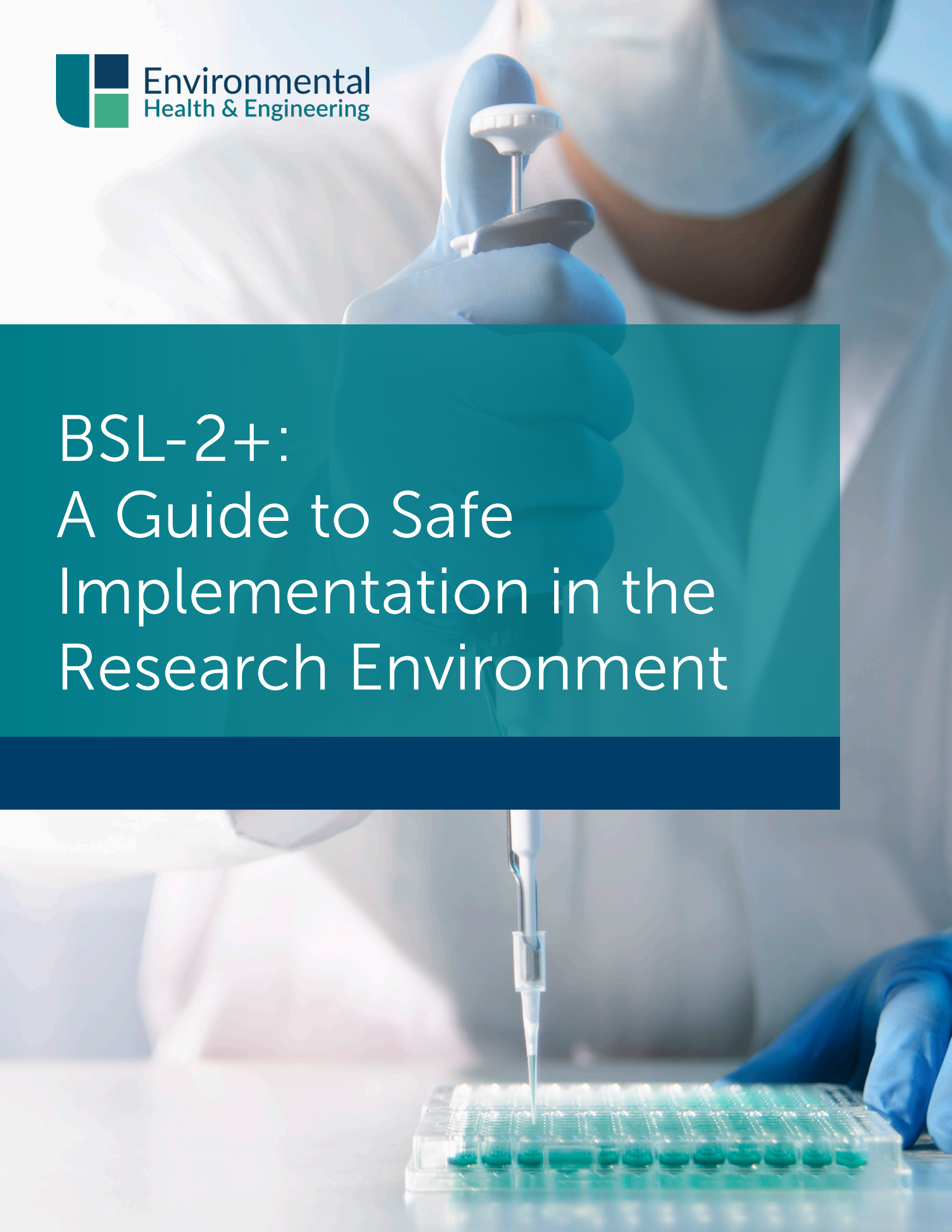
Environmental Health & Engineering

BSL-2+: A Guide to Safe Implementation in the Research Environment



Introduction

There are four formally recognized biosafety levels that describe increasingly stringent containment practices and facilities used to work with biological agents. Biosafety levels are determined by conducting a thorough risk assessment of the biological agents and procedures being used by a specific laboratory. Sometimes, based on the risk assessment, it is determined that a Biosafety Level 2 (BSL-2) facility is appropriate for the work that's being performed, but that more stringent biosafety practices are desirable.

Biosafety Level 2+ (BSL-2+) is the term frequently used to describe laboratories where work with microorganisms is conducted in a BSL- 2 laboratory with selected biosafety practices and procedures that are typically found at BSL-3. In these instances, laboratories are going above requirements by adopting BSL-3 practices as an added safety measure. Since many laboratory facilities do not have a BSL-3 laboratory, the option to do the work in a BSL-3 laboratory is not feasible. Although this hybrid approach has been in use for many years, many research institutions still find it challenging to decide when to use this approach and which BSL-3 practices to use for their experiments. This hybrid approach can facilitate safe science when a thorough risk assessment is performed.

The three greatest challenges to implementation are:

- **Determining what work or projects require BSL-3 practices, and BSL-3 practices to adopt.**
- **Ensuring that the researchers are properly trained in the use of BSL-3 practices in a BSL-2 laboratory.**
- **Ensuring that the Laboratory Supervisor, Principal Investigator (PI), or their designee, develops a project-specific SOP.**

This guide addresses these concerns and outlines a practical approach for successfully implementing BSL-3 practices in a BSL-2 laboratory, specifically for the research environment.

CHAPTER ONE:

What is BSL-2+?

BSL-2+[1] is not recognized as a containment level in the Centers for Disease Control and Prevention's (CDC) *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) guideline or the National Institutes of Health's (NIH) *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* guidance document. However, BMBL acknowledges the need for "enhanced practices, procedures, and facilities" in certain circumstances, and the NIH's Biosafety Considerations for Research with Lentiviral Vectors, mentions "enhanced BL2 containment."

The use of BSL-3 practices and procedures in a BSL-2 laboratory allows for research work with microorganisms including viral vectors to take place in an environment where the safety practices are enhanced over and above the practices required at BSL-2.

To determine how best to implement BSL-2+ procedures, it is critical to begin with a protocol-driven risk assessment. The BMBL guidelines detail the steps involved in this risk assessment process. It further states that, after determination of the appropriate biosafety level, it is important to select additional precautions as indicated by the risk assessment.

[1] For the purposes of this paper, the term "BSL-2+" will be used in reference to a BSL-2 laboratory facility where BSL-3 practices are utilized. In lieu of BSL-2+, some institutions refer to "BSL-2 with enhancements" or "BSL-2 with conditions".

CHAPTER TWO:

Risk Assessment Identifies Biosafety Level and Practices

There is no standardized list of microorganisms, viral vectors, or research projects that should be conducted within BSL-2+ environments. Each decision to use selected BSL-3 practices in a BSL-2 laboratory must be made via a risk assessment process.

The risk assessment process serves to guide the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that will contribute to preventing a laboratory exposure.

As outlined in the BMBL, the steps of the risk assessment process include:

1. IDENTIFY HAZARDOUS CHARACTERISTICS OF THE AGENT AND PERFORM AN ASSESSMENT OF THE INHERENT RISK, WHICH IS THE RISK IN THE ABSENCE OF MITIGATING FACTORS. THIS ASSESSMENT SHOULD ADDRESS THE POSSIBILITY OF TRANSMISSION OF THE AGENT.
2. IDENTIFY LABORATORY PROCEDURE HAZARDS.
3. MAKE A DETERMINATION OF THE APPROPRIATE BIOSAFETY LEVEL AND SELECT ADDITIONAL PRECAUTIONS INDICATED BY THE RISK ASSESSMENT.
4. BEFORE IMPLEMENTING CONTROLS, REVIEW THE RISK ASSESSMENT AND SELECTED SAFEGUARDS WITH A BIOSAFETY PROFESSIONAL, SUBJECT MATTER EXPERT, AND THE INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) OR EQUIVALENT RESOURCE.
5. EVALUATE THE PROFICIENCIES OF STAFF REGARDING SAFE PRACTICES AND THE INTEGRITY OF SAFETY EQUIPMENT. THIS SHOULD BE PART OF AN ONGOING PROCESS.

The BMBL further advises that this process be regularly revisited to verify risk management strategies are effective and determine if changes are necessary. The risk assessment process must also be applied to every new or revised research project. A project registration document serves to detail the risk assessment process.

Factors that Trigger BSL-2+

Examples of when BSL-2+ may be appropriate include:

- **Viral vectors with gene inserts consisting of oncogenes or genes of unknown function.**
- **Second generation lentiviral vectors that have an increased risk in recombination to generating replication-competent lentiviruses.**
- **Drug-resistant Risk Group Two (RG2) bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA).**
- **RG2 organisms with low infectious doses which can cause serious disease (e.g., *Salmonella Typhi, Shigella* spp.).**
- **Organisms where certain factors predispose individuals to infection or negative health outcomes (e.g., Zika virus, *Listeria monocytogenes*).**
- **Low titer and small volumes of Human Immunodeficiency Virus (HIV), an RG3 agent.**
- **High concentrations (>106 PFU/mL) of RG2 viruses.**
- **Work with greater than 10 liters of a RG2 agent.**
- **Organisms that present certain biocontainment and/or biosecurity concerns (e.g., Methicillin-resistant *Staphylococcus aureus*).**

Project Review Process

The project review process is part of the risk assessment process. This review includes several steps that need to take place before work can be approved and commence.

1. Completion and submission of a project registration document by the laboratory supervisor or PI to the Biosafety Officer (BSO). The purpose of the project and the steps to be conducted with the biohazardous material must be documented in detail.

2. Review and discussion of the project registration document with the laboratory supervisor or PI, BSO, and in some cases selected members of the IBC who have expertise in the particular area of research that is

being reviewed. For example, a virologist may be asked to review a project involving viral vectors. An IBC and adherence to the NIH Guidelines are mandatory if the institution receives federal funding and/or is located in a community with a recombinant DNA ordinance. If the institution does not require an IBC, then a similar type of review committee such as a Biosafety Committee should be part of the review process.

3. If the outcome of the review process is that a BSL-3 containment facility is not necessary but BSL-2 practices may not provide adequate safeguards for laboratory personnel, then the use of selected BSL-3 practices in a BSL-2 laboratory may be appropriate. At this stage a suitable BSL-2 laboratory space should be proposed and the BSL-3 practices to be utilized outlined. This is consistent with the BMBL, which indicates that the risk assessment process should allow for a determination of the appropriate biosafety level and selection of additional precautions indicated by the risk assessment.

4. IBC review and consensus must take place prior to initiation of the project. At the IBC meeting, the BSO outlines the proposed project and the BSL-3 practices to be utilized in the BSL-2 laboratory space. Depending on IBC policies and procedures, it may be useful for the laboratory supervisor or PI to attend the IBC meeting to provide additional information on the proposed project and to answer questions that may arise. The IBC members should come to consensus on the appropriate BSL-3 practices that should be applied to the proposed work. In addition, a suitable BSL-2 laboratory space should be decided upon.

5. Risk communication and training must be conducted after IBC approval and before any work is performed in the laboratory. The BSO should review the required BSL-3 procedures with the laboratory supervisor or PI and his/her laboratory staff. Ideally, these should be written in the form of an SOP. Additionally, it is important to review the laboratory space to ensure required BSL-2 elements are in place including, but not limited to, biowaste containers, sink with soap and paper towels, and certified biological safety cabinets (BSCs).

CHAPTER THREE:

Selecting the Lab Space and BSL-3 Practices

Selection of a BSL-2+ Lab Space

It is important to remember that no specialized facilities are required for BSL-2+ and there is no requirement to include BSL-3 facility elements in a BSL-2 space. This designation is only used for materials and procedures that can be worked with safely in a BSL-2 lab space, but where researchers wish to add supplemental safety measures.

BSL-2 laboratories are common in many academic and industrial research facilities. They are often large spaces occupied by many laboratory personnel working on a diverse list of projects and sharing laboratory equipment. In some cases, this scenario may not be conducive to adhering to BSL-3 practices. Dedicating a separate BSL-2 laboratory space to the project that requires BSL-3 practices allows other projects to maintain standard BSL-2 practices.

Practically speaking, creating a BSL-2+ lab space usually means taking a smaller BSL-2 or "tissue culture" laboratory room and dedicating it to the project. This allows for limited access to only those persons who are listed on the research protocol and have received the necessary training.

Selection and Modification of BSL-3 Practices

It is important to keep in mind the BSL-3 requirements for work practices and safety equipment such as personal protective equipment (PPE) and BSCs. Sometimes the appropriate BSL-3 practices determined by the risk assessment may be limited to restricting sharps in the laboratory. In other situations, multiple BSL-3 practices are selected.

Table 1 details selected BSL-3 practices and safety equipment requirements as stated in the BMBL. Each risk assessment and project review should include a review of these practices and equipment requirements to determine the items that will enhance worker and environmental protection. Each item may be subject to discussion and there is room for modifications, provided there is consensus among the IBC members.

Examples of Modifications to BSL-3 Practices

One example of a potential modification to BSL-3 practices for use in a BSL-2+ space is around waste management procedures (table 1; A15). While decontamination within the immediate laboratory is preferable, there is an option to remove materials from the facility for decontamination. Perhaps the autoclave for the facility is not adjacent to the laboratory or the facility does not have an autoclave and relies on a vendor for waste removal and decontamination at an external facility. There is no firm requirement to autoclave all biohazard waste (solids, sharps) on the premises as long as 1) a suitable off-site arrangement is in place that is consistent with local or state requirements and 2) the risk assessment determines there is no additional risk to personnel or the environment if materials are not autoclaved prior to removal from the lab.

Another example concerns the biosafety manual. BSL-3 practices specify that a laboratory-specific biosafety manual must be prepared and adopted as policy (table 1; A4), and the biosafety manual must be available and accessible. For a BSL-3 laboratory, the preparation of a formal biosafety manual as well as numerous specific SOPs is required. However, for a BSL- 2+ laboratory, a more practical approach would be to develop an SOP that details the specific requirements as determined through the risk assessment and with approval of the IBC. This SOP is typically an adjunct to an existing biosafety manual developed for the facility for BSL-1 and BSL-2 laboratories.

This SOP can be posted on the door to the laboratory as well as inside, and makes an excellent training tool that can be used for discussion during laboratory-specific training sessions. A useful addition to the SOP is a flowchart that summarizes the steps involved in the work where BSL-3 practices are necessary and when materials may be safely removed to other laboratory areas where BSL-2 practices are in use. For instance, it may not be practical to house a -80°C freezer in the laboratory room where BSL-3 practices are utilized. Thus, the procedures for removing materials to the freezer are outlined on the flowchart with a notation on how materials are safely packaged and transported in a labeled secondary container to the freezer.

Additional Considerations for Implementing BSL-2+

In many cases, there will be other considerations that should be reviewed in addition to the BSL-3 practices listed in the BMBL, such as simultaneous use of different containment levels and transfer of information in and out of the BSL-2+ laboratory. Examples include:

- **If the BSL-2+ laboratory has adequate space to accommodate additional research projects, a decision may need to be made as to whether to allow other laboratory personnel to work in the space with materials of a lesser hazard. For example, occasional use of the BSC when working with human blood samples that may be handled with BSL-2 practices. Ultimately it is the decision of the IBC, but best practice would likely dictate that the work could take place provided the worker follows the approved BSL-3 practices when conducting the work. Thus, the default is BSL-3 practices when work takes place in the BSL-2+ laboratory. While this may appear to be overly**

cautious, it prevents a double-standard of multiple workers in the laboratory using different levels of PPE and safety practices.

- If the project approved with BSL-3 practices involves work that occurs infrequently, a decision may need to be made as to whether to revert the laboratory back to a standard BSL-2 laboratory with BSL-2 practices. Consideration should be given to the materials in use and whether the laboratory facility and equipment should be decontaminated prior to downgrading. Signage would need to be adjusted as well. If such a practice is allowed, an SOP must be developed to detail the process and training must be provided.

- Consideration must be given as to whether it is appropriate for laboratory personnel to bring laboratory notebooks and portable electronic devices in and out of the BSL-2+ laboratory. Best practice is to prohibit this in order to avoid bringing contamination out of the laboratory, but provide provisions for information to be transmitted to the office through an electronic device that is dedicated for use within the BSL-2+ laboratory. Alternatively a procedure to wipe down a laptop with a disinfectant wipe prior to leaving the lab could be implemented.

- If the BSL-2 laboratory will be renovated or built for a project utilizing BSL-3 practices, it may be useful to incorporate some BSL-3 laboratory facility requirements for convenience. Examples include installing a hands-free or automatically operated sink for hand washing and locating an anteroom between the laboratory and external areas. The anteroom may be useful for storage of PPE.

- If the BSL-2 laboratory is an animal biosafety level two (ABSL-2) facility, then animal biosafety practices, procedures, and safety equipment criteria must be incorporated. The risk assessment guides the decision as to what ABSL-3 practices to incorporate.

Table 1: Selected BSL-3 Requirements from the BMBL and Potential Implementation in a BSL-2 Laboratory

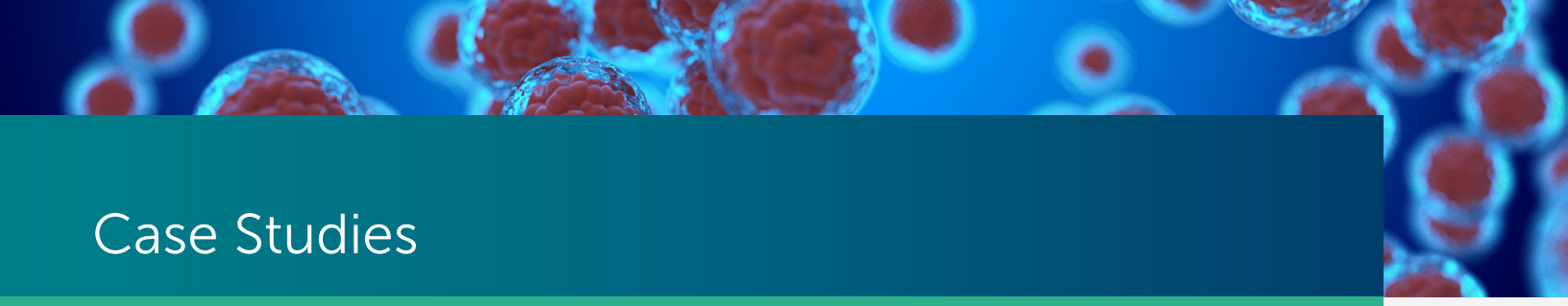
A.	BSL-3 Standard Microbiological Practices	Modified for a BSL-2 Laboratory Facility
A1.	The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.	Limit access to those listed in the approved project and who have received additional training. Card access may be useful.
A2.	The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates or additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.	The PI is ultimately responsible for the safety of his/her personnel. In conjunction with the BSO, appropriate training must be provided.
A3.	Personnel health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.	Occupational health providers should be consulted by lab personnel should there be any questions on the health impact of materials to be worked with.
A4.	A safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.	Develop an SOP that details the specific requirements as determined through the risk assessment and with approval of the IBC. This SOP may serve as an adjunct to an existing biosafety manual developed for the facility for BSL-1 and BSL-2 laboratories.
A5.	A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.	Created by the BSO. Consider adding other languages to allow non-English speaking personnel to read and understand the signage.
A9.	Persons wash their hands after working with potentially hazardous materials and before leaving the laboratory.	A sink for handwashing with paper towels must be available. A hands-free faucet may be useful.
A12.	Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed, implemented, and followed; policies are consistent with applicable state, federal and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries.	A sharps policy is implemented and sharps (e.g., glass Pasteur pipettes, needles) are not allowed. Plasticware is substituted for glassware.
A13.	Perform all procedures to minimize the creation of splashes and/or aerosols.	All work is performed in a BSC.
A15.	Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. Depending on where the decontamination will be performed, the following methods are used prior to transport: (a) Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and secured for transport. For infectious materials, the outer surface of the container is disinfected prior to moving materials and the transport container has a universal biohazard label. (b) Materials to be removed from the facility for decontamination are packed in accordance with applicable local, state, and federal regulations.	Determine whether materials must be autoclaved prior to removal from the facility. If it is deemed optional, consideration may be given to offsite decontamination (e.g., incineration via a vendor).

B.	BSL-3 Special Practices	Modified for a BSL-2 Laboratory Facility
B1.	All persons entering the laboratory are advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or laboratory areas is required for scientific or support purposes are authorized to enter.	Lab-specific training emphasizing the specific BSL-3 practices is provided by the BSO to all lab personnel.
B2.	All persons who enter operational laboratory areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and treatment, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.	Lab personnel must participate if medical surveillance is required per direction of IBC and occupational health physician.
B3.	The laboratory supervisor is responsible for ensuring that laboratory personnel demonstrate proficiency in standard microbiological practices and techniques for working with agents requiring BSL-3 containment.	The PI must provide training to lab personnel who may not have experience working with the materials to be used with BSL-3 practices. E.g., an apprentice program may be established for personnel where they shadow more experienced personnel and are not allowed to work independently until they demonstrate proficiency.
B5.	Incidents that result in exposure to infectious materials are immediately evaluated per institutional policy. All such incidents are reported to the laboratory supervisor, institutional management, and appropriate safety, compliance, and security personnel according to institutional policy. Appropriate records are maintained.	Consider installing a phone in the lab for use in an emergency (e.g., injury or spill). Post emergency contact names and numbers inside the lab.
B7.	All procedures involving the manipulation of infectious materials are conducted within a BSC or other physical containment device, when possible. No work with open vessels is conducted on the bench. If it is not possible to perform a procedure within a BSC or other physical containment device, a combination of personal protective equipment and other administrative and/or engineering controls, such as centrifuge safety cups or sealed rotors, are used, based on a risk assessment. Loading and unloading of the rotors and centrifuge safety cups take place in the BSC or another containment device.	All work is performed in a BSC, including loading/unloading centrifuge rotors/cups.
B8.	Laboratory equipment is routinely decontaminated after spills, splashes, or other potential contamination, and before repair, maintenance, or removal from the laboratory. Equipment or material that might be damaged by high temperatures or steam is decontaminated using an effective and verified method, such as a gaseous or vapor method.	Create a "Spill Kit" and store it within the lab. Decontaminate all equipment prior to servicing within the lab or prior to removal from the lab. Consider a yearly "shut down" for a few days to accommodate servicing and maintenance activities.
C.	**BSL-3 Safety Equipment**	**Modifications for a BSL-2 Laboratory Facility**
C1.	Laboratory workers wear protective clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.	Disposable, solid-front, fluid-resistant gowns are practical. Consider placing hooks inside the lab, near the door so gowns may be hung for additional use if not contaminated.
C2.	Based on work being performed, additional PPE may be required. (a) Eye protection and face protection (e.g., safety glasses, goggles, mask, face shield or other splash guard) are used for manipulations or activities that may result in splashes or sprays of infectious or other hazardous materials. Eye protection and face protection are disposed of with other contaminated laboratory waste or decontaminated after use. (b) Two pairs of gloves are worn when appropriate. (c) Respiratory protection is considered. Staff wearing respiratory protection are enrolled in a properly constituted respiratory protection program. (d) Shoe covers are considered.	Safety glasses with side shields should be worn while in the lab.

Conclusion

In summary, the use of BSL-3 practices in a BSL-2 laboratory may be appropriate for some research projects and may contribute to the safe conduct of that research. As there is no “one size fits all” approach to implementing BSL-2+ practices, a risk assessment is key to determining whether BSL-3 practices are appropriate in a BSL-2 laboratory facility and what practices will be required. The collaboration between the laboratory supervisor or PI, BSO, IBC and laboratory personnel is crucial to the successful outcome.

Continue reading for a few instructive examples on how to implement BSL-2+ for work with lentivirus, Methicillin-resistant Staphylococcus aureus, and other agents.



Case Studies

How you modify BSL-3 practices for work in a BSL-2 laboratory will depend upon the work being conducted, the results of the risk assessment, and your institution's IBC. Depending upon the BSL-3 practice being implemented, there may be more than one acceptable option for modifying the practice in a BSL-2 environment. The following are case studies illustrating the approach some institutions have taken to modifying BSL-3 practices.

Case Study: Work with Lentivirus

A PI requested to use lentivirus to infect human cells in culture. The gene inserts will include oncogenes as well as genes of unknown function. The risk assessment identified the main hazard as sharps injury or skin or mucous membrane contact to material containing the oncogenes and genes of unknown function. An existing tissue culture laboratory was designated as the BSL-2+ laboratory. The project registration document provided a flowchart detailing the work that will be conducted in the BSL-2+ laboratory and included steps that will take place in the general BSL-2 lab (e.g., transport of samples in a secondary container to the -80°C freezer in the main laboratory). The BSO and the IBC specified the following additional practices and procedures:

- **The laboratory has limited access. Only those individuals listed in the project registration, the BSO and some members of the environmental health and safety (EH&S) department are allowed access per signage posted on the door. Janitorial and maintenance staff are required to be escorted by the laboratory supervisor or PI, BSO or project personnel in order to enter the laboratory. Phone numbers for the laboratory supervisor or PI and key laboratory staff are posted in the event of an off-hours emergency.**

- **BSL-2 work is allowed to take place in the BSL-2+ laboratory with the stipulation that the work is performed using the same BSL-3 practices as required for the lentivirus project.**

- **The laboratory supervisor or PI developed an SOP detailing the work practices and procedures as required by the IBC, using a template provided by the BSO. The SOP was reviewed by the BSO and is used for additional training of project personnel.**

- Signage for the laboratory door with the universal biohazard symbol includes: Laboratory supervisor or PI name, list of approved/trained project personnel including others who may have approved access (BSO, EH&S personnel), materials in use (lentivirus and human cells), and emergency contacts and phone numbers. The signage indicates that only approved/trained personnel may enter and visitors must be accompanied by an approved/trained person.

- Based on consultation with the institution's occupational health physician, no medical surveillance is required for the members of the laboratory working on the project. Laboratory staff were instructed to contact the occupational health physician should they have any medical questions or concerns. Since human materials are used in the project, all project personnel were offered Hepatitis B vaccination in accordance with the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogen Standard. Additionally, project personnel were provided with a review of the institution's procedure for immediate reporting of all occupational injuries and illnesses.

- In addition to the two BSCs that were already in the BSL-2+ laboratory, a tabletop centrifuge, a microscope, two incubators, and a laptop computer were purchased and designated for the laboratory. The laptop is used to transmit notes outside of the laboratory, as no notebooks can be taken in and out of the laboratory.

- The PPE consists of a disposable solid front gown with cuffed sleeves, safety glasses with side shields, and nitrile gloves. In the entry area of the laboratory, coat hooks are available so gowns may be hung up for reuse, if deemed not contaminated. A set of hooks immediately outside of the laboratory is available to hang cotton laboratory coats utilized for work in the main BSL-2 laboratory. Each researcher is required to bring a box of gloves into the BSL-2+ laboratory in the appropriate size.

- Sharps such as Pasteur pipettes and needles are prohibited in the BSL-2+ laboratory. Plasticware is substituted for glass. Plastic pipette tips are allowed.

- All work is conducted in the BSC, including loading and unloading of centrifuge safety cups for the tabletop centrifuge located within the laboratory.

- Freshly prepared bleach solutions and 70% ethanol are available and utilized for disinfection of surfaces and equipment in the laboratory.

- There is no autoclave in the BSL-2+ laboratory or the larger BSL-2 laboratory. While there is an autoclave in another location in the building that is used for media preparation, the institution utilizes the services of a vendor for disposal of biomedical waste and sharps. The solid non-sharp waste including but not limited to plastic culture flasks and gloves, is collected within the BSC in a small red biohazard bag contained within a Nalgene container with a lid. When two-thirds full, the bag is removed by the researcher, tied at the top with a rubber band, and placed within a vendor-

supplied large cardboard waste box lined with two red bags. When full, the box is taped, labeled and placed immediately outside of the BSL-2+ laboratory for the vendor to remove from the facility and transport for off-site incineration. The used pipette tips generated in the BSC are immediately put in a plastic sharps container located within the BSC. Liquid waste is treated with mercury-free bleach (1 part bleach to 9 parts liquid waste), allowed to sit for at least 30 minutes, and then carefully disposed of via the sink.

- Materials in labeled secondary containers can be taken out of the laboratory and moved to the main BSL-2 laboratory for storage in the -80°C freezer. In addition, fixed cells may also be removed in a secondary container from the laboratory for cell sorting.

- A laboratory member was appointed to serve as the BSL-2+ Manager and oversee daily operations in the lab, including ensuring that adequate PPE and supplies such as disinfectants are available, monitoring conditions in the lab including PPE usage, and reporting issues that may require retraining. The BSL-2+ manager coordinates with and accompanies the maintenance department and equipment vendors when access to the BSL-2+ laboratory is necessary.

Case Study: Methicillin-resistant Staphylococcus aureus (MRSA)

A PI proposed to work with MRSA, a risk group 2 strain of Staphylococcus aureus that has become resistant to many of the antibiotics that are ordinarily used to treat staph infections. This work should be conducted with additional containment practices above standard BSL-2 due to the agent's virulence and the difficulty in effectively treating it. The BSO formulated the following requirements that were added to the written registration which was presented to the IBC. The IBC members agreed with the risk assessment and enhancements and approved the project.

- BSL-2+ work is conducted in a dedicated small procedure room inside a biosafety cabinet. Unrelated work in the room is discouraged when BL2+ work is being conducted.

- When samples are manipulated outside the biosafety cabinet, they are kept in sealed containment when feasible (e.g., in sealed centrifuge cups or sample containers) that are opened inside the biosafety cabinet.

- If infectious samples must be moved out of the BSL-2+ procedure room, they are enclosed in leakproof secondary containment and the outside of the secondary container is disinfected with an approved disinfectant prior to removing it from the room.

- PPE that is used in the BSL-2+ room is kept in the BSL-2+ room. Non-disposable PPE is disinfected before leaving the room or placed in a clean bag if it will be decontaminated offsite.

- PPE consists of laboratory gown or coat with cuffed sleeves or sleeve covers, nitrile gloves and safety glasses.

- The laboratory has developed SOPs for safe work with MRSA, including surface and equipment decontamination, sample transport, waste disposal, handwashing and new employee training protocols.

- Use of sharps is restricted. When sharps must be used, the procedure is reviewed and a safety protocol is developed.

- Training on SOPs is documented.

- Adherence to SOPS is mandatory, and lab members understand that non-compliance is subject to escalation.

- All staff members, including those who don't directly work with MRSA have been trained on the hazards associated with the agent, routes and symptoms of exposure, drug susceptibility/resistance for the strains that are used in the lab, and reporting requirements.

- Training procedures are reviewed at least annually and when there is a change in the protocol. They are updated as needed to reflect changes in procedures and current best practices.

- Signage for the laboratory door with the universal biohazard symbol includes: PI name, list of approved/trained project personnel including others who may have approved access (BSO, EH&S personnel), materials in use and emergency contacts and phone numbers. The signage indicates that only approved/trained personnel may enter and visitors must be accompanied by an approved/trained person.

- There is an autoclave located near the lab. All solid biohazardous waste is autoclaved by the researchers in a validated autoclave. The autoclaved waste is transported in a covered container to the autoclave, autoclaved, and placed in a vendor-supplied large cardboard waste box lined with two red bags for vendor removal and incineration.

- All new procedures involving the use of MRSA are reviewed and approved by the IBC prior to commencement.

Case Study: Zika Virus

In 2015, a large outbreak of mosquito-borne Zika Virus occurred in Brazil. The disease was found to be associated with Guillain-Barré syndrome and several neurological disorders, including microcephaly in newborns. In 2016, the World Health Organization declared the outbreaks and associated illnesses a Public Health Emergency of International Concern. Shortly after the emergency was declared, several PIs at a research institution proposed protocols to study the disease. Because of the nature of the associated disorders, the increased risk to susceptible populations (e.g., pregnant people), and the discovery of new disorders and complications associated with infection, the IBC required BSL-2+ containment for this research. This included the following:

- **Work in a dedicated laboratory space.**
- **All laboratory personnel were required to have a confidential meeting with the occupational health physician.**
- **Use of sharps was restricted and glassware was replaced with plastic when feasible.**
- **Since this was considered an exotic agent at the time, researchers on this project discussed the merits of taking a baseline serum sample/having a serum storage program.**

Following the conclusion of the WHO public health emergency, and based on a greater understanding of the epidemiology and health risks associated with Zika, many labs now work with Zika Virus following BSL-2 containment practices. Prior to making any change in biosafety containment practices, a new biosafety risk assessment must be completed and the proposed changes and risk assessment should be reviewed by the IBC.

Reference

In 2012, EH&E conducted a survey of academic, biotechnology and healthcare institutions to gain insight into how institutions managed implementation of BSL-3 practices and procedures in the BSL-2 environment and the drivers for using this approach. The survey results were published in an EH&E white paper originally authored by Elizabeth Gilman Duane, MS, RBP, CBSP. That paper was the basis of a published article in Applied Biosafety (cited below), which is the original source of information for this guide. In 2023, this guide was updated by Jessica Healey, MS, RBP, CBSP, and Elizabeth Gilman Duane, MS, RBP, CBSP.

Gilman Duane E. 2013. A Practical Guide to Implementing a BSL-2+ Biosafety Program in a Research Laboratory. Applied Biosafety: Journal of the American Biological Safety Association, 18(1):30-36.

About Environmental Health & Engineering, Inc.

EH&E's experienced biosafety consultants and microbiology experts are adept at developing biosafety programs and training that minimizes the risks of exposure to hazards across the life science, healthcare, academic, and property development and management environments. Through an approach that combines our expertise in investigation, hazard analysis, building engineering, and program support, we help organizations achieve safe environments and full compliance with biosafety guidelines and applicable local, state and federal regulations.

- **Biological Materials Risk Assessment**
- **Biosafety Program Development & Mentoring**
- **Biosafety Program Gap Assessment**
- **Biosafety Support & Staffing**
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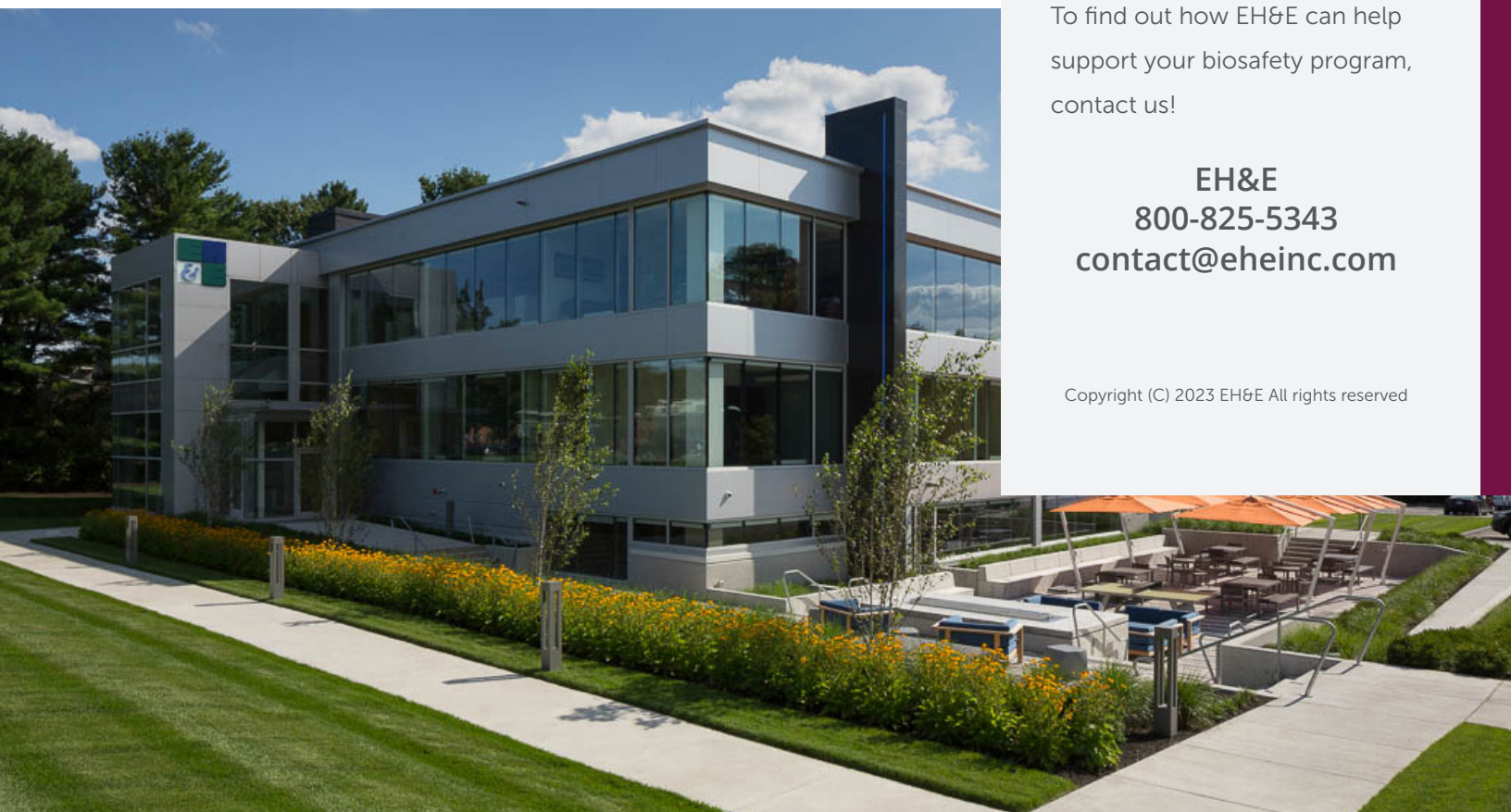
- **Institutional Biosafety Committee and Safety Committee - Development and Support**
- **Permitting & Regulatory Compliance**
- **Training**



To find out how EH&E can help support your biosafety program, contact us!

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