**Setup and Safety Instructions**

*Students will be working with unknown microbes collected from the environment. Even though they are from local ‘safe’ water supplies, samples could contain species that could cause illness. Therefore, all samples should be treated with caution. Guidelines that follow are adapted from the American Society for Microbiology’s Guidelines for Biosafety in Teaching Laboratories and should be followed to ensure safety of all who use the room.*

It may seem pessimistic to begin instructor’s materials with contamination prevention and cleanup. Hopefully you will never need to clean up a spill. Hopefully the samples you collect will never contain cells from a pathogenic microbe that would thrive under laboratory conditions and cause illness. Hopefully. The safety information comes first because the best way to reduce the risk of accidents and of coming into contact with a harmful microorganism is to prepare the workspace and to know how to properly handle and dispose of bacterial waste.

1. Room setup

2. Bacterial trash

3. Wipe down and clean up

4. Disposing of Agar plates

5. Treating bacterial trash

**1. Room Setup:**

Samples containing bacteria should be separated from areas of common use.

Backpacks, notebooks, phones, pens and pencils can transfer bacteria to other areas and this type of contact should be minimized. This lab curriculum suggests that phone cameras can be used to store images of the plates. The students need to know that they should not handle their phones when they have gloves on, nor should they place the phone down on the work area.

*Classroom with desks:* If possible, *all* work with cultures and plates should take place on side tables or countertops, not on desks where students put notebooks and store other materials.

*Classroom with lab benches:* Only the lab materials and a minimal amount writing material should be on the bench tops.

*All classrooms:* Set up a flat work surface containing only the needed materials. Cover work area with flat, absorbent material (bench coat, surface liner, paper towels. If none of these are available use a disposable plastic tablecloth).

**2. Bacterial trash:** Designate a trashcan for ‘bacterial trash only’. Line it with a kitchen garbage bag for easy handling. When a lab day is complete fold the liner in on itself and place it and used gloves in bacterial waste. Students’ gloves should be disposed of in this container only, not in the general trash. *See “Treating Bacterial Trash” below*

**3. Wipe down work areas after routine use:** Work areas should be cleaned with 10% bleach solution *or* 70% alcohol solution (either ethanol or isopropanol). A well-labeled garden spray bottles work well. Spray the area, wipe with paper towels and allow it to air dry. (10% bleach= 100 ml bleach +900 ml water and store in a closed container.)

**Cleaning work areas after a spill:** If a more concentrated bacterial solution, is spilled, *i.e.* one that is cloudy from bacterial growth and smells bad, the area should be cleaned more thoroughly. Place paper towels over the spill and pour 25% bleach on the paper towels. Leave all items in place for 15min then carefully move the paper towels and bench liner to the bacterial trash. (25% bleach solution: 250ml bleach + 750ml water)

**4. Disposing of agar plates:** Plates that have not come in contact with environmental samples are still sterile and can be disposed of in the ‘regular’ trash. Plates that have had lake water added to them must be considered contaminated with microorganisms *even if no growth is visible*. Once plates have had water samples added and been taped shut (on Day 2) they should not be un-taped or opened. Contaminated plates are handled with gloves and placed in the bacterial waste can.

**5. Treating Bacterial Trash:** *If an autoclave is available it should be used.* The bag of bacterially contaminated plates, paper and gloves should be placed inside an autoclave-safe bag and sterilized for 25min.

*If an autoclave is not available* a 10% bleach solutions should be added to the waste bag so that the contents are saturated. After 2hr of treatment the solids (plates, paper, gloves) can be discarded in the ‘regular’ trash and the bleach solution can be poured down the drain. The bleach can removed by placing the bag in a sink, cutting off a corner and allowing the bleach to drain out of the bag.

Safety Setup Information adapted from:

[Appendix to the Guidelines for Biosafety in Teaching Laboratories](http://www.asm.org/images/Education/FINAL_Biosafety_Guidelines_Appendix_Only.pdf) (pdf)

<http://www.asm.org/index.php/education-2/22-education/8308-new-version-available-for-comment-guidelines-for-best-biosafety-practices-in-teaching-laboratories>

Additional Safety materials and student handouts:

James, D.E., 2008. Nine safe practices for the microbiology laboratory. Carolina Biological Supply, Burlington, NC.

<http://www.carolina.com/teacher-resources/Interactive/nine-safe-practices-for-the-microbiology-lab/tr11085.tr>