

# TIPS FOR THE SAFER HANDLING OF MICROORGANISMS IN THE SCIENCE LABORATORY

Approved by NSTA Safety Advisory Board – 31 March 2016

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

Educators need to be aware of the risks involved with the use of microorganisms in the laboratory. The following tips are designed to inform educators of better professional practices to protect the health and well-being of students, faculty, staff and the community.

### I. Tips related to student laboratory work:

1. **A risk assessment should be performed when preparing to do a laboratory activity.** The risk assessment takes into account the type of microorganism that is being considered for use, what is going to be done with this organism, and the risks of the laboratory procedure. Microorganisms are typically handled at a specific biosafety level. Work with microorganisms in the K-12 teaching laboratory and in informal settings are usually biosafety level 1 (BS-1), many settings are not equipped to go beyond this level. The Center for Disease Control (CDC) defines BS-1 as follows:

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. (Wilson & Chosewood, 2009)

2. **Only culture microorganisms that are obtained from known sources.** The only microorganisms that should be used in the science laboratory 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 as well as strain designation. Students should never culture microorganisms from their own bodies or from surfaces around the building, as it is impossible to know whether or not these organisms are pathogenic. Documentation should be kept about the source and handling of stock cultures of microorganisms.
3. **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 if workers have compromised immune systems or if microorganisms are introduced to the body through certain portals of entry (Cowan, 2014). 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° angle to avoid exposing the entire container to the air. Students must be instructed to minimize the amount of time that inoculated growth media are exposed to the environment.

4. **Personal Hygiene.** 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. Dangling jewelry should not be worn while handling microorganisms. Hanging clothing should be secured close to the body. Students should not apply cosmetics, adjust contact lenses or bite nails while in the laboratory.
5. **Personal Protective Equipment.** Sanitized indirectly vented chemicals splash goggles meeting the ANSI Z87.1 Standard, gloves and aprons or lab coats must be worn through all phases of activities involving the handling or use of bacterial cultures: i.e. set-up, hand-son piece and take down.
6. **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. Hand sanitizers containing 60-95 % ethanol in gel are effective in killing most microorganisms on hands, provided the hands are free from dirt, grease, or other materials. If a gel-based hand sanitizer is applied after washing hands with non-disinfectant soap, the incidence of microbial contamination by the hands is significantly reduced (Boyce and Pittet, 2002).
7. What is the right way to wash hands?



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

Gloves can be worn for added protection. Because of the increasing prevalence of latex allergy, vinyl or nitrile gloves are recommended. Wash hands with soap and water after removing gloves.

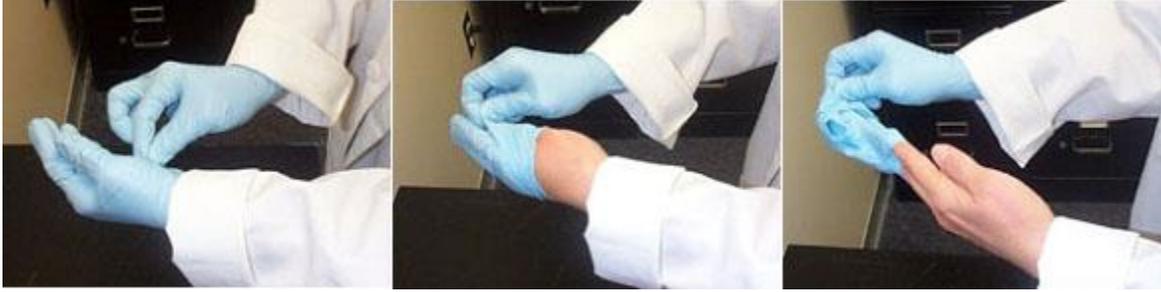
### **How to Remove Gloves Safely**

Teachers should demonstrate for students how to properly remove gloves.

As gloves are removed, 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 a safer way to remove 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, 10% bleach, or other suitable surface disinfectant. Follow the directions on the disinfectant to determine the appropriate contact time. Make sure all sources of flames such as Bunsen burners are shut off before using, 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. Always wear sanitized indirectly vented chemical splash goggles meeting the ANSI Z87.1 Standard when working with disinfectants. 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

are less effective at denaturing proteins in pathogens (Boyce and Pittet, 2002) Do not handle personal items such as cosmetics, cell phones, calculators, pens, pencils, etc. while in the laboratory.

6. 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. An inoculating loop is assumed to be sterile when it glows red hot.

**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 water-proof bandage. Gloves should 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.

**9. Keep only essential papers on the lab bench.** Minimize clutter by keeping only the pages of the lab that is actually being conducted on the lab bench, rather than the whole lab manual. Consider laminating a copy of each lab for the lab bench to be used throughout the day. This copy is easy to wipe clean and disinfect at the end of the lab. If contaminated materials spill onto personal items, the items must be sterilized.

**10. 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. Agar plates should be labeled on the agar side of the petri plate.

**11. 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 sterilized with pressurized steam (autoclaved) at 121°C at 20 pounds of pressure for 30 to 40 minutes. This time may need to be increased if there is a large volume of material to be sterilized.

**12. Clean up spills with care.** Cover any broken or spilled culture tubes with paper towels saturated with 70% ethanol or 10% bleach (or other suitable disinfectant) and let sit for approximately 10 to 15 minutes according to the manufacturer's instructions for disinfection. 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. Contaminated broken glass should be disposed of in a sturdy container that can be autoclaved (an empty liquid laundry detergent bottle with a lid will serve this purpose if a dedicated "sharps" box is unavailable). 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 (if they are immune-compromised or living or caring with someone who is immune-compromised) so an alternative assignment can be made if appropriate. Students should practice all techniques with uninoculated materials (either water or sterile media) before using actual cultures. This practice will allow them to identify problems with technique before these problems are compounded with the danger of cross-contamination.

## II. Tips related to facility and materials maintenance (for instructors)

**Preparation of Growth Media:** If growth media are to be prepared by the instructor on-site, there should be a “clean” area for this purpose. The clean area must have limited access, be free from cross-drafts and dust, and be disinfected after all work (with a germicidal ultraviolet lamp or with chemical disinfectant).

- Growth media should be prepared according to established protocols and sterilized before using. If media is to be autoclaved, containers must not be filled more than halfway. All growth media should be tested for sterility. This is done by holding 5 % of the prepared batch under incubation conditions and checking for contamination. If contamination is detected, the batch of media should be sterilized and discarded.
- Agar plates should always be stored and incubated in the inverted position (“agar up”). Condensation tends to collect on the lid of an agar plate, especially after growth has occurred. If the plate is not inverted, the condensate can drip onto the surface of the plate, potentially causing bacteria to spill out.
- Incubators must be disinfected before and after use using a chemical disinfectant. If incubators are used continuously, they should be disinfected weekly. If cultures are incubated at room temperature, they should be placed in an isolated location, away from other work locations and in a draft free place.

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

- All equipment that is used in the culturing of bacteria should be sterilized using pressurized steam (autoclaving). If an autoclave is unavailable, use pre-sterilized products.
- 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 inoculating tools, 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. The biohazard bag must be sealed and sterilized when it is half full. Overfilling a biohazard bag increases the risk of creating a spill of contaminated materials. 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 and with increased volumes to be sterilized. Autoclaves and pressure cookers present hazards of high temperature and pressure—carefully follow manufacturers’ directions and safety instructions. A log

should be kept next to the autoclave indicating the date, time, user name and contact information, type of load and items autoclaved. Autoclave performance can be monitored using commercially prepared endospore strips and autoclave tape (which changes appearance after effective sterilization).

### **Aseptic Technique**

- Wear gloves and sanitized indirectly vented chemicals splash goggles meeting the current ANSI Z87.1 Standard through all phases of activities involving the handling or use of bacterial cultures: i.e. set-up, hand-on piece and take down. Label cultures (tubes and agar plates) before inoculation. Do not talk or create breezes while working with live 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, sterilize the work area with disinfectant, 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.

### **Laboratory Physical Space Requirements**

Laboratory spaces where bacteria are being cultured should have the following:

- Nonporous floors, bench tops, chairs and stools
- Sinks and running water so that students can wash their hands
- Eyewash station
- Lockable classroom door

Personal belongings, particularly food items should not be in the same room where bacterial are being cultured.

### **Documenting Practices**

- Students should be required to sign a safety agreement specific to working with microorganisms (see [NSTA Safety Portal](#) for an example).
- Keep the signed safety agreement on file at the school.
- Document all injuries and spills, following school district policy.
- Safety Data Sheets (SDS) should be on file for all chemicals that will be used in the laboratory.
- Make the list of cultures to be used in the laboratory available to all students, and encourage them to review the list with their physician. Students and parents should be encouraged to bring to the teacher’s attention any culture that poses a health risk to that student.

Listed below are bacteria and fungi 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 at the elementary/middle schools and introductory level high school science courses.

### **BACTERIA:**

*Aquaspirillum itersonii*

*Aquaspirillum serpens*

*Bacillus cereus*

*Bacillus.coagulans*

*Bac. megaterium*

*Bac. sphaericus*

*Bac. stearothermophilus*

*Bac. subtilis*

*Bac. thuringiensis*

*Clostridium sporogenes*

*Corynebacterium pseudodiphtheriticum*

*Escherichia coli* (only classic strains of K-12, 1776, B and C, and with their indigenous plasmids and phages)

*Lactobacillus acidophilus*

*Lactobacillus lactis*

*Micrococcus luteus*

*Micrococcus roseus*

*Rhizobium spp.*

*Rhodococcus rhodochrous*

*Rhodospirillum rubrum*

*Sarcina aurantiaca*

*Sarcina lutea*

*Spirillum volutans*

*Staphylococcus epidermidis*

*Streptococcus salivarius*

### **FUNGI:**

*Saccaromyces cerevisiae*

## References

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[www.ab.ust.hk/hseo/sftywise/200303/page3.htm](http://www.ab.ust.hk/hseo/sftywise/200303/page3.htm)

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