible, to measure with any accuracy.

c) By cutting the ring to individual discs, one Mastring is enough for two student pairs with one agar plate each. Your purchase of Mastrings goes further.

Teacher note 3 - ZOI

Now introduce students to the standard method for measuring the Zone of Inhibition; in the diagram below, grey represents bacterial growth and white represents the clear area where bacteria have not grown. This area is called the Zone of Inhibition (ZOI). Measure the shortest distance from the edge of the disc to where the bacteria are growing.



Measuring the Zone of Inhibition. Grey represents bacterial growth. White represents a zone of no bacterial growth around a disc. Measure from the edge of the disc to bacterial growth, in millimetres.

Teacher note 4 – Results of antibiotics

Growth on different types of agar may affect the growth and appearance of the bacteria. Nutrient agar (NA) is commonly used in school labs. Depending on the exact recipe you follow, a variable agar concentration in the plates affects the rate of diffusion into the agar, which influences the ZOI. At lower agar concentration, antibiotic diffusion rates are higher and zones of inhibition can be very large and overlapping. This can cause difficulty in measurement and interpretation. For example, see the Mastring Product data sheet supplied by Southern Biological for bacteria on Nutrient agar; some species have large overlapping clear zones that would not enable measurement.

Lab Prep and Sourcing materials for

AOS2 Antibiotics in Action and AOS3 Practical Investigation

These notes cover the materials and preparations for microbiology-based practicals for both the Unit 4 AOS2 practical and Unit 4 AOS3 Practical Investigation.

Links to VCAA Study Design VCE Biology 2017-2021

Outcome 2 - Key knowledge - Biological knowledge and society • the use of chemical agents against pathogens including the distinction between antibiotics and antiviral drugs with reference to their mode of action and biological effectiveness

Outcome 3 - Practical investigation
A student-designed or adapted investigation related to cellular processes and/or biological change and continuity over time is undertaken in either Unit 3 or Unit 4, or across both Units 3 and 4...

Both experiments follow on from the GTAC lessons and activities relating to a food-borne infection "Strategies to deal with an outbreak of food-borne disease" that would be covered in Unit4 AOS2. The AOS2 practical identifies the appropriate antibiotic to treat the infection. In this practical students learn the key skills to undertake an investigation in AOS3 to determine the appropriate concentration of the antibiotic to use. The AOS3 practical is a stepwise process with assessment checkpoints at each step. Students are required to design an experiment. They then conduct a standardised experiment so that you are not trying to accommodate many variables.

The GTAC practicals are designed for students to work in pairs. The materials used for both the AOS2 and AOS3 practicals are illustrated in Figure 1.

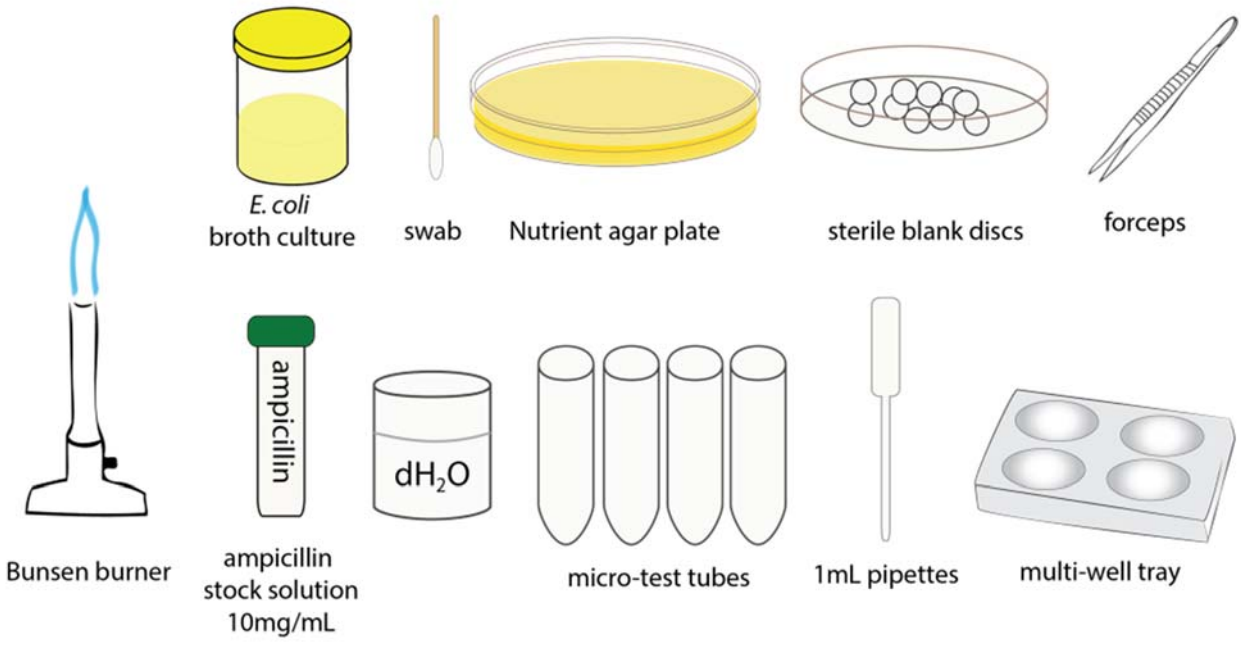


Figure 1: Materials needed for microbiology practical in AOS2 and AOS3

Sourcing materials

Table 1: Where to source the required materials

Material	Supplier - Southern Biological ; item	Cost (in 2016)
Options for nutrient agar:		
(a) Nutrient agar - to make your own plates	MED3.10 - Nutrient agar powder	AU$72.30 Ex GST
(b) Nutrient agar plates – already prepared	MED3.30 - Nutrient agar plates 10 plates	AU$18.50 Ex GST
(c) Nutrient agar – melt & pour	MED20.30 - Nutrient agar gel, 100mL	AU$10.30 Ex GST
Sterile Petri plates for pouring agar	E4.102 - Petri dishes, plastic, sterile, 92 x 16mm; pack of 20 Also available – box of 160 or 480 plates	AU$10.80 Ex GST
Escherichia coli	B1 - Escherichia coli, K-12 strain, live broth	AU$19.40 Ex GST
Mastring antibiotic discs	SB E1.10 - Mastring antibiotic sets, six different antibiotics per ring - 10 Mastrings Also available in sets of 50 and 100 Mastrings	AU$31.80 Ex GST
Sterile swabs	E6.10 - Swabs, sterile, rayon tipped, individually wrapped; pack of 20 swabs	AU$9.85 Ex GST
Ampicillin 30mg vial	BioRad Cat # 1660407EDU http://www.bio-rad.com/en-au/product/pglo-bacterial-transformation-kit this ampicillin is a component of the BioRad pGLO transformation kit and is purchased as a separate item, listed in the refills section for the pGLO kit	AU$17.00

Preparation and testing prior to student practical sessions

Purchase	Nutrient agar, sterile Petri dishes, *E.coli* broth culture, sterile swabs, sterile transfer pipettes (bulb or dropper pipettes), Mastring (AOS2 experiment), ampicillin (AOS3 experiment)
Prepare as required	Nutrient agar plates, sterile test tubes, aliquots of *E.coli* in sterile tubes, Mastring – cut to individual discs, blank paper discs, sterile/clean forceps and multiwell trays, ampicillin stock solution
Test	Serial dilutions of ampicillin and Zones of Inhibition. Adjust concentrations if necessary.

Antibiotic discs from Mastring rings (for AOS2 practical)

Materials:
Instruments should, if possible, be sterilised. If you don't have an autoclave or steriliser, thorough washing and rinsing with distilled water, then a rinse with 70% ethanol followed by air-drying, should be sufficient.

- sterile Petri dishes (autoclaved glass or sterile plastic Petri dishes)
- small scissors (e.g. scissors from a dissecting kit)
- small forceps (e.g. forceps from a dissecting kit)
- Mastring 6-antibiotic rings

Procedure:

1. Work next to the Bunsen burner to minimise contamination
2. Hold the Mastring tab with the forceps
3. Cut the individual antibiotic discs from the ring into the Petri dish (discs from one ring into one dish).

Blank discs (for AOS3 practical)

Materials:

- Whatman filter paper
- Hole punch
- Glass Petri dishes (sterilised) or sterile plastic Petri dishes
- Autoclave (if possible)

Method:

- Punch discs into a glass Petri dish, place on the lid and autoclave (if possible).
- Each pair of students needs a minimum of 4 discs. Allow double that for dropped or damaged discs.
- If you cannot autoclave them, use filter papers from a new packet to minimise contaminants, and punch discs into sterile/cleaned Petri dishes.

Nutrient agar plates

Nutrient Agar Powder - **Storage**: Keep powder at 10-30°C in a tightly sealed container.
Nutrient agar from Southern Biological (Oxoid): use at 28g/L
Nutrient agar from Westlab: use at 28 g/L

Preparation from powder:
Prepare media in Schott bottles or conical flask according to your required volumes and steriliser capacity; make sure you use a bottle or flask with equal volume of air space above the liquid, and a lid or cap that can be left loose in the autoclave. For example, we prepare batches of 300mL in 500 mL Schott bottles. One agar plate needs 15-20mL, so, with some practice, 300mL gives you about 15-20 plates.

To prepare 100mL:

1. Weigh out 2.8 g
2. Add it to 100mL of distilled water in a suitable bottle/flask
3. Place on the lid and swirl to mix; use a cotton wool plug wrapped in gauze or a foil cap for a conical flask
4. Loosen the screw cap, place autoclave tape on the bottle and place in autoclave
5. Autoclave for 20min at 121°C
6. Cool to about 50°C before pouring plates

Pour the plates:

1. Clean a lab bench thoroughly with detergent and water, then spray with 70% ethanol and wipe dry with paper towel.
2. Wash your hands thoroughly.
3. Set up a Bunsen burner; work in the zone around the Bunsen burner – the flame creates an updraft which helps prevent air-borne microbes falling into the agar plates.

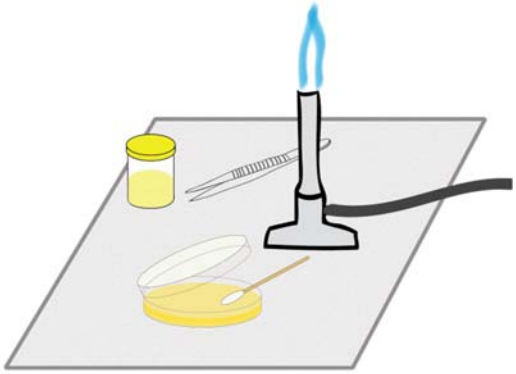


Figure 2: All microbiology work should be conducted around the Bunsen burner

4. Lift the lid of a Petri dish, pour enough molten agar to cover the surface (15-20mL) – pour gently to avoid bubbles, then return the lid.
5. Allow agar to set; it turns slightly opaque as the gel sets, but should still be clear.
6. Turn the plates upside down (agar up). Store in a plastic bag.

If you do not have an autoclave: For these experiments, it is easier to purchase pre-prepared nutrient agar plates or pre-prepared sterile nutrient agar ready to melt and pour into sterile plates.

Alternatively, you can try using a hot-plate-stirrer with a magnetic flea, continuously stirring while heating to dissolve the agar, then boiling for 45min. However, contaminants may still grow in the agar plates and affect the results.

***Storage of nutrient agar plates**:*
Put freshly poured plates in plastic bags, e.g. the sleeves that sterile Petri dishes come in.
NA plates keep ok at room temperature for a few weeks.
Long term storage is best in the fridge
Keep with agar side upwards so condensation falls onto the lid

Spreading bacterial lawns

Materials:

- Bunsen burner
- Sterile swab
- *E. coli* broth culture
- Nutrient agar plate

Method:

1. Set up the Bunsen burner; work within a 30cm radius around the burner
2. Swirl the broth to mix bacteria
3. Open swab package at the stick-end (not the cotton tip end)
4. Dip the swab into the broth
5. Remove the lid from the plate and place it on the bench next to the Bunsen burner
6. Spread the bacterial swab uniformly over the surface of the agar, rotating the plate to cover all areas and reaching the edges

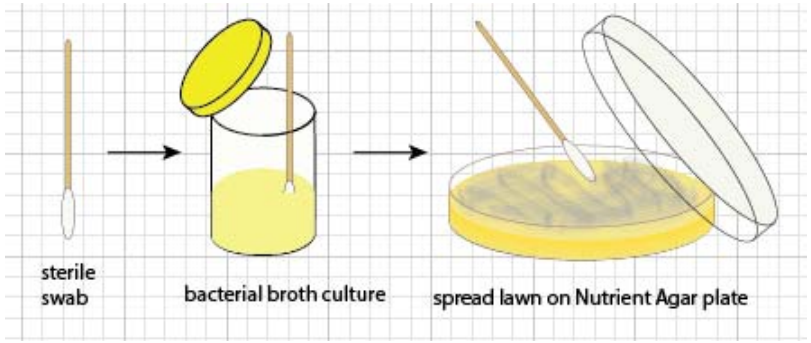


Figure 3 Procedure for spreading a lawn

Apply antibiotic discs to the lawn

Apply antibiotic discs to the bacterial lawn immediately after the lawn is plated and before the plate is incubated.

Materials: bacterial lawn on nutrient agar, forceps, single antibiotic discs (for AOS2 prac) or blank discs and antibiotic solutions (for AOS3 prac).

Method:

1. Label the bottom (agar side) of the plate appropriately
2. Dip forceps into a small volume of ethanol and pass through the flame; ethanol burns off
3. Place discs on lawn
   a. For individual antibiotic discs cut from Mastring - pick up a disc and place onto the lawn
   b. For the different ampicillin dilutions:
      - transfer the solutions to a labelled multiwell plate
      - flame the forceps, pick up a blank disc, dip into the appropriate ampicillin solution, touch the disc to the edge of the tube or well to drain excess solution
      - place disc onto the bacterial lawn. Press gently on the disc to ensure contact with agar
      - tape the lid to the plate – either parafilm around the edge, or sticky tape at two sides.

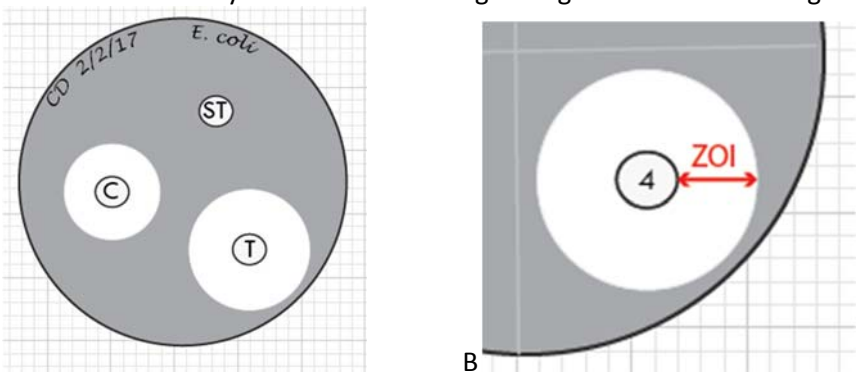
Incubate plates for bacterial growth

Bacterial lawn growth requires overnight incubation.
Place plates with agar side up (the discs should stay in place) into a 30-37°C incubator.

> *If you do not have a 30-37°C incubator, incubate the plates on the lab bench in a warm room. Results should be similar. In our lab the ZOIs are a little larger when identical samples are incubated at room temperature (see Sample Results table 3). Growth at 37°C gives a better lawn than at room temperature.*

Bacterial lawns and Zones of Inhibition

After overnight culture, the bacterial lawn appears as a creamy layer on top of the agar. *E.coli* lawns are sometimes not very obvious unless you have clear zones for comparison. A disc with an effective antibiotic will cause a clear area around the disc. This is the Zone of Inhibition (ZOI). Measure the ZOI from the edge of the disc to where you see the bacteria growing as shown in the diagrams below (Figure 4).



A B

Figure 4: The diagrams represent bacterial growth on agar plates (grey) and a clear zone, the Zone of Inhibition, where bacteria have not grown due to antibiotic action (white). A: Example of three discs on a plate with varying ZOI. B: Measure the ZOI from the edge of the disc to where bacteria are growing.

Ampicillin solution

Prepare ampicillin stock solution

Materials:

- One vial of 30mg ampicillin (BioRad Cat# 1660407EDU)
- Sterile dH_2O
- Sterile (or new clean) 1mL transfer pipettes
- Sterile (or new clean) 20mL test tubes
- Sterile (or ethanol-cleaned) graduated 10mL pipette

1. Add 3mL dH_2O to the vial of ampicillin. Place the cap firmly on the vial and mix by inversion. Continue inverting until all the powder is dissolved. This gives a 10mg/mL solution. Keep this solution on ice if possible.

Keep the remaining concentrated ampicillin (10mg/mL) in a capped tube at 4°C. Do not freeze and thaw repeatedly. This solution retains the same activity for at least 10 days with refrigeration.
Use this solution for initial tests, any required retests, and preparation of student 'Ampicillin Stock solution'.
Store any excess 10mg/mL ampicillin in the freezer at the completion of the student pracs. It is expected to retain activity, but test it first.

***Stability of ampicillin solution**:*
The 10mg/mL ampicillin is stable in the fridge for at least 10 days.
Dilutions down to 500 µg/mL are stable in the fridge for at least 10 days.

Avoid repeated freeze-thaw of the stock solutions.

*NOTE: the following section on testing ampicillin prior to student practical is a preferred step. It is possible to go straight to the section '***Preparation for Student Serial Dilution – 0.5mg/mL Ampicillin'** *(page 9) and test just this range of concentrations that the students will use (0.25, 0.125, 1.063 mg/mL). However, if the result is not as anticipated, then you should test the wider range of concentrations as described in '***Prepare and test ampicillin serial dilutions'**.*

Ampicillin testing prior to student practicals

As everyone will have slightly different batches of agar plates and different incubation conditions (e.g. you may not have an incubator), it is best if you test a wider range of concentrations than the students will use to make sure the results (size of ZOI) work for your conditions.

Prepare and test ampicillin serial dilutions

General description: From the 10mg/mL stock solution first do a 1/5 dilution to get 2mg/mL. Then do serial ½ dilutions to get a range of concentrations for testing on 2 agar plates. It is important to use graduated pipettes for these volumes, preferably 1-2mL graduated glass pipettes, micropipettors, or narrow graduated transfer pipettes with marks for 0.5mL and 1.0mL. [Note: if you have micropipettors, tube 1

below could be prepared with a smaller volume of the 10mg/mL solution (e.g. 250µL + 1000 µL dH_2O) so as to use less of your concentrated ampicillin].

Procedure:

1. Label eight mini test tubes 1-8
2. Add 2mL dH_2O to tube 1
3. Add 1mL dH_2O to tubes 2-8
4. Add 0.5mL of 10mg/mL ampicillin to tube 1. Use the pipette to mix the solution.
5. Using the same pipette, transfer 1mL of Solution 1 to tube 2; mix.
6. Continue in this manner following Table 2.

Table 2: Setting up ampicillin serial dilutions for pre-testing

Tube #	volume of dH_2O (mL)	volume of solution (mL)	Final Concentration (mg/mL)	Final Concentration (µg/mL)
1	2.0	0.5mL of 10mg/mL	2.0	2000
2	1	1mL of #1	1.0	1000
3	1	1 mL of #2	0.5	500
4	1	1 mL of #3	0.25	250
5	1	1 mL of #4	0.125	125
6	1	1 mL of #5	0.063	62.5
7	1	1 mL of #6	0.032	31.25
8	1	-	0.0	0

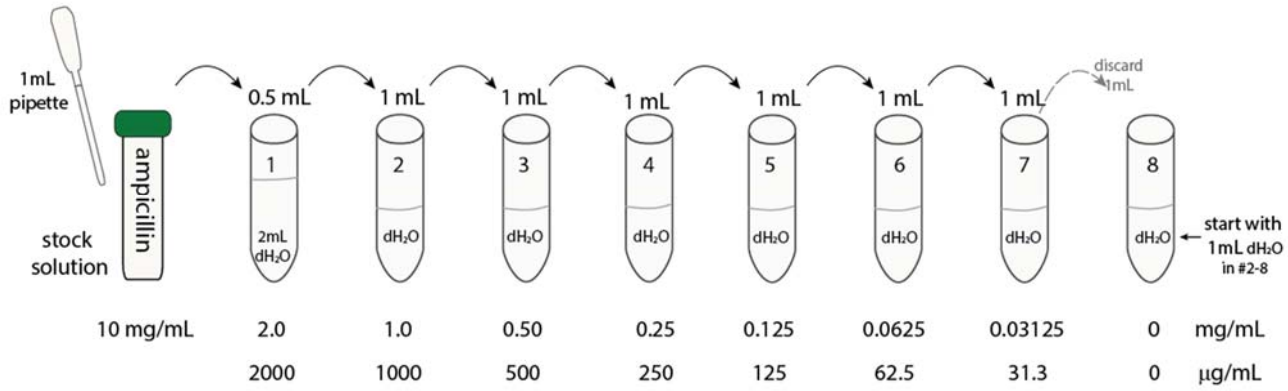


Figure 5: Schematic of procedure described above and Table 2

7. Proceed with spreading lawns on 2 nutrient agar plates. Label the plates.
8. Dip blank discs into each solution and apply to the lawns (such as in Figure 6).

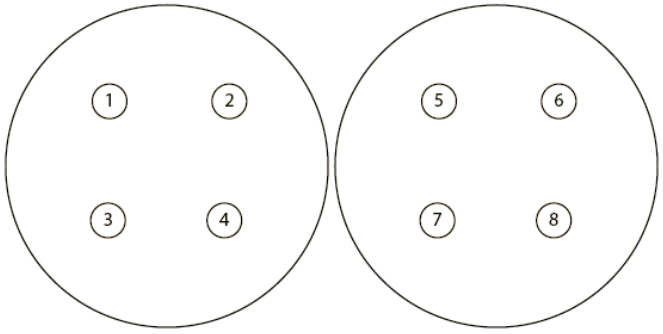


Figure 6: Testing a wide range of ampicillin concentrations. The labels on this diagram are the concentrations.

9. Incubate overnight with the system available in your lab
10. Measure the ZOIs (see section **Bacterial lawns and Zones of Inhibition, page 5**).

Expectation and adjustment if required:

- The bacterial lawn should be obvious
- There should be no clear area around the control (disc 8, 0 ampicillin)
- Check that at least one ZOI is > 6mm and at least one ZOI is <6mm

The tests done at GTAC show that 250, 125 and 62.5 µg/mL is a suitable concentration range. Therefore, the GTAC protocol provides students with a 'stock solution' of 500 µg/mL.

- If your tests are not comparable, then choose three concentrations that give at least one ZOI >6mm and at least one ZOI <6mm.
- Then prepare the student's 'stock solution' to be the concentration 2-fold higher than the highest of the three you choose. If this is necessary, change Steps 1 and 2 in "**Preparation for student serial dilution of ampicillin"** (page 10) accordingly.

Sample results

We tested a range of concentrations in several trials on different agar plates to identify appropriate concentrations that should be feasible to prepare in the school lab. Table 3 shows one set of representative results where the same concentrations were compared on plates cultured at 37°C or at room temperature. Culture at room temperature can result in larger ZOIs.

Based on our tests, we chose 0.5mg/mL (500 µg/mL) as the stock solution for students to start with to prepare serial ½ dilutions, and they test 0.25, 0.125, 0.063 and 0 mg/mL (250, 125, 62.5 and 0 µg/mL) (shaded section of the Table 3). You may need to choose a different range depending on the results of your tests.

Table 3: Sample results of a range of ampicillin concentrations. Identical samples were incubated overnight at 37℃ or at room temperature.

Ampicillin (mg/mL)	ZOI (mm) Overnight incubation at 37°C	ZOI (mm) Overnight incubation at room temperature
10	13	15
5	14	15
2	10.5	15
1	10	13
0.5	8.5	10.5
0.25	7	9.5
0.125	5	6.5
0.0625	3.5	5.5
0.03	1	4.5
0	0	0

Preparation for Student Serial Dilution – 0.5mg/mL Ampicillin

Prepare the 10mg/mL ampicillin from lyophilised powder (as also described previously):

- Add 3mL dH_2O to the vial of ampicillin. Place the cap firmly on the vial and mix by inversion. Continue inverting until all the powder is dissolved. This gives a 10mg/mL solution. Keep this solution on ice if possible.

Prepare 0.5 mg/mL Stock solution:

1. Using a graduated pipette, transfer 1mL of 10mg/mL solution to a clean 20mL test tube (make sure the test tube has a cap/stopper for mixing the solution).
2. Add 19mL dH_2O to give 0.5mg/mL (500 µg/mL) solution. Mix. This is the 'Stock Solution' for students to use.
3. For students, dispense 1.2-1.5mL of the stock solution into clean 2-5mL test tubes, one per pair. Students use 1mL of this stock solution to prepare the serial dilutions, so there should be a little more than 1mL in their tube.

What each pair of students will need and do: Serial dilution of ampicillin

Materials

- 1mL ampicillin stock solution (0.5 mg/ml – *or as determined in your pre-testing*)
- 5 clean small test tubes and rack (2-5mL capacity)
- Marking pen
- 10 mL sterile dH_2O
- 2x sterile 1mL graduated transfer pipettes

Student Procedure:

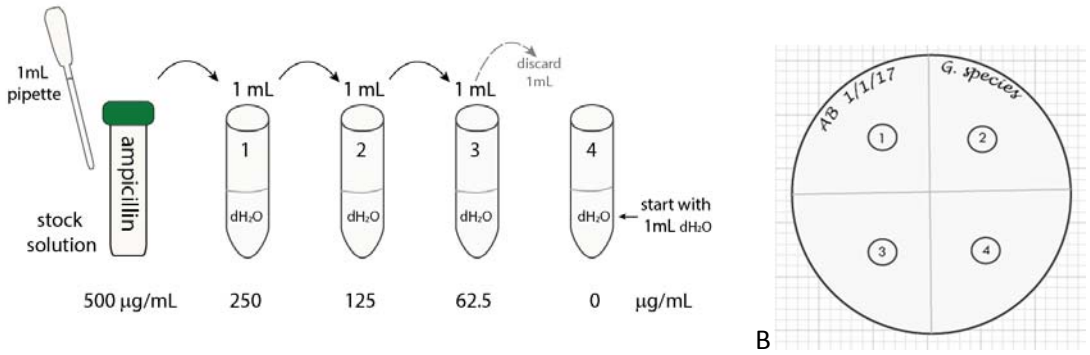


Figure 7: Student practical. A: Serial dilution of ampicillin from 500 µg/mL (0.5mg/mL) stock solution. B: Agar plate with four discs of diluted ampicillin from tubes 1-4.

An alternative to students doing the actual serial dilution is for them to carry out a serial dilution of a coloured solution (food dye in water) so the teacher can judge their technique in comparison to a standard set prepared by you. Figure 8 is an example of a ½ serial dilution of blue food dye.

You would then provide pre-prepared ampicillin dilutions for students to apply to their lawns.

Figure 8: Serial ½ dilution of blue solution

Determine Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the lowest antibiotic concentration considered effective against a bacterium. In standardised microbiology testing (the CDS Antibiotic Susceptibility method) the criterion for effectiveness is set as a ZOI of 6mm or greater.

- Less than 6mm is not inhibitory
- 6mm or more is inhibitory

MIC is determined by plotting ZOI vs ampicillin concentration.

Sample results (Table 4) and graph (Figure 9). Interpolating from the graph shows the MIC is about 180 µg/mL ampicillin.

Ampicillin (µg/mL)	ZOI (mm)
0	0
62.5	3
125	5
250	7

Table 4: Sample data for MIC determination

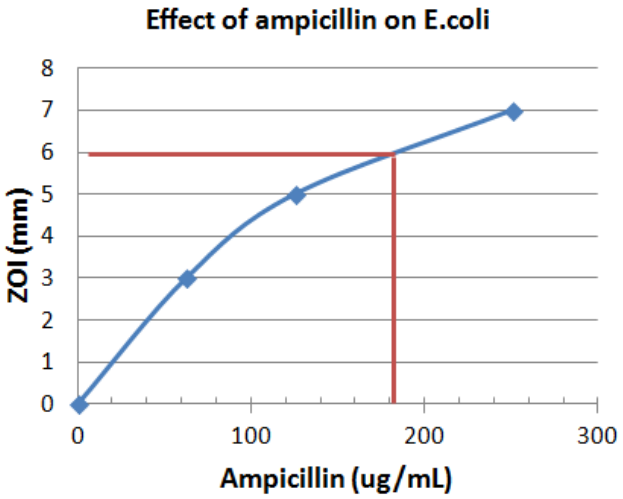


Figure 9: Example of a graph determining minimum inhibitory concentration of ampicillin for E. coli using the conditions outlined in this practical.

The graph is not linear. That is, as you use higher concentrations, the ZOI does not increase in direct proportion to the concentration change. For example, at double the concentration you don't get double the ZOI. Therefore, if you use a series of high concentrations, e.g. 0.5 -10 mg/mL, they will all produce ZOI above 6mm and there may be no difference in ZOI (as seen in Table 3). If that happened in your student's practicals, the interpretation would be that you need to dilute the ampicillin further to determine the MIC.