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Sterilization and Aseptic Technique

Introduction

Microbiology lab activities are common in middle school and high school life science courses. Learning basic microbiology lab techniques and procedures is interesting and relevant to most students—many of them, after all, have had at least one throat culture or other microbiology lab test in their lives. The requirements for safe microbiological work in the classroom include the knowledge and practice of aseptic technique and sterilization procedures.

Standard Microbiology Practices

The federal government has outlined general safety standards for microbiological work in their publication *Biosafety in Microbiological and Biomedical Laboratories*. Biosafety Level I (BSL 1) practices described in this book are appropriate for secondary educational teaching laboratories. BSL 1 work involves using strains of viable, nonpathogenic microorganisms that are known *not* to cause disease in healthy adult humans and are thus of minimal potential hazard to students, teachers or the environment. The level of containment in BSL 1 relies on standard microbiological practices in laboratory settings. Students and lab personnel should receive specific training in the procedures conducted in the laboratory.

Standard microbiological practices include:

- Wash hands thoroughly with soap and water before and after handling viable materials, after performing microbiological transfers and observations or working with potentially hazardous materials, and before leaving the laboratory.
- Eating, drinking, smoking, handling of contact lenses, applying cosmetics, and storing food for human use are not permitted in laboratory work areas.
- Pipetting by mouth is prohibited—use only mechanical pipetting devices.
- There should be policies and procedures for safe disposal of sharps, including broken glassware.
- Perform all procedures to minimize the creation of splashes or aerosols.
- Decontaminate all work surfaces with appropriate disinfectant after completion of work and after any spill or splash of microbiological materials.
- All cultures, stocks, and other potentially infectious materials should be decontaminated prior to disposal using an effective sterilization method.
- Always wear appropriate personal protective equipment, including safety glasses or goggles, lab coats or gowns, and gloves. Alternatives to powdered latex gloves should be available. Persons who wear contact lenses should also wear goggles or safety glasses.
- Change gloves when torn, contaminated or otherwise compromised. Dispose of used gloves with other contaminated laboratory waste. Wash hands after removing gloves—all hand washing protocols must be rigorously followed.

Sterilization Procedures

Safe and successful microbiology lab activities require proper sterilization of all materials before and after each activity. Sterilization is defined as the death of all living things, including spores, in or on an object. Achieving this task is much more difficult than is commonly realized, and it is almost impossible to guarantee total sterility. For practical purposes in secondary educational labs, sterilization can generally be achieved using dry heat, filtration, chemicals, or autoclaving.

Dry heat in a preheated laboratory oven at 160 °C for at least two hours may be used to sterilize glass and metal lab equipment. Inoculating loops and the mouths of culture or test tubes should be sterilized by heating in a Bunsen burner flame.

Microbiological membrane filters provide a useful way of cold-sterilizing materials such as enzyme or vitamin solutions, antibiotics, and cell culture media components that would be damaged by high temperatures or chemical treatment. The filters contain pores small enough to prevent passage of microbes but large enough to allow organism-free fluid to flow through. The sterile liquid is collected in a sterile flask or other sterile container.

Materials that are potentially contaminated with microorganisms must be sterilized before disposal. Examples of microbiological laboratory waste include bacterial cultures and culture tubes, disposable loops, Petri dishes, blood typing materials, biological culture media, and disposable gloves used when handling living materials. Biological culture media are specifically designed to promote the growth of microorganisms. These organisms will continue to grow even after disposal unless they are destroyed. There are two methods for sterilizing biological waste prior to disposal—autoclaving and chemical sterilization. Objects to be autoclaved should be placed into an autoclavable biohazard bag (do not place any sharp objects into the bag, however). The biohazard bag should be placed in an autoclave or pressure cooker if an autoclave is not available. Recommended sterilization conditions are 30 minutes at 121 °C and 15 psi pressure, although the requirement for length of autoclaving varies from state to state. Autoclaves and pressure cookers present hazards of high temperature and pressure—carefully follow manufacturers' directions and safety instructions.

For chemical sterilization of microbiological waste, place culture materials in a 10% bleach solution for 24 hours. Rinse the sterilized materials with water prior to disposal.

Aseptic Technique

Aseptic technique must be implemented at all times when handling bacterial cultures. Wear gloves and safety glasses while handling the cultures. It is important to sterilize metal inoculating loops between "dips" to control cross-contamination, even when working the same bacterial strain, as bacteria from the air may contaminate stock cultures. After opening a culture, briefly sweep the mouth of the tube through a burner flame 2–3 times. This creates airflow outward from the tube, again preventing contamination. Place the inoculating loop in the flame until it glows red and then allow to cool. After finishing work with bacterial cultures, label the tubes, sterilize the work area with 10% bleach solution, and wash hands thoroughly with soap and water. Remember to sterilize any area that may have been touched with your glove, such as an incubator or cabinet handle.

Culturing Environmental Bacteria

Do not culture environmental or indigenous bacteria, which may be infectious, in Petri dishes or above room temperature. Normal amounts of streptococci and staphylococci in the mouth or on the hands do not present a hazard. After incubation, however, a single microbial cell may multiply to over 1,000,000 cells, and at that level may present a risk if a Petri dish is broken or carelessly handled. Closed culture systems such as 3M Petrifilm™ plates are recommended for culturing environmental bacteria. Petrifilm is widely used in the food and beverage industries to monitor microbiological contamination.

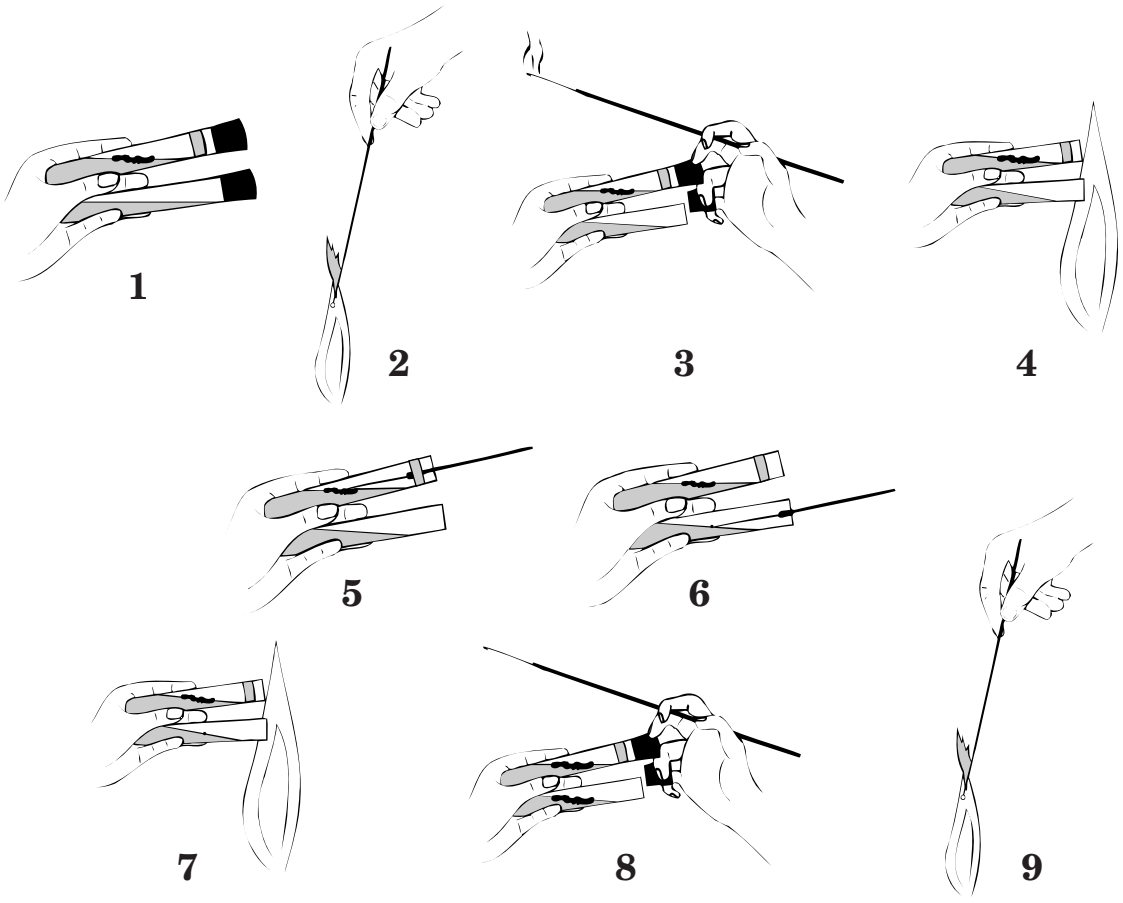
Tips

- An electronic version of the government publication, *Biosafety in Microbiological and Biomedical Laboratories,* may be found at http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf (accessed September 2008).
- Several criteria are specified for laboratory design and facilities under Biosafety Level I. Labs should have doors for access control, they must have sinks for hand washing, and lab floors and furniture should be designed for easy cleaning and disinfection. Bench tops must be impervious to water and resistant to heat, organic solvents, acids and alkalis, and other chemicals.
- To prepare 10% bleach solution, dilute one part household bleach with nine parts water. Prepare within one day of use.

Aseptic Lab Techniques

Transferring Tube Cultures

1. Clean and disinfect your work area. Wash your hands. Hold the culture tube and blank sterile tube in the palm of one hand and keep the tubes in an almost horizontal position during the transfer (see illustrations below).
2. Pick up the inoculating loop with your other hand and flame it in a Bunsen burner flame until it glows red hot. Flame the entire length of the wire.
3. Remove the caps or foam plugs from the two tubes holding the plugs between your fingers while still holding the red hot inoculating loop. Do not lay the loop or caps down or allow them to touch anything.
4. Sterilize the mouths of the two tubes by passing them through the flame several times.
5. Insert the inoculating loop into the stock culture tube. Touch the loop to the top of the slant surface or immerse it in the broth to cool the needle. Pick up a small quantity of live material from the culture tube. Do not touch the sides of the tube.
6. Insert the loop into the sterile culture tube. Streak the loop back and forth from the bottom of the slant to the top, or swirl it in the broth.
7. Withdraw the loop and flame the mouth of the tubes.
8. Replace the caps or plugs.
9. Flame the loop until it glows red. When it cools, lay it down or set it in a holder. Label your culture tubes appropriately and disinfect your work area. Wash your hands.



Test Tube to Petri Dish Transfers

1. Clean and disinfect your work area. Wash your hands. Hold the tube in the palm of your hand.
2. Pick up the inoculating loop with your other hand and flame it in a Bunsen burner flame until it glows red hot. Flame the entire length of the wire.
3. Remove the cap or foam plug from the culture tube holding the plug with the fingers of your hand with the inoculating loop.
4. Sterilize the mouth of the tube by passing it through the flame several times, holding the tube in an almost horizontal plane.
5. Insert the inoculating loop into the culture tube. Touch the loop to the top of the slant surface or immerse it in the broth to cool the needle. Pick up a small quantity of live material from the culture tube. Do not touch the sides of the tube.
6. Withdraw the loop and flame the mouth of the tube and replace the plug into the tube. Place the tube into a test tube rack.
7. Use your free hand to lift the top of the Petri dish straight up without creating any wind currents. Open the lid only enough to allow the insertion of the inoculating loop.
8. Insert the top of loop into the Petri dish and streak the surface of the agar with the tip of the loop. Do not dig into the agar, but simply slide the loop over the surface of the agar in a zig-zag fashion.
9. Remove the loop. Lower the top of the dish over the bottom. Set the dish aside. Flame the loop again until it glows red. When it cools, lay it down or set it into a holder. Label your Petri dish and disinfect your work area. Wash your hands.