Sterile Technique; Preparation of Smears, Simple Stains

Objective
The techniques you will be learning are common techniques used to prevent contamination and promote the safe handling of microorganisms. They will also be applied to the preparation of smears of pure cultures that can be used for a variety of staining techniques. Note that there can be variations in these standard techniques.

Key terms
Aseptic: Transfer of biological material without introducing contamination.
Flaming: Incineration of biological material to sterilize prior to use.
Smear: Thin distribution of bacteria across the surface of a glass slide in distilled water.
Heat fixing: the passage of a smeared slide prep through the flame of a Bunsen burner to kill and affix the bacteria to the slide.

Introduction
A medium that contains living microbes is called a **culture**. If a culture contains a single species it is said to be a **pure culture**. It is essential to transfer microbes from their pure culture to a sterile medium aseptically, that is, without contamination of yourself, others, the environment, the source culture, or the medium being inoculated. In other words you want your pure culture to stay pure, your new culture to be pure, and the surroundings to remain uninoculated.

Stains are solutions consisting of a solvent (water or ethanol) and a colored molecule called a chromogen. Basic stains are attracted to the negative charges on the surface of most bacterial cells.

Materials
Live Organisms: *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Enterococcus fecalis, Klebsiella pneumoniae*
Bunsen burner
Inoculating loop
Glass microscope slides
Distilled water
Stains: Methylene blue, Crystal violet, Safranin
Lab apron
Chemical eye protection

Procedure

Aseptic transfer and inoculation
1. Minimize the potential of contamination. Do not perform transfers over books and papers because you may inadvertently and unknowingly contaminate them with droplets.

2. Be organized. Arrange all media in advance and clearly label them with your name, the date, and the inoculating species. Tubes can be labelled with tape or by writing directly on the glass. Write directly on the base/tube and NOT on the lid of plastic petri plates or tubes. Be sure not to place any labels in such a way as to obscure or obstruct your view of the plates or tubes interior.

3. Place all media tubes in a test tube rack when not in use. Never lay tubes on the table surface as they may leak.

4. Take your time. You are handling potentially dangerous microbes. Working at a frenzied pace leads to carelessness and accidents.

5. Never hold a tube culture by the cap. Caps are generally loose to allow aeration.

6. Hold the inoculating loop like a pencil in your dominant hand.

7. Adjust your Bunsen burner so its flame has an inner and outer cone.

8. Flame the loop and wire from base to tip. Make sure that it gets uniformly orange-hot.

9. Pick up the culture tube in your free hand and remove the tubes cap with the little finger of your loop hand.

10. Flame the tube's lip by passing it quickly through the flame two or three times.

11. Carefully touch the loop to the growth on the agar's surface (approximately 2/3^{rds} down) and gently glide the loop across the surface and pick up the smallest amount of cells you can see with the naked eye from the tube without digging into the surface of the agar.

12. Keeping the loop hand still, flame the tube lip as before and replace the cap. Return the tube to the test tube rack.

13. Pick up the sterile medium tube with your free hand.

14. Keeping the loop hand still, remove the tube's cap with the little finger of your loop hand.

15. Flame the tube's lip by passing it quickly through the flame two or three times.

16. Inoculate the medium directly with the cultured bacteria on the loop.

17. Withdraw the loop from the tube and flame the tube's lip by passing it quickly through the flame two or three times as before and replace the cap. Return the tube to the tube rack.

18. Flame the loop and wire from base to tip. Make sure that it gets uniformly orange-hot.

19. Incubate the inoculated culture at the assigned temperature for the assigned time.

Smears and simple staining

1. Once you have learned the aseptic (sterile) technique, the Instructor will demonstrate how to make a:
(i) bacterial smear*
(ii) clean silde to remove smudges/dirt
(iii) label slide with your name, date, species name, and stain
(iv) place a drop of distilled water in the middle of the slide
(v) touch loop of bacteria to slide and disperse cells from the loop into the water
(vi) flame the loop to kill off any excess bacteria, and cool the loop.
(vii) spread the bacteria in the water around the surface of the slide using the cooled loop.
(viii) dry the smear on a hotplate
(ix) heat fix the smear before performing a simple stain.

2. *The bacterial smear should not be:
(i) too thick, (if you can easily see your smear then you have TOO many cells).
(ii) too thin and/or (iii) clumpy (no visible chunks of bacteria).

3. Heat fixed/unstained slide can be saved in your slide box for staining later.

4. Each student will make at least one smear from the six bacterial cultures available. You may make more smears/stains if time permits.

5. Learn how to take slide pictures using the special microscope cameras.

Simple staining

1. Add stain for ~ 1 min

2. Rinse with water.

3. Blot dry with paper towel

4. Observe at 1000X magnification

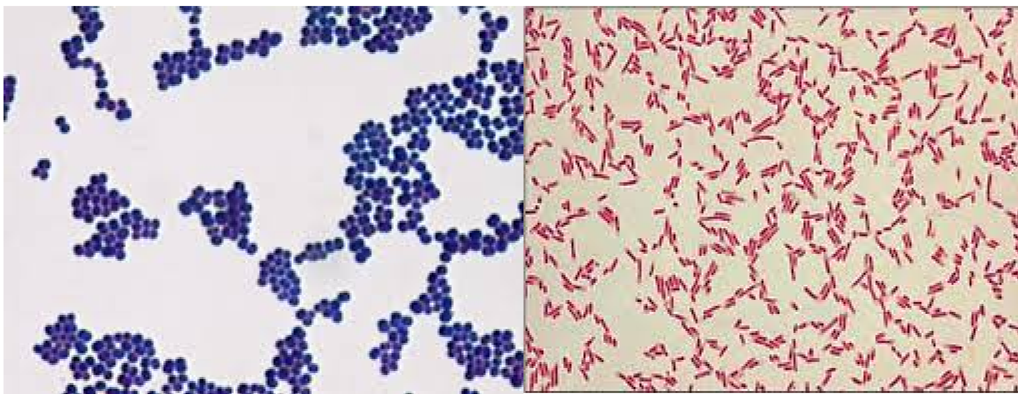
(You can choose any one of the three stains: Methylene blue, Crystal violet, Safranin

Safety and disposal

All used slides that have been fixed and stained may be disposed of in the cardboard box for uncontaminated material at the front of the class. Any slides containing wet unfixed bacteria should be disposed of in the disinfectant glass dish on the side table.

Observations
All stained slides should be originally viewed under low magnification to identify a suitable area of material to examine under higher magnification. After a suitable area has been identified the final observation of the specimen should be concluded under oil immersion at 1000X magnification.

Simple stain allows you to see morphology (rod, coccus, spiral etc.) and arrangement (pairs, tetrads, chains, clusters etc.). Heat fixing kills the bacteria and attaches the smear to the glass slide. You should never hold smear directly under a stream of water when rinsing your stained smears.



Examples of Simple Stains on Bacteria