**Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

# BACTERIAL TRANSFERS

In working with living cultures of organisms, it is essential to observe a procedure known as **aseptic (sterile) technique**.  The goals of this procedure are twofold:

           1.   to prevent contamination of the culture  
            2.  to prevent spread of culture organisms to the lab personnel or the environment.  
   
**STUDENT INSTRUCTIONS**:

The tubes of growing bacteria provided to you are **stock cultures** of common organisms.  These cultures should each be **pure cultures** (only one species of organism in the culture).  Our goal today is to allow you to start your own cultures from these stock cultures, maintaining the purity (avoiding contamination with other species). The organisms you will work with are:

* ***Escherichia coli*** (*E. coli*) is a normal resident in the bowel of man and animals.  It is a single short rod**.** Easily grown under laboratory conditions and has been studied extensively.  Research with *E. coli* has led to advances in our understanding of cellular metabolism as well as genetics.  Genetic engineering using this organism has led to the bulk production of human cell products such as insulin, somototropin, and interferon.
* ***Bacillus subtilis*** (*B. sub*.) is a common soil organism.  Since it is an endospore-forming long rod occurring in chains, it survives many environmental conditions.
* ***Staphylococcus epidermidis*** (*S. epi*.) colonizes the skin surface.  It is a cluster of cocci and tolerates the high salt concentration and relative dryness of such an environment.

In order to appreciate the aseptic technique, you must consider any organism, no matter how common, to be a potential **pathogen** (agent of disease).  Indeed, in great enough numbers and achieving the appropriate **portal of entry**, almost any bacterium can induce an infection.

The organisms grown in pure culture for laboratory study are grown on a **growth medium**, which may be a **liquid broth** or a solid **agar slant or plate**.  A particular medium may be designed to contain only the minimum requirements for growth of a specific organism.  This would be called “ **minimal media**” for that organism.  If one needs to encourage rapid growth of a very small sample of organisms, an **enriched medium** may be used, reinforced with **growth factors** required by **fastidious organisms**.  Typically, common organisms are grown on a standardized beef extract or protein base containing requirements for most bacterial species.  **Nutrient media** and **tryptic soy media** are examples.

READ THIS ENTIRE PROCEDURE AND WATCH IT DEMONSTRATED.  PRACTICE WITH EMPTY TUBES BEFORE ATTEMPTING THE TRANSFER!!!  
   
**PROCEDURE**

You and your partner will be given three test tubes of sterile medium and three plates of agar.

* 1. First, label the tubes with the following information:  Name, date, and the name of organism to be inoculated.
  2. Place the tubes in a rack and turn on the **Bacti-Cinerator** nearest your lab team.  It should heat for ten minutes before use.  Get the **inoculation loop** from your drawer.  (Make sure the loop has an insulated handle)
  3. The inoculation loop is to be inserted into the chamber without touching the sides.  It should be deep enough to heat the loop end of the holder.  ( This end of the holder is often a source of contamination).  Hold the loop in the chamber long enough for the wire to turn red along it’s entire length.  This should take about ten seconds.  As the wire glows red, it is sterilized.  The procedure just described applies to any part of this lab manual wherein the phrase “**heat-sterilize**” the loop occurs.  Do not turn the inoculation loop handle loose while the loop is inside the chamber.  Such a “bad-habit” of resting the loop within the chamber causes the handle to heat to burning temperatures and also damages the chamber.  The Bacti-Cinerator may be left on for the duration of the lab as long as heat- sterilization is required.
  4. Choose a stock culture and a correspondingly labeled sterile tube.  Hold both in the left hand, with the culture closest to you.  (Reverse these instructions if you are left-handed).  The instructor will demonstrate.
  5. Loosen the caps until they will just lift off.  Do not uncover the tubes at this point.
  6. Hold the inoculation loop in your right hand, heat sterilize the wire.  Angle the wire so that it glows red through its entire length.
  7. The loop is now sterile.  Let it cool a few seconds.
  8. Crook the little finger of the right hand around the cap of the stock culture and the third or fourth finger around the cap of the sterile tube.
  9. Lift both simultaneously from the tubes, angle the tops of the tubes into the mouth of the Bacti-Cinerator.  This procedure hits the air at the neck of the tubes so that environmental organisms do not fall into and contaminate the tubes.  Keep the caps in place between your fingers, taking care not to palm them or turn them up.  (Never put the caps down on the lab table, this would contaminate them and also the lab table).
  10. Lower the now-cooled loop just below the surface of a broth stock culture, or gently touch the loop to a bacterial colony if working from a stock agar slant. You are taking a tiny sample of organisms from the stock culture.  This sample is referred to as the **inoculum**.
  11. Withdraw the loop without touching the sides of the tube.
  12. Move the loop directly into the adjacent sterile tube.
  13. Lower the loop below the surface, shake gently, and withdraw as before.
  14. Reheat the tube tops, then recap.
  15. Heat-sterilize the loop, let it cool enough to put down without burning the surface.
  16. Tighten the caps fully, then loosen by one turn and return the tubes to the rack.

General inoculation of a Petri dish containing agar.

* 1. Label the plate with the same information used to label your tubes. Write this information directly on the bottom of the plate, around the edge using a wax pencil or marker.
  2. Heat sterilize your loop and remove the cap from the stock culture.
  3. Heat the top of the tube and reheat your loop is necessary.
  4. Enter the tube, allowing enough time for the loop to cool, enter the broth and remove your inoculum.
  5. Heat the top of the tube again, recap the tube, and place it to the side in a test tube rack.
  6. Open the Petri dish by removing the top. This should only be done when you are ready to inoculate the plate. Air can carry contaminates to the surface of the agar if the plate is left open.
  7. Gentle place the loop on the surface of the agar and move it from side to side while also moving down the length of the plate.
  8. When finished, replace the lid of the dish, sterilize your loop, and place the plates in the appropriate rack **INVERTED** to allow for proper growth.

     Some errors commonly made involve handling of the loop.  If it has not cooled enough before touching broth or agar, a sizzling sound will be heard.  This produces **aerosols** of living bacteria, which contaminate the environment, and can reduce the chance of viability of the transferred specimen.  If the loop drags against the glass on withdrawal, a strong vibration sprays living bacteria into the air. Touching the agar with a loop that is too hot will cause burn scars to appear on the surface and make streaking the plate difficult. Applying too much pressure to the agar surface can lead to digging or pitting of the agar.

     When you finish, place your cultures in the common rack (designated by the instructor) for **incubation at 37°C** . The cultures will be incubated for 48 hours and you will use them for the next two lab sessions in order to learn staining techniques.

**FOR TESTING PURPOSES (DAY 1 of Skills Test):** In order to test the quality of aseptic technique, a **sterile transfer** may be made between two sterile media tubes.  If no growth occurs in either tube following 48 hours of incubation, the technique is adequate.

**After you have incubated your cultures for 48 hours, record your observations below:**

|  |  |  |
| --- | --- | --- |
| **Organism** | **Broth Observations/Turbidity** | **Colony Characteristics on Petri Dish** |
| **E. coli** |  |  |
| **B. sub** |  |  |
| **S. epi** |  |  |