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Barrier Systems: Design Drivers for Animal Research Facilities

by Marco Breuer and Harry van Herck

Designing and building a lab animal facility are complex processes with many stakeholders and drivers. There's a myriad of elements to consider, including the planned species/animals/animal experiments as well as the logistics of clean and dirty materials, animals, personnel and research materials. Safety aspects for all are also necessary, including security, access control, infection control (bio-exclusion and bio-containment), and the control of hazardous substances—such as carcinogenic, mutagenic and reprotoxic substances as well as gases and laboratory animal allergens.

The needed level of microbiological control highly depends on the number and level(s) of microbiologically different categories of animal experiments that need to be facilitated. Examples include germfree, gnotobionts, S(O)PF, ABSL-1, 2, 3, 4 and microbiota versus behavior research. Basic questions include which micro-organisms and which changes of the microbiota of animals are acceptable or needed, how to obtain such animals and how to keep their microbiota unchanged? E.g. the enteric flora of SOPF and SPF animals from different suppliers and breeding units is shown to be quite different. In other words: how to prevent introduction of unwanted micro-organisms in animals? And—on top of that—for infection experiments: how to prevent escape of micro-organisms to other animals, man and the environment?

In general, two main types of animal barrier units can be distinguished: clean animal units and dirty or infected animal units. The basic rule of a clean animal unit is to keep unwanted contaminations out of the unit (gnotobiotic animals and animals with defined and stable microbiota; SOPF and SPF animals). The main rule for a dirty animal unit is to prevent hazardous micro-organisms and/or substances leave the unit in an uncontrolled way (for instance, infection studies in ABSL animal units).

The barrier levels needed highly depend on the protection level needed. Measures taken will generally be higher for gnotobiotic studies than for SPF studies. For BSL-2 infection studies, measures taken to prevent the escape of the micro-organisms used will be considerably lower than for studies using BSL-4 micro-organisms.

Barrier principles

The goal of clean and dirty animal barrier units is basically the same: prevent unwanted contaminants to pass the barrier between dirty and clean. Therefore, the same principles and systems can be applied in both clean and dirty animal barrier units but in a reversed way:

- An airtight and otherwise vermin and micro-organism-tight seal delineating the unit is at least advisable and -depending on the barrier level- sometimes obligatory
- Air pressure differential(s) to create an airflow from clean to dirty inside the unit and over the barrier
- HEPA-filtration of dirty air, indicating the ingoing air of clean animal units and the outgoing air of dirty animal units
- Autoclaving heat-resistant materials from the dirty to the clean side of the barrier (autoclaving in into a clean unit; autoclaving out of a dirty unit). Both the outside and the inside of correctly autoclaved materials will be sterile
- Surface disinfection systems from the dirty to the clean side of the barrier (dunk tanks, spraysluices, gas sluices, UV-light sluices, etc.). Beware: such systems disinfect only the surface of materials. Micro-organisms below the outer surface and in the inside can survive and remain a risk. Therefore, it is advisable to sterilize the inside and to ensure the surfaces are clean before disinfection. Alternatively, infected materials should be transported in a sealed container which is only to be opened in an adequately contained environment by authorized staff or in a bin for incineration.

Personnel:

- Restricted access: only accessible for authorized persons who need to be in the unit
- clear delineation of dirty and clean area; leave dirty outfit on dirty side; step-over bench, water- or air shower; put on clean outfit on the clean side

Live animals:

- Transport in: before allowing animals into an animal unit, assure their microbiological quality meets the minimum requirement defined for that animal unit. Choose a method to import them into the animal unit which will maintain their microbiological quality.
- Transport out: see above surface disinfection systems from the dirty to the clean side of the barrier.

Sometimes different categories of animal experiments can be performed in the same animal barrier unit, either simultaneously or separated in time; others need separate dedicated (barrier) units. If it is decided to combine different categories of animal experiments in the same animal barrier unit, keep in mind the category with the highest demands sets the standard for the other experiments in that unit.

Unwanted infections in lab animals

How unwanted micro-organisms are introduced in animal experiments highly depends on which micro-organisms are unwanted and where those micro-organisms are present. For instance, known rodent pathogens are not present in germfree, gnotobiotic and S(O)PF animals (beware of not yet diagnosed barrier breaches), but opportunistic pathogens may well be present in SPF animals and non-pathogenic micro-organisms are present in every animal (germfree animals excepted), man and the environment. Beware, non-pathogenic micro-organisms can also affect animal experiments. Furthermore, new pathogens are identified regularly. Therefore, the measures to be taken for the different types of animal experiments can vastly differ.

Barrier levels of a lab animal facility

When designing a lab animal facility in outline the following barrier rings can be identified:

- the level of (the outer ring of) the lab animal facility
    - A dedicated design and construction of the building layout, routings to the working area, at the working area, and from the working area to either the next working area or the exit for man; animals (clean, undefined/infected); biological materials (clean, undefined/ infected); other materials & equipment (sterile, clean, dirty, infected)
    - Building and (barrier) unit related technical systems (HVAC, pressure differentials, sewer, showers (wet or dry), autoclaves, cage wash, storage, drinking water systems (automated or not)
- the level of the animal unit(s)
- the room level, including ancillary equipment
    - Animal holding room(s)
    - Animal procedure room(s)
- the cage level

Further important aspects:

- standard operating procedures
- personnel Training, responsibility, culture and awareness

Unwanted micro-organisms can originate from various sources and can infect lab animals via various routes. See Figure 1 for a schematic overview.

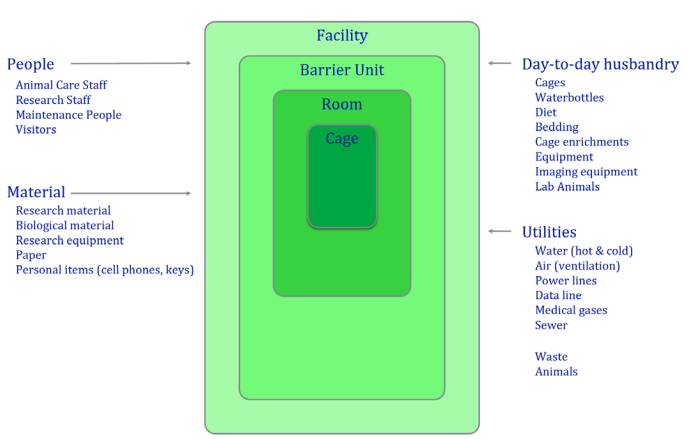


Figure 1. Overview of the level of barriers and most common vectors to introduce unwanted micro-organisms in lab animals.]

Introduction of pathogens via contaminated live animals or biological materials such as cell-lines, tumor material, not-sterilized products (including antibodies) produced in or derived of living (cultured cells of) animals, etc. or the intrusion of wild rodents into the facility are very well known. However, as depicted in figure 1 there are many more sources. Relevant potential vectors must be identified and evaluated, and preventive measures should be decided. Essentially, this comes down to assessing the risks and balancing them against the costs of the measures to reduce the risks to an acceptable level. The infection risk for multiple overlapping long-term experiments with immune deficient animals is probably considered higher compared to a 6h acute toxicity study. As a consequence, more strict barriers, work procedures and caging systems that support a higher degree of bio-exclusion could be decided for the long-term experiments with immune deficient animals. It is important to stress that the effectivity of infection prevention measures is the cumulated effect of the measures taken at the various barrier levels: the building and its installations, the animal unit(s) and their barrier equipment, the animal room, and the animal cage level (animal housing system and ancillary equipment, like animal transfer stations or

biosafety cabinets). In practice, facility policies and work procedures, as well as if/how everybody adheres to these procedures, are at least as important. All installations and equipment should be validated and adequately maintained. And all people entering a lab animal facility should be instructed and be trained adequately. The weakest link in this chain of infection prevention determines the overall efficacy.

Figure 1 shows the enclosure levels at which control measures can be taken. They start at the facility building level. Within each lab animal facility there is at least one, but often two or more different animal units. An animal unit comprises multiple animal holding and possibly procedure rooms. At all levels both architectural and installation measures can be taken to reduce the risk of introduction, escape and/or unwanted spread of infectious diseases, and contaminants, including animal allergens, and odors. Within animal and procedure rooms additional measurements can be taken at the cage level and -during handling—at the level of the individual animal by the choice of the caging system, protective equipment and working procedures.

First level of defense: outer shell of the building

The outside of the building is the first barrier against unwanted visitors. It's surroundings and outer shell should be designed and built to keep out wild rodents and other vermin. It requires a lot of detail in the construction and the use of high-quality finishing materials to realize a vermin proof barrier.

In the ideal situation an animal facility is a standalone building which is not connected to any other building, no underground tunnels and no corridor or other connection from any of the floors of the building. Doors/ openings in the outer are easy access points for vermin and should therefore be kept to the minimum. Such buildings are difficult to design and construct. Keeping them vermin proof is another challenge.

Ideally the building is surrounded by a strip of pebble stones to discourage rodents to approach and to burrow close to the building. Special attention should be paid to the sealants that are often used in constructions, such as polyurethane foam, silicone and fireproof sealants, seals used to close dilatation joints. Standard materials are often not rodent proof. Rodents may eat through and enter the building. Special materials should be used.

Roll-down shutters of loading docks require attention to safeguard the total construction is fully rodent proof. Especially where the shutter closes to the ground, but also to the construction of the sides where the door rolls into the runners and the top. It is needless to say that these doors must be closed always, unless needed during transports. Never leave an opened roll down shutter unattended, nor any door to the outside. Keep outside areas and the inside of a loading dock clean and therefore as empty as possible. Walls should be smooth and cleaned easily. Have a functioning vermin control program in place. Last but not least, the biggest challenge is often to develop adequate barrier procedures and to keep people alert to follow them in order to keep the barrier's function.

Within the animal facility several subareas/functions can be defined. Besides the animal units and rooms, there will be several other function areas like storage for cages and equipment, feed, bedding, consumables, controlled and other drugs, different types of waste, including carcasses, break room(s), staff offices, and likely a washing area for cages and bottles.

To prevent spread of contaminants, microbial and other, it's important to restrict access to individual areas to only the people that need to work there. Keeping doors closed when possible and having rodent proof-door strips (air pressure differentials) or brushes underneath the doors are effective, as well.

Second level of defense: the animal unit(s) inside the building

Often the animal area of an animal facility is subdivided in a number of animal barrier units. Such an animal barrier unit comprises one or more animal holding rooms, corridors, and often procedure rooms and rooms for experimental setups and its own dedicated barrier. The barrier should or—depending on the type and level of the barrier (ABSL 2, 3, 4)—must be designed for a defined purpose and includes dedicated construction measures, equipment and procedures. A SOPF unit with immune-deficient animals demands a stricter barrier and procedures than a unit with conventional animals housed in open cages.

For a reliably functioning barrier, technical design, barrier equipment, working procedures and sufficient space are crucial and must therefore be included in the design of the building. Typically, the following categories cross the barrier of an animal unit and—depending on the desired barrier type and level—require special attention for construction, equipment and working procedures.

People

Animal care staff, research and maintenance staff and possibly visitors will enter and leave the animal unit on a regular basis. To control this in an appropriate way, gowning facilities are needed to support the desired barrier level. Typically, a gowning area consists of a dirty and clean side separated by a stepover bench. Depending on the barrier type and level, the dirty and clean area can also be separated by a shower system. The actual gowning procedure depends on the desired microbiological level (clean conventional, SPF, SOPF) that needs to be maintained, the need to control lab animal allergens, and whether experiments are performed at ABSL level II, III or IV or under other safety measures.

A typical gowning procedure to enter a S(O)PF barrier often looks like: Persons enter the dirty area where they can undress and put these clothes into a locker and wash their hands/arms (hands-free faucet). After stepping over the bench they can put on a coverall, hairnet, facemask, socks and shoes, and gloves. After gowning up, people can enter the animal unit directly (often in SPF research facilities) or via an air shower which is installed in between the gowning area and actual animal unit (for instance in SOPF research animal units and in SPF and SOPF breeding units). An air shower removes particles and attached micro-organisms from smooth surfaces. Using dedicated coveralls etc. improves its effectivity. Air-showering into the barrier strongly reduces the number of micro-organisms present on persons surfaces and in the air through the barrier. Showering out also reduces the animal allergens that were picked up during the stay inside the barrier unit.

To assure that only authorized persons can enter the barrier, access control equipment is at the entrance door of the gowning area. A biometric access control like fingerprint or face recognition is preferred above the traditionally swipe cards. Swipe cards (and mobile phones!) which are used in- and outside can transport infections across the barrier and can be used by non-authorized persons in case of lending, loss and theft. When properly designed, these systems can also be used to deny access to a clean area after entering a dirty area for a defined period—such as no access to a SPF breeding area for a certain

Figure 2. Two air showers built in between the gowning area and a barrier unit.

period (in a research setting often 40-48 hours) after entering a dirty quarantine barrier.

Animals

Animals used within an animal unit originate either from internal breeding (same or other barrier unit) or are obtained from third parties: most times commercial vendors, but also from academia. Newly introduced animals are a well-known risk factor to introduce unwanted infections. Therefore, newly introduced animals MUST meet the microbiological criteria for that specific animal unit. This should be checked before animals are allowed in. How to transfer new animals into that animal unit is another challenge. Most incoming mice and rats are transported in cardboard or polypropylene structures including porous filter material which were sterilized before putting animals in. When carrying animals, the outside surfaces of such transport boxes can hardly or not be adequately disinfected. As a consequence, they are a microbiological risk when introduced into the barrier. To have a controlled entry of animals, several vendors developed animal transfer hatches (ATH), based on biological safety cabinets (BSC-II), that are either built into the barrier wall or placed into a transfer room. The transport box with

a

b

Figure 3. Example of an animal transfer hatch built into a transfer room between the animal barrier and outside.

animals in it is placed on the dirty or loading side of the ATH by Person A. (S)he opens one end of the transport box. An animal caretaker sitting in front of the ATH/ BSC-II (Person B) takes all animals out of the transport box without touching the outside transport box and places them in a clean cage. Next, Person A pulls back the transport box and discards it. Person B passes the clean cage with animals to a second animal caretaker (Person C) inside the barrier unit and brings the animals to their holding room(s).

Materials

A lot of materials are needed inside a barrier unit on a daily basis: materials for animal husbandry and

general consumables, but also study-specific research materials, such as cell lines, tumor materials, special drugs etc.

Figure 4. Example of two pass-through disinfection chambers built into the barrier wall.

Often, research materials are transported by researchers into the animal unit. Research materials, especially non-sterilized biologicals like antibodies, cells and body fluids are a notorious risk for introducing unwanted micro-organisms. It is important to ensure that such research materials are certified to be free of defined human and animal pathogens before they are allowed into an animal unit. Furthermore, the outside of the tubes, vials and other materials need to be disinfected/decontaminated when entering the animal unit. This can be achieved in a quick, controlled and validated way by using a small pass-through disinfection chamber (disinfectant spray) built in a wall of the barrier.

Husbandry materials like cages, water bottles, enrichment material and general consumables are more voluminous and more plannable. Such materials are typically introduced into a clean animal barrier unit via either an autoclave or via a large disinfection chamber. Autoclaving is preferred because it sterilizes both the inside and outside of materials. But this is only possible if materials withstand the heat and moisture of this process. Material that cannot be autoclaved can be safely introduced by a disinfection chamber where the number of sensitive micro-organisms present on the surfaces of sterile, bagged materials are strongly reduced via a fumigation process, most often with vaporized hydrogen peroxide as a disinfectant.

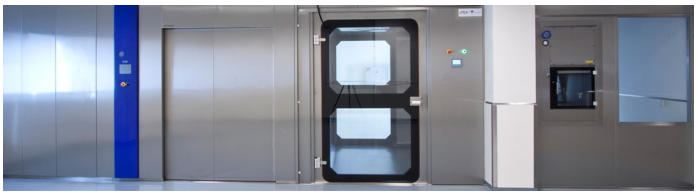


Figure 5. Example of a large pass-through sterilizer (left) and large pass-through VHP disinfection chamber (right).

Casings, utilities and cables

During the construction process, many holes are made in the barrier walls, floor and ceiling for doors, autoclaves, disinfections chambers, etc., and to enter or exit casings of pipes and cables. To safeguard that these penetrations of the barrier do not cause infection risks, special attention is needed during the design, construction, use and later changes of an animal barrier unit. Several types of rodent and fireproof sealings and systems for cable and piping transits are available.

An example of a high-end product is shown in Figure 6. After installation, a fire proof, airtight, and water-tight transfer can be achieved. The ease of installation and flexibility of this system—also for the various different diameters of cables—make this modular system very convenient.

When casings are used in poured concrete floor and walls, every single cable has to be sealed in the

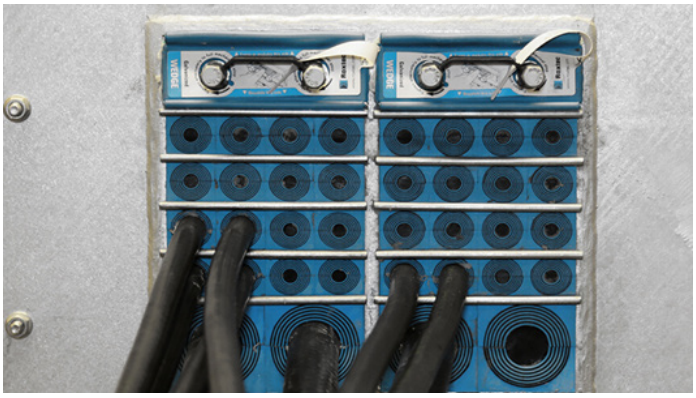


Figure 6. Several types of rodent and fireproof sealings and systems for cable and piping transits are available that ensure a fire proof, airtight, and water-tight transfer.

casing itself. A special rodent-proof sealant has to be used to create a rodent-proof, air-tight barrier. Because they have a high risk for contamination, the layout and design of sewer pipings need special attention. If sewer is needed, it should be prevented at all times that different barriers use the same sewer line. Water seal siphons and other water locks lose their function when fallen dry or can be overruled by incidental over- or under-pressures, thereby creating an open connection between two barriers via a connected sewer pipe

Third level of defense: the animal room

A barrier unit normally comprises multiple rooms, including animal holding rooms, procedure rooms, rooms for experimental setups, and storage, connected via one or more corridors. Rooms and corridors should be designed, built and used in such a way that they are easy to clean and disinfect. For some units, fumigation of individual or multiple rooms connected at the corridor level should be taken into account. Intense and frequent traffic of large and heavy materials dictates requirements for the floor, wall and ceiling finishes as well as additional wall and door protection to prevent damage by different types of carts and trolleys that are moved around.

To prevent cross-contamination between rooms in case of infection, it is advisable to treat each animal holding room as its own entity. This implies as little cross trafficking between rooms as possible, dedicated equipment for each room, and restriction of each researcher or type of research to its own dedicated room. An access control system at room level can be helpful, only allowing in persons that need to be in a specific room. As animal caretakers most times service multiple rooms a workflow should be created to service rooms and perform activities in a decided sequence.

Fourth level of defense: rodent housing systems

Nowadays, individual ventilated cages (IVCs) are the most used housing type in recently built/refurbished rodent facilities. They provide an extra barrier against infections and containment of lab allergens. IVCs are often used in combination with animal transfer stations (ATS) or biosafety cabinets (BSL-II), which add to the control during cage changing and other animal procedures.

In recent decades, single use IVCs have entered the market and are increasingly used compared to the traditional cages made from durable plastics. They have fundamentally different strategies regarding cross-contamination control, and logistics and processing.

During their life time, traditional cages, lids, bottles, etc., from durable plastic cages repeat the circle depicted in Figure 7.

Due to high-volume, this is a logistically challenging process. From the perspective of cross contamination risks, this cycle is heavily dependent on a reliable and effective washing and autoclaving process. All links of the chain must operate correctly, and

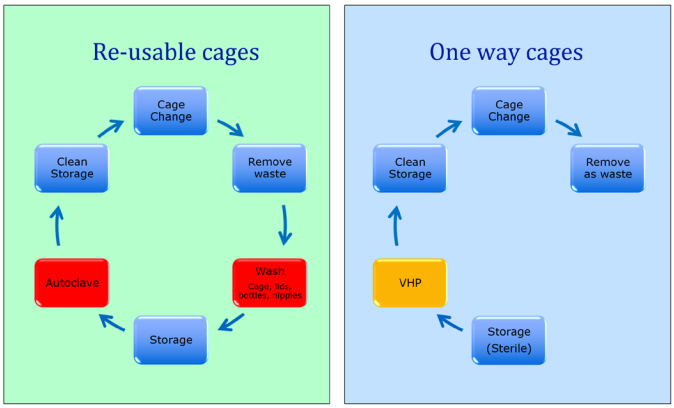


Figure 7. Schematic representation of cycle of re-usable cages versus the linear use of one-way caging systems.

backup plans must constantly be available: maintenance and validation of the equipment, availability of well trained and motivated support staff to keep up to the strict working procedures and back up plans for failing equipment. Too often, the washing machine, autoclave or staffing are points of failure. Also, insufficient capacity is regularly a bottleneck resulting in failure.

Fifteen years ago, a different concept was developed, in which users are supplied by an external producer with already sterile cages filled with bedding, lids, filled water bottles, all for single use. The concept is an analogy of the replacement of re-use glass syringes and needles by single use prepacked sterilized syringes and needles. The same happened with the glass petri dishes and inoculation needles used in the earlier days in microbiology/tissue culture. Single-use IVCs are put in the market by a limited (but growing) number of producers. Sterile, single-use cages eliminate the risk of cross contamination via inappropriate washing/autoclaving processes. They also eliminate the need for expensive equipment, like bulk autoclaves and washing areas, as well as the space and infrastructure to place and operate them. As always, there are downsides, such as the amount of plastic used and discarded and the dependence on timely availability of new cages. However, vendors are very active to support local recycling or (in the U.S.) by picking up used cages for recycling the plastic and composting the bedding.

Importance of a well-designed HVAC system

A well designed and functioning heating, ventilation, and air conditioning (HVAC) system is one of the most prominent prerequisites for a lab animal facility. It must provide environmental (temperature [T], relative humidity [RH], air quality) and space pressurization control in a reliable but also flexible manner:

- Supply of fresh air—typically, 100% fresh are is used and recirculation are avoided to prevent cross-contamination with microorganisms of volatile agents used within a vivarium. Intake of fresh air should be positioned in such a way that the air is free of fumes of vehicles and other buildings/industry.
- Defined T and RH requirements (setpoints and range) must be met throughout the year. It should be possible to modify them with changes in animal species, number of animals, equipment (T and RH in IVCs often exceed those in the animal room, IVCs air intake and outlet can be from the animal room, but also via a separate HVAC system), special requirements of animal species, strains (e.g. nude/hairless mice) and experiments. Therefore, T & RH are often primarily controlled at central installation level and subsequentlythe temperatute and sometimes the relative humidity is fine-tuned at a second room level.
- Reduce the risk of spread of odors, allergens and other toxic substances as well as of

airborne micro-organisms present in animals between rooms and animal units. This can be achieved by pressure differences between units and rooms, thereby creating an unidirectional air flow from clean to dirty or contaminated. To this end, both the desired relative air pressure intervals and the possibility to change them between animal room and corridor, animal barrier units, animal areas, support areas, office areas and areas outside of the animal facility/building should be well defined, designed and engineered. Beware of connections via the sewer, between floors, and possible effects of wind force and directions. It is desirable to have the possibility to switch a room that is run in positive pressure to negative pressure in case of a calamity contamination, thereby containing the infection to that room.

- Remove CO_2, odors, allergens and potentially harmful chemicals from the animal room, animal barrier unit and building. Appropriate ventilation must provide the accumulation of the CO_2, odors and allergens produced by the animals and humans, and—in case of ABSL conditions—the exhaust air should be HEPA-filtered to prevent GMOs from entering the environment.

Ventilation is an utmost critical installation within the animal facility and should be robust and function 24/7/365 without interruptions. A continuous steady airflow and pressure within the total system is required.

During design, engineers should anticipate for regular and curative maintenance of the installations, including changing the HEPA filters, decontamination of animal barrier units and animal rooms, and subsequent testing. Planned and other power outages should be taken into the design. As a consequence, the HVAC system must have adequate redundancy, flexibility, and emergency power supply systems that cover periods from the start of power outages until the HVAC systems functions normal and all its functions have become normal and stable. This requires knowledgeable—skilled and experienced technical maintenance staff. Power dips and outages, as well as other HVAC failures, often result in a major changes in air changes per hour and of the relative pressure interval between animal rooms, barrier units and even between the in- and the outside of the animal facility. S(O)PF animal rooms and units can very well become in negative pressure to the outside of the building and ABSL containment can become in positive pressure to their direct environment.

Multiple design approaches exist to reach these requirements, ranging from dedicated HVAC system with full redundancy (2N) per barrier unit to multiple HVAC systems that run in parallel, which could be considered as one very big system where redundancy is achieved by shutting down or reducing ventilation of non-essential areas. For every design, it is critical to ensure that the ventilation system itself cannot become a route for contamination from room to room, or from animal unit to animal unit. Not even in case of a power failure or loss of one of the fans in the supply/exhaust system.

As mentioned, ventilation is done with fresh air. The level of ventilation needed is mostly driven by the occupancy of the animal rooms. In general, a 15 to 20-fold ventilation is used. With the use of IVC—and especially where IVC systems are connected to the room ventilation—exhaust system ventilation folds can possibly be reduced to a 7 to 8-fold level. In some institutes with IVC systems connected to (a) separate HVAC-system, the ventilation fold is reduced during the time when there are no people in the room/not working with animals to save energy and money.

Lastly, an adequate number of air changes per hour alone isn't a guarantee for good room ventilation. For an optimal air exchange of all areas inside a room, other aspects should be taken into account, such as the type of air-diffusors or air-socks used, air-volumes and position of supply and exhaust points. These are essential for a correct air distribution inside the room to prevent draft and dead spots that are poorly ventilated, resulting in rising levels of CO_2, ammonia, allergens and other undesired chemical substances and micro-organisms.

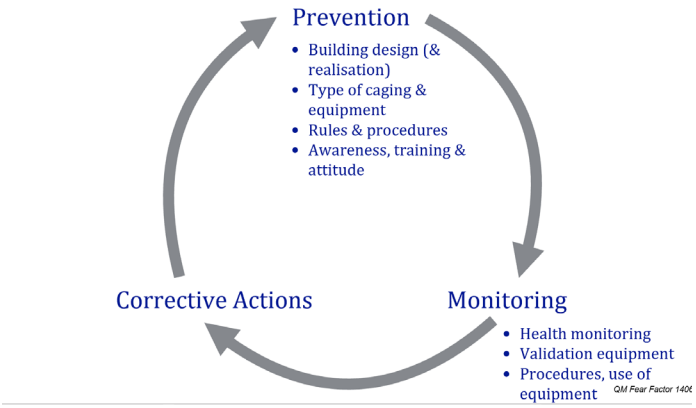


Figure 8. The cycle of prevention, monitoring and corrective action should be implemented in regards to an animal lab building, infrastructure, equipment and training.

Conclusion

In this paper, we discussed that a vivarium is a specially designed lab building that accommodate extensively controlled environments. This to avoid the introduction of unwanted microbiological contaminants, reduce the risk of infectious outbreaks, and avoid the transmission of odors and lab animal allergens. However, the result will strongly depend on the design of the building, to what level this design is realized during construction, the choice of housing systems and ancillary equipment and—most importantly—the implementation of appropriate work procedures and how animal care staff and researchers are trained and act in these procedures. Only if there is a seamless integration of these aspects can the desired result be achieved.

This is not a static process and both the building and infrastructure, as well as equipment, regular maintenance and validations followed by corrective actions are needed. A similar process holds for work procedures that have to be kept up-to-date, in addition to the training and competence of animal care and research staff.

Author Biographies

***Marco Breuer Ph.D.**, is a trained biologist, completing his Ph.D. at the Netherlands Cancer Institute. After his Ph.D., he set up a Transgenic Core Facility and became the head of the Animal Facility in the Leiden University Medical Center. In 2000, he earned his degree as an Animal Welfare Officer, and returned to the Netherlands Cancer Institute as head of the Animal Facility the following year. While there, he was responsible for the design and construction of a new high-end facility that was opened in 2014. In 2020, he also became head of the Animal Facility of the Netherlands Institute of Neuroscience. Breuer also works as a consultant.*

***Harry van Herck Ph.D.**, Dipl. ECLAM, is DVM. He completed his Ph.D. and earned his degree as an Animal Welfare officer at the Department of LAS at Utrecht University. From 1990 until 2003 he was responsible for Biosafety, Veterinary Care, Quality Management and daily operations of the Animal facility of Utrecht University. From 2003 until 2020 he was head and Biosafety Officer of the Animal Facility of the Academic Medical Centre Amsterdam. Where he designed and operated a new BSL-3 and a Large Animal unit and reconstructed the existing facilities into a SOPF breeding unit and an SPF research unit.*

Conducting Safe COVID-19 Research in the Vivarium

Co-authored by Michelle Taylor

One day, historians will write the dramatic account of COVID-19's successful vaccines and therapeutics. In that story several types of heroes should be praised: the researchers working in white lab coats, the average people who volunteered for clinical trials, and the other important research subjects—countless laboratory research animals.

The virus that causes COVID-19 is classified as a 098iourisk group (RG) 3 pathogen. As a result, all animal work related to this pathogen must be performed in a high containment laboratory, either biosafety level 3 or 4. Animal work is critical to understanding this new disease and for the further development of vaccines and therapeutics for COVID-19.

Organizations carrying out work with this virus in animals must do so in a vivarium that has an RG-3

Mouse models have been instrumental in the testing and development of COVID-19 vaccines, antibodies and other treatments.

Pathogen and Toxin License, authorizing work with SARS-CoV-2, according to Geoffroy Legault-Thivierge, spokesperson for the Public Health Agency of Canada (PHAC). This license is issued by PHAC to animal containment zones that meet the rigorous physical and operational requirements described in the Canadian Biosafety Standard. This ensures that all of the necessary measures are in place to contain the virus within the facility and to prevent vivarium workers from becoming exposed or infected in the course of their work, thereby reducing the potential infection of others in the community.

Within a vivarium, animals are often housed in ventilated cage rack systems, with each cage serving as a primary containment device. The equipment selected must meet these rigorous requirements:

- The ventilated cage rack system and individual cages must contain all air being supplied to and exhausted from the system (typically with HEPA filtration);
- The individual cages must remain sealed or HEPA filtered during all potential operational modes (i.e., connected to ventilated cage rack, transportation of the animal from the cage rack to a biosafety cabinet, transportation for cleaning and/or waste decontamination); and

- Ensure the laboratory has available space to isolate, clean and decontaminate the ventilated cage rack system following an experiment.

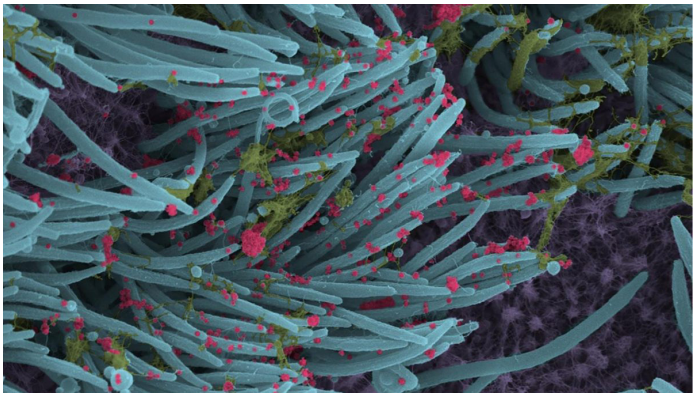
Work with pathogens or toxins in live animals poses a substantially higher risk as compared to in vitro work. Animals can behave unpredictably, especially if they are ill. In addition, infected animals may be symptomatic or asymptomatic, or may be carriers of zoonotic pathogens also capable of causing disease in humans. Pathogens or toxins may be present in the large volumes of waste produced by animals, and can also be shed from their bodies. Exposure to pathogens that animals may harbor can occur as a result of animal bites, scratches, aerosols, or through direct contact with animal waste and bodily fluids.

Cutting-edge organizations follow strict biosafety protocols at all of laboratories, as PHAC does at their National Microbiology Laboratory (NML). These protocols are based on international standards developed by the World Health Organization and the Canadian Biosafety Standards. The NML is internationally renowned for its world class bio-containment systems.

"Safety and security are paramount at the NML, and the lab continues to meet or exceed all national and international standards for containment laboratories," said Legault-Thivierge says.

Personnel at NML are required to have regular medical check-ups and are unable to enter the lab if they are ill. Security clearance is also required for all staff entering and participating in high containment research, as well as clearance for working with human toxins or pathogens.

Additionally, the Centre for Biosecurity continues to prioritize pathogen and toxin licenses for work with SARS-CoV-2, helping to increase Canada's containment level 3 capacity and therefore actively contributing to the Government of Canada's response to the COVID-19 outbreak.



SARS-CoV-2 virions (in red). Credit: Coutehre Lab, UNC School of Medicine

ABSL3

Canada is, of course, only one place in North America where the standards are high and the training is systematic. For example, scientists at NYU Langone Health were among the first to redirect their research efforts to COVID-19—early enough in the pandemic that there were only a couple hundred cases in the U.S. and none in NYC, which would later become a hot spot.

"We initially developed in vitro assays with patient samples in particular. But to unravel complex disease mechanisms, one must rely on animal models," said Ludovic Desvignes, professor and director of high containment laboratories at NYU Langone Health.

And while most patient samples either do not contain the virus itself or can be treated to be handled in a conventional laboratory, live SARS-CoV-2 and infected animals—as per CDC guidelines—must be

manipulated in a high-containment facility to protect scientists and the community.

NYU Langone Health has operated an Animal Biosafety Level 3 (ABSL3) facility for more than 15 years and the current facility has been open since 2016. Their current ABSL3 vivarium houses mice, allowing researchers to study not only diseases and immune responses, but also to test candidate therapeutic or prophylactic treatments in a pre-clinical setting."

"High-containment facilities represent a dedicated space, with tightly restricted access, where only trained and authorized scientific and animal care staff can enter after clearing multiple layers of security," said Desvignes.

The layout of NYU's ABSL3 vivarium allows these select few to safely don and remove personal protective equipment, including full-body suits and respiratory protection. Within the facility itself, work is conducted inside biosafety cabinets, according to rigorously enforced high containment standard operating procedures (SOPs). Also, mice are housed in isolated cages, with a closed and HEPA-filtered ventilation system. This ventilation system has multiple redundancies, alarms and failsafe mechanisms, which are tested and certified on a regular basis, as is the rest of the facility.

In addition to these safety measures, the SOPs also define procedures for complete decontamination of all the waste generated, including the animal waste, through chemical treatment or/and autoclaving. The university also has a dedicated occupational health program under which the ABSL3 facility staff's health is actively monitored and tested regularly.

Researchers at NYU Langone Health were among the first to redirect research efforts to COVID-19 in the early days of the pandemic. Credit: NYU Langone Health

The value of animal research

Having an animal model that exhibits a similar course of illness to what is seen in humans is one of the most valuable tools scientists have for researching and battling disease. For example, in August 2020, researchers at the University of North Carolina developed a COVID-19 mouse model that captures many of the features of the human disease, and helped advance a vaccine candidate to clinical trial.

As scientists geared up to research COVID-19, all eyes were on ACE2—a protein that sits on the surface of many types of cells in the human body, including cells in the heart, gut, lungs and nasal cavity. SARS-CoV-2 latches onto the ACE2 receptor and uses it to enter cells and begin growing, leading to infection. Researchers discovered, however, that SARS-CoV-2 cannot latch onto the mouse version of the ACE2 receptor. For a time, this difference rendered mouse studies essentially useless in the fight against the pandemic.

However, that changed thanks to scientists in the lab of virologist Ralph Baric at UNC, which has a history of generating mouse models for other coronaviruses like SARS-CoV and MERS-CoV. By changing two amino acid positions in the viral genome, they

generated a mouse-adapted virus capable of infecting standard laboratory mice with COVID-19. By spring, the researchers were able to begin multiple studies involving mice and now, the model is being used to test antibodies as therapeutic possibilities.

Elsewhere on the UNC campus, scientists implanted human lung tissue (LoM)—that allowed for replication of SARS-CoV-2—in immune-deficient mice in order to test the experimental drug EIDD-2801. To evaluate therapeutic efficacy for COVID-19, the researchers administered EIDD-2801 to LoM starting 24 hours or 48 hours post SARS-CoV-2 exposure and every 12 hours thereafter.

"We found that EIDD-2801 had a remarkable effect on virus replication after only two days of treatment—a dramatic, more than 25,000-fold reduction in the number of infectious particles in human lung tissue when treatment was initiated 24 hours post-exposure," said senior author J. Victor Garcia, UNC professor of medicine and director of the International Center for the Advancement of Translational Science. "Virus titers were significantly reduced by 96% when treatment was started 48 hours post-exposure."

Next, the researchers tested the ability of EIDD-2801 to prevent SARS-CoV-2 infection by administering the drug 12 hours prior to SARS-CoV-2 exposure and every 12 hours thereafter.

"We found that EIDD-2801 pre-exposure prophylaxis significantly inhibited SARS-CoV-2 replication, reducing virus titers in the human lung tissues of LoM by over 100,000 fold in two independent experiments," said co-first author Angela Wahl, assistant professor of medicine at UNC and assistant director of the International Center for the Advancement of Translational Science.

As of March 2021, Phase 2 and 3 clinical trials are ongoing to evaluate EIDD-2801 safety in humans and its effect on viral shedding in COVID-19 patients.

From old to new

When COVID-19 showed no signs of slowing down in 2020, University of Iowa researchers Stanley Perlman and Paul McCray realized that a mouse model they created a decade earlier to study SARS might be an invaluable tool for understanding the disease and for testing potential treatments.

In a study published in December 2020, Perlman, McCray and colleagues presented a detailed characterization of the effects of SARS-CoV-2 infection in K18-hACE2 mouse model.

"The mouse develops pretty robust lung disease that is on the severe end of the spectrum. That gives us an opportunity to investigate what's going on with lung disease with COVID," says McCray, University of Iowa professor of pediatrics-pulmonology. "Also, people who die from this disease often have vasculitis, which is unusual for coronavirus infections, and we found that the mice may develop signs of vasculitis in the liver, lung, and brain."

One particularly interesting finding was that the infected mice lost their sense of smell. This effect, also known as anosmia, is seen in a large proportion of people who get COVID-19, but is still not well understood. The study showed that K18-hACE2 mice treated with convalescent plasma and then infected with SARS-CoV-2 infection did not succumb to the infection but, like many infected patients with mild disease, had loss of smell as a major symptom.

Further investigation of the cells in the nasal passage suggested that the anosmia results from initial infection and damage to a type of cell that helps to support the function of neighboring sensory neurons thIt would be difficult to overemphasize the importance of animal research facilities and staff in the struggle against COVID-19. Every major health care breakthrough, every treatment, owes a debt to animal research, and this pandemic fight is no different. Throughout these events, vivaria have largely continued to operate, with essential research and animal care staff present daily to carry out this vital work and provide top quality care for the research animals.

"Research teams have responded to this crisis by rapidly pivoting their work toward understanding the mechanisms and physiology of this novel coronavirus. The science of solving the coronavirus threat is multifaceted, from examining the mechanisms of infection in the full body system or cellular/molecular level, to understanding the differences in individual response to infection, to evaluation of potential treatments and vaccines. And each step involves the use of animals," concluded Donna Clemons, DVM, MS, DACLAM, president of the American College of Laboratory Animal Medicine.

Author Biography

Michelle Taylor *is the Editor-in-Chief of the Lab Group at CompareNetworks, comprising Laboratory Equipment, Forensic and Labcompare. She has more than 10 years of experience researching, interviewing and writing about laboratory science. She can be reached at mtaylor@laboratoryequipment.com.*

Training and Best Practices in The Vivarium

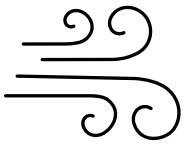
by Shawn Coleman

Judiciously controlling the environments that reside in most businesses allows these organizations to operate at efficiencies necessary to achieve the best possible results. This fact is especially salient within the vivarium, where complex and highly specialized tasks endemic to in vivo research activities occur every day.

In these results-oriented collectives, the consequences of failure are much more draconian because failures could negatively impact not only schedules, budgets, and contracts, but, more importantly, animal and human lives.

Marshaling the resources needed to maintain the proper level of control in the vivarium is a direct function of the effectiveness of that organization's written procedures. Equally important are codified training plans to frequently disseminate this written information. Simply stated, training is of paramount importance.

Here are a few vivarium priority parameters and associated best practices:

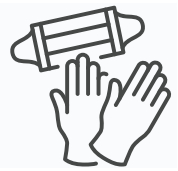


The importance of airflow supersedes just breathing. The controlled movement of air protects everyone. Filters, air curtains and exchanges are critical airflow topics. Keep these elemetnts in mind as well:

- Trust your airflow system, but verify daily. A calibrated anemometer will help.
- Each piece of equipment is designed to work well with your airflow system. Understand this interaction as well as proper equipment operation.
- Know where YOU should be. Task specific body positioning enables you to maximize the protections afforded by your airflow system.
- Know the location of each activity. These locations are not arbitrary and they are often dictated by your airflow system.

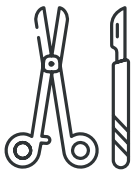
Tasks related to caging are performed frequently. Attention to the items below will help to preclude any mishaps:

- You must understand that your highest exposure risk occurs when a dirty cage is open. Stay especially focused during this time.
- Before moving cages, review whether an ATS or a BSC will be used. The latter offers more protection.
- Properly managing dirty cages is critical to maintaining a healthy environment. Awareness of cage dumping, staging areas, and cleaning procedures will enhance operational performance.



Even in the mature vivarium, a certain level of personal protective equipment (PPE) is mandatory. These items represent minimum levels of PPE:

- To protect your eyes, safety glasses with side shields are a necessity. Goggles must be used for splash hazards.
- Because of its surface area, your skin has an inherently higher risk of contact exposure. Protection is afforded through the use of long sleeves, long pants, covered shoes, lab coats, sleeves, and gloves.
- Some activities are noisy and violate OSHA's 85dB occupational exposure level. Ear plugs and/or ear muffs provide adequate hearing protection.
- In cases where your airflow system does not provide sufficient respiratory protection, a paper respirator (N95), a rubber mask with cartridges, or a supplied air device (PAPR) an be used. Respirator training and fit tests are required.



Diagnostic procedures involving anesthesia and surgery are particularly complex. These tasks are performed by highly trained professionals who should also be cognizant of the following items:

- Cleanliness is another trust but verify parameter. Microbiological testing helps with cleanroom and aseptic procedure oversight.
- Ensure you have a balanced anesthesia protocol. A multimodal approach will enable you to avoid using a high dose of one drug.
- The discipline of surgery is amenable to the adage, "the right tool for the job." Check the applicable veterinary surgical set for your procedure.

Author Biography

Shawn Coleman *is a chemist, environmental/health/safety professional, technical writer, inventor, and entrepreneur who, in his 38-year career, has excelled with Fortune 500 employers. He also founded, owned, and operated two corporations. The first one was a commercial testing laboratory specializing in soil, water, and air samples. His current business provides user-friendly, practical, and innovative EHS solutions to industrial clients. One of Shawn's inventions, a unique packaging system that saves space, time, labor, and money, was recently patented.*

How to Avoid Cross Contamination in the Vivarium

by A.B Ebeling

Cross contamination can pose problems across many different laboratory settings. Avoiding cross contamination when performing any kind of biological research, especially in a vivarium or animal research facility, is of paramount importance to protect the integrity of research results and pharmaceutical products alike. Protecting laboratory personnel and ensuring their health and safety is equally critical in any lab operation.

The downward pattern of airflow significantly minimizes cross-contamination in the work zone.

Options exist on multiple levels to address these concerns and offer choices to limit the chances of cross contamination in a vivarium setting. These options range from how you set up your lab to how you set up your experiment, as well as choosing to invest in the right equipment for your specific research needs. Implementing strategies to avoid cross contamination help to protect lab workers from occupational hazards and could end up saving you both valuable time and resources by preserving the accuracy of your research data.

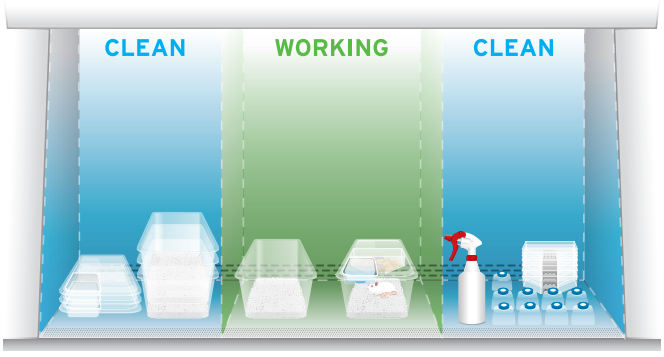
Lab managers and, in fact, the entire team need to be aware of specific considerations when working in an animal research facility and must understand best practices of how to avoid cross contamination. As established in the cover article, the first step is the design of the animal facility itself. Animal facilities are often set apart from other areas of a research institution in a limited access environment. The purpose of this is to safely and securely house the animals there, but also to restrict access by other research teams as multiple groups from various areas of a research facility sharing the same equipment would pose a serious cross contamination risk.

Vivaria also have stricter heating, ventilation and air-conditioning (HVAC) considerations as well as higher air quality standards than other spaces in a research facility, and may have entirely separate HVAC

and mechanical systems altogether. Animal facilities often utilize high energy particulate air (HEPA) filters in their HVAC design, with the goal of eliminating contaminated air recirculating either within the animal facility or to other areas in the building. While these needs can be a lot to handle when designing the space initially, they make all the difference in ensuring the vivarium is a safe, contained and isolated space where transfer of air, particulates, pathogens and research agents are strictly controlled.

Cage Changing Procedures

When cage changing small animals in a vivarium setting, a changing station works to ensure both laboratory personnel and the research animal are being protected. An animal transfer station (ATS) is a popular option in vivaria for performing cage changing procedures because it is an ergonomic and easy to use unit with a number of features that optimize workflow and ease of use, depending on a lab's specific needs. An ATS is usually a mobile unit, which provides for easy transport throughout the vivarium, with models offered either with single-sided access or dual-sided openings. Having an ATS with access on both sides allows for multiple lab members to more easily share the same workspace and maximizes flexibility with regards to ergonomic and workflow options.



A workflow with a clean area on the left and right sides and a working zone in the middle.

Meanwhile, an animal transfer station with single side access offers a higher level of personnel, product and environmental protection during cage changing procedures than a unit with dual-sided access. This is illustrated by NuAire's AllerGard ES NU-620 Containment Animal Transfer Station, which achieves a higher level of protection with the single-sided option through laminar air flow, creating an active air barrier, and a vacuum system along the front grill of the transfer station's front opening. Room air is drawn through the supply pre-filter located on top of the station and passes through a HEPA filter, which filters 99.99% of particulates 0.3 microns in size.

This contaminant-free air moves uniformly over the work surface, maintaining sterility of the work zone inside the ATS. The "dirty" air from the work surface moves through a grille of perforated vacuum slots around the perimeter of the unit. It then passes through an exhaust HEPA filter, minimizing the amount of allergens and irritants created from animal dander, hair or waste produced during the cage change being dispersed back into the laboratory. The laminar air flow pattern provides an active air barrier of 75 fpm (0.38 m/s) at the front of the unit that separates the user from the work zone inside the unit.

Choosing an Animal Transfer Station

An animal transfer station with a variety of customization options offers choices with the ability to improve ergonomics and workflow when cage changing small animals. When performing multiple cage changes or working for an extended period of time in uncomfortable positions, lab personnel may become prone to mistakes, which can easily lead to contamination issues. Having a large, clear workspace with easy access and an ergonomic setup are

NuAire's AllerGard ES NU-620 ATS has a variety of customization options to provide accessibility and improve ergonomics for the user.

key in optimizing workflow and lowering the risk of cross contamination.

When cage changing a small animal, there should be enough room to accommodate separate spaces for clean and dirty cages, as well as clean and dirty instruments and supplies. For example, the AllerGard ES NU-620 ATS has a variety of options to provide accessibility and improve ergonomics for the user, including the option of three different widths to allow lab managers to choose the right size for their facility.

The large surface area of the work environment and 14-inch access opening, along with add-on options such as a folding shelf or embedded base stand shelf for storage, create options for optimizing workspace and by extension, workflow. The NU-620 also features the ability to add a cage top holder, bottle rack stand or hand sanitizer dispenser built into the unit, all of which can help reduce the risk of cross contamination. Knowing the needs of your research and choosing the appropriate configuration and options that laboratory equipment manufacturers offer will strengthen protection for the laboratory worker, the research animal, and the work surface inside the unit.

Just as the overall design of a vivarium is important, where the ATS is placed within a laboratory is also a factor in mitigating cross contamination. Avoid placing the changing station near a door or other high traffic area as this can disrupt the laminar airflow and compromise the air barrier of the unit. Only perform procedures in the ATS when the sash is open to the designated height. In addition, do not use the changing station to store any unrelated instruments or equipment while performing manipulations on small animals, and do not leave anything inside the ATS when it is not in use. Take care not to block the pre-exhaust mesh with any equipment, as this can disrupt the equilibrium of the airflow.

Other Considerations

While an animal transfer station utilizing HEPA-filtered air and laminar airflow provide high degree of protections for both personnel and product, depending on your specific research needs, hazardous and infectious agents designated at biosafety level 2 or higher may have to be used during the course of your work. If this is the case, an ATS will not be sufficient and a biological safety cabinet (BSC) should be utilized.

When beginning a small animal cage change, always use the appropriate level of personal protective equipment (PPE) required for the task. First, turn on the ATS and allow the blower to operate for the amount of time recommended by the manufacturer to allow the airflow to normalize. Use a chemical disinfectant to clean and sanitize the interior of the

transfer station before commencing work. While performing manipulations inside the ATS, movement in and out of the work area and other activity taking place within the room should be kept to a minimum to avoid altering the airflow throughout the facility and within the changing station. Lab personnel should avoid rapid movements or activities like vigorously dumping bedding, which can create splashes and aerosolize particulates and microorganisms, creating a greater risk of cross contamination and exposing the lab worker to potentially harmful irritants. Once the procedure is finished, decontaminate the workspace again and discard all waste properly. The AllerGard ES NU-620 Containment ATS includes a built-in waste chute to conveniently dispose of waste produced during a cage changing procedure.

A lab team with a strong commitment to avoiding cross contamination is just as critical to the success of a lab as any equipment used. Consistently using best practices ensures that cage changes are performed safely and that the benefits of an ATS are properly utilized. In a vivarium, developing and following a clearly defined standard operating procedure (SOP) for small animal cage changing procedures is vital to using an ATS in a way that maximizes environmental, personnel and research product protection.

Author Biography

A.B. Ebeling *has a Bachelor's degree in cell biology and neuroscience from Rutgers, the State University of New Jersey. He has completed graduate level coursework in molecular and cell biology, anatomy, physiology, pathology, immunology and infectious diseases. With research positions held at The W.M. Keck Center for Collaborative Neuroscience, the University of Pennsylvania School of Medicine, and Sidney Kimmel Medical College, he has training in general research principles and practices, scientific protocols and research techniques across multiple settings.*

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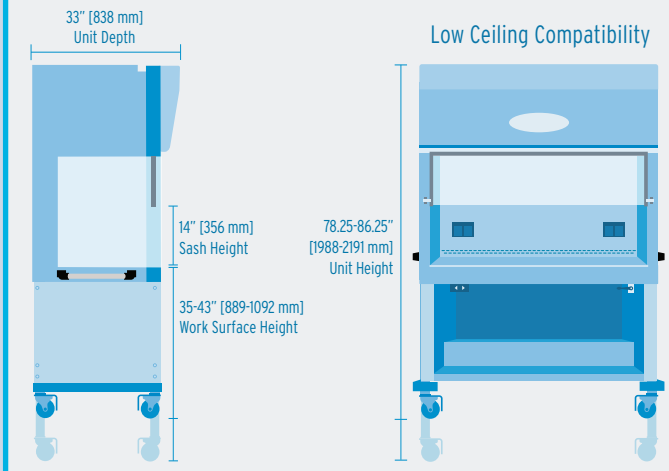

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