Exercise 2.2 – Aseptic Transfer

LEARNING OUTCOMES

1. Explain the importance of working aseptically when handling microorganisms.
2. Use aseptic technique to inoculate solid and liquid media from a bacterial culture.

Whenever it is necessary to transfer growing organisms to a sterile medium, microbiologists use a method called *aseptic technique***.** Aseptic technique prevents the introduction of unwanted contaminants and is good lab practice for handling bacteria and other microbes in the lab.

In this exercise, you will practice using aseptic technique to transfer two microorganisms, *Escherichia coli* and *Staphylococcus aureus*, between various forms of liquid and solid media (Figure 2.5). *E. coli* is a predominant member of the enteric, or intestinal, microbiome of humans and animals. While many species of *Staphylococcus* are typically present on the skin and mucous membranes, particularly the respiratory tract, *S. aureus* is only carried by some people. It is important to note, however, that both *E. coli* and *S. aureus* are potential pathogens and frequently implicated in infections, particularly those that are healthcare associated.

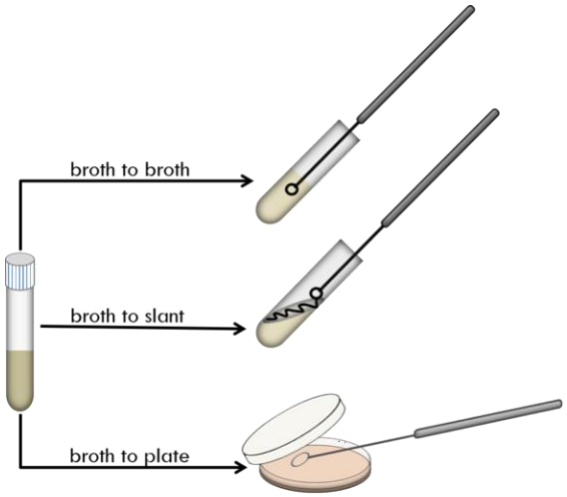

Figure 2.5: Transferring bacteria from a broth culture to liquid and semisolid media.

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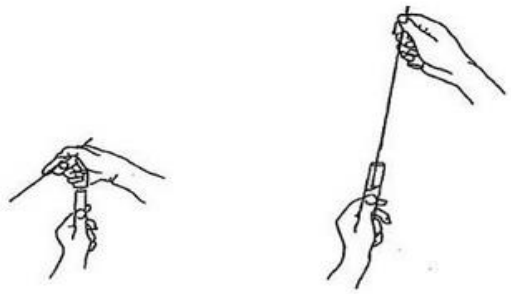
General Procedure for Aseptic Transfer

Note: Aseptic transfer is done without the help of a partner, so always close one tube before opening another. By doing this, you will always have a free hand with which to work.

1. Sterilize the inoculating loop (for broth cultures) or needle (for agar cultures) by inserting the wire in the incinerator for about ten seconds until it turns bright orange, then removing it. **To avoid a serious burn, never leave the loop unattended in the incinerator.**
2. Pick up the bacterial culture tube with your free hand and wrap the pinky of the hand holding the loop around the cap (Figure 2.6a). Remove the cap by turning the tube rather than the cap, always keeping the cap in your pinky (Figure 2.6b).
3. Heat the mouth of the culture tube by holding it against the incinerator opening and rotating it once, keeping the tube upright.
4. Obtain bacteria by dipping the loop just once in broth or lightly touching the needle to bacteria growing on a semisolid surface.
5. Reheat the mouth of the culture tube and recap it, turning the tube rather than the cap, then return the tube to the rack so that you have a free hand.
6. Pick up and carefully uncap the sterile media tube using the pinky of the hand holding the inoculating loop or needle with bacteria.
7. Heat the mouth of the sterile media tube by turning it against the incinerator opening.
8. Inoculate the sterile media tube by dipping the loop once into broth or by spreading the needle on the surface of an agar slant, being careful not to cut into the agar.
9. Reheat the mouth of the sterile media tube and recap it.
10. Sterilize the loop by placing it in the incinerator for 10 seconds.



(a) (b)

Figure 2.6: (a) Aseptically obtaining bacteria; (b) The cap always remains in the hand holding the loop.

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OBJECTIVE

Practice aseptic transfer technique using agar slants and broths.

MATERIALS

- MEDIA: Trypticase soy agar slant and broth (2 each)
- SOLUTIONS: Sterile water
- CULTURES: *Escherichia coli* slant*, Staphylococcus aureus* broth
- EQUIPMENT: Inoculating loop and needle, incinerator, vortex, marking pen, labeling tape, test tube rack

NOTE: Loops are intended for transferring bacteria that are growing in broth while needles are used for bacteria on solid media. However, when first practicing aseptic transfer, students are often more comfortable using a loop rather than a needle for obtaining bacteria.

PROCEDURE – STUDENTS WORK INDIVIDUALLY

1. Practice with a tube of sterile water before working with bacteria.
2. When you are ready, label one sterile slant and one sterile broth tube for each organism using small pieces of labeling tape with your initials, date, and organism number and placing the tape on the glass portion of the tubes near the cap; do not write directly on tubes.
3. Tighten the cap of the *S. aureus* broth culture and vortex the tube briefly to mix.
4. Using aseptic technique, transfer a loopful of *S. aureus* to a sterile tube of broth and finger-tightening the cap to close.
5. Beginning anew, use aseptic technique to transfer a loopful of *S. aureus* to a sterile slant by dragging the loop up on the agar and finger-tightening the cap to close.
6. Repeat steps 4 through 6 to transfer *E. coli* to a sterile broth tube and agar slant.
7. Place the four inoculated tubes in a common rack for incubation at 37^{o}C for 18-24 hours.
8. Place the two bacterial cultures and water tube in a common discard rack for autoclaving.
9. Following incubation, observe growth patterns and complete the report.