BUGSS Safety Manual

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

Revision 2.0 May l 2025

Change History

Version	Date	Author	Notes
1.1	July 28, 2012	Thomas Burkett, Ph.D.	Initial version from Biocurious safety manual with Modifications
1.2	June 11, 2019	Edmund Wonilowicz, CSP, CHMM	Changes to allow some animal work. Editorial changes for consistency
2.0	May 1, 2025	Edmund Wonilowicz, CSP, CHMM	Rewrite to reflect current practices

Contact Information

911	Emergency Call Number
443-452-8498	BUGSS Emergency Contact (Lisa Scheifele)
safety@bugssonline.org	General questions, requests for clarification on specific substances/ experiments, requests for new capabilities
safety@bugssonline.org	Ed Wonilowicz, 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. It is also important to protect 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 these obligations and responsibility to the larger community of citizen scientists to operate in an exemplary and transparent manner. To participate in BUGSS each member or guest worker must adopt these values.

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 scientists to individuals with no formal scientific training. We celebrate and embrace that diversity and seek to provide a space where people can come together to learn about and practice biotechnology. To some the idea of a community laboratory may cause concern so we must strive to be shining examples of safe and responsible laboratory practice.

Our Goals

BUGSS wants 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 commensurate 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 has existed for some time and we have developed a level of comfort with the types of work which individuals desire to perform. However, we are always learning about other areas of interest to our members. Consequently, our safety practices must evolve as needed. Emailing your questions and concerns to safety@bugssonline.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 Areas

The BUGSS wet-lab areas are distinctly marked with signage and are separate from the rest of the facility. The policies in this section pertain to the wet -lab areas.

Wet-Lab Area Access

Access to the Wet-Lab Areas 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. Guests may not conduct any lab work until they have completed the BUGSS Safety Orientation course.

Food/Drink

The consumption or storage of food/drink is not permitted in the wet-lab areas, 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 areas.

Smoking

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

Clothing

Closed-toe footwear will be worn at all times in the laboratory. Long pants and 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)

BUGSS keeps a stock of gloves, protective eyewear and lab coats. BUGSS encourages members and guests to wear PPE at all times when working in the lab, but they 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, especially when working with hazardous materials. Everyone working in the lab should be well trained in emergency procedures and the location of safety equipment in the facility.

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. 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 6th Edition:

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and that 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.

Requests may be made to work at BSL-2 in restricted areas of the lab. These will be considered on a case-by-case basis and standard operating procedures would be developed in conjunction with the Safety Officer. 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 list of microbial species that may be worked on our inventory (https://docs.google.com/spreadsheets/d/1XF9I7uUJXn4b7biRnr2EfA_Onyko1wBjz_VKF3teXpA/edit?usp=sharing).

Microbial samples from nature may not be cultured at BUGSS unless specific permission has been obtained from the Safety Officer and a Standard Operating Procedure agreed upon. Such samples are by definition not well characterized, and doing so often 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 not cultured in the lab.

Mammalian

The culturing of mammalian cells is restricted to a specific area and biosafety cabinet at BUGSS. All rules applying to other organisms with respect to safety and sufficient characterization would apply to mammalian cells. Contact safety@bugssonline.org with questions pertaining to work with mammalian cells

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, as described in the sections below.

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 that they affirm that fact. Samples must be analyzed immediately once brought into BUGSS and cells may not be stored and/or incubated. Participants may only work with autologous (their own) cells, and may not exchange samples with other individuals .

Blood and other human fluids

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

Animals

Work with live animals at BUGSS is strictly controlled. Only animals which carry no diseases or conditions which are communicable to either humans or animals are allowed. Transgenic animals or those which have been genetically engineered are permitted on a case-by-case

basis at the discretion of the Safety Officer and/or Board. The production of such animals is not allowed. Experiments such as natural breeding of animals, dissection for study of anatomical structures, and other non-recombinant experiments are permitted.

To bring animals into the BUGSS facility, you must provide documentation as to the source and identity of the organism, describe any regulatory approvals (IACUC, etc) that have been obtained from other institutions, and describe methods that will be used for the disposal or removal of the animals from the BUGSS facility. If using preserved specimens, individuals should provide information on the type and manner of fixation. Individuals must also provide a protocol that describes how the fixative as well as the ventilation and PPE to be used complies with BUGSS' chemical safety policy.

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: https://www.selectagents.gov/sat/list.htm

3. You may not genetically engineer any animal.

Chemical Safety

BUGSS maintains a chemical inventory (https://docs.google.com/spreadsheets/d/1XF9I7uUJXn4b7biRnr2EfA_Onyko1wBjz_VKF3teXpA/edit?usp=sharing) and some of the materials are hazardous. The chemical inventory will be updated annually and lists PPE and precautions that should be taken when working with hazardous chemicals. Chemicals not already in inventory may not be brought into the facility by members without approval from the BUGSS safety officer and provision of a Safety Data Sheet for that chemical. Any precautions listed on the inventory for working with a chemical or limits to quantities must be followed.

Safety Data Sheets (SDS) for chemicals in the inventory list can be found at https://chemicalsafety.com/sds-search/ or https://www.sigmaaldrich.com/US/en/documents-search?tab=sds and should be consulted when working with any unknown chemical.

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 pH when applicable. Hazardous chemicals have designated storage cabinets in the lab and should always be stored there.

Flammables

Flammable chemicals with Globally Harmonized System fire ratings of less than 2 may not be brought into the facility at present (note that in this system, lower ratings indicate greater risk). Ratings can be found on the SDS for any chemical. 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. The total volume of stock flammable materials at the BUGSS facility may not exceed what fits in the flammables cabinet.

Corrosives

BUGSS maintains a small quantity of acid and base for use in pH adjustment. Types of acids/bases and concentrations may not exceed those listed on the inventory (https://docs.google.com/spreadsheets/d/1XF9I7uUJXn4b7biRnr2EfA_Onyko1wBjz_VKF3teXpA/edit?usp=sharing) and volumes should be as small as possible. Acids and bases should be diluted to their final working concentrations as soon as possible, subject to space limitations and storage compatibility.

Oxidizers

Oxidizers may not be brought into the facility at present.

Explosives

There are no plans to allow explosives to be brought into the BUGSS facility, nor would any chemical with any degree of explosivity be allowed in inventory.

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 currently has limited capability to dispose of hazardous waste. As such chemicals that cannot be disposed of down the drain or in the municipal trash must be discussed with the BUGSS safety officer before they are brought into the facility so that proper disposal through a contracted company can be arranged and the storage of waste can be planned for.

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 in the BUGSS organism/chemical inventory (https://docs.google.com/spreadsheets/d/1XF9I7uUJXn4b7biRnr2EfA_Onyko1wBjz_VKF3teXpA/edit?usp=sharing), either explicitly or categorically. Nonhazardous chemicals such as salts without heavy metals, vitamins, media components, etc. are allowed. If in doubt, consult the BUGSS Safety Officer at safety@buggsonline.org.

2. If the chemical is not in inventory, but you believe it to be allowable based on this safety manual, email safety@bugssonline.org with your request for approval or (preferable) use the online approval form at (https://docs.google.com/forms/d/e/1FAIpQLSfGO54buX9PFp7NxEFH6_17WBvQEvYlkSfjlGoBf_CAeOsQdw/viewform?usp=header). Include the SDS for any requested chemical, and specific strain information for any organism. Items not already in inventory may not be brought into the facility without explicit written approval from the BUGSS safety officer. When an item newly approved by the Safety Officer is brought into the facility, it must have an original manufacturer's label specifying the compound, concentration and hazard warnings. It should also be checked in with a BUGSS staff member to ensure that it is stored properly.

4.If you ship chemicals or organisms to BUGSS, they will be held for 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. Chemicals removed from the facility by members must not be regulated as hazardous materials by the U.S. Department of Transportation.

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. Plasmids containing fully infectious agents are forbidden.

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 inventory grows

sufficiently large, and categorical rules can be created that make requests to bring materials into the facility rarely necessary. Until then, we'll err on the side of caution and respond promptly for requests to allow chemicals in inventory.

Material Supply Store:

BUGSS 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.

You may also elect to bring your own supplies 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. Persons may not bring hazardous materials into the facility without proper approvals and by following U.S. Department of Transportation regulations for shipment. In other words, some materials may not be carried into the facility in personally owned vehicles but must be ordered and delivered bv a commercial carrier licensed to transport such materials.

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.

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 that is unlabeled or 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:

As a BUGSS member you will be provided with space in the -80^0 C freezer for long term storage of your cells and cultures. 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

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. Note that plastic pipette tips are not treated as sharps – they should be discarded as dry waste.

Dry Biohazradous Waste

Dry biohazardous waste should be discarded in the biohazardous waste containers, lined with orange or red 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 ¾ full, they should be closed and sealed with tape. . The cardboard carton and bag should then be replaced with a new carton and red biohazard autoclave bag. Biohazardous waste bags should not be emptied before becoming ¾ full unless there is reason to do so (i.e. an unpleasant odor). Biohazardous waste must remain segregated from regular trash.

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

Hazardous waste which cannot legally be disposed of down the drain or in the municipal trash must be approved by the safety officer and waste disposal must be planned for in advance. 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 (10% bleach, paper towels, biohazard bags and forceps)
2. Put on appropriate PPE, including lab coat, gloves and eye protection.
3. Initiate cleanup with 10% bleach as follows:
    1. Place paper towels or other absorbent material over spill area
    2. Carefully pour 10% bleach around the edges of 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 10% bleach
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 the area.

Chemical Hazardous Waste Spill

A plan for hazardous waste spills must be described as part of the approval process by the BUGSS Safety Officer. 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.

1. Evacuate area
2. If fire is small and you are trained and comfortable, 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 Lisa Scheifele at the BUGSS emergency number (443-452-8498) to report

Medical Emergency:

1. Call 911
2. Initiate First Aid
3. Call Lisa Scheifele at the BUGSS emergency number (443-452-8498) to report

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