STUDENT STERILE TECHNIQUE PRACTICE
Module 3 Supplement

ONLY FOR PRACTICE you should

Re-use the loop and pipette and re-use their "sterile" wrappings
Do not dispose of a culture dish even if you accidentally contaminate it.

If you were really doing a lab with bacteria you would

- **Never** reuse any loop or pipette.
- **Always** dispose of used loops and pipettes in the "biological trash beaker."
- **Throw away** any culture dish or other item that is accidentally contaminated, and ask for a replacement.
- **Wash** hands immediately if they contact any liquids containing bacteria.

Each member of the team should practice each of the following:

1. **Open, hold, and close** a sterile 15 ml culture tube.

2. **Open and close** a sterile 1.5 ml test tube.

3. **Open** a culture dish, observe it without breathing on it, and close it.

After each team member has practiced 1-3, use (practice) sterile technique as you

4. Transfer 250 l of liquid from the 1.5 ml test tube to the culture tube.

5. Pretend to **transfer** a colony from the surface of the culture dish to the liquid in the culture tube.

6. Use the pipette to **suspend** the imaginary bacteria in the culture tube liquid.

After each team member has practiced 4-6, use (practice) sterile technique as you

7. **Transfer** 100 l of culture tube contents to culture dish and **spread** it.

POSSIBLE SEQUENCE OF STEPS FOR STERILE TECHNIQUE DEMONSTRATION/PRACTICE

Delete parts as time requires

1. a. Teacher demonstrates opening/holding/closing of sterile 1.5 ml test tube, sterile 15 ml culture tube, sterile pipettes, measuring with pipettes.

   b. Students practice with test tubes, caps, droppers. (opening, closing, transferring)

2. a. Teacher demonstrates cleaning lab bench, opening/closing of petri dish, how not-to-breathe-on an open plate.

   b. Teacher passes around sample culture dish for touching and looking.

   c. Teacher demonstrates labeling, opening inoculating loop, using loop, disposition of loop and other trash.

   d. Students practice cleaning lab bench, label/open/close plate.

3. a. Teacher demonstrates sterilization of spreader with ethanol and flame, spreading sample on medium, closing, taping, waiting, then inverting.

   b. Students practice spreading, waiting, taping, etc.

Transfer technique for new starter culture plates -Transformation Lab

Streaking is best done with a wire loop. Isolated colonies should occur in the section of the plate streaked last.

Step (1)-Streak the inoculum over the surface of the plate. Start near the edge of the plate.

Step (2)-Flame the loop to get rid of the original bacteria. Cool the loop by gently touching a sterile portion of the agar.

Step (3)-Rotate the agar plate 1/4 of a turn. Streak across the agar by transferring bacteria from the original streak(A).

Step (4)-Rotate agar plate again 1/4 of a turn. Flame and cool the loop. Streak again by transferring bacteria from section "B".

Step (5)-At this step do not flame the loop. Just rotate the plate and streak again as in step (4) this time by carrying bacteria from section C