

BUGSS Safety Manual

Practices and Operating Procedures for members and guests of the Baltimore Under Ground Science Space

Revision 1.1 July 2012

Change History

Version	Date	Author	Notes
1.1	July 28,2012	Thomas Burkett, Ph.D.	Initial version from Biocurious safety manual with modifications

Contact Information

911	Emergency Call Number
410-292-0710	BUGSS Emergency Contact
safety@bugssonline.org	General questions, requests for clarification on specific substances/ experiments, requests for new capabilities
TBurkett@bugsonline.org 410-292-0710	Thomas Burkett BUGSS Safety Officer

Safety at BUGSS

What we are protecting

At BUGSS we are concerned about protecting the safety of our members and guests using our facility, the community in which we reside, the environment in which we live, and our continued ability to operate a community laboratory. We believe as practitioners of DIY-Biology that we have an obligation to these groups and to the larger community of citizen scientists to operate in an exemplary and transparent manner. To participate in BUGSS so must you.

Why we are different

At BUGSS we believe in learning by doing and the right of ordinary citizens to participate and learn about biotechnology. Our members range from Ph.D. level scientist to individuals with no formal training in the sciences. We celebrate and embrace that diversity and seek to provide a space where individuals can come together to learn about and practice biotechnology. To some the idea of a community laboratory is scary so we must strive to be shining examples of safe and responsible laboratory practice.

Our Goals

We want to create an environment where curiosity is encouraged, innovation is celebrated and learning can take place. We desire to create a community laboratory that operates safely without requiring everyone to become safety experts. Beyond operating safely ourselves, we want to not only set an example, but create repeatable processes for future community laboratories.

Our philosophy

We will design practices commiserate with the risks associated with activities taking place at BUGSS. We will be creative in designing practices for our unique environment, but will not compromise on safety. We do not want to review each experiment that members want to perform. Instead, we will focus on developing general policies, promoting true understanding of them (and why they exist) with our members, and expect our members to make responsible decisions, with basic oversight. We will also provide help where it is needed in assessing risks of experiments, explaining procedures, and determining whether and how experiments can be done safely.

This is a work in progress

BUGSS is still in the boot-up phase. We are still learning what types of experiments members will want to perform, and still working on setting up capabilities we know we want, such as using hazardous chemicals. We will evolve our safety policies as needed. Emailing your questions and concerns to safety@bugsonline.org will help uncover where this manual is unclear, what safety areas have been missed, where policies are too burdensome, etc. Additionally if you want to perform work not permitted under these policies, please let us know. Your input will help us prioritize what capabilities to support next.

General Lab Policies

Lab Area

The BUGSS wet-lab area is distinctly marked with signage, and is separate from the rest of the facility. The policies in this section pertain to the wet -lab Area.

Wet-Lab Area Access

Access to the Wet-Lab Area is restricted to individuals who have completed the BUGSS Safety Orientation course, individuals taking part in a class (and therefore under the supervision of an instructor/BUGSS facilitator), and guests under the supervision of members who have taken the Safety Orientation course.

Food/Drink

The consumption or storage of food/drink is not permitted in the wet-Lab Area, including water bottles even if they are closed. Food may be consumed in the classroom, lounge, and other areas of the facility. No food or drink is permitted at any time in the refrigerator or microwave in the wet lab area.

Smoking

Smoking is not permitted anywhere within the BUGSS facility, or within 20 feet of either entranceway.

Clothing

Closed-toe footwear and long pants and/or a lab coat should be worn when working with corrosive substances (e.g. strong acids/bases) or other hazardous chemicals and with any biological agents.

Personal Protective Equipment (PPE)

Gloves, protective eyewear and lab coats must be worn when:

1. There is anticipated exposure to potentially infectious material
2. Working with corrosive substances such as strong acids and bases
3. Working with other hazardous chemicals

It is the responsibility of a BUGSS member to ensure that their guests in the lab are provided with and use appropriate PPE.

Working Alone

Working alone in the lab is not recommended. During operating hours, there will always be at least one BUGSS staffer present.

Use Common Sense!

Avoid exposure to chemicals and biohazards by using safe work practices. Plan for the disposal of waste before you begin any experiment, keep your work area and aisles free from clutter, confine loose hair and clothing, wash your hands with soap and water before

leaving the lab, never ingest or smell lab materials, and never eat, drink or apply cosmetics in the lab.

Biological Safety

General

BUGSS operates at a BSL1 containment level. Basically this level applies to organisms that can be worked with on an open bench using standard microbial practices. No particular safety equipment such as biological safety cabinets or PPE is required. According to the CDC/NIH manual on biosafety in biomedical laboratories 4th Edition:

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.

That is a broad definition open to interpretation. Because we don't expect everyone to be biosafety experts, and there are additional safety and regulatory requirements beyond the basic BSL1 classification, we have created these more precise policies. Again, if you have questions as to whether a specific experiment is permitted, or would like to do something beyond our current capabilities, please email safety@bugssonline.org If something is not expressly permitted below, categorically or otherwise, you should email safety@bugssonline.org prior to beginning your experiment and obtaining samples.

Microbial

Culturing and manipulation of well-characterized bacteria, Archaea, and yeast strains that are known not to be pathogenic to immunocompetent adults (e.g. lab *E. coli* K12 strains, *Saccharomyces* spp.) is permitted at BUGSS. Well-characterized means you must have a documented source for these microbes, such as obtaining them from another laboratory or commercial provider. If you bring a culture into the BUGSS facility you will be asked to provide documentation as to the source and identity of the organism.

BUGSS maintains a white list of microbial species that may be worked with in our facility. The white list can be found on the member resource pages.

Samples from nature may not be cultured at BUGSS. As such samples are by definition not well characterized, doing so requires a BSL2 facility. Indeed such samples are often pathogenic, and while they may not cause disease in the quantities found in nature, they can become dangerous when cultured.

Samples from nature are permitted for analysis (e.g. examining under a microscope, DNA extraction), provided that there is no reason to suspect the sample to be pathogenic, and it is not cultured.

Mammalian

The culturing of mammalian cells is not permitted at this time. However we are working on having the capability to culture mammalian cells in the future.

Human Samples

Working with human samples is generally a BSL2 activity. As we realize it is of particular interest to many of our members, we've scoped out one way to allow it.

Buccal/Saliva Samples

Human buccal and saliva samples may be analyzed, so long as, to the best of their knowledge, the provider of such samples is not infected with a disease/virus which would present a hazard to the lab, and signs a statement to that effect. The specific statement and infection list is currently being developed. Samples must be analyzed immediately once brought into BUGSS, and may not be stored and/or incubated.

Autologous Cells

Working with autologous cells (i.e. your own cells) can be hazardous, especially if they are cultured or manipulated. Working with autologous buccal/saliva samples is permitted so long as they are expediently analyzed, and not stored, incubated, or cultured at BUGSS.

Blood and other human fluids

Working with blood or other human fluids is not currently permitted.

Animals

Live animals are not permitted in the BUGSS facility.

Recombinant DNA

In general, the manipulation and recombination of DNA and/or RNA is permitted at BUGSS. The only restrictions are:

1. You may not deliberately work to create organisms that would be pathogenic to humans or not fall under CDC/NIH BSL1 guidelines.
2. You may not work with DNA and DNA sequences obtained from organisms on the US Select Agents list:

(<http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>)

Chemical Safety

Chemicals used in the BUGSS facility are non-hazardous and for the most part can be disposed of down the drain. A white list of approved chemicals is available on the member resource page. If you wish to bring a chemical into the facility not on this white list you must obtain prior approval from the BUGSS safety officer and supply a Material Safety Data Sheet for that chemical.

Material Safety Data Sheets (MSDS) are maintained for all chemicals in the BUGSS inventory and are available for member inspection.

All solutions/reagents used in the BUGSS facility must be labeled to include; the chemical name and concentration, the owner/preparer, any applicable hazard warnings, date prepared, and any additional steps such as pH adjustment or filter sterilization.

Flammables

Flammable chemicals with NFPA fire ratings of 2+ may not be brought into the facility at present. BUGSS does maintain a small quantity of isopropyl and ethyl alcohol used in DNA extraction and in surface disinfection. These chemicals are stored in the flammable storage cabinet and no more than 500 ml is allowed in the wet-lab area at any one time.

Corrosives

Concentrated strong acids or bases may not be brought into the facility at present. BUGSS maintains a small quantity of diluted acid and base for use in pH adjustment.

Oxidizers

Oxidizers may not be brought into the facility at present. .

Explosives

Explosives and chemicals with a NFPA reactivity rating of 2+ may not be brought into the facility. There are no plans to allow explosives to be brought into the BUGSS facility.

Radioactivity

Radioactive chemicals may not be brought into the facility. There are no plans to allow radioactive substances to be brought into the BUGSS facility.

Hazardous Waste Disposal

BUGSS does not currently have the capability to dispose of hazardous waste. As such chemicals that cannot be disposed of down the drain or in the municipal trash may not be brought into the facility.

Inventory & Chemical/Biological Control

In order to safely operate BUGSS, maintain our insurance coverage, and meet important legal and regulatory requirements, the following items must be controlled and tracked while inside the BUGSS facility:

1. Living organisms
2. Lab chemicals/reagents

This is true regardless of how long they will be inside the facility. Biological enzymes are not subject to these requirements.

The procedure for bringing organisms and chemicals into the facility is as follows:

1. Check whether the organism or chemical is on the BUGSS organism/chemical white list, either explicitly or categorically.
2. If it is not, but you believe it to be allowable based on this safety manual, email safety@bugssonline.org with your request to add it to the white list. Include the MSDS for any requested chemical, and specific strain information for any organism. For items not on the white list you will not be allowed to bring it into the facility without explicit written approval from the BUGSS safety officer.
3. All chemicals brought into the facility must have an original manufacturer's label specifying the compound, concentration and hazard warnings. When you bring a chemical or organism into BUGSS, you must check it in with the staff members on duty. The staff member will verify that it is on the white list, and will add it to the BUGSS facility inventory, marking you as the owner. You may elect to keep the chemical or organism private for your use only or allow other members of the facility to access them.
4. For long term storage of cells and cultures you may elect to have the culture stored at -80°C. In that case you will be asked to provide a suitably prepared sample for archiving in the BUGSS master cell bank. Unless specified by the owner, that culture will be made available for other members to use.
4. If you ship chemicals or organisms to BUGSS, they will be held for you. When you arrive, the staff member will go through the check-in procedures with you.
5. When removing organisms or chemicals from the BUGSS facility you must notify a staff member who will check the material out of the facility. If a culture / chemical is disposed of

or otherwise used up while in the facility you must notify a staff member who will remove it from the facility inventory

6. DNA sequences and plasmids may be stored in the facility as long as they comply with the above restrictions governing recombinant DNA. Any plasmids or DNA sequences brought into the facility need to be characterized at the sequence level and a DNA sequence of the plasmid needs to be provided. As with chemicals and organisms, when you bring DNA into the facility you will be asked if this is for your personal use or if it can be shared with other members.

We realize that these procedures may be somewhat burdensome, but have not yet found a better way to ensure the proper safety procedures are in place for arbitrary chemicals which members wish to bring in. It is hoped that over time the white list grows sufficiently large, and categorical rules can be created, that requests to white list materials are rarely necessary. It is also anticipated that a web application may aid this process. Until then, we'll err on the side of caution, and respond promptly for requests to white list materials.

Material Supply Store:

The BUGSS supply store maintains a collection of general lab supplies, chemicals and chemical solutions, enzymes, strains, plasmids and other general molecular biology reagents for use by BUGSS members. Supplies and materials for group projects and courses are included as part of the course / project fee. Materials for personal projects are available for purchase from the BUGSS store. Lists of materials available in the store can be found on the BUGSS web site under member resources.

You may also elect to bring your own materials into the BUGSS facility. When you check the material in you can indicate whether the material is for your use only or may be used by other members.

The rationale for having a central repository for solutions/ reagents, and supplies is to 1) Insure that reagents and solutions used in experiments are correctly made 2) Keep an accurate inventory of supplies and materials so that adequate stocks can be on hand without over buying 3) Provide members with the cheaper prices available by buying larger quantities and repackaging into smaller usable quantities. 4) Provide a ready source of materials so that you can spend your time doing experiments rather than making solutions 5) Reduce the risk of contamination and cross contamination.

Use and Labeling of Solutions and Chemicals:

Dry chemicals, chemical stock solutions, and diluted solutions are maintained in the BUGSS supply store and available for purchase by members for use in personal projects.

To obtain an in stock chemical/solution:

1. Fill out a requisition slip and give it to the BUGSS staff member
2. The staff member will obtain the solution and your account will be charged for the price of the solution.

If you desire to make a solution from scratch or using a different recipe than used at BUGSS, contact a BUGSS staff member who will obtain the necessary starting materials for you. You will be asked to provide a recipe for the solution and amount of stock or raw chemical used and will be billed accordingly.

Storage of Materials:

As a member of BUGSS you will have access to room temperature, refrigerated, and frozen storage space. We ask that all solutions/reagents/supplies that you are using in your experiments be kept in the designated storage space. Material left out will be disposed of.

All solutions/reagents used in the BUGSS facility must be labeled to include; the chemical name and concentration, the owner/preparer, any applicable hazard warnings, date prepared, and any additional steps such as pH adjustment or filter sterilization. Any unlabeled solutions will be disposed of.

Biological Materials:

Biological materials (bacterial strains, plasmids, etc) are available from the BUGSS supply store, or you may bring in your own materials as long as they meet the source documentation criteria outlined above.

As a BUGSS member you will be provided with space in the -80°C freezer for long term storage of your cells and cultures. To remove strains from the -80°C freezer contact a BUGSS staff member who will assist you. If you have elected to make your strain available to other BUGSS members you will be asked to provide a suitably prepared sample for archiving in the BUGSS master cell bank and use by other members.

Incubators:

Anything placed in an incubator or growth chamber must be labeled with (either on plate or rack):

- Your name
- Your phone number
- The substance/cell line
- Date placed in incubator

Waste

Biohazardous Waste

In Maryland Biohazardous waste is officially referred to as Special Medical Waste and is regulated under statutory authority by both the Department of Health and Mental Hygiene (DHMH) and Department of the Environment (MDE). According to Maryland Statute any of the following is considered to be Special Medical Waste and must be disposed of in an approved manner:

1. Any living cells incubated or experimented on in the laboratory
2. Any human samples/fluids used in laboratory procedures/experiments
3. Any waste materials in contact with said cells/samples (i.e. pipette tips, centrifuge tubes, incubation plates, swabs, etc.)

When in doubt, treat it as biohazardous!

Sharps Waste

Sharps waste is anything which has the potential to cut or puncture human skin and includes broken glass, needles, scalpel blades, microscope slides, and glass transfer pipettes. All sharps waste needs to be placed in a puncture resistant container for safe disposal. If the sharps has been in contact with biological material it should be treated as biohazardous sharps waste and placed in the proper container for disinfection and disposal. If the sharps waste is not contaminated it can be placed in the broken glass receptacle for disposal. Note that plastic pipette tips are not treated as sharps – they should be discarded as dry waste.

Dry Biohazardous Waste

Dry biohazardous waste should be discarded in the red biohazardous waste containers, lined with orange biohazard autoclave bags. Tubes with small amounts (<1-2 ml) of liquid present may also be discarded as dry waste as well, which is often more convenient. If several tubes with small amounts (< 1-2 ml) of liquid biohazardous waste are being discarded place several paper towels in the autoclave bag to soak up any spilled liquid.

The lid on biohazard waste bins must remain closed. The lid on tabletop biohazard waste bins may be left open when actively supervised (i.e. during pipetting operations frequently generating waste tips), but must otherwise be kept closed.

Once biohazardous waste bags are $\frac{3}{4}$ full, they should be closed and placed in the large biohazardous waste bin kept near the autoclave. The bag should then be replaced with a new red biohazard autoclave bag.

Biohazardous waste bags should not be emptied before becoming $\frac{3}{4}$ full unless there is reason to do so (i.e. an unpleasant odor). Biohazardous waste must remain segregated from regular trash and should only be disposed of by a BUGSS staff member.

Liquid Biohazardous Waste

Liquid biohazardous waste should be diluted 1:10 with bleach (such that the solution contains 10% bleach), covered and left to sit for 10 minutes. After 10 minutes, the solution may be flushed down the drain with cold running water. If the container is disposable (and thus waste), it should be disposed of as dry biohazardous waste.

Chemical Hazardous Waste

BUGSS cannot currently dispose of hazardous chemical waste. Therefore hazardous waste which cannot legally be disposed of down the drain or in the municipal trash must not be generated on site. If you are unsure of whether your waste would be considered hazardous, please email safety@bugssonline.org first.

Emergency Procedures

Biohazardous Waste Spill

1. Assemble clean-up materials (bleach, paper towels, biohazard bags and forceps)
2. Put on appropriate PPE, including lab coat, gloves and eye protection.
3. Initiate cleanup with bleach as follows:
 1. Place paper towels or other absorbent material over spill area
 2. Carefully pour disinfectant around the edges of the spill and then onto the paper towels. Avoid splashing or generating aerosol droplets.
 3. Allow disinfectant to remain in contact with spill for at least 20 minutes
 4. Apply more paper towels to wipe up spill
 5. Clean spill area with fresh towels soaked in disinfectant
 6. Dispose of all towels or absorbent materials using appropriate biohazardous waste disposal procedures. If any sharp objects are present, use forceps and discard in a sharps container.
7. Remove protective clothing and segregate for disposal or cleaning.
8. Wash hands with soap prior to leaving area.
9. Call the BUGSS safety officer (410-292-0710) to report a spill

Chemical Hazardous Waste Spill

Since hazardous chemicals are not yet permitted at BUGSS, there should not be any

chemical hazardous waste spills. BUGSS is not yet equipped to handle them. Spills of dilute acids or bases should be treated by flushing the affected area with water.

Fire

The following is a general fire response procedure. It is not meant for flammable liquid fires, as flammable liquids are not yet permitted in the facility.

1. Evacuate area
2. If fire is small, attempt to extinguish with fire extinguisher
3. If fire is large, or extinguishing with a fire extinguisher is not immediately successful, call 911
4. Call the BUGSS safety officer/ emergency number (410-292-0710) to report

Medical Emergency:

1. Call 911
 2. Initiate First Aid
 3. Call the BUGSS safety officer (410-292-0710) to report
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