

Practical 8 - S(d) The Effect of Penicillin on Bacterial Growth

In this practical focuses on the practical skills of: **Planning – defining the problem**

You will be developing other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Pour agar plates using aseptic technique
- Experience growing microorganisms
- Use the disc diffusion technique needed to undertake a microbiological investigation
- Use vernier callipers to measure clear zone diameters.
- Assess the reliability of results

Safety Information

See below and the next page.



Culturing microorganisms may be hazardous

Background information

- Penicillin is an antibiotic that prevents the synthesis of mucopeptides in bacterial cell walls by preventing the formation of peptide bonds; it is bactericidal.
- *Staphylococcus epidermidis (albus)* is a gram-positive bacterium which forms small white colonies.
- Penicillin is effective against gram-positive bacteria.
- Discs containing penicillin are placed on an agar plate containing *Staphylococcus epidermidis (albus)*.
- If the penicillin has been effective there will be a clear zone around the disc.
- The size of the clear zone is a measure of the effectiveness of the penicillin

All microorganisms should be treated as potential pathogens so it is important to follow safety procedures when working with *Staphylococcus epidermidis (albus)*.

- Read the information above.
- Identify and write down the independent and dependent variables.
- Write down the hypothesis.
- List the variables that should be controlled.
- Outline how each such variable might be controlled.

Safety in the use of microorganisms**Rules for working with microorganisms:**

- 1 BEFORE STARTING WORK cover all cut or broken skin with a waterproof dressing.
- 2 WEAR tightly fitting disposable gloves when working with all live cultures.
- 3 WEAR a clean laboratory coat with all the fastenings closed.
- 4 BEFORE AND AFTER each working session wash the bench surface with bactericidal disinfectant.
- 5 AFTER each working session dispose of gloves into the sterilin bag or other disposal container provided. WASH your hands with bactericidal soap.
- 6 SWAB any spillages with bactericidal disinfectant. DISPOSE of any contaminated paper in the sterilin bag or other disposal container provided.
- 7 NEVER place anything in your mouth whilst working with microorganisms. This includes foods, liquids, gummed labels, pipettes etc.
- 8 ALL CULTURES should be labelled clearly with the following information:
 - Your name
 - Name of the organism
 - Type of nutrient medium used
 - Date
- 9 NO CULTURE should be left for more than one week. Incubators are checked daily and outdated cultures will be removed for safe disposal.
- 10 WHEN you have completed your work with a culture the petri dish should be placed in a sterilin bag or into a container of bactericidal disinfectant.

Method**Preliminary study – Safety and Plate Pouring**

After watching the demonstration on plate pouring and aseptic technique prepare a pour plate as follows.

- 1 Read the safety rules for working with microorganisms.
- 2 Use a sterile 1cm³ pipette to place 0.5cm³ of *Staphylococcus epidermidis (albus)* into a sterile petri dish aseptically.
- 3 Label the base of the petri dish with your name, name of the organism, type of nutrient agar and the date.
- 4 Add 10cm³ of nutrient agar to the petri dish using aseptic technique and leave to set.
- 5 Using aseptic technique place an antibiotic disc on the surface of the agar using flamed and cooled forceps. Note the concentration of antibiotic on the disc.
- 6 Seal the petri dish.
- 7 Incubate the petri dish at 25°C for 2 to 3 days.

Preliminary Study – Measuring the Clear Zone

- 1 Watch a demonstration on measuring the size of the clear zone
- 2 Measure the diameter of the clear zone around the disc without opening the lid.

Day One – Preparation of the Pour Plate

Draw lines on the base of the petri dish so that the base is split into 3 equal parts. Label the sections 1 to 3.

- 1 Using aseptic technique prepare a pour plate containing *Staphylococcus epidermis*. (Use the method above.)
- 2 Prepare a control disc by soaking a sterile disc in sterile water.
- 3 Place the control disc in the centre of section 1 using flamed and cooled forceps.
- 4 You have 2 penicillin discs which contain 2 different penicillin concentrations. Using flamed and cooled forceps place the disc containing the lowest concentration in section 2 and the higher concentration disc in section 3.
- 5 Seal the petri dish and incubate the petri dish at 25°C for 2 to 3 days.

Follow Up – Data Collection

- 1 Without opening the lid measure the diameter of the clear zone around each disc using vernier callipers. If vernier callipers are not available, use a pair of dividers and measure against a mm rule
- 2 Tabulate your results to show the effect of penicillin on *Staphylococcus epidermidis (albus)* growth.

Write-up

- Using the class results, plot a bar chart to show the mean diameter of clear zone for each disc.
- (Optional - add error bars to your graph.)
- Do a t-test to see if there is a significant difference between the results obtained at the 2 different concentrations.
- Make an evaluation considering:
 - the limitations of the methods used,
 - anomalous values if any,
 - replication and range of values of independent variable,
 - effectiveness of control of selected variables,
 - the reliability of the results
 - the confidence with which conclusions should be drawn
- Draw conclusions considering:
 - detailed description of the features of the results,
 - the meaning of the results in relation to the hypothesis,
 - scientific explanation of the results and conclusions,
 - potential improvements and further predictions

Lesson Plan**The Effect of Penicillin on Bacteria****Context**

A practical set in the context of 9700 Syllabus – a timed practical investigation to determine the minimum effective concentration of penicillin against *Staphylococcus epidermidis* (*albus*).

It is anticipated that students will have completed an AS practical course and so they will have good basic practical skills. It is also assumed that they will have undertaken work on the nature of bacteria and the methods by which bacterial growth can be investigated.

Key aims of lesson

This practical is designed to develop practical skills and carry out an investigation over a number of lessons.

Intended learning outcomes

By the end of this practical and by answering the questions the student should be able to:

- Pour agar plates using aseptic technique
- Experience growing microorganisms
- Use the disc diffusion technique needed to undertake a microbiological investigation
- Use vernier callipers to measure clear zone diameters.
- Assess the reliability of results

Resources required

White board or flipchart and suitable pens or chalkboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

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

Timings/ minutes	Teacher / Student Activities
Previous lesson	Preparation – students to practice / learn aseptic technique by pouring an agar plate and creating a bacterial lawn. It may also be prudent for students to practice the disc diffusion test during this lesson. Student should research the mode of action of penicillin for homework as well as considering suitable controls that should be observed during such an investigation.
0-5	Introduction to the aims, intended outcomes and background information - teacher led oral presentation.
5-10	Context - review disc diffusion test, bacterial lawn and aseptic technique - teacher led class discussion / question and answer session.

10-15	Introduction to method - teacher to go through method step by step, emphasising the importance of aseptic technique throughout.
15-50	Carrying out the practical - students carry out the practical work.
50-60	Drawing together the threads – students to complete questions and begin write up no results will be available at this stage so students should evaluate the method and apparatus used.
Lesson 2	Gathering results -
0 – 10	Teacher demonstration of the correct procedure for measuring 'clear zones' produced by the disc diffusion method either using vernier callipers or other appropriate measuring device.
10 – 30	Students to measure clear zones produced in their investigation and record their results in a suitable table.
30 - 60	Students to complete write up of investigation.

Useful Information

- Students may require some practice in mastering aseptic technique. If possible they should attempt various bacterial culturing methods during the practical lessons prior to this investigation.
- A bunsen burner creates a sterile environment in a limited area around the Bunsen. Students should work as close as possible to the flame.
- The gas inlet is a useful prop for sterilised equipment
- Students should be encouraged to treat all organisms being cultured as potential pathogens.

Technical Information - The Effect of Penicillin on Bacteria

- 1 It is possible to purchase filter paper discs which are already impregnated with penicillin; these are very useful for this investigation. However, if these are not available students may impregnate filter paper discs themselves by soaking them in a solution of antibiotic, using aseptic technique.
- 2 Bacterial cultures should be prepared at least two days in advance by growing bacteria in sterilised nutrient broth.
- 3 Much of the equipment used should be sterilised before student use, these items are marked with * on the list below (other items either do not need to be sterile or are purchased already sterilized. They may be sterilised by using an autoclave. If an autoclave is not available a chemical antiseptic may be used to clean the equipment although this will not be as effective.
- 4 Students should wear lab coats and latex gloves throughout the procedure, if available.
- 5 It is advisable although not essential to incubate the bacterial lawn plates at 25°C to encourage bacterial growth, suitable growth usually takes 2-3 days at this temperature (longer if not in an incubator).
- 6 Each bacterial lawn will require 10cm³ molten nutrient agar, the agar should be prepared, sterilised and decanted into individual sterile vessels prior to the practical so that each student has access to their own bottle containing 10cm³ sterile, molten agar.
- 7 Agar can be prepared well in advance and has a shelf life of years if kept sterile but should be melted down prior to the practical using a boiling water bath.
- 8 Variables that should be controlled include concentration and volume of bacterial culture, concentration and volume of agar used, size of filter paper disc, weight of filter paper and concentration of penicillin solution.
- 9 A suitable control for the experiment would be the presence of a filter paper disc soaked in sterile water.

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

- 1 Bunsen burner, to enable good aseptic conditions
- 2 10 cm³ bacterial culture in nutrient broth
- 3 10 cm³ molten nutrient agar*
- 4 10 cm³ sterile water*
- 5 Sterile 90 mm Petri dish
- 6 1 cm³ pipette*
- 7 Filter paper discs* or prepared penicillin discs
- 8 Forceps
- 9 10 cm³ 1% penicillin solution (students may carry out dilution of this to achieve several concentrations for testing)
- 10 20 cm³ absolute ethanol, to enable sterilisation of forceps
- 11 50 cm³ bactericidal disinfectant for containment of used forceps and pipettes, also to clean work surfaces.

Safety Precautions/Risks.

It is recommended that the following sources be used to ensure the safety of all microbiological work by teachers, support staff/technicians (if any) and students.

Topics in Safety (3rd Edition, 2001), publisher Association for Safety Education, College Lane, Hatfield, Herts, AL10 9AA. ISBN 0863571042

MISAC Safety guidelines

<http://www.ncbe.reading.ac.uk/NCBE/SAFETY/PDF/Topics15.pdf>

Society for General Microbiology Safety website including GMLP (Good Microbiology laboratory Practice) and Risk Assessment.

<http://www.microbiologyonline.org.uk/safety.html>

Follow the safety precautions as given below.

Safety in the use of microorganisms



Rules for working with microorganisms:

- 1 BEFORE STARTING WORK cover all cut or broken skin with a waterproof dressing.
- 2 WEAR tightly fitting disposable gloves when working with all live cultures.
- 3 WEAR a clean laboratory coat with all the fastenings closed.
- 4 BEFORE AND AFTER each working session wash the bench surface with bactericidal disinfectant.
- 5 AFTER each working session dispose of gloves into the sterilin bag or other disposal container provided. WASH your hands with bactericidal soap.
- 6 SWAB any spillages with bactericidal disinfectant. DISPOSE of any contaminated paper in the sterilin bag or other disposal container provided.
- 7 NEVER place anything in your mouth whilst working with microorganisms. This includes foods, liquids, gummed labels, pipettes etc.
- 8 ALL CULTURES should be labelled clearly with the following information:
 - Your name
 - Name of the organism
 - Type of nutrient medium used
 - Date
- 9 NO CULTURE should be left for more than one week. Incubators are checked daily and outdated cultures will be removed for safe disposal.
- 10 WHEN you have completed your work with a culture the petri dish should be placed in a sterilin bag or into a container of bactericidal disinfectant.

A risk assessment should be carried out as a matter of course.