Exercise 3
(Ed. Fall 2010)
Microorganisms Are Everywhere (or almost everywhere)

INTRODUCTION:
Student Learning Objectives: After completing this exercise students will:

a. Demonstrate the presence of microorganisms on inanimate objects in the environment by growing them in cultures.
b. Demonstrate the presence of microorganisms on different locations on the human body by growing them in cultures.
c. Evaluate the effects of antibacterial agents on skin bacteria.
d. Define the aseptic technique and demonstrate the aseptic transfer of microorganisms from one medium to another.

Activities for today:

- Culturing microorganisms from soil.
- Culturing microorganisms from air.
- Culturing microorganisms from the table surface.
- Culturing microorganisms from various body surfaces.
- Aseptic transfer of microorganisms from different culture media.

Materials

Work in groups of 4 per table. This is a group activity. Each group needs the following:
Tube racks
1 Nutrient broth 5ml tube
3 Nutrient Agar Plates
4 Tryptic Soy Agar Plates
1 Sterile saline (0.85% NaCl) 3ml tube
Beaker with Disinfectant

Per person:
Work individually on the Aseptic Technique; this is NOT a group activity.
Inoculating loop and needle
Bunsen burner and striker
Slant, broth, and agar plate cultures of *Serratia marcescens* (one of each per table).
Sterile nutrient broth tubes, slants, and agar plates. (one of each/student)
Non-sterile tubes and Petri dishes for dry runs.

Introduction:

Important for today only: Prior to starting your lab exercise, do NOT disinfect your table working area, until you have swabbed the surface for microorganisms.

Microorganisms (of all types) are present just about everywhere in the environment, on live as well as inanimate surfaces, in the soil, suspended in the air, and even inside the body (or is it really inside?). Today you will demonstrate their presence by growing culturable microorganisms, such as certain bacteria and fungi. Viruses are excluded from this exercise, since they cannot be grown on the media available. However, this does not exclude them from being present on these surfaces.

Always label your plates with the plate name (type of agar), date, table #, and source of inoculation or culture.

1. Culturing microorganisms from your tabletop.
   - Label two Nutrient Agar plates: Tabletop, Pre-disinfection and Tabletop, Post disinfection. Also include your table number, date, and type of medium (NA).
   - "Aseptically", without contamination, remove a sterile cotton swab from the tube and dip it in the sterile saline tube and squeeze against the tube wall to avoid oversaturation.
   - Touch the tabletop and rub the swab several times across the surface.
   - Inoculate the Nutrient Agar plate labeled (Tabletop, Pre-disinfection) by gently rubbing the swab across its surface in a rolling motion. Close the plate immediately and incubate upside down at 30 C for 24-48 hours.
   - Place the used swab in the beaker with the disinfectant
   - Now go ahead and disinfect the surface of the table, and repeat the procedure for the plate labeled (Tabletop, Post-disinfection) and incubate inverted at 30 C.
   - Observe plates and note for differences in the amount of growth on each

2. Culturing microorganisms from the air in the room
   - Label a Nutrient Agar plate; Room air
   - Place the plate right side up with the lid open in a corner of the room for 30 minutes.
   - Close the plate and incubate <u>at room temperature</u> for 48 hours.
   - Observe (closed) for growth.

3. Culturing microorganisms from your body ("inside surface)
   - Label a TSA plate; (Tongue, Cheeks, etc.)
   - "Aseptically", without contamination, remove a sterile cotton swab from the tube and dip it in the sterile saline tube and squeeze against the tube wall to avoid oversaturation.
   - Rub the swab on the surface of your tongue or inside cheeks.
   - Roll the swab on the surface of the TSA plate.
   - Close the plate and incubate inverted at 37 C for 24 hours.
   - Observe plate for growth.

4. The Aseptic Technique for transer of Bacteria

As demonstrated in the exercises above, microorganisms can be present almost everywhere in the environment, as well as body surfaces. Contamination of otherwise sterile areas is inevitable, unless great care is used to keep those areas from accidental contamination. Bacteria in the air, on body surfaces, and on inanimate objects can contaminate sterile media plates and tubes. Using the aseptic technique can prevent unwanted contamination of surfaces, people, as well as culture media, where a pure culture of a specific species is desired. Pure cultures are those that grow a particular species of bacteria, used for specific purposes in research or medicine.

Your instructor will demonstrate the following:

1. Aseptic transfer of live Bacteria from a broth/slant culture to a slant tube.
2. Aseptic transfer of live Bacteria from a broth/slant culture to an agar plate.
3. Aseptic transfer of live Bacteria from a plate to a slant or broth tube.

Please keep in mind that you will be using live bacterial cultures. Although the organisms used in this exercise are not highly pathogenic, assume that they are.
You will also use the Bunsen burner, and inoculating loops and needles. Please make sure that long hair is tied back, and keep loose clothing away from the flames. Never leave your Bunsen burner on unattended, and keep all flammable materials away from the flame.
Pay close attention to the demonstrations by your instructor, and practice the techniques using the empty tubes and Petri dishes before using the live organisms. Write detailed notes, and ask questions as needed. This is probably the most important technique you will have to master for this course.
Use the loop to transfer bacteria from tube cultures, and use only the needle to transfer bacteria from the agar plate, since you will be picking a single colony from this medium. Always flame the loop/needle before and after inoculation.