**The effect of antibiotics on bacterial growth STUDENT**

**Introduction**

Antibiotics can be used to treat or inhibit a bacterial infection. They work by killing the bacteria or inhibiting their growth. This activity allows you to investigate the effect of different antibiotics on the growth of *Bacillus subtilis*.

**Aim**

To investigate the effect of various antibiotic preparations on bacterial lawns*.*

**Intended class time**

* 45 minute session to spread the plate and place antibiotic discs
* 30 minutes three days later to observe results

**Chemicals**

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| --- | --- |
| 70% ethanol | HSE warning symbol Highly flammableHarmful if swallowed |
| *Bacillus subtilis* | Although *Bacillus subtilis* is not considered pathogenic the possibility of mutation or contamination means all cultures should be treated as potentially pathogenic. Spills must be disinfected. Used cultures must be disposed of by sterilisation.  |
| Antibacterial (1% Virkon®) | No known health hazard but avoid cotact with skin and eyes |

**Equipment**

* Bunsen burner
* Antibacterial spray
* Antibacterial waste pot
* Bactericidal hand-wash
* Paper towels
* Forceps
* Sterile nutrient agar plate
* 5 ml culture of *Bacillus subtilis*
* Tray
* 100 µl pipette and sterile tips
* Glass spreader
* Marker pen
* 3 different antibiotic discs, **A** , **B** and **C**
* Sterile filter paper disc soaked in sterile distilled water (**D**)
* 70% ethanol provided in a glass Petri dish with a lid
* Tape
* Graph or squared paper

**Health and Safety**

* Ethanol is highly flammable and you have a naked flame on your bench. Great care is therefore needed to minimise the risk of fire. Have only a small volume of ethanol in your wide dish, keep it covered when not in use and well away from the Bunsen burner.
* Wear a lab coat and goggles when working with microorganisms.
* Cover any skin cuts or abrasions.
* All spills must be immediately disinfected using 1% Virkon® solution left in place for 10 minutes.

**Procedure**

**Part 1 – spreading the plate and placing the discs**

*Note: At all times in this investigation, a roaring Bunsen flame should be burning on the bench. This is needed for sterile working. However, it is a fire hazard. Keep your dish of ethanol well away from the Bunsen burner and keep the dish covered when not in use.*

1. Wipe your bench with antibacterial spray and a paper towel. Pour 1% Virkon® solution into your tray to cover the bottom with a thin layer, leave in place for 10 minutes and then wipe away.
2. Take a sterile agar plate and a 5 ml culture of *Bacillus subtilis* and place in your tray.
3. Label the agar plate with the date and your name.
4. On the bottom of the plate, draw a cross and label the 4 sections “**A**”, “**B**”, “**C**” and “**D**” using the marker pen.

**B**

**A**

**C**

**D**

1. Wash your hands thoroughly using anti-bacterial hand wash and dry with a paper towel.
2. Put a sterile tip on to your pipette and then, working near the flame, carefully open the culture and use the pipette to remove 100 µl of culture, flaming the top of the culture bottle when opening and closing.
3. Lift the lid of the agar plate very slightly and pipette the 100 µl of culture into the centre of the plate, close the lid. Discard the pipette tip into the antibacterial waste pot.
4. Next, take the glass spreader, dip it in ethanol and flame it in a blue Bunsen flame, hold it close to the flame until it is cool, then use it to spread the culture evenly across the surface of the agar plate.
5. Dip the tips of the forceps in ethanol and flame them in a blue Bunsen flame and allow them to cool.
6. Take a disc of the first antibiotic to be tested in the forceps.
7. Lift the lid of the agar plate very slightly, only enough to be able to slide the forceps in and gently place the disc in the centre of area **A**.
8. Dip the forceps in ethanol and flame them in a blue Bunsen flame.
9. Repeat for the other two antibiotic discs and place them in areas **B** and **C**, remembering to flame the forceps each time.
10. In area **D**, place a sterile filter paper disc that has been soaked in sterile distilled water, flaming the forceps as before.
11. Use 2-4 small pieces of sticky tape to tape the lid of the plate securely onto the base but do not seal it completely.
12. Disinfect your tray with 1% Virkon®, and wash your hands thoroughly with bactericidal hand-wash.
13. Incubate the plate for three days at 20-25°C.

**Part 2 – observing the effects of the antibiotics**

1. After three days, look at the plate but do not open it. Draw and annotate a diagram of the agar plate indicating any clear zones that have appeared in the four areas.
2. Measure any clear zones around the antibiotic and water discs (graph or squared paper can be used to help you).
3. Use your drawing and measurements to draw conclusions about the anti-bacterial properties of each antibiotic.

**Extension questions**

1. What was the purpose of the lit Bunsen burner while you were working?
2. Why did you only lift the lid slightly from the agar plate when placing the discs on the agar?
3. Why did you not seal the plate completely?
4. Why is 20-25°C a more suitable incubation temperature than 37°C?
5. What was the purpose of using a filter paper disc soaked in distilled water in area **D**?

**To submit**

For this piece of work to count towards Practical Activity Group 7 of the GCE Biology Practical Endorsement, you need to have evidence of a drawing of the agar plate with its clear zones around the antibiotic and filter paper discs, measurements of these clear zones and have drawn conclusions about the anti-bacterial properties of each antibiotic. You also need to have considered the above questions as the answers to these questions will aid you in preparation for your written examinations.