Institutional Responsibilities in Contamination Control for Research Animals and In Occupational Health and Safety for Animal Handlers

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Abstract

The mechanisms for controlling microbial contamination in research animals are similar to those for preventing exposure among animal handlers to naturally occurring pathogens, research-related biohazards, or animal allergens. Research and resource preservation are the primary goals of each approach, and an appropriate assessment of risk is their foundation. The identification of potential risks enables the implementation of relevant risk management or control measures. This article summarizes the components of an occupational health and safety program for animal handlers, including screening, training, work practices, effective use of engineering controls, selection and use of personal protective equipment, and emergency response protocols. The features of a risk assessment and risk management program and the level of interaction and training required to implement and sustain the program correlate well with programs designed to control microbial contamination in laboratory animals. This article includes an explanation of the "five Ps of risk assessment and risk management." *Pathogens* and the proposed experimental *procedures* account for the first two Ps; the other three focus on training and awareness of the *personnel* involved in the experiment, *protective* equipment and work practices, and factors associated with the *place* (or facility) where the research will be conducted. Animal handlers' comprehension and knowledge determine the success of any containment program, and so this review also includes a discussion of critical teaching points for animal handlers and of the importance of evaluating personnel to verify their proficiency and competence in required protocols.

Key Words: animal biosafety; biohazard; biosafety; biosafety training; containment

Numerous research institutions worldwide use a broad variety of species of laboratory animals, placing significant responsibility on employers to ensure both the appropriate level of animal care and the protection of the expanding number of employees involved in animal research. In this article I review the foundation of an occupational health and safety program, with emphasis on the importance of risk assessment, training, and documentation of employee competence in containment work practices.

The US Occupational Safety and Health Act (PL 91-596, 1970) requires that employers provide a workplace free of recognized hazard and, as necessary, use control measures for such hazards if technologically and economically feasible. Numerous reports document the hazards that confront those who handle animals and the mechanisms to protect them (Acha and Szyfres 1991; Fleming 2006; Harrison 2001; NRC 1989, 1997; Phipatanakul and Wood 2006; Reeb-Whittaker et al. 1999; Richmond 1991a,b; Richmond et al. 2003). However, a job-specific hazard analysis is necessary to identify the tasks and procedures that involve risk in order to select adequate controls. In addition, it is important to determine whether proposed animal handlers have elevated susceptibility to either innate animal hazards or hazards related to the research. Once hazards are identified, measures can be put in place to control microbial contamination of animals and prevent animal handlers' exposure.

The following risk assessment elements are crucial to an effective identification of potential hazards:

- pathogenicity
- virulence
- route of exposure (fecal-oral, percutaneous, through mucous membranes, inhalation)
- transmission (between animals, from animals to handler, and from handler to animals)
- infectious dose
- availability of pre- and postexposure prophylaxis
- environmental stability
- hazards associated with the animal species

The following staff members and groups should participate in the risk assessment process:

- the principal investigator (PI), responsible for the initial risk assessment involving biohazard protocols
- veterinary care staff
- animal resources staff
- the biosafety officer
- the occupational health physician
- the institutional animal care and use committee (IACUC)
- the institutional biosafety committee (IBC)

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Occupational Health Program

Employers must provide both facilities that are appropriately designed to maintain and contain research animals and the specialized equipment and controls needed to protect both handlers and animals. Those who use the facilities and equipment must receive (1) adequate training to ensure effective operation and, before working in an animal facility and having contact with animals, (2) medical clearance for their proposed work assignments (Goldman 1995; NRC 1997). No individual should have access to an animal facility without medical clearance from the occupational health program.

An effective occupational health program screens those with animal contact in order to identify individuals who may be particularly susceptible to animal allergens or the infectious disease under study, or who may present an elevated risk to the animals. This medical evaluation, which is part of the individual's medical record, must be private and confidential.

The occupational health program may include a baseline serum collection and administration of applicable immunizations with antibody titer quantification to document sufficient protection. In general, immunization against tetanus should be provided every 10 years, and rabies vaccination is recommended for those who may handle wild-caught or other animals of unknown health status used in research. Occupational health services may also be called upon for periodic screening and surveillance.

Animal handlers must report to occupational health services for medical evaluation after an injury or exposure. The occupational health provider will assess the need for postexposure prophylaxis, instruct the injured handler on postincident care, and describe symptoms that will require additional attention. All exposures to animals and research-related materials should be addressed through immediate cleaning with antiseptic soap and water for percutaneous injuries and/or immediate flushing of mucous membranes with potable water in an eye wash for 15 minutes. The importance of immediate postexposure response and reporting must be stressed to animal handlers at the time of hire and periodically throughout employment.

Exposures to research-related materials and to animals, their wastes, and particles shed by them may sensitize handlers to allergens or immune system stimulants used in research. Because repeated exposures to a sensitizing agent may lead to more significant reactions, occupational health services must evaluate such reactions. Additional controls may be necessary to protect sensitized employees, including minimizing the level of involvement in high-risk procedures.

Occupational health services will also evaluate physical injuries such as burns, sprains, strains, and fractures. After an affected handler's initial treatment, rest, and therapy, occupational health will determine the employee's schedule for returning to work in order to limit additional risk to the individual, and will report the incident and injury to the health and safety office for evaluation to help prevent further injuries among animal care staff.

Personnel Training

Regulations designed for the humane care and use of laboratory animals, along with those for worker protection, mandate a variety of training programs, and properly trained animal handlers are the foundation of an institution's compliance program. State-of-the-art animal facilities, the latest in containment cage technology, and the most advanced biological safety cabinets are not sufficient to provide the requisite containment and protection of both animals and employees. Thus although animal handlers may feel inundated with training, managers, supervisors, and safety representatives must provide both initial and ongoing training for all personnel who will handle animals.

Content and Methods

Training should be tailored to the group in question, not a generic or "one-size-fits-all" program taken off the shelf each year to review the basics. Trainers should be familiar with the specific operations performed by employees, understand the equipment they use, know the design and layout of the work space, be aware of the hazards posed by the species handled or research agents in use, and target the educational level of the group. A trainer who is not knowledgeable in these areas must spend time onsite observing or participating in daily activities to gain an understanding of the operations. Alternatively, the trainer can recruit a colleague with the appropriate knowledge or work with a member of the group to develop training materials.

Animal welfare training guidelines generally call for extensive classroom and hands-on field training for animal care staff (NRC 1996). In addition, US Occupational Safety and Health Administration (OSHA) standards may include training in the following subjects based on each individual's job function:

- hazard communication
- laboratory chemical safety
- bloodborne pathogens
- noise and hearing conservation
- radiation protection
- personal protective clothing, including selection, use, fit, donning, doffing, storage, disposal, and decontamination
- respiratory protection and fit testing
- forklifts/powered industrial vehicles
- emergency response

Special standards for extremely hazardous chemical compounds, such as ethylene oxide and formaldehyde, require

focused training for use in situations that may exceed a defined exposure limit.

It is also prudent to provide additional training to cover risks or hazards faced by animal handlers even if it is not mandated by regulation. Examples of training programs in this category include

- ergonomics and slips, trips, or falls
- effective use of the biosafety cabinet or chemical fume hood
- safe handling of sharps
- sterilization/disinfection

Employees must also receive training for the use of any equipment that may involve risk or present a hazard to the employee; autoclaves, rack washers, containment equipment, ventilated caging systems, and precision vaporizers used by researchers and veterinary care personnel are a few examples of such equipment.

Documentation and Verification of Proficiency

The initial training session, any refresher or update classes, manufacturer demonstrations, and all opportunities for hands-on practical experience must be documented and included in individual training records for each employee. From a regulatory viewpoint, "if it isn't documented, it didn't happen."

A database is an effective way to track training information and should include a complete training record: the title and outline of the training class, the name and qualifications of the trainer, the date and time of the session, and the names of the attendees. Although required by only a few regulations (including those that govern select agents and shipping/transport), it is prudent to maintain documentation that training class participants understood the key learning points. Quizzes, case studies, and proficiency checklists are examples of methods to verify the required level of comprehension.

Verification that an animal handler is proficient in the required animal care and use techniques, understands the written safety protocols, knows how to properly use related equipment, and can follow prescribed emergency response procedures is critical. Such verification not only will help the institution's regulatory compliance program but also will protect its investment in its human, animal, and physical resources. Managers, supervisors, and/or safety representatives can verify employee proficiency by developing core competency checklists for each task and procedure and by creating internships for hands-on experience under qualified supervision, in either mock or actual locations depending on the level of risk. Supervisors must perform a critical evaluation of each employee during the observation to determine competency in new tasks.

An individual who has never worked with infected animals should demonstrate proficiency with the required biosafety work practices and the institution's peer-reviewed standard operating procedures (SOP) with noninfectious animals before proceeding to practice sessions with infected animals. The trainee should also have sufficient time to observe an experienced person performing each task. This practice can actually improve a safety procedure with fresh insights from the trainee. However the verification process is set up, trainers should avoid the "see one, do one, teach one" approach to transitioning employees into new assignments.

Last, it is useful to bear in mind that what is customary in an industry may become standardized and required by regulatory groups, so it is important to keep abreast of the direction in which peer institutions are heading. Today's best practices are tomorrow's guidelines and standards.

The Five Ps of Risk Assessment and Risk Management

The "five Ps of risk assessment and risk management" are a simple way to teach principal investigators and animal care managers the principles of effective practices in these areas. Adapted from the five Ps used by sports coaches ("prior planning prevents poor performance"), the five Ps of risk assessment and risk management group risk factors in the following categories: pathogens; procedures; protective equipment; personnel; and place.

Pathogens

The first step in effective risk assessment and management is to identify the risk factors associated with the pathogenic agent involved in the research or with the animal species. **Red flags** for discussion are agents that

- have been involved in previous laboratory-acquired infections,
- have an airborne route of transmission,
- have a low infectious dose,
- present a significant risk to the individual or other animals,
- have unique medical surveillance requirements or recommendations,
- may persist outside containment if released, and/or
- present decontamination challenges.

It is also important to ensure that all personnel involved in the project are fully aware of both naturally occurring zoonotic agents common to the species and any physical hazards associated with the animal.

Box 1 contains an exercise that biosafety officers and other risk assessment/risk management trainers can use to

Box 1 Risk assessment/risk management trainer's corner

It is critical that all individuals working with a biohazard understand not only how they may become infected but also the signs and symptoms of infection. Individuals who handle an infectious agent must have a high degree of suspicion if they experience symptoms that are similar to the agents under study, even in the absence of a known exposure. The pioneer epidemiologists who reviewed laboratory-acquired infections (LAIs), Pike and Sulkin, found that only 20% of the LAIs reviewed had a known route of exposure (Pike 1976, 1979; Sulkin and Pike 1951).[a]

A useful training exercise to emphasize this incredible statistic is to place a small colored sticker on the back of 1 out of 5 (20%) training handouts. As part of a stand/sit exercise, have the class stand while you summarize the Pike and Sulkin findings as well as the update by Harding and Byers (2006). Tell the trainees that they represent the number of LAIs identified in these reviews (the aggregate number from the summaries is over 5,000) and ask them to identify what percentage of those infected knew how they got infected. Collect the guesses and then ask the group (still standing) to flip over their handouts. Those with a colored sticker should sit down. The group will have a vivid demonstration of the import of this research and is likely to better understand that protection against biohazards is not as simple as they may have thought.

The trainer can extend the discussion by asking those standing what route of exposure they think was most likely involved in infecting the 80% who remain standing. Of the four routes of exposure, the airborne route of transmission is usually the most likely. The trainer can use this as a lead-in for a discussion of aerosols (what they are, how they are created and disseminated, terminal settling velocity, respiratory deposition) and give examples of laboratory-acquired infections.

[a](1) Harding AL, Byers KB. 2006. Epidemiology of laboratory-associated infections. In: Fleming DO, Hunt DL, eds. Biological Safety: Principles and Practices, 4th ed. Washington: ASM Press. p 53-80; (2) Pike RM. 1976. Laboratory-associated infections: Summary and analysis of 3,921 cases. Health Lab Sci 13:105-214; (3) Pike RM. 1979. Laboratory-associated infections: Incidence, fatalities, causes, and prevention. Ann Rev Microbiol 33:41-66; (4) Sulkin SE, Pike RM. 1951. Survey of laboratory-acquired infections. Am J Pub Hlth 41:769-781.

demonstrate that laboratory-acquired infections do occur and are frequently associated with a route of exposure not identified by those infected. This exercise can be a useful introductory component of group training sessions to help reinforce the importance of biocontainment and strict adherence to established biosafety protocols.

Procedures

It is essential to outline all procedures that will be used in research or animal handling, especially those that may involve potential routes of exposure; and the work practices specified by the researcher or in the animal care standard operating procedures must minimize the risk of exposure. **Red flags** for discussion are procedures that may involve the creation of aerosols or droplets, the use of sharps, or opportunities for bites or scratches.

Basic biosafety work practices are summarized in Box 2, and a simplified method for explaining the disinfection process is provided in Box 3. A sample set of standard operating procedures for safely inoculating research animals with a biosafety level (BSL) 2 agent is provided in the section below on Risk Management Measures.

Box 2 Seven foundational work practices in biosafety[a]

1. Do not eat, drink, smoke, or store food in the laboratory. Keep your hands away from your face (avoid touching your eyes, nose, or mouth with gloved hands).
2. Do not pipette liquids by mouth.
3. Wear personal protective clothing in the laboratory (e.g., lab coats, gloves, and face protection).
4. Eliminate or work very carefully with sharp objects.
5. Work carefully to minimize the potential for aerosol formation. Confine aerosols as close as possible to their source of generation (i.e., through the use of a biosafety cabinet).
6. Disinfect work surfaces and equipment after use.
7. Wash your hands after removing protective clothing, after contact with contaminated materials, and before leaving the laboratory.

[a]Adapted from NRC [National Research Council]. 1989. Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials. Washington: National Academy Press.

Box 3 The three Cs of disinfection

1. Chemical. Select a chemical that can inactivate the microorganism(s) or agent(s) of concern (both those that are in use and those that may be present). Many charts are available for further information[a] and can also provide other essential information such as factors that could inhibit effectiveness (e.g., pH, temperature, or presence of organic material).
2. Concentration. Follow the manufacturer's preparation guidelines for dilution and use, or use the concentrations listed on one of the summary charts from the NIH or WHO. Not all disinfectants increase in effectiveness with elevated concentrations; for example, alcohol (which requires the presence of water for protein denaturation) and iodine (which needs water to disassociate iodine bonds) work much better at dilute concentrations than in concentrated forms.
3. Contact time. Allow the disinfectant to be in contact with the microorganism for a sufficient duration to ensure decontamination; use a peer-reviewed reference to determine the contact time needed for the agent in question. The EPA uses a minimum contact time of 10 minutes for testing disinfectants against a spectrum of microorganisms, and this contact time is prudent to ensure decontamination of most agents. However, always check with your biosafety officer for confirmation of your decontamination procedures to verify your protocol.

[a](1) EPA [US Environmental Protection Agency] lists of Registered Disinfectants, Sterilants and Tuberculocidal Disinfectants (www.epa.gov/oppadd001/chemregindex.htm); (2) NIH [National Institutes of Health], US Department of Health, Education, and Welfare, Office of Research Safety, National Cancer Institute, and Special Committee of Safety and Health Experts. 1979. Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research, 2nd ed. Available from the NIH Office of Biotechnology Activities and online at http://www.twu.edu/research/IBC/LabSafetyMonograph.pdf; (3) Characteristics of Selected Disinfectants, the Center for Food Security & Public Health, Iowa State University (available online at www.cfsph.iastate.edu/BRM/resources/disinfectants/characteristicsselecteddisinfectants.pdf); and (4) Heinson PA, Jacobs RR, Concoby BA, eds. 1995. Biosafety Reference Manual, 2nd ed. Fairfax VA: American Industrial Hygiene Association Publications. p 104-106.

After the development of standardized work practices, the next step in the risk assessment and management process is the establishment of protocol-specific emergency response procedures. These should be developed by experienced staff representing animal resources, occupational health and safety, veterinary care, occupational health services, and security, and they should account for each anticipated event that could create a risk of exposure, spread of contamination, or harm to the animals. Fires, floods, power loss, terrorist events, missing animals, dropped cages, and spills or releases outside of primary containment are examples of incidents that require emergency planning. It is crucial to ensure that personnel have a firm knowledge of all emergency response procedures.

The first requirement for the development of written incident response procedures is a clear understanding of the risk factors associated with a pathogen, its route of transmission to other animals and handlers, its stability outside the host animal, and effective disinfectants to control it. Every protocol should detail immediate response actions to protect handlers, reduce contamination spread, and minimize building damage, followed by a series of notifications to relevant groups (environmental health and safety, animal facility management, veterinary care, occupational health, and the principal investigator). These groups will also be subsequently notified of the resolution, findings, and recommendations for preventing future incidents.

It is important to review the specific factors involved in each incident before beginning any mitigation effort. Representatives of animal resources, occupational health and safety, veterinary care, occupational health services, and security should participate in the incident evaluation and provide guidance for the response. For example, the response to a dropped cage that contains animals infected with a pathogen will depend on whether the animals were shedding the organism; if they were, immediate evacuation of the room would be necessary to avoid handlers' inhalation of (or other contamination from) aerosols generated by the incident. The next step involves cleaning and decontamination of personnel, as needed; a team of at least two trained individuals should participate in this step. Access to the laboratory should be restricted until aerosols have settled or been removed by the room ventilation system. Spill remediation may require supplementary protective clothing and equipment, including replacement cages for released animals.

Development of the decontamination strategy is the responsibility of a team of representatives from related groups (environmental health and safety, animal facility management, veterinary care, occupational health, and the principal investigator) for each incident (e.g., a dropped cage of animals, dropped vial of inoculum, failure of containment equipment). However, it is advisable to develop in advance generic response procedures to address the possible decontamination of floors, walls, cages, racks, and other equipment. It is similarly prudent to preassemble and maintain spill kits in a clean area of the facility.

Protective Equipment

In addition to the work practices and procedures for protecting animals and handlers, the selection and use of protective equipment are important in effective risk management.

Engineering controls provide a barrier both between the individual and a potential hazard and between an animal and potential or known contaminants. Microisolator cages, individually ventilated cage racks, biological safety cabinets, chemical fume hoods, sharps containers, and forceps are examples of devices that can help protect the animal handler.

A **red flag** for discussion concerns animal procedures performed outside primary containment, as they could expose the handler to allergens or biohazards or expose the animal(s) to contaminants in the room.

Protective clothing options include a wide array of both disposable and reusable styles of personal protective equipment. Lab coats, back-fastening gowns, and jumpsuits provide increasing levels of coverage for personal clothes or scrubs. Disposable examination gloves in a variety of sizes must be available in all animal facilities. Disposable sleeve covers prevent exposure to the wrist (when gloves pull away from lab coat sleeves) and protect the lab coat or gown sleeve from contamination when working in the biosafety cabinet. Cut-resistant gloves and sleeves may be useful for protection when working with large research animals; thinner versions of Kevlar gloves provide cut resistance without significantly sacrificing tactility and dexterity. (Although cut-resistant gloves do not prevent a direct needle stick through the material, they do minimize exposure from cuts or slices.) Face or respiratory protection may be required depending on the potential for splash, droplet, or aerosol formation. Hair covers may be indicated for further protection of the animals. Booties can prevent contamination between rooms.

The safe handling of chemicals in the laboratory may require safety glasses, goggles, or a full face shield, and an impact-resistant face shield and goggles are necessary for opening the autoclave, where pressure could cause the rapid release of contents. Surgical masks protect the nose and mouth from splashes or splatters and also block the spread of droplets from the animal handler's mouth and nose to the animal. Respiratory protection may be required with agents that have an inhalation route of exposure. Individuals whose responsibilities require them to wear a respirator must enroll in the institution's respiratory protection program, be medically cleared to wear the respirator, have it selected and fitted by an occupational health and safety professional, pass a qualitative or quantitative fit test, and receive training on the donning, use, doffing, disinfection, and cleaning or disposal of the respirator.

A **red flag** for discussion concerns any written safety protocols in which a PI has indicated generically that "personal protective clothing will be worn." The researcher must specify the garments needed and these must be consistent with the required level of containment.

Personnel

It is important to verify that individuals have successfully completed all required initial and refresher training programs and have qualified work experience with the proposed procedures. All personnel should be comfortable with and competent in the proposed work (which may include performing procedures on laboratory animals) and established safety protocol, and all animal handlers must demonstrate proficiency in the techniques associated with the care and use of laboratory animals. Animal handlers must also receive clearance from the occupational health program for each protocol. Such clearance is not a "blanket" authorization to work in any animal room or with any agent but requires reissuance for other agents.

Red flags for discussion are animal handlers who have a poor safety or performance record or who may be at greater risk of infection if exposed (e.g., because they are immunocompromised or have contraindications for immunizations).

Place (Laboratory Features)

The proposed housing and research location and setup must be appropriate to protect susceptible species by containing research-related hazards and/or adequately excluding contaminants. Facility conditions may require that the animal room be operated under negative pressure for containment or under positive pressure for additional protection of the animals.

It is important for animal handlers to understand that the institutional animal care and use committee or animal care division is responsible for determining animal housing and use locations. And researchers must be familiar with the institution's policies for room assignments and be aware that the space needed to safely perform higher-risk experiments may not be available. The unauthorized transfer of animals between rooms may have significant consequences to the institution's contamination control program or put animal handlers (or other personnel) at risk.

Red flags for discussion are experiments with Risk Group 3 agents, any other agents that have unique facility design requirements, and unusual research animals with special housing and/or handling requirements.

Risk Management Measures

The information gained in the risk assessment process is essential but incomplete without a set of corresponding risk management measures to ensure the protection of handlers and adequate containment of pathogens. In addition to the components described above, key risk management measures include a startup meeting to promote effective communication, preparation for possible unknown hazards, prudent practices with sharps and biosafety cabinets, and strict adherence to animal biosafety level (ABSL[1]) 2 standard operating procedures and requirements.

[1]Abbreviations used in this article: ABSL, animal biosafety level; HEPA, high-efficiency particulate air

Startup Meeting

One effective mechanism for ensuring communication about risk assessment and risk management decisions is a "startup meeting" for all involved parties before initiation of an experiment that involves animals; the IACUC approval letter for an experiment that involves hazardous agents should include a requirement for such a meeting. Along with the principal investigator (or her designee) and a biosafety officer, representatives from the veterinary care group, animal resources center technical staff, and animal handlers should attend the meeting to review the proposed safe operating procedures. The startup meeting is also an opportunity for the animal handlers to meet the research group, so all involved will know whom to consult if questions arise.

Using a standard document as a starting point, such as the CDC/NIH guide to *Biosafety in Microbiological and Biomedical Laboratories* (BMBL; Wilson and Chosewood 2007), participants should review both the proposed steps in the research protocol to create a site-specific safety procedure for the experiment and special restrictions to minimize the chance for mistakes.

An important message to convey at the startup meeting is that the protocol SOP cannot be changed without another group meeting. The room location, type of caging, and type of biosafety cabinet required for the experiment all ensure maximum protection. Changes in the SOP that are not reviewed can jeopardize the health of both the research animals and the handlers and may also interfere with experimental results. Animal care and safety personnel should authorize and document all changes before implementation.

Unknown Hazards

The review and approval process provides an opportunity to determine what constitutes an acceptable risk; but establishing control measures for a known risk or hazard is easier than for unknown or potentially infectious materials. When it is not possible to identify all the answers in a risk assessment review, an institution must determine whether it is appropriate to conduct the experiment.

Once an institution has accepted the risk it can apply the "block all routes of exposure" (BARE) approach, which involves assessing the animal, the agents that may be present, and the proposed procedures, and then applying the best controls available. For ABSL-3 experiments it is appropriate to consider protective clothing and equipment such as a back-fastening gown or jumpsuit, double gloves, full face protection, and an N-95 or high-efficiency particulate air (HEPA[1])–filtered respirator. All work must be conducted in a biological safety cabinet or equivalent containment device. Animals should be housed in microisolator or individually ventilated cage racks equipped with supply and exhaust HEPA filters.

Rigorous work practice controls can prevent the spread of contaminants within a room or between rooms. A complete change out of protective clothing and scrubs, followed by a shower, may be necessary. The room selected will be operated under negative pressure; the risk assessment team determines other facility design features, such as the need for clean and dirty anterooms and HEPA-filtered exhaust air. The individuals performing the work must be completely proficient in the proposed procedures and the required biosafety containment practices.

It is important to ensure that all involved in the protocol understand why the controls have been selected, how they work, what their limitations are, and what can happen if they are not used effectively.

Once the best controls are in place, the greatest risk to the individual is through a percutaneous injury from a contaminated sharp. The greatest risk to the animal would likely be from failure to use the biological safety cabinet appropriately or from improper removal of personal protective equipment.

Safe Handling of Sharps

Animal experiments frequently require sharps for the inoculation of research materials and for drawing samples; procedures on small laboratory animals may require reusable sharps (e.g., fine-tipped forceps, scissors, scalpels). The use of safe sharps devices and practices can minimize the risk of exposure and reduce the risk from reusable sharps during cleaning and disinfection after use.

The first step in a safe sharps program is to attempt to eliminate the need for sharps in the procedure. Is a sharp required? Can a nonsharp device be used? For example, a nonsharp alternative can easily replace a needle and syringe as a pipette. Glass also should be eliminated from the experiment: replace glass Pasteur pipettes with plastic alternatives, glass vacutainer tubes with plastic, and glass capillary tubes with plastic or mylar-wrapped capillary tubes. Box 4 provides a summary of safe sharps work practices.

Effective Use of the Biological Safety Cabinet

Cage changing, bedding disposal, and experimental procedures on research animals can expose animal handlers to allergens and known biohazards or potentially infectious material (Harrison 2001; Reeb-Whittaker et al. 1999), but a variety of primary containment devices can protect animals and animal handlers (Chosewood and Wilson 2007). Engineering controls such as microisolator cages, individually ventilated cage racks, and sealed biological waste containers are examples of primary containment equipment that can minimize personal exposure and prevent the spread of contaminants among laboratory animals.

Positive pressure ventilated enclosures—such as clean benches, which direct clean air across the work surface and

Box 4 Safe sharps considerations[a]

- Place the needle box or sharps container close at hand (e.g., in the biosafety cabinet) to facilitate disposal. All users should be able to see the top opening for disposal.
- Never recap needles.
- Never bend, break, or otherwise manipulate needles by hand.
- Discard the needle and syringe as an intact unit immediately after use.
- Never allow sharps containers to overfill; empty them when they are ⅔ to ¾ full.
- Use tongs, two dust pans, or other devices to collect sharps such as broken glass. Never collect sharps by hand.
- Never remove scalpel blades by hand; use a forceps, clamp, or other device.
- Load needles and syringes just before use in the animal room. Avoid the transport of a loaded syringe as it requires removal of the cap from a contaminated needle.
- To secure an animal during necropsy, replace pins with tape.
- Use a 50 ml Falcon tube or small plastic beaker to safely cover the sharp end of the needle if needed between uses; never recap for this purpose.

Reusable sharps

- Set up a collection tray for processing reusable sharps such as scissors and forceps after use. Close the puncture-proof tray after use and transfer it to the autoclave.

For chemical disinfection:

- Place sharps in the disinfectant with sharp ends pointing in the same direction. Leave scissors open to ensure contact with all surfaces.
- Allow sufficient contact time for initial disinfection.
- Carefully remove sharps after 10 minutes by lifting them with their handles or with tongs.
- Clean instruments with a bristle brush to remove residual debris.
- Return to tray for final disinfection soak.

Safe sharps devices

- Retractable needles, needles with sliding shields
- Disposable scalpels, scalpels with sliding shields
- Scalpel removal devices
- Mylar-wrapped capillary tubes
- Plastic vacutainer tubes

[a]The University of Virginia Health System office posts an updated list of sharps safety devices on its website (www.healthsystem.virginia.edu/internet/epinet/safetydevice.cfm).

directly out the front of the enclosure (usually toward the worker)—protect research materials and the animal but not the worker from aerosols or biohazards. Negative pressure ventilated enclosures, such as the Class I biosafety cabinet, draw room air inside the unit and protect the animal handler but not the animal from contaminants that may be present in the room. The Class II biosafety cabinet combines the features of both units and protects the research materials, animals, personnel (the animal handler), and environment (room air/outdoor air) as the air both entering and exiting the biosafety cabinet passes through HEPA filters. For a detailed description of biological safety cabinets, please refer to the CDC/NIH BMBL and other resources (Chosewood and Wilson 2007; Rake 1978; Wilson and Chosewood 2007).

It is important to educate users of biological safety cabinets on how they work, what can impair proper function, and the best practices for use. Although the Class II biosafety cabinet is the most significant primary device for research with biohazards in laboratory and animal rooms, it is not a total containment barrier as air flows into the cabinet through a front opening. In addition, how the cabinet is used determines its effectiveness (Rake 1978). It is essential that animal handlers who use a biological safety cabinet adhere to the following practices:

- Make sure the biological safety cabinet is tested and certified before use and at least annually thereafter. The person performing the certification should possess the necessary professional qualifications.
- Never move a biosafety cabinet. Its location, determined by health and safety professionals, is carefully set away from overhead supply diffusers, door openings, and heavy traffic patterns in the room. Moving a cabinet can also interfere with a filter cabinet seal, which could compromise safety or sterility.
- Never store items on top of the biosafety cabinet as they may interfere with air flow or damage the exhaust filter.
- Avoid walking or working directly behind a person using a biosafety cabinet, as the resulting air movement can interfere with the inward protective curtain of air at the opening.

- Disinfect the biosafety cabinet's interior and work surfaces before and after every use.
- Load all materials beforehand that will be needed during the experiment to minimize hand movement in and out of the cabinet once the work begins.
- The front and rear grilles of the biosafety cabinet must remain clear of blockages, which can interfere with effective operation.
- Collect and seal waste within the cabinet.
- Disinfect the exterior of items before their removal from the biosafety cabinet.

Training and demonstration ideas that stress the importance of adherence to safe working precautions in a biological safety cabinet are provided in Box 5.

Sample Standard Operating Procedure for an ABSL-2 Research Experiment

1. Transport to the animal room all necessary supplies that are not already stored there. Disinfect the exterior of all materials before bringing them into the room to avoid the accidental transfer of adventitious agents. For the transport of biohazardous material for inoculation of research animals:
   a. Wear gloves that adequately cover the wrist and a lab coat with tight-fitting cuffs. Face protection is necessary to prevent any contact with chemicals, such as the standard disinfectants used in the lab.
   b. Use a biosafety cabinet for preparing the sample for transport.
   c. Seal the primary container and wipe its exterior with disinfectant.
   d. Place the primary sample container inside a secondary leak-proof plastic container labeled with the biohazard symbol. Also label the exterior of the container with your contact information, the biosafety level, and name of the agent (unless restricted by institutional policy).
   e. Wipe the exterior container with disinfectant before transfer.
2. Remove your lab coat and place it on a hook inside the BSL-2 laboratory. Remove your gloves and place them in the biomedical waste receptacle. Remove your face protection.
3. Wash your hands with soap and water for 30 seconds. If a hands-free sink is not available, leave the water running while you wash your hands. Dry your hands with paper towels and use them to cover the faucet handles when you turn off the water to minimize the possibility of contaminating your hands.
4. Collect any supplies that you will need for the experiment, including syringes and needles. It is safer to load a syringe inside a biosafety cabinet in the animal facility than to transfer loaded syringes and needles from the laboratory.
5. Transfer the sealed leak-proof containers and your supplies to the animal facility.
6. Don the ensemble of protective clothing required for entry to the ABSL-2 room. This usually consists of a lab coat or gown, gloves, and face protection. Additional protective clothing, such as booties, may be required at your institution.
7. Always check the door for new postings and the lab biohazard door sign for updated entry requirements.
8. Once inside the room, turn on the biological safety cabinet and disinfect all interior surfaces.
   a. Set up your work area in the biosafety cabinet to facilitate your experiment. Generally you will work from a clean (supply) side to a dirty (waste) side, with the work area in the middle. Keep the front and rear grilles of the biosafety cabinet clear of obstruction. Place a sharps container in the biosafety cabinet for prompt disposal of used sharps. Also place a small biohazard bag in the biosafety cabinet for the collection of soft waste (e.g., used wipes, disposable gloves, and other contaminated materials that will not puncture the bag).

Box 5 Biological safety cabinet trainer's corner

The following two demonstration exercises can help animal handlers understand that a biosafety cabinet is only a partial containment barrier (because of the long opening across the face of the cabinet) and that it is very sensitive to interference.

1. Bring a tray of dry ice into the room and place it on a cart or chair in front of the biosafety cabinet. Add warm water to the dry ice to form more vapor. Once a visible vapor stream is flowing into the cabinet, walk back and forth across the front of the cabinet behind the tray. The turbulence created by walking past should disrupt the inward flow of dry ice vapor to the biosafety cabinet. This exercise emphasizes the importance of minimizing activity behind the operator.
2. The average airflow into the front of a Class II biosafety cabinet is 100 FPM (feet per minute). This sounds impressive, but it is actually slower than most people walk. To demonstrate the rate of airflow into the cabinet, bring a tape measure and a watch with a second hand to your class. Have two trainees mark off 25 feet and stand at each end. Start at one end and take 15 seconds to walk off the distance. Practice this so you can walk at an even pace and hit 25 feet right at the 15-second mark. You and your trainees will be amazed at how slow it seems. This is a great take-home exercise to get the group focused on how easily other factors—such as the movement of arms in and out of the cabinet, the location of supply air diffusers, and foot traffic behind the operator—can interfere with safety and sterility.

b. The placement of everything inside the biosafety cabinet, including waste receptacles, helps minimize disruption of the protective curtain of air that flows into the front grille. Moving hands in and out of the cabinet can create turbulence that can allow contaminants in or out of the cabinet.

9. Transfer the animal cage to the biosafety cabinet.
10. Remove a new syringe from its packaging and load the desired amount of the agent into the syringe. Remove a needle from its packaging and place it on the end of the syringe. Remove the cap from the needle and place the loaded needle/syringe inside a clean, open 50 ml Falcon tube on the work surface. Place the cap in the sharps container.
11. Open one or two alcohol wipes and set them down in the clean work area.
12. Open the animal cage and carefully collect and restrain one mouse to prepare for inoculation. (Chemical or physical restraint can be used if the handler is not proficient with the live restraint technique.)
13. Wipe the inoculation site with an alcohol wipe and put the used wipe in the biohazard bag.
14. Remove the loaded syringe from the Falcon tube with your free hand and carefully inoculate the animal. Discard the needle and syringe as an intact unit directly into the sharps container in the biosafety cabinet if you are done using the needle. If you are inoculating another animal, place the needle/syringe back inside the Falcon tube to prevent accidental puncture.
15. Wipe the inoculation site with an alcohol wipe to disinfect any leakage of the agent after the injection. Put the used wipe in the biohazard bag.
16. Place the animal back in the cage.
17. Repeat this procedure for the remaining animals in the cage.
18. When you have completed the animal work, disinfect all exterior surfaces of the cage (including the bottom) with an appropriate disinfectant.
19. Allow sufficient contact time for disinfection before removing the cage from the biosafety cabinet.
20. Because the exterior of the gloves is likely contaminated (e.g., by aerosols, droplets), they should also be sprayed with disinfectant. Note that bleach and alcohol degrade latex gloves and thus limit their effectiveness (Klein et al. 1990). It is therefore essential to use gloves that will hold up to the disinfectant; nitrile gloves, for example, can be washed with bleach and alcohol. Alternatively, some researchers double glove and then discard the outer pair of gloves in the biohazard bag in the biosafety cabinet before exiting.
21. Place the cage back on the holding rack in the animal room.
22. Spray or wipe all surfaces in the biosafety cabinet—sides, rear wall, inside the front view screen, back grille, work surface, and front grille—with disinfectant. Also disinfect any items that will remain in the biosafety cabinet, such as the sharps container or disinfectant bottle.
23. Put used wipes in the biohazard bag and seal with tape. Spray the outside of the bag with disinfectant, allow sufficient contact time, then remove from the biosafety cabinet and place in the biological waste receptacle for the room. (Disinfect your gloves as noted in step #20.) The fact that steam cannot penetrate sealed bags may prolong the autoclave treatment time; ensure that there is sufficient moisture in the bag to generate steam, otherwise you may need to add water to the bag before sealing it to ensure that its interior reaches the appropriate temperature for the minimum time during the run (Vesley et al. 2000).
24. Remove your gown, then gloves (taking care not to touch the outside of your gloves with your bare hands during removal). Remove your face protection last so you won't risk touching your face with gloved hands.
25. Wash hands with soap and water as described in step #3.

Risk Assessment and Risk Management Resources

Regulatory and advisory groups all over the world have established criteria and published risk group definitions and assignments for a variety of biological and infectious agents on the basis of hazard level. The American Biological Safety Association (ABSA) has compiled a summary of published risk group assignments on its website (www.absa.org, under Biosafety Resources: Risk Group Classifications for Infectious Agents). These risk groups, from the low risk level of 1 to Risk Group 4 (for agents that represent a grave risk to an individual and a high risk to the community), are an excellent starting point for determining the risk potential of a particular microorganism.

With respect to risk management, the US Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established containment recommendations categorized in four biosafety levels (BSLs) that correlate with the risk groups (Wilson and Chosewood 2007). The 5th edition of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (Wilson and Chosewood 2007) provides a comprehensive framework for laboratory and animal research at each of the four levels (biosafety and animal biosafety levels, respectively) of containment. Another excellent resource for risk assessment and risk management are the Material Safety Data Sheets (MSDS) developed by the Public Health Agency of Canada, Office of Laboratory Security (available at the ABSA website under Biosafety Resources).

Concluding Thoughts

The success of any containment program in controlling contamination of laboratory rodents or in protecting workers

from exposure to potential biohazards usually hinges on how well the primary messages are disseminated and retained by employees. Periodic repetition of the core training themes ensures the continued success of the program. Supervisors, managers, safety officers, and others responsible for training animal handlers must also verify retention and proficiency in the desired practices.

The challenges in training and retraining are to identify the most important messages to communicate and to develop a program that involves trainee interaction and thus provides direct experience. Trainee participation (as opposed to traditional screen- or slide-based teaching methods) also enhances retention. In addition, to ensure the retention of core messages, programs should be broken down into blocks of information.

The continued growth in the number of individuals working with animals, participatory training sessions, and the breakdown of information into blocks all require smaller group sizes, more sessions, and, ideally, a concomitant growth in the number of trainers. As most trainers have multiple job opportunities, a future challenge will be to develop a sufficient number of competent trainers to meet the increasing demand that lies ahead.

For further information about the protection of handlers and laboratory animals, readers may wish to consult *Occupational Health and Safety in the Care and Use of Research Animals* (NRC 1997) and the *Guide for the Care and Use of Laboratory Animals* (NRC 1996).

Acknowledgments and Additional Resources

Dr. Diane Fleming, one of the founding members of the American Biological Safety Association (ABSA), gifted teacher, mentor for many, and tireless advocate for the use of safe microbiological work practices, quoted from a sermon that "we are all warmed by fires started by another." This perfectly describes how many of us learn biological safety given the few academic programs that focus on this important field. ABSA has helped to keep the "fire of learning" going for its members, those involved in the profession, and anyone with an interest in biosafety. The ABSA website (www.absa.org) is an excellent starting point for resources and educational opportunities related to the profession.

Another of ABSA's founding members, Dr. Jonathan Richmond, former Director of the Office of Health and Safety at the US Centers for Disease Control and Prevention, has fed the fire through training and mentoring, and by establishing a Biennial International Symposium on Biosafety that has helped to advance the profession. He will also be forever linked to *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), which he helped to advance through two editions. This text is the primary reference document for biosafety officers and is referred to as the "biosafety bible." Dr. Richmond has also served as principal editor for ABSA's Biosafety Anthologies. The latest in this series, *ABSA Anthology X: Animal Biosafety*, is another principal resource for health and safety professionals.

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