Laboratory Biosafety in Rodent Biocontainment

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Michael Sidelsky, RLATG

The rapid growth in funding for research and vaccine development for infectious diseases and pathogens is causing academic, government, and private research institutions to look for new biocontainment facility and equipment solutions. For some institutions, the solution to the growing demand for biocontainment facilities is space conversion or upgrade, an undertaking further impacted by existing building systems.

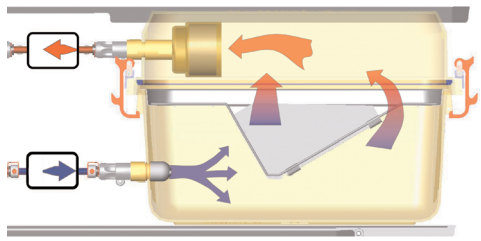
The term "biocontainment" is used to describe safe methods for managing infectious materials or agents, including those specific to mice and rats, in the laboratory and animal facility where they are being handled or maintained. Biocontainment reduces or eliminates exposure of laboratory staff and the macro-environment to potentially hazardous agents.

Biocontainment Solutions for Rodents

Rodent biocontainment environments encompass a number of engineering, design, safety, equipment, and ventilation considerations. To ensure the safety and protection of personnel and animals, biocontainment is achieved through the creation and implementation of an overarching containment culture within a facility. Put simply, the goal of that culture is to protect the staff and the lab environment from the animals and to protect the animals from the lab environment and other animals.

From the facility protocol and equipment to their appropriate use by laboratory management and personnel, all aspects of biocontainment must be congruous to provide a maximum level of protection. Once the animals are subjected to infectious agents such as influenza, rabies, herpes, and other indigenous or exotic agents, they need to be prevented from exposing other animals and clearly prevented from exposing personnel through adherence to established laboratory biocontainment practice, techniques, equipment, and biosafety procedures.

This article is meant to advance the understanding and techniques that can be applied up to and including BSL-3 in a fashion that is user-friendly and safe. As a result of adhering to biosafety procedures and equipment, I personally never witnessed or had reported by health care professionals monitoring our staff on an annual basis, a staff incident of anyone contracting any of the viruses we worked with in nearly three decades of lab experience.



BCU Airflow: Illustrates the movement of cage air in a sealed system.

Biosafety Levels

Four biosafety levels (BSLs) have been established by the federal Centers for Disease Control and Prevention which consist of combinations of microbiological practices and techniques, safety equipment, and laboratory facility design when working with potentially infectious microorganisms. Each combination level is appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory activity.

BSL-1 involves practices and equipment that are appropriate for laboratories working with microorganisms not known to consistently cause disease in healthy adult humans such as Bacillus subtilis, Naegleria gruberi, and infectious canine hepatitis virus.

BSL-2 involves agents that have moderate potential hazard to personnel and the environment such as Measles, Salmonellea, Toxoplasma spp., and Hepatitis B virus. These agents can be used safely in activities conducted in a Class II biosafety cabinet (BSC), even though the potential for producing splashes or aerosols may be low. Primary hazards to personnel in BSL-2 relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments at this level and in BSL-3.

BSL-3 includes infectious agents which may cause serious

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disease and which have the potential for aerosol transmission through inhalation. Among these agents are M. tuberculosis, St. Louis encephalitis virus, and Coxiella burnetti. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in the environment and contiguous areas from exposure to potentially infectious aerosols. All laboratory manipulations must be performed in a Class II BSC or other enclosed equipment.

BSL-4 entails work with dangerous agents that have a high risk for life-threatening disease, which may be transmitted via aerosol, and for which there is no vaccine or therapy. BSL-4 requires that the cage and animals manipulated be inside a glovebox or that personnel are wearing a one-piece positive pressure suit, similar to an astronaut's space suit, ventilated with a dedicated life support system. BSL-4 requires strict adherence to CDC guidelines, regardless of any marketplace information that would imply otherwise.

Biological Safety Cabinets

The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by microbiological procedures. Used in conjunction with injections, manipulation, readings, and data, a BSC should always be tested to NSF standards and/or be NSF-certified.

There are three types of biological safety cabinets (Class I, II, III) used in microbiological laboratories. Open-fronted Class I and Class II BSCs offer significant levels of protection to laboratory personnel and to the environment when used with sound microbiological techniques.

The Class II BSC, which is typically used in BSL-2 and BSL-3, also provides protection from external contamination of the materials being manipulated inside the cabinet. Ambient or room air is drawn into the front grill of the BSC, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides personnel and product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air is pulled down, over, and off the work surface then passed through a certified HEPA filter, it is contaminant-free and may be partially recirculated and/or totally exhausted out of the building.

The gas-tight Class III biological safety cabinet provides the highest level of protection to personnel and the environment and must be used in BSL-4.

Solution by Dilution

Preparation and decontamination of the working surface inside the BSC hood is necessary during and after bringing a cage to the BSC. I favor a technique learned from Dr. Roger Orcutt 25 years ago. For a cage change, a fiber towel is wet with chlorine dioxide solution which is an agent that kills many viruses on contact.

Once the cage is opened on the work surface of the BSC, microbial contaminants on the exterior of the cage and on the animals are forced downward to the surface by the vertical laminar flow of HEPA-filtered air. A towel laying on the work surface, wetted with chlorine dioxide solution, serves to collect and kill many potential pathogens. Airborne microbial taxis transporting pathogens are pushed down, via the curtain of laminar flow air, onto the towel. Wiping forceps with a chlorine dioxide solution is another important aspect of eliminating cross-contamination. Next, move the animals from soiled to clean sterile cage using forceps. Wipe gloves with chlorine dioxide before and after each cage change. After transferring the mice or rats to the clean sterile cage, close it and then close the soiled cage.

The concept is solution by dilution. Following this procedure assists in contaminate control because each and every cage is exposed to the chlorine dioxide solution.

Biosafety Precautions

Persons working with infectious agents must be aware of potential hazards, and be trained and proficient in the practices required to handle such material safely. The director of the laboratory is responsible for providing or arranging the appropriate training of personnel.

Each laboratory should develop or adopt a biosafety operations manual that identifies hazards that will or may occur, and that specifies practices and procedures designed to minimize or eliminate exposures.

Personal protective equipment is often used in combination with BSCs. Personal protection includes items such as gowns (closed front), gloves (taped to the gown at the wrist), shoe covers, N-95 masks or respirators, face shields, safety glasses, or goggles.

The exposure risks for most laboratory work in BSL-2 facilities are direct contact with the agents or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, as in BSL-3, higher levels of containment become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory.

To review the CDC's comprehensive guidelines for each biosafety level, a Web-enabled edition of *Biosafety in Microbiological and Biomedical Laboratories* can be found at www.cdc.gov/.

The ABCs of BCUs

My experience with sealed cage level biocontainment

Biosafety Levels at a Glance

Biosafety Level 1

Infectious Agents:

- Not ordinarily associated with disease in healthy human adults

Procedures:

- Accepted animal management and microbiological practices

Safety Equipment:

- As required for species-appropriate care

Facility:

- Sink for hand washing

Biosafety Level 2

Infectious Agents:

- Known to cause human disease
- Exposure: percutaneous, mucous membrane, ingestion

Procedures:

- Lab access limited
- Post biohazard sign
- Sharps safeguards
- Biosafety manual
- Hazardous wastes and cages decontaminated
- Medical surveillance

Safety Equipment:

- Species-appropriate containment (e.g. biocontainment cage rack)
- Personal protective equipment

Facility:

- Autoclave capability
- Hand washing sink in vivarium

Biosafety Level 3

Agents:

- May cause serious disease
- Potential for aerosol transmission

Procedures:

In addition to BSL-2 practices:

- Restricted access
- Clothing decontaminated prior to laundering
- Decontaminate cages before bedding changes
- Disinfectant foot bath as necessary

Safety Equipment:

In addition to BSL-2 equipment:

- Containment of cage changing
- Class I or II BSCs for manipulation that may cause aerosols
- Personal respiratory protection

Facility:

In addition to BSL-2 facility:

- Physical separation of lab from unrestricted areas
- Access through a series of two self-closing doors
- Sealed and impermeable interior surfaces
- Sealed windows

Biosafety Level 4

Agents:

- Dangerous agents known to cause lethal disease, aerosol transmission or unknown risk

Procedures:

In addition to BSL-3 practices:

- Change clothing before entering vivarium
- Shower on exit
- Wastes decontaminated prior to removal from facility

Safety Equipment:

In addition to BSL-3 equipment:

- Class III BSC or Class II BSC in combination with air-supplied positive pressure personnel suit

Facility:

In addition to BSL-3 facility:

- Separate facility or isolated area
- Dedicated supply and exhaust, vacuum, and decon systems
- Additional requirements outlined in CDC guidelines

units (BCU) for animal housing began in 2000. A true Biological Containment Unit cage rack system provides absolute separation of animals from their external environment. Offering protection comparable to flexible film isolators or gloveboxes, the ideal BCU is a totally sealed system with HEPA-filtered air supply and HEPA-filtered exhaust. The negative operating pressure of the BCU seals each cage from the other cages and from the ambient environment, affording the end user a superior level of containment that avoids both micro- and macro-environment contamination.

This type of biocontainment unit system is applicable for:

- Infectious disease experiments (BSL-2 and BSL-3 agents)*
- cytotoxic/potent compound experiments*
- rats and mice housed in quarantine
- pheromone control when connected to facility exhaust
- housing of immune-deficient and immune-competent animals in the same room
- allergen control
- breeding Tg, KO, and standard mice, murine virus or pathogen free and/or infected with same

* Consult with Safety Officer and/or IACUC for risk assessment.

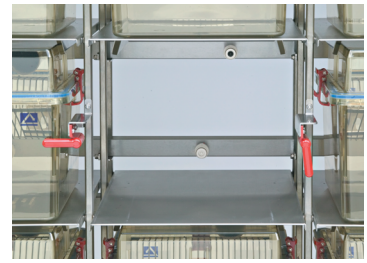
Because of these potential applications, the integrity of the housing system must not be taken lightly. The system must consistently and repeatedly provide a sealed environment, despite the rigors of life in a vivarium, with rough handling for bedding removal, washing, and exposure to heat or chemical sterilization.

The Cage

To provide a seal, the BCU cage should have a durable gasket material between the lid and base, as well as sealed air supply

BCU-Mouse cage: This illustrates the description of a biocontainment cage: sealed cage, solid top, locked top, gasketed, exhaust pre filter, sealed intake and exhaust ports.

BCU Rack: Illustrates a Biocontainment rack configuration, size, shape.



Empty CU: Illustrates how a rack is sealed from the room: Rack mounted sealing intake and exhaust ports sealing rack from the room when cages are removed.

and exhaust ports. Furthermore, a system to regularly confirm the integrity of these seals, such as a check station, is a wise option.

The Rack

The BCU rack also needs to feature seals and extreme durability. When cages are removed, the rack itself is a potential source of contamination, so every effort should be made to maintain containment, and preserve the filtration. To that point, the HEPA filtered airflow path is very critical. All connections between the HEPA filters and the air delivery system must be rigidly sealed, with no opportunity for a break in connections or ductwork. The HEPA filters themselves should be independently certified, and removed from harm's way, as HEPA filters are almost exclusively constructed with paper. HEPA filters should never be washed, or handled by non-expert personnel.

Further levels of protection can be added to the system, such as a roughing filter at the cage level, can continue to protect the integrity of the system by keeping particles out of cage- and rack-level exhaust valves ensuring a continued seal (and also prolonging the life of the HEPA filter.) This type of "prefilter" may be used as a method of sampling the cage environment. For select studies, or as an adjunct to your animal health monitoring program, the pre-filter may be transferred, with the same animals, into the new clean sterile cage assembly. After several weeks of the pre-filter being exposed to the cage environment it may be used for PCR analysis. This technique reduces the need for invasive procedures on or euthanizing of the mouse or rat. Testing for virus, bacteria, parasite, or fungal contamination can be done via the cage environment, simply by utilizing the throw-away rough filter.

An alarm system and battery back-up is a necessity for a truly sealed cage system. The alarm system should be capable of contacting animal facility personnel 24/7 in the event of a power or equipment failure. The battery back-up should be able operate the air flow to the cages for a time period that is sufficient for personnel to react to and correct the problem.

Controlling viruses or other pathogens at the cage level through the use of sealed cages and a biocontainment cage rack system is the alpha of biosafety equipment. Techniques which are then applied during the entire process create the omega of the total biocontainment procedure.

Everyone is looking for a magic bullet, but the easiest method is rarely the safest method. Safety is paramount at any BSL level and certainly as you move into BSL-3 and beyond. You cannot lower the mentality of safety "at all times." Cage rack biocontainment (CRB) systems are not all the same. They are easier than flexible film and glove box isolators to operate, however their construction and design is critical to cage level containment and must be given serious consideration. Safety requires protecting personnel, animals, and the environment around the clock.

While biosafety techniques and operational procedures used in concert with biocontainment equipment have proved effective in protecting personnel, animals, and environment, they should be further customized for each individual laboratory and in conjunction with other available scientific information.

If you decide to work at higher biosafety levels, investigate or develop protocol procedures with your Safety Officer or Lab Director in conjunction with CDC guidelines and test-drive important equipment like BSCs and BCUs prior to purchase.

***Michael Sidelsky, RLATG**, Rodent Facility and Housing Specialist at Allentown, Inc has over 40 years experience in laboratory animal colony operations and management. He has served on Institutional Animal Care and Use Committees, met regulatory requirements, and maintained accreditation by AAALAC International.*