Issues Related to the Use of Animals in Biocontainment Research Facilities

John Copps

Abstract

The expansion and improvement of high-containment animal facilities has been driven by terrorism, economics, the emergence of new pathogens, and the re-emergence of other pathogens in new areas. Working with highly infectious viral agents requires a team of trained scientists, laboratory technicians, veterinarians, animal care staff, biological safety officers, engineers, and physical plant staff to ensure safety, biocontainment, and the animals’ well being, while providing essential scientific data. The challenges of working with infectious disease agents in high levels of containment, and some solutions to these challenges, are described from an animal care point of view.

Key Words: animal care; animal facility; biological containment; infectious disease; safety

Basic Need for Expansion and Improvement of Present Animal Facilities

Since the early 1990s, it has become increasingly apparent that there is a growing need for a wide range of biocontainment facilities that are properly designed to accommodate animals safely for infectious disease research. Since the 1980s, several countries, including Australia, the Netherlands, and Canada, have built state-of-the-art biological containment laboratories (Murray 1998); and the United States has embarked on a major regional construction program for new biocontainment laboratories. The defection of senior Soviet scientists Vladimir Pasechnik and Kanatjan Alibekov, and subsequent revelations of the Soviet research programs on biological weapons, awakened the world to the need for more research into biological defense (Albeck 1998). The events of September 11, 2001, and anthrax-related terrorism have changed the entire focus of many governments, especially the United Kingdom and the United States, as defense and homeland security have become an important focus of government funding agencies (Baker et al. 2004; BRA 2002; NIAID 2002). These events illustrated many weaknesses within the biological defense policies of governments, including prevention, diagnosis, disinfection, emergency response, and security planning (BRA 2002; NIAID 2002).

Several nations have drafted reports on the needs and direction for research to meet biosecurity and defense requirements (BRA 2002; NIAID 2002), with subsequent large budgetary allotments. The need was accentuated by long-term severe underfunding of biological containment facilities in the United States and around the world, which led to a huge deficiency in biocontainment infrastructure capacity that was incapable of meeting the growing demand. Constructed in the late 20th century, the facilities generally lacked in space and quickly became overwhelmed with projects. The deficit was most marked in the higher levels of biological containment, specifically Canadian containment levels (CLs$^1$)-3 and -4 (BRA 2002) and US biosafety containment levels (BSLs$^3$)-3 and -4 (NIAID 2002).

The increasing occurrence of new or re-emerging pathogens such as bovine spongiform encephalopathy, new variant Creutzfeldt-Jakob disease, West Nile virus (WNV$^1$), severe acute respiratory syndrome (SARS$^1$), foot and mouth disease, and H5 and H7 avian influenza (AI$^1$) has further heightened the need for CL-3 and -4 research space. It is important to note that each agent may require different research and animal facility characteristics. A variety of standards must be met in the design and operation of these facilities, which can be quite challenging and must include current and emerging construction guidelines and regulations. Pressure comes not only from government agencies for more accountability, but also from commercial companies vying for the profits of biodefense products. Facilities must also comply with new, existing, and increasingly rigorous and changing standards to qualify for certificates of good laboratory practices, good manufacturing practices, or quality assurance programs such as the International Organisation for Standards.

The public has become more aware of the risks associated with performing research with biohazardous agents. Through their elected officials, the general public has demanded higher standards of security. These demands include more rapid and accurate reporting of incidents, higher

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1Abbreviations used in this article: AI, avian influenza; BSL, biosafety level; CL, biological containment level; HEPA, high-efficiency particulate air; HVAC, heating, ventilation, air conditioning; MSDS, material safety data sheet; SARS, severe acute respiratory syndrome; SOP, standard operating procedure; SPF, specific pathogen-free; WNV, West Nile virus.
levels of containment and security of the agents, and more accountability by the research community. The frontline workers—research facility occupational health and safety committees, research scientists, laboratory technicians, and animal care staff—all demand higher levels of protection when working with these new and emerging diseases. These increasing demands must be met through revised standard operating procedures (SOPs) and upgrading of existing infrastructure.

**Basic Needs of the Scientist in the Biological Containment Animal Facility**

**Fulfillment of Legal Requirements**

Scientists face a number of regulatory barriers before they can perform research on many infectious agents. The legal requirements for permits and acquisition of the newest strain of the organism can be problematic. The research facility and animal housing space must be certified for use of the infectious agent. Permits can take months, if not years, if regulatory agencies are overwhelmed with work or if existing regulations no longer apply. In Canada, for example, it is necessary to fulfill both a Health Standard and a Veterinary Standard, which are similar but not exactly the same. This is also the case in the United States, where there can be differences between, for example, Biosafety in Microbiological and Biomedical Laboratories (Richmond and McKinney 1999) and US Department of Agriculture (USDA/APHIS 1999) standards. These standards and other guidelines (e.g., CCAC 1984, 1993; NRC 1996) have been developed over the years as a result of much discussion among scientists, and considering the interests of the public, the scientist, the research staff, and the animals. Naturally, this necessary process may seem cumbersome to the scientist wanting to perform research.

The needs of the scientist may be vastly different from those of the research animal model criteria. The basic goal for most scientists is the observation or sample required to achieve the objective of the research. Samples and procedures must be scientifically and statistically justified, reviewed, and approved in the initial planning phases. Animal infectious disease models often require frequent sampling, which can engender debate with institutional animal care and use committees. A thorough knowledge of the laws and guidelines of each country, coupled with in-depth justification for the frequency, nature, and volume of samples, will help with this review process. Facing uncertainty with limited knowledge of a newly emerged organism, as well as the uncertainty of working with agents in new or unproven animal models for which the behavior of the agent may be unpredictable, can also challenge the scientist to devise with a working protocol. A mechanism must also be in place to ensure rapid access to animals in the face of an outbreak of a serious human or animal pathogen. Standing approved protocols can allow an animal care committee to review the document before the animals are required.

The route of inoculation can be problematic when dealing with many highly contagious diseases such as tuberculosis, WNV, and AI, as well as less contagious agents such as prions. Research on the agents of tuberculosis, AI, SARS, and other respiratory diseases may require nasal or aerosol challenge; others such as WNV may require intravenous inoculation, and intracranial inoculation is common with agents such as prions. Each route and agent requires different animal care facilities, SOPs, training of personnel, and personal protection equipment. When working with scrapie in hamsters, for example, it is important to develop SOPs before the work is undertaken to satisfy the permitting agencies, local animal care committees, local safety officers, and animal care staff. The detail requirements may include a description of a calibrated injector, gauge of needle, requirement for general gaseous anesthesia (including safety associated with the anesthetic agent), and personal protection.

**Sampling Procedures**

Before the project starts, the goals of the scientist must be clearly articulated and understood by all technical and support personnel to achieve success. Most commonly, infectious disease research requires mucosal surface swabs, blood, and tissues. To accomplish these tasks, it is often necessary to develop novel approaches or search the literature for methodology. The “holistic” sampling method—a request for everything from everywhere at every point in time—is common. In CL-3 and -4, the personal safety requirements for protective equipment will affect the ability of the individual to obtain samples. Some sampling procedures may be considered too risky to perform; it is always important to keep the time factor in mind. For example, to take one blood sample from one pig will take about 15 min in a level 2 animal facility, 30 min at level 3 (including the time it takes to don and doff protective equipment and shower out), and 1 hr at level 4 because of the need to check all systems, change, get suited, restrain the animal, have a chemical shower, remove the suit and shower out. Therefore, the general rule is as the CL level increases, the number of procedures and frequency of sampling should decrease. It may be necessary to replace full necropsies with a preselected set of samples.

Alternatively, it may be necessary to increase the number of trained staff needed to complete the work. Scientists enjoy overseeing experiments and dropping in to ensure that the project is proceeding well. To do so, the scientist, and even the director, must be adequately trained in high-containment safety procedures to be in the room with the animals. Initial training can take years, and all procedures that will be carried out must be reviewed, practiced, and discussed to ensure that safety is not compromised, even by these scientific observers.

Once the sample is taken from the animals, it is important to have planned where the sample will be manipulated and how it will be treated before the samples leave the

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animal room. First, the scientist must remember that removal of samples must follow the local, regional, and national biological safety regulations, as well as the guidelines for the transport of dangerous goods. Second, it may be necessary for the sample to be treated before it is removed from a higher level of containment to a lower level. For example, serum from CL-3 is frequently gamma irradiated at 2 mega rads to remove any possible contaminating pathogens. The irradiation can alter the protein configuration, which may negate its usefulness for analyzing antibody or antigen in the native configuration. Third, it may be necessary to fix samples in formalin for a length of time before they are eligible for removal, and the analysis will be unavoidably delayed. All staff, including animal care staff, must be aware in advance of how the samples are to be processed. Photographs and video clips of animals exhibiting signs or lesions of infectious diseases are extremely useful and easily accomplished. High-quality publishable digital pictures can be produced within 1 day and can be sent to as many sites as required.

The use of any sharp object must be kept to a minimum. If sharps are used, it is critical to know the location of all staff, as well as the sharps container; constant control is vital to maintain a safe working environment. The use of sharps should be limited to a select few highly trained, highly experienced individuals to minimize potential accidents. Exposure can also be decreased by continuous improvement and review of procedures to eliminate the use of any sharp. For example, the elimination of use of a scalpel in favor of blunt scissors during a necropsy procedure greatly enhances safety. However, not all modifications can be adopted. Vanish Point® needles can be used for injections; the needle retracts into the barrel of the syringe. For small animals that do not move, the use of these needles increases safety. However, if larger animals move, Vanish Point® needles may bend or break more easily than conventional needles and cannot be retracted, decreasing the safety of the procedure. Vacutainer bleeding systems are used for both large and small animal bleeding techniques. The availability of sharps containers for the removal of the needle sharps will allow the staff member to dispose of the needle without handling the needle hub or fumbling when removing the needle from the holder. Alternatively, the entire needle and holder can be discarded.

Basic Needs of the Animal in the Biological Containment Animal Facility

Housing

Housing animals within a high-containment laboratory is a difficult task. The building design frequently dictates how the animals can be housed, what kinds of animals can be used, and what kind of environment they can live in. Buildings with inadequate design for certain types of animals simply cannot be used for those animals despite the desire of the scientist. When designing an animal facility, it is important to consult with a wide range of experts, and it is vital to solicit input from the animal care staff. Only animals that are of the highest quality and free of adventitious pathogens should be used in high-containment facilities. The cost of operating a high-containment facility on a daily basis far exceeds the extra cost of quality animals. The facility must always yield the highest quality of results. Rodents, for example, should be selected for infectious disease studies based on a complete health profile, which includes serological panels and analysis for pathogenic parasites, fungi, and bacteria. When possible, it is beneficial to use calm strains that are noted for ease of handling, rather than strains known to bite or that are intractable. All animals must be properly acclimated to the high air flows, room temperature, feed, lighting regimes, and noise.

Larger animals such as nonhuman primates, domestic pigs, sheep, goats, and poultry pose unique housing problems. For example, the volume of manure an adult cow generates is not the same as an adult mouse. It may be necessary to increase the frequency of cleaning or to keep the stocking density to a minimum to accommodate the volume of feces produced. The type of infectious disease agent also affects the requirement regarding treatment or sterilization of the waste. In Canada, the effluent human CL-3 from a facility may be treated directly within the laboratory, whereas an agricultural CL-3 pathogen requires secondary effluent treatment (CSVF 1996; Murray 1998). Most agricultural animals are governed separately if they are inoculated with CL-3 agricultural agents such as foot and mouth disease, classical swine fever, and capripox. Pre-treatment of large volumes of organic material with disinfectants such as sodium hypochlorite, Virkon®, or iodine-containing compounds is widely advocated, but difficult to justify scientifically. It is well known that organic matter decreases or inactivates many disinfectants (Vesley et al. 2000). Heat and pressure in cookers provide one of the best methods of treating organic material (Edwards et al. 2002; Murray 1998).

Specific pathogen-free (SPF1) large animal species are rare and expensive. One SPF goat, for example, can cost up to $5,000 Canadian, but may be necessary for polyclonal antibody production destined for humans. Prior acclimation in conventional housing or a farm will decrease the amount of time for the animals to be housed in containment. It is possible to decrease certain acclimation periods from 2 to 3 wk to 1 wk, which increases annual program output. For example, nonhuman primates can be tested for tuberculosis while being acclimated to new caging systems, feed, cage mates, and personnel before they enter the high-containment area. Sheep can be shorn, dewormed, vaccinated, acclimated to pelleted feed or hay cubes, and treated for parasites. Furthermore, this period can be used to assess the temperament of larger animals, and thus their suitability, for use in the containment facility.

Species, size, sex, and containment level all are factors for consideration. Planning work with infectious agents in
animals requires the selection of the correct animal model. Literature searches and web-based articles can provide the scientist with vital information on a wide range of animal models. The most common species used for infectious disease studies are mice, rats, guinea pigs, and rabbits. Many guidelines and SOPs have been written for their well-being. Problems may be encountered when items such as novel caging systems, special bedding, new kinds of food, and environmental enrichment devices are needed. All items must be able to withstand frequent disinfection, if not sterilization, and some environmental enrichment devices may be unworkable or create hazards.

Caging Systems

Cages must be manipulated within a biological safety cabinet, which requires a great deal of coordination, training, and man power. Often, the cage cleaning equipment is not located near the CL-3 or -4 areas. The cages are cleaned and autoclaved within the CL-3 or -4 area and then sent to the cage wash area for disinfection. Single housing of animals may be required to ensure that animals do not grab, bite, or touch other cage wash area for disinfection. Single housing of animals may be required to ensure that animals do not grab, bite, or touch the staff member during the cleaning, feeding, observing, or sampling of the animal. Ethically, group housing must always be considered if personal safety is not compromised.

It is easily possible to maintain small animal comfort with the use of bedding, hiding places, proper animal density per cage, and cage change frequency, as is commonly undertaken in level 2. Many different caging systems are available for rodents in CL-3 and -4. Basic rodent microisolators can be used for many agents and can be disassembled, sterilized, and stored very easily. Ventilated racks and cages offer the added advantage of more animal protection, which helps eliminate cross-contamination and increases safety for the animal care staff. Automatic watering decreases the work for staff and means less time in containment safety apparel. However, all parts of the ventilated racks and watering systems must be able to be sterilized and not simply sanitized. Prion caging requires high-temperature plastics that can be autoclaved at or above 132°C (Prusiner et al. 1984).

SOPs are required to decrease tracking of the agent as well as to ensure proper bedding and cage sterilization. Procedures address the pretreatment, rinsing, and removal of soiled bedding within a biological safety cabinet. The cage life will be extended if the cage is rinsed before sterilization. Quality assurance testing of every autoclave run is vital to the safety of the staff and helps eliminate cross-contamination. Extended autoclave run times are required to ensure penetration of the steam and heat into the bedding. The SOP must be part of the safety protocol, and test runs are invaluable. Once sterilized, waste may require a second step such as an additional autoclave run, alkaline digestion, or incineration, depending on the local, state, or federal laws. The goal after each cage change is to eliminate the infectious agent from the environment as completely as possible and to work in a manner that eliminates possible contamination of the laboratory.

Washing Systems

Animal species used in the room, cost, and availability of water treatment dictate the installation of a particular washing system. Three types of systems are used within containment facilities: (1) The most common system uses no floor drains and requires no floor mopping. Cages are sterilized with an autoclave and run through a classical cage wash unit. (2) The high-volume low-pressure washing system is used for large animal facilities. Typically, this system is characterized by a wide-bore 5- to 10-cm-diameter fire hose with a line pressure of 40 to 60 psi. The advantages are ease of cleaning large volumes of feces with few side effects for staff. The disadvantage is the significant amount of water consumption, the necessity of treating large volumes of water, and the potential smell from holding lagoons. (3) The high-pressure (up to 1200 psi) low-volume washing system shaves the manure off the flooring. The advantages of low water consumption and treatment must be weighed against the potential for occupational health hazards. Grasping high-pressure spray guns for extended periods of time can result in repetitive strain injuries. The high-pressure spray can also generate a mist, potentially aerosolizing the infectious agent.

Waste Treatment and Disposal

In large animal facilities, all waste must be treated by heating under pressure or for extended periods of time. Separate treatment protocols and holding tanks for different agents or containment levels are also in use (Edwards et al. 2002). The large animal enclosure must be easily disinfected and preferably decontaminated with a strong anti-infective agent such as formaldehyde gas. Epoxy finishes are preferred. The pen is cleaned daily to reduce the contamination rate within the room while total decontamination procedures are left to the end of the study.

One of the most contentious problems encountered is the cleaning method for the large animal pens. Gang-housed nonhuman primates can be housed on litter if there is a substantial investment in man power and if incineration is available. Also with chickens, bedding (litter) can be removed and sterilized on a regular basis. However, bedding is generally not used within large animal rooms. Most large animal waste treatment systems do not readily allow for the flushing of wood shavings, straw, or hay down the drainage system. The total elimination of bedding poses an ethical problem. Providing soft rubber mats for large animals is
common; however, animals tend to defecate on the mat and become soiled and uncomfortable. Rubber inlaid flooring that contains channels will decrease but not eliminate soiling while providing a drier place for the animal to rest. It is important to keep in mind that large adult animals such as pigs can chew through rubber flooring. Tie stalls can successfully restrain the animal while the manure is channeled away, and soft rubber mats can provide comfort for the animals. Tie stalls decrease the animal’s mobility, which may cause bed sores and is an ethical concern.

Doors

Sealed doors and directional air flow maintain the infectious material within the room (Murray 1998). The animal care staff must be consulted on the style of door to be used within the facility. Large heavy doors that require substantial force to open may lead to repetitive strain injuries if staff must open and shut numerous doors during the work day. Increased numbers of staff and mechanical advantage levers may be needed to work within a facility using large door seals.

Doors with sills or elevated thresholds should not be used where there is a significant amount of movement of animals or heavy items. Air gaskets can seal efficiently; however, they break down, requiring excessive compressed air capacity, directional airflow, and automated alarms to ensure the safety of the enclosure (Murray 1998).

Additional Needs of the Animals

Nonhuman primates can easily be maintained with automatic or bottled waterers. However, there is no simple answer for watering other species. Water for large animals can be from wall-mounted automatic waterers as well as rubber water troughs; both are common. Wall-mounted units must be flushed regularly and can rupture or jam, causing flooding of the pen and excessive waste treatment. Fixed troughs are more rigid and decrease the workable space, yet plastic water buckets can be tipped over easily or the water can become contaminated. It is imperative to be able to decontaminate all watering systems, which involves the use of a suitable disinfectant agent and an appropriate contact time. A device to prevent back flow and filters within the line are important to ensure that there is no retrograde movement of the water in the lines (Edwards et al. 2002).

Feeding practices for nonhuman primates are well established and must be strictly regulated within the high-containment laboratories. A set schedule of food pellets, fruits, and treats must be established to ensure the health and well-being of the animals, as well as to avoid interfering with the goals of the study. Feeding sheep, pigs, cattle, and other large animal species is easily established by using a complete pelleted ration supplemented with mineral blocks and cubed hay. The ration can be presented in a fixed trough, a rubber trough, or a removable bucket. Food storage, and preparation to the extent possible, must be kept to a minimum within containment. For example, cutting fruit for nonhuman primates, pigs, or cattle within CL-3 or -4 is much more dangerous than in CL-2. The food can be transported into containment in batches in disposable containers such as plastic bags, which provide the added benefit of easily disposing of the original packaging.

Environmental enrichment should be maintained within the CL-3 and -4 areas when working with large animals. Boredom in a stark environment will affect the well-being of the animals, which in turn can compromise the study. Nevertheless, devices must not pose a safety hazard. Numerous devices have sharp edges, can clutter the cage, and may be difficult to sterilize between animals. It is essential to maintain a balance between the number of devices used and the safety requirements. Smooth chain, rubber balls, and cleaning equipment make excellent environmental enrichment devices. It is critical to consult both the scientist and the safety personnel before using any environmental enrichment device.

Restraint

By far, one of the largest differences between the containment levels is how to restrain and manipulate animals. Restraint starts with manipulating the animal within the home cage. As a general rule of thumb, manipulations of all animal species infected with a CL-3 or -4 human pathogen should be kept to a minimum, well thought out in advance, and performed according to SOPs. Animal nails, teeth, beaks, and horns should be considered sharps. Each species must be considered individually and the peculiarities understood. For example, mice can be manipulated with forceps, avoiding any contact with the animal, whereas this procedure would be impossible for Syrian hamsters, which have no tail. It is possible to move hamsters easily from one area to another by using a strainer. Rats and guinea pigs require the animal care staff to pick up the animals physically when moving between areas or cages. Armored (Figure 1) or Kevlar® (Figure 2) gloves can keep the individual staff member safe, but have the disadvantage of being bulky (Table 1). It is also difficult to gauge the pressure required to grasp the animal safely, which can be an ethical problem. Numerous standard commercial restraint devices that are suitable for high containment are available for mice, rats, rabbits, and guinea pigs.

In the author’s opinion, nonhuman primates should not be handled in CL-4 without chemical restraint. Frequently, in CL-3, leather restraining gloves are worn. Leather is not easily decontaminated, which should lead to serious consideration for the use of chemical restraint or armored gloves when using infectious CL-3 agents. The most common drugs used for restraint in nonhuman primates are Telazol® and ketamine in combination with xylazine (Popilskis and Kohn 1997). The animal can be restrained within the home...
cage with a squeeze, and then given an injection intramuscularly. Once the animal appears sedate, the door is opened, the animal can be gently prodded or touched with a plastic or metal rod, and then picked up while wearing restraint gloves.

Gates and Enclosures

With the use of a gating system, larger animals such as cattle and sheep can be coaxed into a head gate and a halter for most procedures. Kicking, head butting, and trampling are the main concerns. Restraint when inoculating a CL-3 or -4 agent into sensitive areas such as the feet is best completed under sedation. Xylazine is the most common and reliable drug for sedation of both of these species. Sedation allows for the accurate and safe placement of the pathogen into the animal.

Pigs, however, are more complex. Several good methods for working with small pigs in CL-4 have been published (Abraham et al. 2002). The pig must first be placed in a confined area or a crush to immobilize the animal temporarily. An intramuscular injection using a 20- to 30-cm plastic catheter allows for injection while the pig is moving. When this method is used in CL-3 or -4, it is necessary to wear armored gloves or Kevlar because the needle may spring back toward the person injecting. Catheters made with tubing that has no memory are very useful. Small pigs can be squeezed into a tight chute that allows for easy direct injection with little movement. The animal can also be picked up and placed into a sling. The sling alone can act as the restraint device, or intramuscular sedation with Telazol® or xylazine can be easily accomplished. One last alternative for the pig is to mask the animal down with halothane or isoflurane. Both procedures work quickly and effectively on most strains of pigs, and do not require needles. However, it is important to wear protective gloves to avoid being bitten. Pigs are also commonly restrained with wire or rope snares.

Exotic animal handling may require novel enclosures and modifications in common techniques used in domesticated animal species or at zoos. For example, caging and housing of crows for West Nile virus studies have been accomplished using fish netting, poultry isolators, and high-tension wire. Feed has been placed in stainless steel dog food dishes, cattle chutes used as perches, and plastic tubs used as bird baths and as a source of water. Fish nets have been used to capture the birds. Kevlar gloves aided in the handling, and the birds were easily anesthetised using a small animal gas anesthetic machine.

Endpoints

For animals infected with CL3 or -4 agents, it is extremely important to establish endpoints. In most animal models, animals show clinical signs of infection, and some of the agents cause severe clinical signs, including death. Endpoints must be established to protect the animal from pain and distress while maintaining the integrity of the research. An excellent summary of setting endpoints for infectious disease work has been published (Bhasin et al. 1998). An endpoint can be established using a series of observations or measurements and scoring the animal as the infection progresses. Once a predetermined endpoint is reached, the animal is euthanized. Endpoints other than death allow for high-quality fresh samples to be obtained, to the benefit of the animal.

Basic Needs of the Staff Working in the Biological Containment Animal Facility

Team Approach

The scientist must be trained in all safety and animal care techniques required to obtain the necessary samples from
the animals in CL-3 and -4. Alternatively, trained veterinarians, technicians, or veterinary technicians can collect the samples. The best team approach developed in our laboratory is to use a combination of all staff members, which maximizes their training. For example, the scientist completes the animal use document, explains the rationale for the sampling techniques, and assists with sample selection at necropsy. The veterinarian develops the sampling techniques so they can be practiced easily and safely, trains staff in techniques, and possibly completes the necropsy. The laboratory technicians aid with restraint, sample identification, preservation, and decontamination, and conduct the laboratory testing. The animal care staff member takes care of the animals, changes cages, makes daily observations, provides sedation, and takes various samples including swabs, blood, and feces. A means of communication from inside the animal rooms must be available to staff members, particularly if they are working alone.

Counseling, extensive training, and ongoing education of all staff are essential for success. Mock exercises and training with the live animals before they are infected or in low-containment levels are essential. After the exercise, the entire group will understand and appreciate the time, energy, and effort that is required for each procedure. Slow, methodical, well-planned experiments are much safer than hurried, ill-prepared ventures. All staff must be enrolled and trained in an effective occupational health and safety program.

**Biological Safety Officers**

Biological safety officers are pillars for safe work within the animal facility if they understand animal-based research. A biological safety officer can aid with the animal experimentation in many ways. The most important way is to compile a safety document that clearly outlines the following information: agent, dose, route, safety requirements, relevant SOPs, decontamination procedures, signage, relevant material safety data sheets (MSDSs), verification of training, and finally, a signature indicating informed consent by all involved. MSDSs are available for most infectious pathogens, but they may be outdated. Regular meetings with the animal care attendants will quickly earn trust and foster a safe work ethic. Not all biological safety officers are comfortable working within high containment, and they must be willing to accept guidance from the scientist and animal care staff to keep them safe when they enter the CL-3 and -4 animal facilities.

**Personal Protection**

Protective clothing varies with agent, procedure, and local biological safety requirements. In CL-3, most rodent work requires the following wardrobe: long-sleeved scrubs and dedicated footwear covered with booties, high-efficiency particulate air (HEPA)-filtered face protection, face shield or glasses, a long-sleeved laboratory coat with impervious front and arms, and one or two pairs of disposable gloves. All of the equipment is important, but gloves and respiratory protection are the most critical. As stated above, the addition of safety gloves makes animal manipulations much safer. Kevlar and armored gloves are recommended over chain mail. Kevlar gloves are not needle stick-resistant or -proof.

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**Table 1 Suggested hand protection for mouse procedures**

| Procedure     | CL-2 infectious | CL-3 zoonotic/infectious | Prions |
|---------------|-----------------|--------------------------|--------|
|               | Gloves | Anesthesia | Restraint | Gloves | Anesthesia | Restraint | Gloves | Anesthesia | Restraint |
| IV injection  | Nitrile | — | D | AG-R | — | D | # | — | — |
| IM injection  | AG-R | — | P | AG-R | — | P | # | — | — |
| IP injection  | AG-R | — | P | AG-R | — | P | AG-R | — | P |
| SQ injection  | AG-R | — | P | AG-R | — | P | AG-R | — | P |
| ICr injection | AG-R | — | P | AG-R | — | P | AG-R | — | P |
| IN injection  | Nitrile | — | P | # | — | — | # | — | — |
| Oral injection | AG-R | — | P | AG-R | — | P | AG-R | — | P |
| Orbital bleed | AG-R | X | — | AG-R | X | P | # | — | — |
| Saphenous bleed | — | — | — | # | — | — | # | — | — |
| IC bleed      | AG-R | X | — | AG-R | X | P | # | — | — |
| Ear notch     | Nitrile | — | P | # | — | — | # | — | — |

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*Table notes:*

- **Gloves types:** AG-R, armored; Nitrile, Nitrile.
- **Anesthesia:** X, required.
- **Restraint:** P, second personnel; D, device.
- #, procedure not carried out at the facility.
but are highly cut-resistant and moderately flexible. Armored gloves are needle stick- and cut-resistant but have poor flexibility. Multiple layers of gloves can increase safety but the benefit is tempered by the decreased ability to complete fine motor skill tasks.

Personal respiratory protection must be tailored to the agent and local biological safety requirements (McCullough 2000). Within the animal pen or facility, the use of a powered air-purifying respirator with a hood or face piece is comfortable and provides protection for many hours (McCullough 2000). When using the powered air-purifying respirator, a completely charged, well-maintained spare unit must be readily available. In CL-4, a full supplied-air pressurized suit and chemical showers to clean the outside of the suit are required (Abraham et al. 2002; Wihelmson et al. 2002). Selection of personal protective equipment and clothing must also take comfort into account, to ensure that it is readily used and not circumvented.

Clothing in the large animal facility varies with the organism and the potential for human infection. Most CL-3 agricultural agents are not zoonotic and require only rubber boots, coveralls, and gloves. Zoonotic CL-3 and human CL-3 pathogens require extra protection, especially when necropsies are performed. When working in an infected large animal room, all staff should consider themselves to be in the biological safety cabinet with the organism. Commonly, hip waders and plastic rain coats (Figure 3) cover the pathologist so they can be rinsed and disinfected after the procedure. Within large animal facilities, the hoses and HEPA filter unit can be damaged by the pens, animals, and fellow workers.

Sampling the animals at the endpoint is probably the most important and can be the most dangerous procedure. Comprehensive necropsy procedures have been published for CL-4 (Abraham et al. 2002). These guidelines can be a starting point for most CL-3 infectious agents. Every project should have a predesigned sampling strategy. For example, in a project involving pigs and chickens infected with SARS, the animal care technician cleaned the room and bled the animals. A veterinary pathologist completed the necropsy and selected the tissue samples. The scientist ensured that all samples were taken and placed in the appropriate containers, and transferred them to the high-containment laboratory. The laboratory technician immediately performed the tests as well as preserved and catalogued the samples.

**Operational Needs Within the Animal Facility**

**Design Features**

The “box within a box” classical theory of biological containment is well known, and many programs are in place for various kinds of agents such as viruses, bacteria, myco-

![Figure 3 Example of protective clothing for Canadian containment level 3 work.](image-url)
The requirement for body showers when entering or leaving a containment area is a very contentious issue. However, from an animal care perspective, they are very important for the following reasons: (1) Body showers help stop fomite spread between animal rooms, which is essential if they are infected with different agents or even different strains of the same organism. In short, it forces staff to change clothing to prevent the spread of organisms. (2) Exit showers provide a mechanism to help remove any pathogen on the surface of the body. (3) The showers prevent quick access, which can lead to circumvention of safety protocols; and finally (4) showers refresh the staff members after a long experimental procedure.

**Summary**

Work with highly infectious disease organisms in animal model systems will continue to be required in the future. A team of highly motivated individuals can work safely within CL-3 and -4 if they plan ahead, use basic safety principles, and communicate effectively. The scientist, laboratory technician, veterinarian, biological safety officer, animal care staff, engineering staff, and physical plant staff all must understand the ethical use of animals, animal use protocol, and the building design.

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