Science ASSIST Standard Operating Procedure

Preparing a bacterial lawn culture

1. Introduction

A bacterial lawn culture is where an agar plate is inoculated with bacteria to produce a heavy uniform layer of growth over the whole surface of the agar without any individual colonies visible. This is often referred to as confluent growth. Lawn cultures are used to test the susceptibility of bacteria to antimicrobial substances such as antibiotics, antiseptics and disinfectants.

Bacterial lawn cultures can be produced by 3 different methods:

A pour plate technique where a bacterial suspension is flooded over the surface of the agar plate and any excess removed with a pipette.
A spread plate method where the agar is inoculated with a sterile swab soaked with a bacterial suspension.
A spread plate method where a bacterial suspension is spread over the surface of the agar using a sterile spreader.

In this activity students will produce a lawn culture using the spread plate method and a sterile swab soaked in a bacterial suspension.

Teachers and laboratory technicians should check the microbiological guidelines in their jurisdiction before proceeding as some microbiological activities are permitted in some jurisdictions and not others.

2. Context

- This activity aligns with **School Level 3** of Science ASSIST Microbiology Guidelines.
- This practical activity should be supervised by science teachers and technicians who are highly trained in microbiological techniques. Staff need to be trained and experienced in aseptic technique, subculturing, recognising contamination and sterilisation and decontamination procedures.
- PC1 facilities required. Autoclave or pressure cooker with gauge required for sterile preparation of media, equipment and for decontamination.
- Microorganisms (Risk group 1 bacteria) obtained as pure cultures from school biological suppliers
- Additional staff are recommended to enable a high level of student supervision when undertaking this activity.

3. Safety Notes

To be undertaken only by trained personnel in conjunction with a current Safety Data Sheet (SDS) and site-specific risk assessment. Before proceeding, ensure that this activity is permitted within your school jurisdiction.

- Do not commence work unless a site specific risk assessment is completed and control measures are implemented. Immunosuppressed staff and students must be considered.
- Regard all microorganisms as potential pathogens and treat them with standard microbiological practices to minimise risk to laboratory staff and the environment.
- Use only a pure culture of a Risk Group 1 (RG1) microorganism.
- No subculturing from plates or broths inoculated by students.
- Observe good hygiene practices and aseptic techniques at all times.
    - Wash hands thoroughly with soap and water before commencing activity and on leaving the laboratory.
    - Cover any cuts or wounds on the hands with a waterproof dressing or Band aid.

- Keep hands away from the mouth, nose, eyes and face during and after this activity.
- Decontaminate workbenches with disinfectant (70% v/v ethanol or hospital grade disinfectant) before and after microbiological activities.
- Keep 70% v/v ethanol away from heat and sources of ignition.
- Use aseptic techniques to minimise the production of microbial aerosols.
- Conduct all work close to a Bunsen flame.
- Only open Petri dishes, tubes and bottles for the minimum amount of time.

- Waste must be sterilised before disposal at 15psi, 121˚C for 15-30 minutes.
- Use caution when working with a Bunsen burner, the neck of culture tubes may be hot.
- Disposable plastic aprons are not recommended when Bunsen burners are used.
- Care must be taken if there is a great deal of condensate in the lid of the agar plate. This can easily drip out carrying microorganisms with it.
- Count plates out and in.
- Follow all general laboratory safety guidelines and wear appropriate PPE.

4. Regulations, licences and permits

Consult the science guidelines in your jurisdiction for policies regarding the subculture of microorganisms..

5. Equipment

- PPE: safety glasses, laboratory coat; non-slip, non-porous closed in shoes.
- 70% v/v ethanol
- 0.5-1% bleach solution freshly prepared. 1 beaker per group of students.
- Pure culture of a Risk Group 1 microorganism in nutrient broth (slight turbidity), prepared aseptically or purchased commercially. This can be in a test tube with lid or small screw capped bottle. Suggest *Escherichia coli* K-12 strain or *Micrococcus luteus*.
- 1 sterile nutrient agar plate
- 1 purchased sterile cotton swab stick or cotton buds sterilised in a beaker with a foil cover.
- Bunsen burner
- Sticky tape or laboratory sealing film (e.g., Parafilm® M)
- Incubator or suitable area for incubation that is away from sunlight and between room temperature (22-25˚C) and a maximum of 30˚C.
- Paper towel

6. Operating procedure

1. Prepare the laboratory by closing all windows and doors to prevent draughts and the entrance of staff and students.
2. Remove all non-essential books and equipment from the workspace and disinfect the work bench with 70% v/v ethanol and paper towel. Allow the ethanol to evaporate and ensure all alcohol vapours have dissipated before lighting Bunsen burners
3. Collect all equipment required including cultures, agar plates, and sterile swab stick.
4. Light the Bunsen to create a hot blue flame which provides an updraft for the surrounding area to help reduce contamination
5. Loosen the lid of the broth culture.
6. Place the agar plate upside down on the bench so that the lid is on the bench and the base of the plate containing the agar is uppermost.
7. Gently tear the swab packaging at the handle end so that the handle is slightly exposed. See figure 1. Alternatively, loosen the foil cover on the beaker containing the sterile cotton buds.

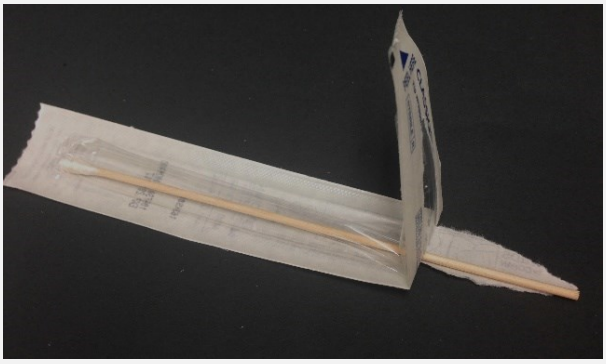

Figure 1: Purchased sterile cotton swab stick with handle exposed immediately prior to sampling.

8. Aseptically remove the sterile cotton swab from the packaging or beaker without touching the cotton tip to anything. Hold it in one hand.
9. Working close to the Bunsen flame, hold the well mixed broth culture in the other hand, remove and hold the lid with the little finger on the hand holding the swab stick. Do not place the lid down on the bench. Flame the opening of the tube/bottle by passing it through the flame 3 times. This creates an expansion and upward rising of air in the tube to minimise microorganisms entering the tube from the environment.
10. Moisten the cotton swab in the broth culture. Remove the swab and flame the neck of the tube as before and replace the lid.
11. Working close to the Bunsen lift the base of the agar plate and turn it so the agar surface is facing you. Gently swab the agar surface with the cotton swab in a rolling motion or in a backwards and forwards motion over the entire surface of the agar, turning the plate 3 times to distribute the bacteria evenly across it. This must be done very gently so the swab does not break the agar surface.
12. Immediately replace the culture plate into the lid of the Petri dish. It is important that the Petri dish is kept open for the minimum amount of time to reduce the risk of contamination.
13. Place the used swab into the container of bleach.
14. The lawn inoculated plate is now ready for susceptibility testing of antiseptics and disinfectants. See SOP: Susceptibility testing of antiseptics and disinfectants

Alternatively:

15. After spreading the culture over the surface of the agar, replace the culture plate into the lid of the Petri dish. Tape the agar plate closed using four pieces of sticky tape at 12 o'clock, 3 o'clock, 6 o'clock and 9 o'clock. Alternatively, use one strip of laboratory sealing film by wrapping it once around the perimeter of the agar plate where the two halves join. The laboratory sealing film must not overlap at any section.
16. Incubate the agar plates upside down for 24 to 48 hours between room temperature (22-25˚C) and a maximum of 30˚C in an aerobic environment. This can be in an incubator or an area in the lab away from direct sunlight.
17. After incubation, plates should be sealed around the whole circumference with sticky tape or laboratory sealing film before distribution to students for examination. This reduces the risk of students opening the plates and prevents any liquid escaping that may contain microorganisms. The plates can be stored in the refrigerator upside down for up to 1 week.
18. These plates should remain sealed whilst students examine them for an even and complete spread of growth (confluent) over the surface of the agar plate (a lawn). There should be no single colonies visible. See Figure 2. Have the students comment on the growth.

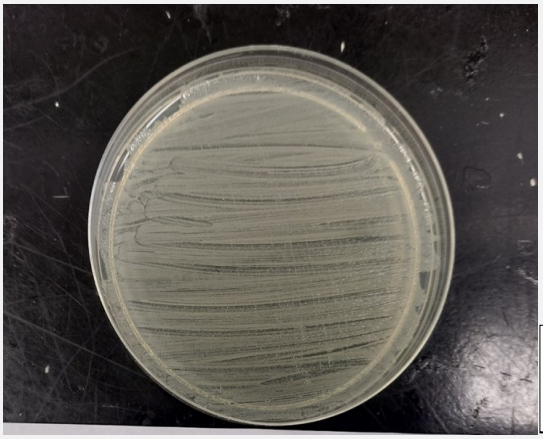

Figure 2: Lawn culture of *E. coli* on nutrient agar

19. After the plates have been examined, decontaminate the work bench with 70% v/v ethanol.
20. Remove PPE and wash hands before leaving the laboratory or starting another activity.
21. Ensure the agar plates are sterilised in an autoclave or pressure cooker (15psi, 121˚C for 15 -30 minutes) before disposal. Once autoclaved place into a garbage bag and into the normal waste bin.

7. Trouble shooting/emergencies

- **First Aid:** See latest SDS for ethanol, hospital grade disinfectant, and sodium hypochlorite (bleach) for more detailed information
  - **If swallowed**: Do not induce vomiting. Rinse mouth with water. Seek medical attention.
  - **If in eyes**: Hold open and irrigate with copious quantity of water. Seek medical attention.
  - **If on skin or hair:** Flush skin and hair with running water (and soap if available). Seek medical attention if irritation occurs.
  - **If inhaled:** Remove to fresh air and seek medical attention if symptoms persist.
  - For further advice contact the Poisons Information Centre on 131 126.

- **No lawn culture produced but single colonies evident**:
  - Insufficient culture picked up with the swab and placed onto the surface of the agar or concentration of organisms in the broth is too low.
- **Different shapes, sizes and coloured colonies growing on the surface of the agar plate** indicates contaminating organisms. This may have resulted from:
  - Nutrient agar not sterile
  - Broth culture containing the RG1 microorganism is not pure, it has been contaminated.
  - Breach of aseptic technique allowing unwanted microorganisms to contaminate the broth, nutrient agar plate and/or swab sticks.

8. Waste disposal

- Swab sticks should be placed in a 0.5-1% bleach solution for 2 hours prior to disposing into the regular waste.
- Paper towel should be placed into an autoclave or oven bag and sterilised at 15psi, 121ºC for 15-30 minutes, before being disposed of in the regular waste bin.
- Culture media containing microorganisms such as broth or agar plates should be autoclaved in their original containers prior to disposing in the regular waste. It is very important that bottles with lids should be loosened before putting through an autoclave or pressure cooker.
- Do not autoclave paper towel or any item that has been in contact with ethanol. These can be placed directly into the general waste bin.

9. Related material

- Risk Assessment
- Safety data sheets for microorganisms used, nutrient agar, bleach and ethanol
- See the following Science ASSIST material on:
  - SOP: Operating a pressure cooker and autoclave
  - SOP: Preparing nutrient and plain agar plates

References and further reading

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