A Practical Guide to Implementing a BSL-2+ Biosafety Program in a Research Laboratory

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Abstract

Biosafety Level Two Plus (BSL-2+) is a term frequently used to describe laboratories where work with microorganisms is conducted in a BSL-2 laboratory with biosafety practices and procedures typically found at BSL-3. The risk assessment process may determine that safety practices over and above those required at BSL-2 are needed for a research project, yet a more complex BSL-3 laboratory facility is not necessary.

This hybrid approach has been used for many years; however, many research institutions still find it challenging to decide when to use this approach and which BSL-3 practices to use. This is due to the fact that it is not a recognized containment level in biosafety guidance documents such as the Centers for Disease Control and Prevention's (CDC) Biosafety in Microbiological and Biomedical Laboratories *(*BMBL*) (U.S. Department of Health and Human Services, 2009) or the National Institutes of Health's (NIH)* Guidelines for Recombinant DNA Research *(the* Guidelines*) (NIH, 2011).*

This article aims to assist environmental health and safety and biosafety professionals by detailing a practical approach to implementing BSL-2+, including how to identify what projects may benefit from BSL-3 practices and how to modify BSL-3 practices and selected facility requirements for the BSL-2 environment based on institutional needs. In addition, strategies to ensure researchers are properly trained in the use of BSL-3 practices in a BSL-2 laboratory and for developing a project-specific Standard Operating Procedure (SOP) are detailed. Implementing BSL-2+ can be successful when there is a collaboration among the Principal Investigator, Biosafety Officer, Institutional Biosafety Committee, and laboratory personnel.

Introduction

In 2012, Environmental Health & Engineering, Inc. (EH&E, Needham, MA) conducted a survey of academic, biotechnology, and healthcare institutions to gain an understanding of how institutions are managing implementation of BSL-3 practices and procedures in BSL-2 labs, the deciding factors that prompt them to implement this approach, and how they handle Standard Operating Procedures (SOPs) and training. Based on the survey, the following three areas were rated most challenging:

1. Determining what work or projects require BSL-3 practices.
2. Ensuring that researchers are properly trained in the use of BSL-3 practices in a BSL-2 laboratory.
3. Ensuring that the Principal Investigator (or his/her designee) develops a project-specific SOP.

What is BSL-2+?

BSL-2+ is not a recognized containment level in biosafety guidance documents such as the Centers for Disease Control and Prevention's (CDC) *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*) (U.S. Department of Health and Human Services, 2011) or the National Institutes of Health's (NIH) *Guidelines for Research Involving Recombinant DNA Molecules* (NIH, 2011), although other biosafety guidance documents such as the NIH's *Biosafety Considerations for Research with Lentiviral Vectors* refer to "enhanced BL2 containment" (NIH, 2006).

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. However, BSL-2+ may not always be appropriate for pathogens that are infectious via the inhalation route. For example, *Burkholderia mallei* is infectious via an inhalation route, and the *BMBL* states that procedures with *Burkholderia mallei* must be performed under BSL-3 containment whenever infectious aerosols or droplets are generated, such as during centrifugation or handling infected animals, or when large quantities of the agent are produced. However, for primary isolations of the agent from patient fluids or tissues, these tasks may be performed with BSL-2 practices, containment equipment, and facilities in a biosafety cabinet (BSC) (U.S. Department of Health and Human Services, 2009).

The critical first step to implementing BSL-2+ is the risk assessment process. The *BMBL* details the steps involved in the risk assessment process and states that after determining the appropriate biosafety level, additional precautions must be selected as indicated by the risk assessment (U.S. Department of Health and Human Services, 2009).

Risk Assessment Identifies Biosafety Level and Practices

No standardized list of microorganisms, viral vectors, or research projects that should be conducted at BSL-2+ exists. Each decision to use BSL-3 practices in a BSL-2 laboratory must be made via the risk assessment process. The risk assessment guides 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* (U.S. Department of Health and Human Services, 2009), the steps of the risk assessment process include:

1. Identify agent hazards and perform an initial assessment of risk.
2. Identify laboratory procedure hazards.
3. Determine the appropriate biosafety level and select additional precautions as indicated by the risk assessment.
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional, subject matter expert, and the Institutional Biosafety Committee (IBC). IBC review is not required by the NIH unless the work involves recombinant DNA. Some IBCs may choose to review non-recombinant work. Some institutions that do not have recombinant work and therefore do not have an IBC have chosen to establish a Biosafety Committee. The risk assessment process must be applied to every new or revised research project.

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

- Viral vectors with gene inserts consisting of oncogenes or genes of unknown function that may have the potential to disrupt cellular processes.
- Drug-resistant Risk Group Two (RG2) bacteria such as methicillin-resistant Staphylococcus aureus (MRSA).
- Low titer and small volumes of Human Immunodeficiency Virus (HIV), a Risk Group Three (RG3) agent.
- High concentrations of RG2 viruses. Note that the values assigned to "high concentrations" must be established through the risk assessment process and may be tied to the infectious dose and the characteristics of the agent.
- Work with greater than 10 liters of an RG2 agent.

Project Review Process

The project review process is part of the risk assessment and a project registration document details the risk assessment. The project review process includes several steps that take place before the work can be approved and commence:

- *Completion and submission of a project registration document* by the Principal Investigator (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.
- *Review and discussion of the project registration document* with the 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 works with recombinant DNA and receives federal funding and/or is located in a community with a recombinant DNA ordinance (NIH, 2011). 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.
- 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, then the use of 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 determines the appropriate biosafety level and selection of additional precautions indicated by the risk assessment.
- *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 PI to attend the IBC meeting to provide additional information on the proposed project and to answer questions. 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. Again, depending on the institution and whether recombinant DNA is used, there may or may not be an IBC. In lieu of an IBC, a Biosafety Committee could be established.
- *Risk communication and training* must be conducted after IBC approval and before any work is performed in the laboratory. The BSO reviews the required BSL-3 procedures with the PI and his/her laboratory staff; ideally these procedures are written in the form of a Standard Operating Procedure (SOP). SOP training is also documented to establish that personnel were trained. Additionally, it is important to review the laboratory space through a lab audit to ensure the required BSL-2 elements are in place. These include, but are not limited to, biological waste containers, sink with soap and paper towels, and certified biosafety cabinets (BSC).

Selection of Laboratory Space for BSL-2+

BSL-2 laboratories are common in most academic and industrial research facilities. Often they are 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. Therefore, a separate BSL-2 laboratory space may need to be dedicated to the project that requires BSL-3 practices. Practically speaking, this usually means dedicating a smaller BSL-2 or "tissue culture" laboratory room 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. In some cases, new construction or renovation of a laboratory facility affords the opportunity to select certain BSL-3 facility features that may add value to the BSL-2+ lab, such as hands-free sinks.

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 working in a BSC and 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* (U.S. Department of Health and Human Services, 2009). Each risk assessment and project review should include a review of these practices and equipment requirements to determine the items that enhance worker and environmental protection. Each item may be subject to discussion and may be modified, providing the IBC members concur.

Examples of Modifications to BSL-3 Practices

One example is waste management procedures (Table 1; A8). While decontamination within the immediate laboratory is preferable, removing materials from the facility for decontamination is also an option. 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 offsite arrangement is in place that is consistent with local or state requirements; and 2) the risk assessment determines that no additional risk to personnel or the environment exists if materials are not autoclaved.

Another example is that BSL-3 practices specify that a laboratory-specific biosafety manual must be prepared and adopted as policy (Table 1; B4), and that 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. The 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, housing a -80ºC freezer in the laboratory room where BSL-3 practices are utilized may not be practical. 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 secondary container to the freezer.

Additional Considerations for Implementing BSL-2+

In many cases, other considerations should be reviewed in addition to the BSL-3 practices listed in the *BMBL*. These include simultaneous use of the BSL-2+ laboratory for multiple projects, each with 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, the IBC makes this decision, but best practice would likely dictate that the work could take place provided the worker follows 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 seem 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.
- Consider whether laboratory personnel may bring laboratory notebooks and portable electronic devices in and out of the BSL-2+ laboratory. Best practice is to prohibit this to avoid bringing contamination out of the laboratory but also to provide ways for information to be transmitted to the office area via a fax machine or a computer that is dedicated to the BSL-2+ laboratory. However, in lieu of a fax machine or computer, an alternative may be acceptable such as developing a SOP that details how paper records can safely be removed from the laboratory.
- If the BSL-2 laboratory will be built or renovated for a project utilizing BSL-3 practices, consider incorporating some BSL-3 laboratory facility requirements. 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 risk assessment requires autoclaving of all wastes, a pass-through autoclave might be part of the laboratory.
- 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 about which ABSL-3 practices to incorporate. For example, sharps precautions may be required. Since some procedures may require the use of a needle and syringe, the review of safer sharps devices must be included in the risk assessment process. Sharps precautions are then included in the written ABSL-2 SOP. The use of appropriate animal restraint devices coupled with training on the safe use of sharps is incorporated into the SOP training.

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 must enforce the institutional policies that control access to the laboratory.	Limit access to those listed in the approved project registration and who have received additional training. Card access may be useful.
A2.	Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.	A sink for hand-washing with paper towels must be available. A hands-free faucet may be useful.
A5.	Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should 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.
A6.	Perform all procedures to minimize the creation of splashes and/or aerosols.	All work is performed in a BSC.
A8.	Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport: a) Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport; b) Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state and federal regulations.	Determine whether materials must be autoclaved prior to removal from the facility. If this is deemed optional, consideration may be given to offsite decontamination (e.g., incineration via a vendor).
A9.	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 name (or other responsible personnel), telephone number, 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.
A11.	The PI or their designee (e.g., laboratory supervisor) must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations, or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.	The PI is ultimately responsible for the safety of his/her personnel. In conjunction with the BSO, appropriate training must be provided. Occupational health providers should be consulted by lab personnel should any questions arise about the health impact of materials with which personnel will work.
B.	**BSL-3 Special Practices**	**Modified for a BSL-2 Laboratory Facility**
B1.	All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.	Lab-specific training emphasizing the specific BSL-3 practices is provided by the BSO to all lab personnel.
B2.	Laboratory personnel must be provided medical surveillance and offered appropriate 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.	Each institution should consider the need for collection and storage of serum samples from at-risk personnel.	Lab personnel participate if serum storage program is required per direction of IBC and occupational health physician.

Table 1 (continued)

Selected BSL-3 Requirements from the *BMBL* and Potential Implementation in a BSL-2 Laboratory.

B.	BSL-3 Special Practices	Modified for a BSL-2 Laboratory Facility
B4.	A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.	In addition to the institution's biosafety manual, a specific SOP is developed by the PI to detail appropriate BSL-3 practices for the project.
B5.	The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.	The PI must provide training to lab personnel who may not have experience working with the materials to be used with BSL-3 practices. For example, 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.
B7.	Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination. a) Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material. b) Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.	Create a "spill kit" and store 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.
B8.	Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records 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.
B10.	All procedures involving the manipulation of infectious material must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.	All work is performed in a BSC, including loading/unloading centrifuge rotors/cups.
C.	**BSL-3 Safety Equipment**	**Modifications for a BSL-2 Laboratory Facility**
C2.	Workers in the laboratory wear protective laboratory 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.
C3.	Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.	Safety glasses with side-shields should be worn while in the lab.
C4.	Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers: a) Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate. b) Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory. c) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.	Each worker should store a supply of disposable gloves inside the lab in appropriate size/style. Consider double-gloving if dexterity is not compromised.

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, the risk assessment is key to determining whether BSL-3 practices are appropriate in a BSL-2 laboratory and what practices are required. The collaboration between the PI, BSO, IBC, or Biosafety Committee and laboratory personnel is crucial to the successful outcome.

Case Studies: Implementing BSL-2+

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

Case Study: Work with Lentivirus

A PI proposed the use of lentiviral vectors to transduce human cells in culture. The gene inserts 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 to be conducted in the BSL-2+ laboratory and included steps to take place in the general BSL-2 lab (e.g., transport of samples in a secondary container to the -80ºC 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 staffs are required to be escorted by the PI, BSO, or project personnel to enter the laboratory. Phone numbers for the PI and laboratory manager are posted in the event of an off-hours emergency.
- BSL-2 work may take place in the BSL-2+ laboratory with the stipulation that the work is performed using the same BSL-3 practices required for the lentivirus project.
- The PI develops an SOP detailing the work practices and procedures as required by the IBC, using a template provided by the BSO. The SOP is reviewed by the BSO and is used for additional training of project personnel.
- Signage for the laboratory door with the universal biohazard symbol includes PI name, list of approved/trained project personnel and 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 are 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 must complete annual bloodborne pathogen training and are offered Hepatitis B vaccination in accordance with the Occupational Safety and Health Administration's (OSHA) Bloodborne Pathogen Standard. Additionally, project personnel are provided with a review of the institution's procedure for immediate reporting of all occupational injuries and illnesses. In addition to the two BSCs already in the BSL-2+ laboratory, a tabletop centrifuge, a microscope, two incubators, and a laptop computer are purchased and designated for the laboratory. The laptop is used to transmit notes outside of the laboratory, since in this case the SOP stated that no papers or notebooks can be taken in and out of the laboratory.
- 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 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 stock a box of gloves in 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. The PI informs all project personnel that any future use of sharps must be reviewed and approved in advance by the PI and the BSO. 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 an autoclave used for media preparation is located in another place in the building, 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 (Thermo Fisher Scientific, New York, NY) container with a lid. When two-thirds full, the bag is removed by the researcher, tied at the top with a rubberband, 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 the BSL-2+ laboratory for the vendor to remove from

the facility and transport for offsite 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 secondary containers can be taken out of the laboratory and moved to the main BSL-2 laboratory for storage in the -80ºC freezer. In addition, fixed cells may also be removed in a secondary container from the laboratory for cell sorting.
- A laboratory member serves as the "BSL-2+ manager" and oversees 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: A Project with *Neisseria meningitidis*, serogroup B strain

A PI proposed to work with *Neisseria meningitidis*, serogroup B strain, a Risk Group Two (RG2) agent. To do this work safely, enhancements over and above the standard BSL-2 practices are required because the infectious dose is unknown and the bacteria can be infectious via injection, ingestion, and droplet exposure to mucous membranes. Additionally, there is no vaccine for the serogroup B strain. 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.

- The laboratory has keycard access and only those individuals listed in the project registration, the BSO, and some members of the EH&S department have access. The researchers received additional training in techniques and biosafety. Janitorial and maintenance staffs are required to be escorted by the PI, BSO, or project personnel when entering the laboratory. All "guests" log their name, date of entry, and purpose of entry into a logbook located on the laboratory door. Phone numbers for the PI and laboratory manager are posted in the event of an off-hours emergency.
- BSL-2 work is not allowed in the BSL-2+ laboratory due to the nature of the work and the fact that the lab contains only one BSC.
- The PI develops the SOP that is used for additional training of project personnel.
- Signage for the laboratory door with the universal biohazard symbol includes PI name, list of approved/trained project personnel and others who may have approved access (BSO, EH&S personnel), materials in use (*Neisseria meningitidis*, serogroup B), 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.
- The institution's occupational health physician reviews the project and the antibiotic susceptibility pattern for the agent, in the event that an exposure occurs. The physician meets with the PI and laboratory personnel and reviews the signs and symptoms of infection with *Neisseria meningitidis* and emphasizes the importance of reporting any illness immediately.
- In addition to the BSC that was already in the BSL-2+ laboratory, a tabletop centrifuge, an incubator, and a fax machine are purchased and designated for the laboratory. The fax machine is used to transmit laboratory notes to office areas.
- The PPE consists of a disposable solid-front gown with cuffed sleeves, safety glasses with side-shields, and nitrile gloves.
- Plastic is substituted for glass. Pasteur pipettes and needles are prohibited.
- All work is conducted in the BSC, including loading and unloading of centrifuge safety cups.
- A ready-mix bleach product and 70% ethanol are available and utilized to disinfect surfaces and equipment in the laboratory.
- 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.
- An autoclave is located near the lab. All solid biohazardous waste is autoclaved by the researchers in a validated autoclave. The waste is transported in a covered container to the autoclave, then autoclaved and placed in a vendor-supplied, large, cardboard waste box lined with two red bags for vendor removal and incineration.
- Materials in secondary containers can be taken out of the laboratory and moved to the main BSL-2 laboratory for storage in the -80ºC freezer.

Acknowledgment

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