Edexcel B Biology A-Level

Core Practical 13

Isolate an individual species from a mixed culture of bacteria using streak plating

Aseptic technique is used to **avoid contamination** of the sample from outside substances, such as microorganisms. This is important to get **reliable** and **repeatable** data.

Streak plating is a technique in which bacteria are spread out on a nutrient agar plate so that **distinct individual colonies** can be seen. These colonies can then be grown on clean agar plates to produce non-contaminated **samples of one species of bacteria**. The colonies can be identified as a particular species of bacteria via indicators such as the **size, colour and texture** of the colony. Streak plating is an alternative to pour or spread plating.

Aseptic Technique

- Wipe down surfaces with **antibacterial cleaner** both **before and after** experiment.
- Use a **Bunsen burner** in the work space so that **convection currents** draw microbes away from the culture.
- **Flame the wire hoop** before using to transfer bacteria.
- **Flame the neck of any bottles** before use to prevent any bacteria entering the vessel (air moves out so unwanted organisms don't move in).
- Keep all vessels containing bacteria **open for the minimum amount of time.**
- **Close windows and doors** to limit air currents.

Equipment

- Bench disinfectant
- Paper towels
- Bunsen burner
- Inoculating loop
- Mixed culture of bacteria
- Three nutrient agar plates
- Adhesive tape

Method

1. Loosen the cap of the mixed culture tube.
2. Flame the **inoculating loop** to **sterilise** (by holding it in the Bunsen burner flame until the loop turns bright orange). Allow to cool.
3. Flame the neck of the mixed culture tube to sterilise (by holding it in the Bunsen burner flame).
4. **Dip the inoculating loop** into the mixed culture.
5. Flame the neck of the mixed culture tube again and replace the lid.
6. Open the petri dish lid as little as possible. Make four **streaks** from one 'corner' of the plate to an adjacent 'corner'. Streak lightly and do not scratch the agar.

7. Turn the plate **ninety degrees** and make another three/four streaks at a nine degree angle from the first. **Subsequent streaks should overlap**, but the fourth streaks should not overlap with the first.
8. Flame the inoculating loop again.
9. **Tape the lid** of the Petri dish (not all the way around, so the conditions in the dish are not **anoxic**) and leave for 24 hours in an incubator.
10. The following day, **observe and sketch** the plates and take a sample of a white colony using an inoculating loop which has grown on the plate and repeat the process on a fresh nutrient agar plate.
11. Take a sample of a yellow colony using an inoculating loop which has grown on the plate and repeat the process on a fresh nutrient agar plate.
12. Tape the lid of the Petri dishes (not all the way around, so the conditions in the dish are not anoxic) and leave for 24 hours in an incubator.
13. Observe and sketch the plates.

Note: white colonies are likely to be ***Salmonella***. Yellow colonies are likely to be ***Staphylococcus***.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Disinfectant	Flammable	Keep away from naked flame	Put out fire; seek assistance	Low
Biohazard	Contamination ; infection	Use disinfectant; wash hands with soap after dissection; do not incubate at human body temperature; do not open agar plate post incubation	Seek assistance	Low/medium (depends on likeliness of bacteria sample used to cause infection)
Naked flame	Fire hazard; burns	Keep away from flammable materials; tie up long hair; keep away from edge of desk	Put out fire; seek assistance;run burns under cold water immediately	Low