Equipment


Nutrient agar plate/Petri dish (pre-prepared)


Filter paper discs


Clear tape


Antibacterial handwash


Ruler


Three antiseptics


Disinfectant bench spray


Forceps


Wax pencil/ Permanent marker

Learning Objectives -

→ Investigate the effect of antiseptics on bacterial growth using agar plates and measuring zone of inhibition.

Aseptic Techniques

Essential to avoid contamination


Surroundings get into your experiment, spoiling results.

Experiment getting into surroundings, causing a hazard.

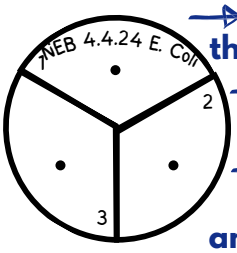
Method:

Pre-use sterilisation of petri fishes and agar gel in an autoclave
Kills any present bacteria

Set up

1) Thoroughly wash your hands.
Spray and wipe the bench with **disinfectant**.

2) Mark the **underneath** of a nutrient agar plate with the wax pencil:



→ Divide the plate into three, numbered 1,2 & 3.

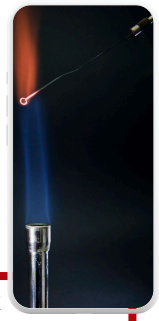
→ Draw a dot into the middle of each.

→ Write your initials, the date and name of bacteria.

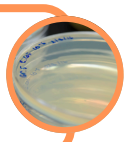
3) Rewash your hands with antibacterial hand wash.

Sterilisation technique to create uncontaminated cultures:

1. **Petri dish** and **agar** must be sterilised
2. **Inoculating loop** is passed through a Bunsen burner flame. Once cooled it is used to transfer to the agar.



Make sure the lid stays in place.
Avoids contamination



Sterilisation of neck and glass spreader in Bunsen burner
Kills any present bacteria

Testing antiseptics

4) Soak filter paper discs in your 3 antiseptics.

5) Lift the lid of the Petri dish at an angle facing away from you. Avoid fully opening it.

Soak each for the same duration

6) Use the sterilised forceps to place each disc onto dots drawn on with the wax pencil.

7) Secure the lid of the Petri dish using two small pieces of tape.

8) Incubate the plate at 25°C for 48 hours.

9) Measure the diameter of the clear zone around each disc with a ruler - horizontally and vertically (90°)
Calculate the mean diameter.

10) Record you results

REQUIRED PRACTICAL 2
MICROBIOLOGY

What can grow on an agar plate?

→ Fungi ✓

→ Bacteria ✓

→ Viruses ✗ (they need living cells to reproduce)

Keep the lid in your hand
Replace all lids as soon as possible
Prevents airborne bacteria contaminating the bottles/plate

Do not seal the lid as this creates anaerobic conditions, preventing *E. coli* growth.

Zones of inhibition
The 'clear zone' you measure around the disc has no bacteria, so a larger zone indicates the antiseptic was more effective.

Incubation temperatures
Reduce chance of growing harmful pathogens.
Higher in industry to increase the rate of growth.

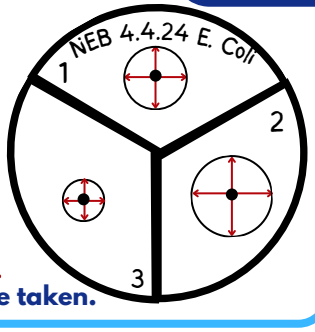
Math Skills
Cross sectional area = πr^2
r = diameter ÷ 2

Type of antiseptic	Diameter of clear zone in mm			
	1	2	Mean	Area
Mouthwash (1)				
Antiseptic gel (2)				
Antiseptic cream (3)				

Conclusion example:

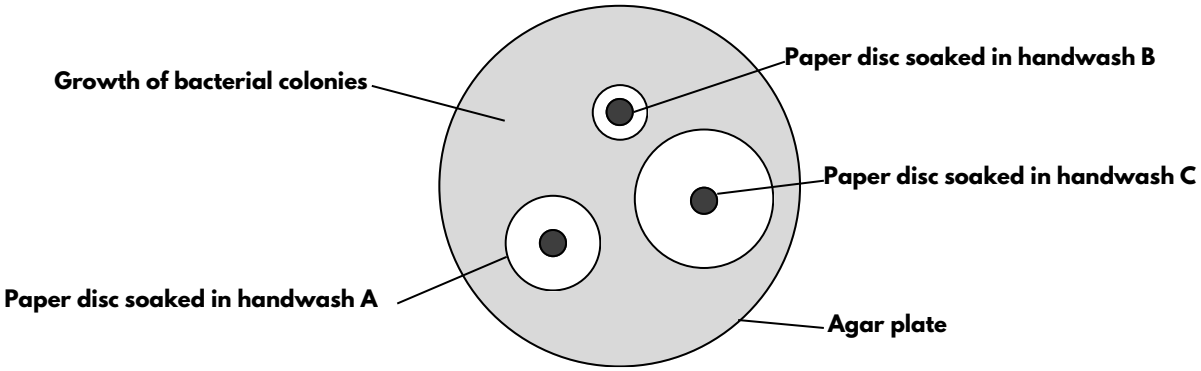
"The most effective antibiotic was antiseptic 2 because it has the largest clear zone around the disc with an area of ____"

Measurements to be taken.



Exam Style Questions - Microbiology

1. A student investigated the effectiveness of three handwash brands (A, B, C) at preventing the growth of bacteria.
Figure 1 shows the way the investigation was set up.



a) Describe two aseptic techniques the student should have used. (2 marks)

b) What measurement would the scientist need to take to calculate the area where no bacteria were growing? (1 mark)

c) Which antiseptic is the most effective at preventing the growth of bacteria?
Give a reason for you answer. (2 marks)

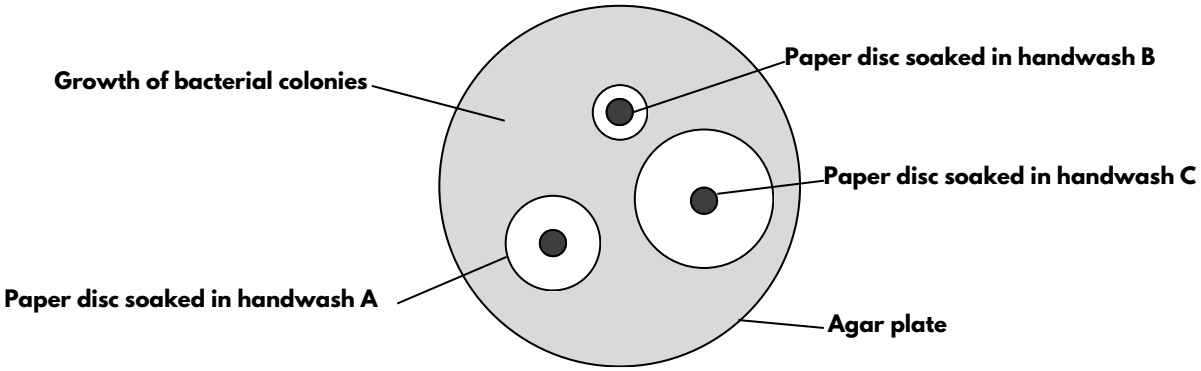
d) Give one change to the investigation that would allow the scientist to check is the results are repeatable. (1 mark)

2) Some students investigated antibiotics. To do this they set up a sterile Petri dish containing an agar culture media with five paper discs with different antibiotics. They incubated the dish for 48 hours at 25˚C. Give one safety precaution that the student used in their investigation. State why this safety precaution is needed. (2 marks)

3) Scientists grow microorganisms in industrial conditions at a higher temperature (40˚C) than in school laboratories. What is the advantage of using a higher temperature? (1 mark)

Exam Style Questions - Microbiology (Answers)

1. A student investigated the effectiveness of three handwash brands (A, B, C) at preventing the growth of bacteria.
Figure 1 shows the way the investigation was set up.



a) Describe two aseptic techniques the student should have used. (2 marks)

One mark for each of the following, up to a maximum of two marks:
- **Sterilise equipment/surfaces (before use).**
- **Use sterilised agar.**
- **Secure lid of the Petri dish with adhesive tape.**
- **Only lift the lid of the Petri dish a little bit.**
- **Lift the Petri dish at an angle.**

b) What measurement would the scientist need to take to calculate the area where no bacteria were growing? (1 mark)

Mark One - Radius (of area with no bacteria growing). Allow diameter, but ignore πr^2.

c) Which antiseptic is the most effective at preventing the growth of bacteria?
Give a reason for you answer. (2 marks)

Mark One - Handwash C.
Mark Two - It has the largest area with no bacteria growing (allow largest inhibition zone or description of largest inhibition zone).

d) Give one change to the investigation that would allow the scientist to check is the results are repeatable. (1 mark)

Mark One - Repeat and look to see if results are similar/different or if there are anomalies.

2) Some students investigated antibiotics. To do this they set up a sterile Petri dish containing an agar culture media with five paper discs with different antibiotics. They incubated the dish for 48 hours at 25˚C. Give one safety precaution that the student used in their investigation. State why this safety precaution is needed. (2 marks)

Mark One for the correct precaution and Mark Two for the corresponding reason:
- **Precaution: Petri dish/culture media/agar sterile. Reason: So no harmful microorganisms grow.**
- **Precaution: Petri dish/lid secured with tape. Reason: So microorganisms cannot get out.**
- **Precaution: Incubated at 25˚C. Reason: To prevent/reduce growth of pathogens.**

3) Scientists grow microorganisms in industrial conditions at a higher temperature (40˚C) than in school laboratories. What is the advantage of using a higher temperature? (1 mark)

One mark for each of the following, up to a maximum of two marks:
- **Microorganisms grow/work/reproduce/act faster.**
- **Results/product acquired sooner.**