Tips for the Safer Handling of Microorganisms in the School Science Laboratory

1. **Only culture microorganisms that are obtained from known sources**. The only microorganisms that should be used in the classroom are those that have been obtained from a known source such as a biological supply house or university laboratory. Organisms should be identified with their genus and species name. Students should never culture microorganisms from their own bodies, from surfaces around the building or any other locations/sites, as it is impossible to know whether or not these organisms are pathogenic.
2. **Treat all microorganisms as potential pathogens**. All microorganisms, especially unknown cultures should be treated as if they are pathogens. The majority of microorganisms that will be used in the science laboratory are not pathogenic and have never been shown to cause disease in humans (James, 2008); but it is always possible for a microorganism to exhibit pathogenic properties. Students with compromised immune systems, or those who have recently been ill should consult with their teacher, the school nurse, or their physician before participating in any microbiology laboratory. When transferring bacterial colonies, lift the Petri dish cover at a 45^{o} angle to avoid exposing the entire container to the air.
3. **Personal Protective Equipment & Personal Hygiene**. Students should use gloves, chemical splash safety goggles, and aprons. Students should not wear open toed shoes (sandals or flip flops), long hair should be tied back, and hands should be kept away from the face at all times.
4. **Wash your hands**. Hands should be washed before and after performing any experiment in the laboratory using disinfectant soap. Non-disinfectant soap can be used if it is the only soap available. Gloves can be worn for added protection. Wash hands with soap and water after removing gloves.

What is the right way to wash your hands?

- Wet your hands with clean, running water (warm or cold) and apply soap.
- Rub your hands together to make a lather and scrub them well; be sure to scrub the backs of your hands, between your fingers, and under your nails.
- Continue rubbing your hands for at least 20 seconds. Need a timer? Hum the "Happy Birthday" song from beginning to end twice.
- Rinse your hands well under running water.
- Dry your hands using a clean towel or air dry them.

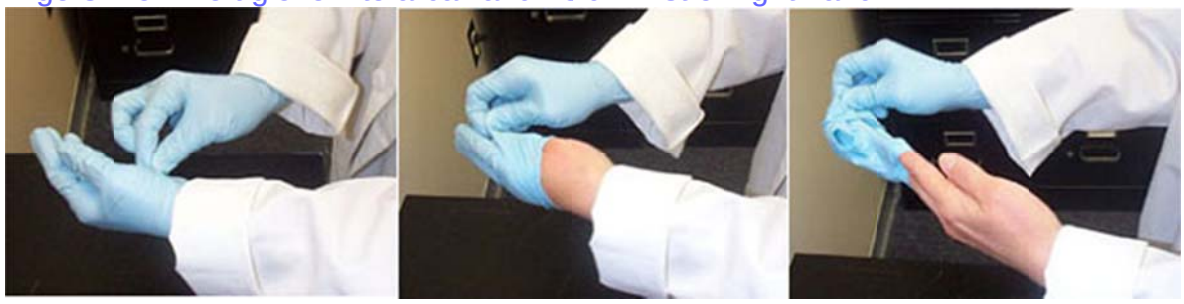
How to Remove Gloves Safely

Teachers should demonstrate for students how to properly remove gloves.

As you remove the gloves, avoid allowing the outside surface of the gloves to come in contact with your skin, because the outer surface may have become contaminated. Avoid letting gloves snap, as this may cause contaminants to fly into your eyes or mouth or onto your skin or other people in the area. Remove used gloves before touching anything. Counter tops, faucets, pens and pencils are often contaminated because workers wearing gloves touch them.

The following is one way to safely removing gloves.

Step 1. With right hand, pinch palm of glove on left hand and pull left glove down and off fingers. Form left glove into a ball and hold in fist of right hand.



Step 2. Insert one or two fingers of left ungloved hand under inside rim of right glove on palm side; push glove inside out and down onto fingers and over balled left glove.

Step 3. Grasp gloves, which are now together and inside out, with left hand and remove from right hand.

Step 4. Discard gloves in autoclave biohazard bag.

Step 5. Wash hands thoroughly with soap and water.

5. Disinfect work areas before and after use. Benches and work areas should be wiped down before and after working with bacteria. First apply a green cleaner to the surface. Then apply a solution of 70% ethanol or 10% bleach. A contact time of approximately 10 to 15 minutes should be effected (follow manufacturer's recommended application time). Alcohol should not be used around Bunsen burners, and paper towels that are used to wipe counters should not be disposed of with the regular trash to prevent the fumes from catching fire. Bleach, if spilled, will stain clothing. Both ethanol and bleach are dangerous to the eyes; students should know the location of the eye wash station before they begin the experiment. Note: 70% alcohol is the optimum concentration that should be used because higher concentrations evaporate too quickly and do not expose microbes to the alcohol for a long enough time to kill them.

6. Sterilize equipment. All equipment that is used in the culturing of bacteria should be sterilized by autoclaving. If an autoclave is unavailable, use pre-sterilized products.

7. Never pipette by mouth. Liquid cultures should be transferred using a pipette bulb or pipetting device.

8. Do not eat or drink in the laboratory or store food in a refrigerator where microorganisms are being stored. Food and beverages should not be consumed in any laboratory environment. Cuts, broken skin or wounds on the hands should be covered by a bandage. Gloves can be worn for extra protection. Food or beverages for human consumption should never be stored in a refrigerator with bacterial cultures to prevent the possibility of cross contamination. Refrigerators must be labeled relative to allowable contents – e.g., Food for human consumption only; Lab biological/chemicals only!

9. Label everything clearly. All cultures, media, chemicals and disinfectant should be clearly labeled with their names and the date. Hazardous substances should be labeled as such with their hazard information clearly marked.

10. Autoclave or disinfect all waste material. All items to be discarded after class should be placed in an autoclaveable biohazard bag. Such materials include culture tubes, culture plates, swabs, toothpicks, plastic pipettes, and plastic gloves. The bag should be autoclaved at 121^{o}C at 20 pounds of pressure for 30 to 40 minutes. If an autoclave is not available, cultures should be covered in bleach for 1-2 hours before being discarded.

11. Clean up spills with care. Cover any broken or spilled culture tubes with 70% ethanol or 10% bleach and let sit for approximately 10 to 15 minutes. Clean up the spill with paper towels and throw all waste into the biohazard bag. Wash area with disinfectant. Clean up glass spills with a broom and dustpan. Keep in mind that we are responsible for the safety of everyone who may come in contact with materials that are being disposed of, including the custodial staff.

NOTE: Teachers should always do a run through of any experiment they plan to perform with their students. This will ensure that all needed equipment for running the lab and cleaning up are available. Teachers should make students and parents aware of potential health and safety hazards in working in the laboratory with bacteria using a Safety Acknowledgement Form. Students should be encouraged under strict confidentiality to let the teacher know their status so an alternative assignment can be made if appropriate.

Sterilization Techniques: Beyond the measures mentioned above, the following are additional sterilization techniques that can be used:

- Safer and successful microbiology lab activities require proper sterilization of materials before and after each activity. Sterilization is defined as the death of all living things, including spores, in or on an object. It is almost impossible to guarantee total sterility. For practical purposes in secondary education 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 container.

- Materials that are potentially contaminated with microorganisms must be sterilized before disposal. Examples of microbiological waste include bacterial cultures and culture tubes, disposable loops, Petri dishes, 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 autoclaveable 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. The requirement for length of autoclaving and temperature increases at higher altitudes. Autoclaves and pressure cookers present hazards of high temperature and pressure—carefully follow manufacturers' directions and safety instructions.

Aseptic Technique

- Wear gloves and indirectly vented chemicals splash goggles while working with the cultures. It is important to sterilize metal inoculating loops between "dips" to control cross-contamination, even when working with the same bacterial strain. 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, preventing contamination. Place the inoculating loop in the flame until it glows red and then allow it 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 areas that may have been touched with your glove. Further information on aseptic technique can be found from the following resources: Recombinant DNA and Biotechnology (Kreuzer & Massey); American Association for Microbiology, 1996; and DNA Science (Micklos & Freyer); Cold Spring Harbor Press, 2003.

Listed below are bacteria considered safer for advanced high school level science laboratory course activities following appropriate legal safety standards and professional best practices (American Society for Microbiology). Culturing and use of live bacteria is not recommended and should not be done at the elementary/middle schools and introductory level high school science courses.

BACTERIA: Acetobacter aceti (vinegar), Bacillus cereus (cocoa, tofu), Bac. licheniformis (cocoa), Bac. megaterium (cocoa), Bac. pumilus (cocoa), Bac. subtilis (cocoa, rice natto), Erwinia dissolvens (coffee), Lactobacillus acidophilus (acidophilus

milk; yogurt), Lact. bulgaricus (yogurt), Lact. casei (many cheeses), Lact. delbrückii (pickles, soy sauce), Lact. helveticus (many cheeses), Lact. lactis (most cheeses), Leuconostoc (many cheeses), Leucon. mesenteroides (pickles; sauerkraut), Pediococcus (sauerkraut, ensilages, pickles), Propionibacterium acidipropionici (Emmenthaler cheese), Prop. freundenreichii (Swiss cheese), Prop. jensenii (buttermilk), Prop. shermanii (Emmental and Swiss cheeses), Prop. technicum (Edam cheese), Prop. thoenii (Emmenthaler cheese), Streptococcus cremoris (many cheeses), Strep. diacetilactis (sour cream, and butter products), Strep. faecalis (pickles), Strep. lactis (many cheeses, sour milk), Strep. thermophilus (yogurt and many cheeses).

BACTERIA and associated natural plasmids and lysogenized natural phages: Aerococcus, Agrobacterium radiobacter, Alcaligenes eutrophus (degrades 2,4D), Alcal. faecalis, Alcal. viscolactis, Alicyclobacillus acidocaldarius, Ali. acidoterrestris, Ali. cycloheptanicus, Aquaspirillum itersonii, Aquaspirillum polymorphum, Aquaspirillum serpens, Aquaspirillum sinuosum, Arthobacter globiformis, Azotobacter chrooccum, Az. vinelandii, Bacillus apiarius (bee symbiont), Bac. azotofixans (N-cycle), Bac. brevis, Bac. circulans (rumen), Bac. coagulans, Bac. laterosporus (rumen), Bac. macerans (rumen), Bac. marinus, Bac. pasteurii, Bac. polymyxa (N-cycle), Bac. pulvifaciens (insect symbiont), Bac. schlegelii, Bac. sphaericus (mosquito control), Bac. stearothermophilus, Bac. thiaminolyticus (insect symbiont), Bac. thuringiensis, Bac. tusciae, Beggiatoa (S-cycle), Brevibacterium linens, Butyrivibrio (rumen), Caulobacter, Cellumonas, Corynebacterium pseudo-diphtheriticum, C. xerosis, Epulopiscium spp., Escherichia coli (only classic strains of K-12, 1776, B and C, and with their indiginous plasmids and phages), Kurtha zopfi, Lucibacterium spp., Metabacterium polyspora, Micrococcus luteus, Micro. roseus, Neisseria flava, Neis. sicca, Photobacterium, Pseudomonas fragi, Rhizobium, Rhodococcus rhodochrous, Rhodospirillum rubrum, Ruminococcus (rumen), Sarcina aurantiaca, Sarc. flava, Sarc. lutea, Selenomonas (rumen), Serratia liquefaciens, Spirillum serpens, Spir. volutans, Sporosarcina ureae, Staphylococcus epidermidis, Staph. saprophyticus, M. mutans, M. salivarius, M. stereothermophilus, Streptomyces albus, Strep. antibioticus, Strep. venezuelae, Succinomonas (rumen), Sulfolobus (S-cycle), Thermoplasma, Thiobacillus thioparus, Vibrio anguillarum, Vib. fischeri, and Zymomonas

References

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

Ewald, H., Brashears, J., Huynh, C., Freeman, E., Corvini, M., Davis, M., Femenia, E., Hart, B., and Vermeulen, C.(1997). Micro-Organisms for Education. Presented at the general meeting of the American Society for Microbiology, Miami, FL.

Science Department Safety Notes – Microbiology Safety. (2011). Flinn Scientific, Inc.

Internet Resources

Putting It On, Taking It Off - www.ab.ust.hk/hseo/sftywise/200303/page3.htm

Center for Disease Control & Prevention – Handwashing: Clean hands Save Lives: http://www.cdc.gov/handwashing/