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BIOSAFETY
RESOURCE GUIDE

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Questions to Ask When Buying a Biological Safety Cabinet

Biological safety cabinets (BSCs) are designed to protect the operator and environment from the contaminants contained within, as well as to protect the contents from potential contamination. There are different classes of cabinets, ranging from Class I cabinets that provide basic protection, to Class III cabinets that offer protection from the most lethal biohazards.

Is the primary function of the BSC sterility, biosafety, or both?

Class I BSCs offer operator protection, but no product protection as laboratory air is drawn in and comes in contact with samples and the workspace. Alternatively, Class II BSCs use inflow to provide operator protection and filtered downflow to maintain sterility within the cabinet. A Class III cabinet is entirely enclosed, with multiple features to ensure operator safety and sterility within the cabinet.

What chemicals and reagents will be used inside the BSC?

BSCs are equipped with HEPA (high efficiency particulate air) or ULPA (ultra low particulate air) filters designed to filter particulates only, they do not trap gases and vapors. Class II Type A1 and A2 BSCs recirculate air into the laboratory, and if hazardous vapors are present in the BSC, they pass through the exhaust filter and into laboratory air. Therefore, it is important to ensure no chemical vapors will be used inside these BSCs.

How much laboratory space is available?

When comparing BSCs, it is important to consider how much laboratory space is available as well as how much space will be required to contain the equipment and work that is to be carried out inside the BSC.

Is the BSC ergonomically designed to ensure good working posture?

Prolonged periods spent in poor working postures contributes to a greater incidence of neck, back, shoulder, and wrist injuries. Features such as footrests, armrests, easy to reach fixtures and controls, and the ability to adjust the height of the worksurface ensure operators maintain good working posture and prevent injury.

Maintenance Tip

When work is completed, all items in the BSC including equipment should be decontaminated and removed to prevent microbial growth. The interior walls and glass should be wiped daily to destroy any organisms found inside the cabinet using a solution of 70% alcohol.

Working with Biohazards

Fundamentals of a comprehensive exposure control plan

By Vince McLeod

Working with human pathogens or biohazards poses serious risks, not only for employees, but for the public and communities as well. Infectious agents such as microorganisms, viruses, recombinant or synthetic nucleic acid molecules, and biological toxins present a potential for severe or lethal disease, adverse health effects, or contamination. Any unplanned exposure or release has the potential to cause extensive harm or damage to people, the environment, and society.

The foundation for safe handling and research with infectious/biohazardous agents is an effective exposure control plan (ECP). This article discusses the basic elements of a comprehensive exposure control plan, what each element should address, and advice for successful implementation.

The ECP is essentially a biohazard safety manual developed to address the unique conditions of the current research, facility design, and personnel operations necessary to carry out the laboratory's mission. One excellent free reference is the CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL), which contains comprehensive information on biological risk assessment and summary statements on many common infectious agents.

An excellent ECP is comprehensive, clearly written, and well organized. A good companion to the BMBL is OSHA's model ECP contained in Appendix D of 29CFR1910.1030. However, effectiveness is ensured only when all persons who must enter or work in the containment areas are trained on and understand the key elements.

ECP Critical Elements

Your exposure control plan should contain main sections that address ECP administration, employee exposure determination, implementation and control methods, and health and medical monitoring requirements, including appropriate pre-exposure prophylaxis, emergency procedures, and postexposure evaluation, employee training, and record keeping. Below we describe what each of these sections should address.

ECP Administration

The opening section should provide a clear organization of personnel and assign responsibilities for implementation and support for the facility. The responsibilities of positions and/or departments are outlined for maintaining, reviewing, and updating the ECP. In addition, responsibilities for maintaining and providing necessary personal protective equipment (PPE), engineering controls, and other infrastructure and equipment are contained here. Finally, responsibilities for medical actions, employee training, incident follow-up, and record keeping should also be listed.

Employee Exposure Determination

All employees who are determined to have potential occupational exposure, and thus need to comply with the ECP, are defined in this section. Provide a list of all job classifications at the facility that have potential for exposures. Conduct job hazard analyses and exposure assessments where needed and as necessary.

Implementation and Control Methods

This section contains all the specific procedures for working safely. Everything from universal precautions to engineering controls to PPE is detailed and described. Specific laboratory

layout and operations are also explained in this section. Controlling access is extremely important, and access should be restricted to only certified persons who are absolutely necessary. Certified means they understand the potential biohazard, have demonstrated proficiency in the laboratory's procedures, and comply with the health and medical entry requirements.

Proper entry and exiting procedures for staff, visitors, and maintenance/custodial workers are clearly established in this section. Included are security access mechanisms, such as self-closing, lockable doors, and other security measures.

Proper signage indicating agents present, contact information for the principal investigator and other responsible persons, and any special requirements are posted at all access points.

Engineering controls, such as interlocks and positive pressure airflow, and the means for checking they are properly functioning are spelled out in detail. The handling and disposal of sharps and other biohazardous waste are addressed. Ensure proper labeling is clearly described, including use of warning labels and red bags.

PPE is one of the most important parts of the exposure control plan and discussed thoroughly in this section. The personal protective equipment that must be worn is listed for each position. Describe where PPE is stored as well as when and where it is used and how it is removed and discarded. This section should cover the proper types of gloves, eyewear, and gowns or lab coats to be used.

This section also addresses proper use and maintenance of the lab's safety equipment, such as autoclaves, biosafety cabinets, eyewash stations, safety showers, ventilation alarms, and other specially designed containment equipment. Procedures for decontaminating equipment prior to maintenance work should be included.

The implementation and control section should address safe handling and storing of viable material, including biological safety cabinet use, handling frozen samples, and use of secondary containers. Procedures for housekeeping (e.g., cleaning up at the end of the day or after finishing a research protocol) are discussed, along with special instructions for laundry.

Health and Medical Monitoring

The purpose of this section is to provide another level of protection against laboratory-acquired illness, and it documents necessary immunizations. Immune-suppressed individuals or persons at increased risk should be strongly discouraged from entering the facility. Depending on the agents present, vaccinations (hepatitis B), antibody testing (TB skin test), or serum storage may be required. The ECP should clearly define what is required and who is covered, with well-documented rationale.

Emergency Procedures

This section describes procedures for an accident, exposure incident, or spill or release that injures laboratory staff or contaminates the environment. A good reference for putting this section together is OSHA's bloodborne pathogens standard, 29CFR1910.1030. Follow initial first aid procedures and document the routes of exposure and how it occurred. Ensure spill kits are available and biohazard spills decontaminated and cleaned up as soon as possible by properly trained and equipped staff. Any incident should be completely documented with a written report, and a postexposure evaluation and follow-up should be performed.

Employee Training and Incident Reporting

The final section of a comprehensive exposure control plan covers employee training and record keeping. First, ensure everyone who will be working in the containment facility has been trained on and understands the ECP. Inform employees about each infectious agent present, the risks associated with these agents, and the signs and symptoms of infection or disease. Make sure procedures for identifying, reporting, and correcting exposures, incidents, near misses, or violations of protocol are covered in detail. Finally, the training should be renewed annually, and written documentation should be kept on file.

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Biological Safety Cabinets and Personnel Must Work Together

When working with a biological safety cabinet (BSC), safety comes first. Nonetheless, it's easy to make mistakes that can compromise a BSC's performance

by Mike May

One of the most common mistakes users make when working in the cabinet is to cover the front grill where the air enters. This disrupts the airflow, and compromises both the safety of personnel and product protection. It might seem convenient to set something—like a rack of test tubes—over the grill, but it's definitely not safe.

It is also important to be aware of the movements made by personnel in and around the BSC. For example, one should move their arms in and out of the cabinet slowly, as arm movements will disrupt unidirectional laminar airflow within the work zone and the barrier at the front of the cabinet. That starts a chain reaction of problems. This disruption could cause air turbulence, increasing the odds of product cross contamination within the work zone. In general, keeping down the turbulence inside a BSC improves safety inside and out, largely by preventing the unwanted spread of aerosols or liquid splatter.

Also, be sure to take a look inside your BSC to see whether you are making any mistakes there. For instance, users often clutter the inside of the cabinet with items that aren't needed. Even worse, Bunsen burners placed inside generate large amounts of heat that disrupt airflow and can lead to explosions.

To use a BSC safely, consider how it works. Once users understand the airflows and how fragile the filters are, it is easy to see what not to do.

Prepare a Plan

To ensure BSC safety, labs need a plan that encompasses every aspect of moving in and out of the BSC.

The plan must include how users will handle items being used inside the BSC. For example, one should never open pipettes outside the cabinet before using them inside the cabinet, as this introduces contamination.

Users should also always wear gloves. Many believe that since the cabinet is clean, gloves are unnecessary but this is not true. Gloves should be worn, and they should be sterile (which can be achieved by spraying them with 70 percent ethanol or bleach). This will further prevent contamination.

In addition to cleaning gloves, the BSC's surface should also be cleaned. It is important to use an appropriate cleaning solution or 70 percent ethanol, and to let surfaces soak for one to five minutes. Wipe the BSC dry but allow the cabinet to run for a few more minutes to resettle the airflow.

The plan should also include what to do if something goes wrong. Imagine that a BSC indicator shows that a filter is no longer working properly. What do you do? Under these circumstances, work must should cease, the window sash should be closed, power turned off, and a note should be placed on the cabinet stating that it is out of service. Then call the appropriate service provider for further assistance.

Up to Speed

In general, keeping a BSC running keeps it safer but can consume more energy. Some BSCs come with a night mode, though, in which the viewing screen can be closed, the lights are shut off, and the airflow is reduced.

The longer a BSC runs the more impact on the filter, which gets loaded over time. Some BSCs have a magnehelic gauge that gives a general idea of how much filter loading has taken place. As a filter loads with particulates, some BSCs automatically adjust the fan speed as needed to ensure adequate airflow for safety.

Knowing when a BSC last received service also impacts its safety. There must be clear communication among users regarding maintenance. Only then can all users be certain that they are working with a safe device.

Nonetheless, even different organizations recommend different maintenance cycles. For example, NSF recommends cabinets be certified annually, while USP 797 recommends certification twice per year. In a certification, the cabinet will be adjusted to maximize safety through airflows, and a certifier can also recommend when it might be time to replace a BSC, or whether new parts are required.

If a BSC does need repairs, it could go beyond adjusting the airflow or replacing filters. Some BSCs also include audible and visual alarms. These need to be kept in working order too.

In some cases, the result of a certification or repair will be that a BSC cannot meet the necessary airflows. This is bad news, but it must be heard to keep a lab and its personnel safe. If a cabinet is unable to meet specified downflow and inflow air, it often means it is time for new supply and exhaust HEPA filters.

At some point, staying safe means replacing an old BSC with a new one. In a lab that keeps the personnel properly trained and the BSCs properly certified, though, everyone can be safe for a long time.

The Right Biological Safety Cabinet for Your Job

When selecting a new biological safety cabinet (BSC), many factors should be considered

by Mike May

When selecting a new biological safety cabinet (BSC), many factors should be considered, and it all starts with picking the right class—broadly speaking, I, II, or III. The right choice arises from a risk assessment, which depends largely on what is used in your experiments.

The first question should be, what kind of protection do you need: product, personnel, or both?

Next, the American Biological Safety Association's Risk Group Database may be used to determine the biosafety level (BSL) of the agent(s) you will be working with—1, 2, 3, or 4—according to various organizations, including the U.S. National Institutes of Health and the European Union.

It may also be valuable for labs to work with a biosafety professional to help identify the lab's long-term needs and review purchasing options. This consultation can help save money and time.

BSC Basics

The least risky BSL is 1. For scientists working with low-risk agents—like Escherichia coli K12—the BSC protects the sample more than the scientist, really. Here, a class I or II BSC works fine.

Scientists working on human diseases, such as Staphylococcus aureus or E. coli O157:H7, must meet BSL-2 containment. Here, a BSC must be used, but a class I or II could suffice.

At BSL-3, the agents, such as Mycobacterium tuberculosis, pose a danger through inhalation. In addition to a variety of increasingly complex criteria, the CDC recommends a class II or III BSC for this work.

The most dangerous agents can be worked on only in a BSL-4 facility, and there are only a few dozen in the world. These labs work on the worst of the worst, like Ebola and Marburg viruses. At BSL-4, an operator must either wear a one-piece positive pressure suit and work in a class II BSC or use a class III BSC with a lower level of personal protective equipment.

Keep in mind, too, that it's not just the biological agents that matter. Working with hazardous chemicals with a biological load will have an impact on your selection.

This overview provides some general guidelines, but this is an area in which expertise should be sought before deciding. Some labs also need a flexible option— something that can be adapted to an evolving space.

Diving into the Details

While there are three classes of BSCs, the vast majority are class II. Class I BSCs only protect personnel and the environment, not the product, and class III BSCs can be challenging to work in and are only used with the most hazardous agents.

For most lab managers, the most likely question will be which type of class II BSC is needed? Class II BSCs are available in five types: A1, A2, B1, B2, and C1. Where volatile toxic chemicals are not used, an A1 or A2 with air filtration and venting to the lab will often suffice. When using volatile toxic chemicals and biological agents, a class II BSC connected to an external exhaust, such as a canopy- or thimble-connected A2, B1, B2, or C1 is required. Often, a thimble or canopy-connected A2 is the popular choice.

Consider the Construction

Even with a given BSC class, there's much more to consider to ensure the required level of safety, and a key factor is how the product is constructed.

When assessing how a BSC is made, begin with the material. Is it constructed from cold-rolled or stainless steel? You'll need to balance risk, investment, and equipment life. Then, buy a BSC constructed of the material that keeps your scientists as safe as possible, but doesn't unnecessarily break the bank.

How long a BSC keeps your team safe, though, also depends on what holds the pieces together. Some manufacturers use rivets and sealants, and others use welds.

Overall, a properly constructed cabinet made from stainless-steel pieces welded together to create a monolithic core will provide years of safety, and users will not have to worry about gaskets and sealants degrading over time.

Know the Flow

Any BSC's safety depends on it doing what it's supposed to do, such as providing the specified flow. Usually, the flow is monitored inside the plenum, which measures the static pressure, or with an airflow probe. Airflow probes offer the added benefit of using the same technology certifiers use to certify cabinets. The static measurement works fine to ensure that a BSC is at the proper pressure, but will not provide any information about the actual airflow.

Whether monitoring the pressure or the airflow, a BSC should let you know if something goes wrong. An alarm should indicate when the system is not working safely or if the sash is at a level that makes the system useless.

To select the right BSC for your task, you'll need to think about how you'll use it, study the requirements for the agents that you have in mind, and make sure to get the features that ensure the performance you need.

Best Practice Tips for Biological Safety Cabinets

Keeping your cells healthy and happy

by Angelo DePalma

In creating a life-sustaining "bubble" within a hostile laboratory environment, biological safety cabinets (BSCs) protect workers, the environment, and processes involving cells or biological tissues.

The first best-practice tip, directed mostly at chemists and analytical scientists, is that BSCs are not fume hoods. Air flow into hoods is more robust and does not protect products or processes from ambient air and its many contaminants. Fume hoods also vent to the outside, whereas BSCs may vent or recycle laboratory air through a HEPA filter, returning the scrubbed air to the laboratory. Knowing the type of protection required for product, process, and worker is the key to selecting the right BSC.

You're not at the bench

If asked, most users would recognize that the spaces inside BSCs are potentially hazardous, but complacency easily replaces caution.

Users need to be aware that the principal safety feature on all BSCs is inward air flow. Unlike fume hoods, which only draw air through or away from the operator's breathing zone, BSCs must also balance the HEPA filtered downflow air. The balance may be affected by user location and movement, as well as the placement of the BSC within the room.

Work planning should include each step of a workflow or experiment, including how to handle waste and avoid cross contamination. It is recommended to work from "clean to dirty" in a direction depending on the operator's handedness, and avoid moving materials and equipment in the opposite direction.

Avoiding airborne contaminants

BSCs work by purifying non-sterile ambient laboratory air through HEPA filtration. Purified air is then blown downward, toward the product or process on the BSC's work surface. Since the product or process may have contaminated this air, it passes through a second HEPA filter before being exhausted to the outside or back into the lab. So, as a general tip, labs need to maintain those filters and assure that air flow remains within specifications.

The following is recommended to avoid most sources of contamination:

- Disinfect work surfaces before and after working to minimize or eliminate surface contamination
- Disinfect or sterilize materials brought into the hood
- Wear personal protective equipment to prevent the introduction of contaminants from the operator's skin
- Practice aseptic technique
- Do not block the air intake grills in the front or back of the work surface
- Do not store materials in the BSC as they may disrupt airflow
- Disinfect gloves after touching a non-sterile surface outside the BSC
- Do not place BSCs in high traffic areas or near doors
- Avoid using volatile chemicals in the BSC

Only as safe as the user

As with any laboratory operation, safety is the primary concern when operating a BSC. Regardless of the nature of the work performed in the BSC, it is only as safe as the operator performing the work. It is important to perform routine maintenance and certification, and maintain a clean, disinfected cabinet. To clean a spill, cover with a disinfectant towel to prevent aerosolization and use sterile water to rinse the area to prevent corrosion inside the cabinet. Waste from spills should be put in a biohazard bag inside the cabinet.

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Biohazard Management

Level 1, 2, 3, or 4? Know the risks and procedures

by Vince McLeod

The explosion of biotech companies and research into fighting cancer and other diseases, combined with the ever-present threat of biological weapons of mass destruction have propelled concern and discussion of biohazards into prominence. Given the complexities and potential for harm, it is paramount that we promote understanding and sensible approaches to managing biohazards.

Definition, levels, and examples

A biological hazard, biohazard for short, is a biological material that poses potential harm to the health of other living organisms. Most importantly, we are concerned with those that pose threat to humans. However, biohazards may also potentially harm animals, aquatic life, and plants.

Biohazard material could be bacterial, viral, or toxic. The United States Centers for Disease Control and Prevention (CDC) ranks biohazards into four levels, with Level 1 being minimum risk and Level 4 being extreme risk. Here are brief definitions of the levels with examples of biohazards ranked for each:

- **Biohazard Level 1:** Well-characterized bacteria and viruses not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Examples include *Escherichia coli* varicella (chicken pox), and feline leukemia virus.
- **Biohazard Level 2:** Similar to Level 1 agents, but known to cause mild disease in humans or are difficult to contract. Examples include measles, mumps, salmonella, influenza A strains.
- **Biohazard Level 3:** Indigenous or exotic bacteria and viruses that can cause serious or fatal disease in humans, but for which vaccines or other treatments exist. Examples include anthrax, tuberculosis, yellow fever, and malaria.
- **Biohazard Level 4:** Biological agents that are likely to cause serious or fatal human disease for which vaccines, preventive, or therapeutic interventions are not available. Examples include Ebola virus, Marburg virus, Lassa fever virus, and other hemorrhagic diseases.

Regulatory Compliance

The list of select biological agents includes approximately 40 viruses, bacteria, rickettsia, fungi, and toxins. In the United States, any work with or transfer of these agents is controlled due to their capacity to cause considerable harm to human health. The Federal Select Agent Program developed by the Department of Health and Human Services (HHS) along with the CDC regulates all work with and transfer of select agent material, be it for agriculture, animals or animal products, or public health.

The public health regulations contain five main components:

- The list of biological "select agents"
- Procedures for registration of facilities that transfer or use these agents. Any organization that transfers or obtains these agents must register with HHS and provide sufficient information that the facility meets all the biosafety level requirements for working with the particular agent or agents

- Process for documenting successful transfer of agents requiring both shipping and receiving parties to complete written record forms
- Audit, quality control, and accountability verification procedures
- Procedures for appropriate disposal of select agents

Other parts of the regulations address exemptions, designation of a facility responsible official, restricted access and security, training, and notification of any theft, loss, or release of material.

Exposure prevention—the primary goal

Efficient and proper management of biohazards begins with preventing exposures. If we are handling or working with biological agents with known human etiology, and especially any agents in biohazard class 2, 3, or 4, we should have the necessary infrastructure in place and a comprehensive written exposure control plan in effect.

The exposure control plan (ECP) is written to address the unique conditions of the current operations, research, facility design, and personnel actions necessary to carry out the facility's mission. One exceptional reference for developing your ECP is the CDC's *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), which contains ample information on biological risk assessment. A good companion to the BMBL is OSHA's model ECP contained in Appendix D of 29 CFR 1910.1030. A good ECP should address the following key elements:

- **Administration, responsibilities, and accountability:** Provides a clear organization of personnel and assigns responsibilities for implementation and support, and identifies positions and/or departments responsible for reviewing and updating the ECP, maintaining and providing necessary personal protective equipment, engineering controls, and other infrastructure and equipment
- **Determination of employee exposure:** Identifies all employees that have potential occupational exposure and conducts job hazard analyses and exposure assessments
- **Exposure control methods:** Details specific procedures for safe work including engineering controls, PPE, universal precautions, signage, access, and security
- **Health and medical monitoring:** Protects against laboratory-acquired illness by documenting and certifying the necessary immunizations and vaccinations are attained
- **Emergency procedures:** Describes procedures for reporting and investigating any accident, exposure incident, spill, or release that injures laboratory staff or contaminates the environment
- **Employee training:** Ensures that everyone working in the containment facility has been trained on and understands the ECP. Informs employees about each infectious agent present, the associated risks, and the signs and symptoms of infection or disease
- **Reporting and recordkeeping:** Ensures procedures for identifying, reporting, and correcting exposures, incidents, near misses, or violations of protocol are recorded, investigated, and corrected

Biohazardous wastes

A final major element of managing biohazards is the task of handling the wastes properly. Questions abound due to biohazardous waste versus medical waste and the inevitable mixing of the two. You need to know in which category to place the waste and the proper protocols for treatment and disposal.

If a waste meets any of the criteria below, it is usually considered a biohazardous waste:

1. Laboratory waste, including but not limited to:
    - Human or animal specimen cultures from medical or pathology labs
    - Research laboratory cultures and stocks of infectious agents
    - Waste from the production of bacteria, viruses, spores
    - Discarded live and attenuated vaccines used in healthcare or research
    - Discarded animal vaccines
    - Culture dishes and devices used to transfer, inoculate, or mix cultures
2. Human surgery or autopsy specimens or tissues suspected of being contaminated with infectious agents
3. Animal parts, tissues, fluids, or carcasses suspected of being contaminated with infectious agents
4. Waste that contains fluid blood, blood products
5. Containers or equipment containing blood

6. Blood from animals known to be infected with diseases highly communicable to humans

Biohazardous waste disposal

If you are disposing of any CDC-listed select agent waste, be sure to follow all regulations of the Federal Select Agent Program. In addition, be sure to check with all local agencies (e.g. sanitary landfill or sewer departments) for approval prior to disposal.

In general, solid biohazardous waste should be sterilized or otherwise inactivated and rendered non-infectious prior to disposal as normal sanitary waste. Inactivation is achieved by autoclaving or by vapor sterilization with ethylene oxide, formaldehyde, glutaraldehyde, hydrogen peroxide, or other approved methods. Liquid biohazardous waste must be inactivated using appropriate chemical disinfection prior to discharge to the sanitary sewer. Animal carcasses and other large solids should be disposed through an approved vendor or other special means such as incineration or chemical decomposition.

Medical wastes and their disposal

Medical wastes are generally defined as biohazardous waste (see above) or sharps and are generated or produced by the following activities:

1. Diagnosis, treatment, or immunization of humans or animals

2. Research associated with the above

3. The production or testing of medicinal serums, vaccines, antigens, or antitoxins made from living organisms

Generally, all medical wastes are disposed through approved, contracted specialty vendors. Any other means of disposal must be authorized and approved by all federal, state, and local agencies.