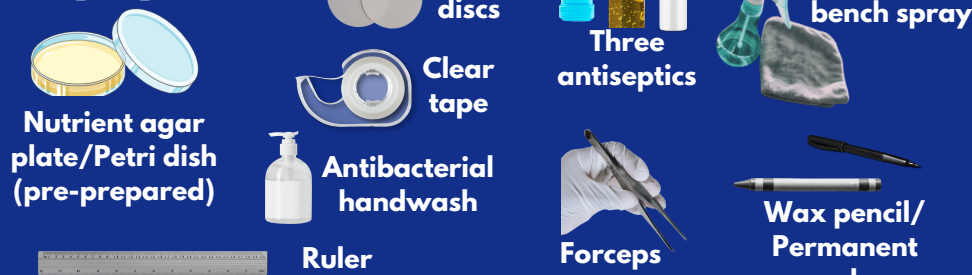


# Equipment



**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:

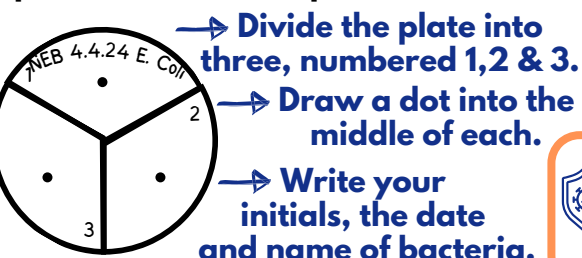
**Pre-use sterilisation of petri dishes and agar gel in an autoclave**  
 Kills any present bacteria

### Set up

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

**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.

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



Make sure the lid stays in place. **Avoids contamination**

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

3) Rewash your hands with antibacterial hand wash.

### Testing antiseptics

4) **Soak** filter paper discs in your **3 antiseptics**.

# REQUIRED PRACTICAL 2 MICROBIOLOGY

**What can grow on an agar plate?**  
 → Fungi ✓  
 → Bacteria ✓  
 → Viruses ✗ (they need living cells to reproduce)

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

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

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**.

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.

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

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

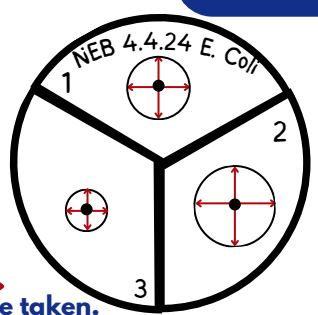
9) Measure the **diameter** of the clear zone around each disc with a ruler - **horizontally** and **vertically (90°)**. Calculate the **mean diameter**.

**Math Skills**  
 Cross sectional area =  $\pi r^2$   
 r = diameter ÷ 2

10) Record your results

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 \_\_\_\_"

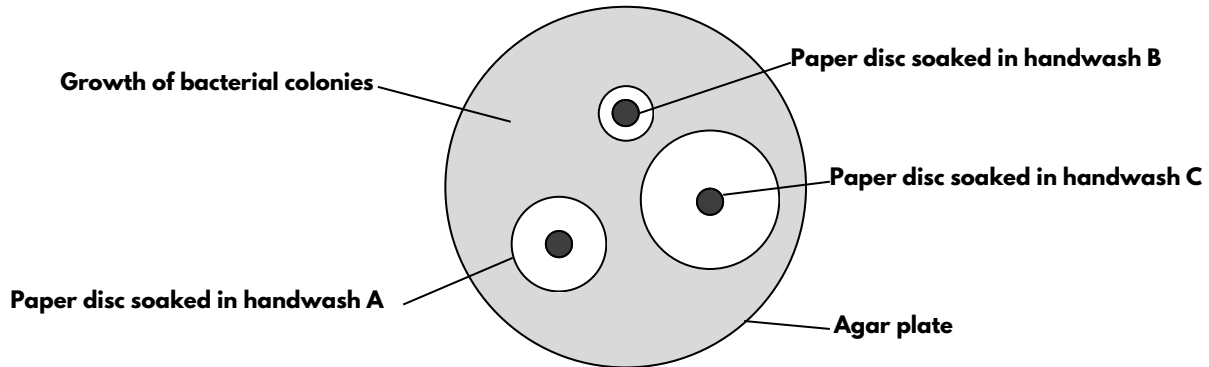


Measurements to be taken.

# 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)

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

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

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

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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)

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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)

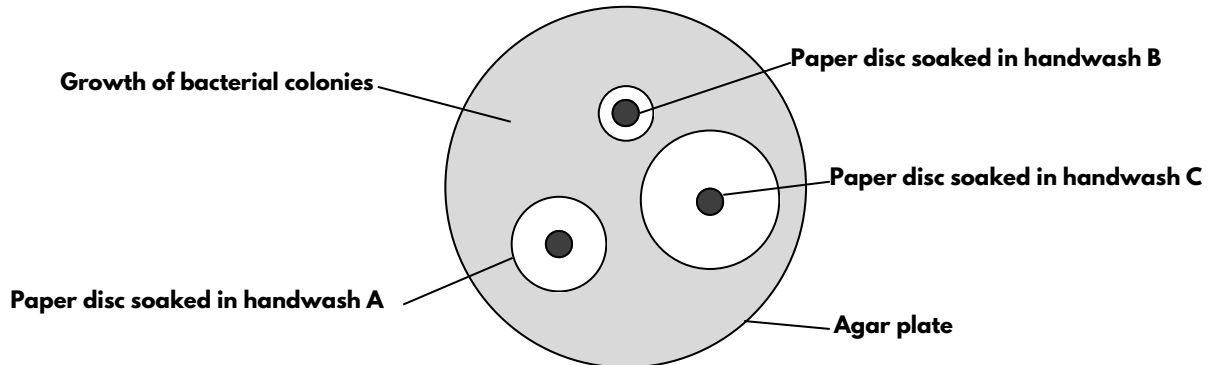
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# Exam Style Questions - Microbiology (Answers)

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  $\pi r^2$ .

c) Which antiseptic is the most effective at preventing the growth of bacteria?

Give a reason for your 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 if 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.