Chapter 30

BIOSAFETY

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INTRODUCTION

Biosafety

Biological safety, or "biosafety," is the application of concepts pertaining to risk assessment, engineering technology, personal protective equipment (PPE), policies, training, and preventive medicine to promote safe laboratory practices, procedures, and the proper use of containment equipment and facilities. In biomedicine, laboratory workers apply these tenets to prevent laboratory-acquired infections and the release of pathogenic organisms into the environment. A biohazard is defined as any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa); parasite; vector; biological toxin; infectious substance; or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance that is capable of causing the following:

- death, disease, or other biological malfunction in humans, animals, plants, or other living organisms;
- deleterious alteration of the environment; or
- an adverse impact on commerce or trade agreements.

These hazardous agents may be handled safely through careful integration of accepted microbiological practices and primary and secondary containments of potential biohazards.

Primary containment involves placing a barrier at the level of the hazard, confining the material to protect laboratory personnel and the immediate laboratory environment by adhering to prudent laboratory practices and appropriate use of engineering controls. Examples of primary containment include biological safety cabinets (BSCs), ventilated animal cages, and associated equipment. Secondary containment involves protecting the environment external to the laboratory from exposure to infectious or biohazardous materials through facility design and operational practices.

Combinations of laboratory practices, containment equipment, and special laboratory design are used to achieve different levels of physical containment. The current terminology in the United States is "biosafety level" (BSL), though historically, the designation "P" was used to indicate the level of physical containment (such as P-1 through P-4).[1] BSL is used in *Biosafety in Microbiological and Biomedical Laboratories*,[1] which focuses on protecting laboratory employees. Biosafety level may also be abbreviated "BL," which is used in Appendix G of the National Institutes of Health (NIH) publication Guidelines for Research Involving Recombinant DNA Molecules (also known as the NIH Guidelines).[2] However, Appendix G of the NIH Guidelines focuses primarily on physical containment involving work with recombinant deoxyribonucleic acid (DNA) molecules and organisms and viruses containing recombinant DNA molecules.

There are four levels of biosafety (designated 1 through 4) that define the parameters of containment necessary to protect personnel and the environment.[1] BSL-1 is the least restrictive, whereas BSL-4 requires a special containment or maximum containment laboratory facility. Positive-pressure encapsulating suits (PPES), primarily manufactured by ILC Dover (ILC Dover LP, Frederica, DE) or Honeywell Safety Products (Smithfield, RI), or gas-tight Class III BSC systems are used in a maximum containment (BSL-4) laboratory environment. Biosafety is not possible without proper and extensive training. The principal investigator or laboratory supervisor is responsible for providing or arranging for appropriate training of all personnel within the laboratory to maintain and sustain a safe working environment.

Evolution of Biosafety

Steps to limit the spread of infection have been practiced in the field of biomedicine since human illness was associated with infectious microorganisms and biologically derived toxins. However, Fort Detrick (in Frederick, MD) is considered the birthplace (beginning in the 1940s) of modern biosafety as a discrete discipline. During the early years of biosafety, safer working practices, principles, and engineering controls were developed,[3,4] as individuals conducting biomedical research commonly became infected with the organism being studied. As the hazard of working with organisms increased, so did the need to protect laboratory personnel conducting the research. Contributions to the field of biosafety were a direct result of the innovations and extensive experiences of Fort Detrick personnel who worked with a variety of infectious microorganisms and biological toxins. Dr Arnold Wedum, director of industrial health and safety at Fort Detrick—and regarded by many as the father of the US biosafety profession—promoted the attitude that biosafety should be an integral part of biomedical research.[5]

To enhance worker safety and environmental protection, Wedum[4] promoted use of the following:

- class III gas-tight BSC;
- noninfectious microorganisms in recombinant DNA research;
- P-4 (today's BSL-4) principles, practices, and PPES facilities when working with potentially aerosol-transmitted zoonotic microorganisms (eg, those causing tularemia and Q fever if a class III cabinet system was not available); and
- vaccination or immunization of laboratory workers.

Another safety enhancement was demonstrating and publicizing the importance of prohibiting mouth pipetting for fluid transfers involving hazardous material.[6,7] Dr Emmett Barkley[8] reiterated the hazard of oral pipetting, which should not be practiced in the laboratory. Barkley was chief of the Safety Division of the National Cancer Institute (Bethesda, MD) and subsequently director of research safety at NIH when the NIH Guidelines were developed and adopted. He was instrumental in developing physical containment parameters for recombinant DNA research.[9]

Critical to the advancement of modern biosafety was the development of air filtration technology. During the early 1940s, the US Army Chemical Warfare Service Laboratories (Edgewood, MD) studied the composition of filter paper captured from German gas mask canisters in search of better smoke filters. These early studies resulted in the design of collective protection filter units for use at the particulate-removal stage by a combined chemical, biological, and radiological purification unit of the US armed services. In the late 1940s, the Atomic Energy Commission (precursor of the Nuclear Regulatory Commission) adopted this type of filter to confine airborne radioactive particles in the exhaust ventilation systems of experimental reactors and in other areas of nuclear research. Subsequently, Arthur D Little Company, Incorporated (Boston, MA) and the US Naval Research Laboratory (Washington, DC) developed a prototype glass-fiber filter paper. Eventually, thin, corrugated, aluminum-alloy separators replaced the original asbestos, thermoplastics, and resin-treated papers. Throughout this development period, military specifications were developed and implemented to ensure the safe operating and optimal conditions of filters,[10] ultimately leading to the production of high-efficiency particulate air (HEPA) filters, which are used today in a variety of engineering controls, as well as in laboratory heating, ventilation, and air conditioning systems.

HEPA filters are constructed of paper-thin sheets of borosilicate medium that are pleated to increase their surface area. The borosilicate sheets are tightly pleated over aluminum separators for added stability and affixed to a frame.[10] A BSC first developed in 1964 for a pharmaceutical company used HEPA filter technology to provide clean air in the work area and containment as the primary barrier placed at the source of hazardous powders. Subsequent research led to the development of a class II, type A BSC that was delivered to the National Cancer Institute by the Baker Company (Sanford, ME).[11] The National Cancer Institute also developed a specification for the first class II, type B console BSC. HEPA filters have been proven to be effective, economical, and reliable devices for removing radioactive and nonradioactive particulate aerosols at a high rate of collection frequency.[10]

Operation and retention efficiency of HEPA filters has been documented. Three mechanisms account for the collection (retention) of particles within HEPA filters:

1. Small particles ranging from 0.01 to 0.2 µm in diameter are collected in a HEPA filter by diffusion and are retained at an efficiency approaching 100%.
2. Particles in the respirable range (those of a size that may be inhaled and retained in the lungs, 0.5 to 5.0 µm in diameter) are retained in a HEPA filter by a combination of impaction and interception at an efficiency approaching 100%.
3. Particles with an intermediate size range (between 0.2 and 0.5 µm in diameter) are retained by a combination of diffusion and impaction.

The HEPA filter is least efficient at retaining particles with a diameter of 0.3 µm, with a minimum collection efficiency of 99.97%. Hence, a standard test of HEPA filter efficiency uses a generated aerosol of particles that are 0.3 µm in diameter; to pass the test, the HEPA filter must retain 99.97% of the particles.[12]

All the air exhausted from BSCs, within which infectious materials must be manipulated, is directed through a HEPA filter before recirculation to a laboratory room or discharge to the outside environment through the building exhaust system. Therefore, in addition to adherence to rigorous work practice controls, HEPA filtration of laboratory exhaust air provides an extra margin of safety for workers, the laboratory areas, and the outside environment.

RISK GROUPS AND BIOSAFETY LEVELS

Risk Groups

Agents infectious to humans, including those used in research, are placed into risk groups based on the danger they pose to human health. Risk group assignment helps researchers determine the containment condition (or BSL) appropriate for handling a particular agent (Table 30-1). Multiple schemes for assigning risk groups have been developed. The NIH Guidelines, Health Canada (Ottawa, Ontario, Canada),[13] other nations, and the World Health Organization (Geneva, Switzerland)[14] all have risk group paradigms. The World Health Organization has categorized infectious agents and biological toxins into four risk groups. These risk groups relate to, but do not equate to, the BSLs of laboratories designed to work with organisms in each risk group.[14] Risk group 1 (no or low individual and community risk) comprises microorganisms including *Escherichia coli* K12 and *Candida albicans* that are unlikely to cause human or animal disease in healthy adult individuals. Risk group 2 (moderate individual risk, low community risk) includes pathogens that can cause human or animal disease, but are unlikely to be serious hazards to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available, and the risk of infection spreading is limited. An example is a causative agent of viral hepatitis, Hepatitis B virus, in humans and animals. Risk group 3 (high individual risk, low community risk) includes pathogens that usually cause serious human or animal disease, but do not ordinarily spread from one infected individual to another efficiently. Effective treatment and preventive measures are likely available. An example is the causative agent of tularemia, *Francisella tularensis*, in humans and animals. Risk group 4 (high individual and community risk) pathogens usually cause serious human or animal disease and can be readily transmitted from one individual to another, either directly or indirectly. Effective treatment and preventive measures are not normally available. Examples include Variola virus, Ebola virus, Lassa fever virus, and Marburg fever virus.

Assigning Agents to Risk Groups

It is important to understand how microorganisms are placed in risk groups and how that knowledge is used to develop procedures and physical infrastructure to contain these agents. The following criteria must be considered to assess risk while working in a laboratory or animal environment with a specific microorganism.

- **Number of past laboratory infections**. The most frequent laboratory-associated infections in humans are caused by the Brucella species. Extra caution must be taken when working with this agent because of its low

TABLE 30-1

RELATIONSHIPS OF RISK GROUPS, BIOSAFETY LEVELS, PRACTICES, AND EQUIPMENT

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic: BSL-1	Basic teaching; research	Good microbiological techniques	None; open bench work
2	Basic: BSL-2	Primary health services; diagnostic services; research	Universal precautions plus protective clothing and biohazard sign	Open bench plus BSC for potential aerosols
3	Containment: BSL-3	Special diagnostic services; research	As level 2 plus controlled access, double door entry, special clothing, and directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment: BSL-4	Dangerous pathogens; research	As level 3 plus airlock entry, shower exit and special waste disposal	Class III BSC, or positive-pressure protective suits in conjunction with class II BSCs, double-door autoclave (through the wall), and filtered air

BSC: biological safety cabinet
BSL: biosafety level

TABLE 30-2

CASE-FATALITY RATE BY DISEASE

Disease (Untreated)	Organism	Case-Fatality Rate
Plague, bubonic	*Yersinia pestis*	50%–60%
Cholera	*Vibrio cholerae*	50% or more
Tularemia, pulmonary	*Francisella tularensis*	30%–60%
Anthrax, cutaneous	*Bacillus anthracis*	5%–20%
Tularemia, typhoidal	*Francisella tularensis*	5%–15%
Brucellosis	*Brucella* species	2% or less
Q fever	*Coxiella burnetii*	1%–2.4%

infectious dose for humans. About 10 to 100 organisms can cause an infection in a susceptible human host.[15]

- **Natural mortality rate**. The natural mortality or case-fatality rate of diseases varies widely (Table 30-2).[15]
- **Human infectious dose**. Working with an organism having a low infectious dose for humans will place the laboratory worker at a greater risk than working with an organism having a higher infectious dose. The infectious dose of organisms for humans varies and is also dependent on the immunological competency of the host (Table 30-3). Although the literature contains information about the potential infectious dose for humans as extrapolated from animal data (see Table 30-3), an attempt to provide quantitative human infectious doses is not possible.[16]

TABLE 30-3

HUMAN INFECTIOUS DOSE BY ORGANISM

Organism	Infectious Dose	Route of Exposure
Vibrio cholerae	108	Ingestion[1]
Yersinia pestis	100–20,000	Inhalation[2]
Bacillus anthracis	~ 1,300	Inhalation[3]
Brucella species	10–500	Inhalation[2]
Francisella tularensis	10	Inhalation[4]
Coxiella burnetii	1	Inhalation[5]

Data sources: (1) Sack DA, Sack RB, Nair GB, Siddique AK. Cholera. *Lancet*. 2004;363:223–233. (2) Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*. 1997;278:399–411. (3) Dull PM, Wilson KE, Kournikakis B, et al. *Bacillus anthracis* aerosolization associated with a contaminated mail sorting machine. *Emerg Infect Dis*. 2002;8:1044–1047. (4) Jones RM, Nicas M, Hubbard A, Sylvester MD, Reingold A. The infectious dose of *Francisella tularensis* (tularemia). *Appl Biosafety*. 2005;10:227–239. (5) Jones RM, Nicas N, Hubbard A, Reingold A. The infectious dose of *Coxiella burnetti* (Q-fever). *Appl Biosafety.* 2006;11:32–41.

- **Efficacy of vaccination and treatment (if available)**. Vaccines are available for some of the agents studied within the laboratory. Receiving a vaccination must be based on a risk assessment. Only those individuals who are considered at risk should be offered the vaccination. However, the potential risk of the adverse effects from the vaccination might outweigh the risk of acquiring an infection. In addition, a vaccination might not provide 100% protection; an overwhelming infectious dose can overcome the protective capacity of a vaccination. Therefore, a vaccination should be considered only as an adjunct to safety, not as a substitute for safety and prudent practices. Treatment (chemoprophylaxis) in the form of antibiotic therapy may also be available to treat illnesses caused by many of the microorganisms being manipulated in the laboratory, specifically by the bacterial and rickettsial agents. It is necessary to determine the antibiotic sensitivity and resistance pattern (antibiogram) of the agent under investigation. The rationale is that treatment will be known in advance if an inadvertent laboratory exposure occurs. Treatment for exposure to a virus might be problematic because only symptomatic treatment may be available. There are few available antiviral agents that may be effective for postexposure prophylaxis. Specific antiviral agents include the following:
  - Rabies: rabies immune globulin for passive therapy, followed by the human diploid cell rabies vaccine or rabies vaccine, adsorbed for active vaccination.
  - Macacine herpesvirus 1 (formerly Cercopithecine herpesvirus; B virus): valacyclovir hydrochloride (VALTREX; GlaxoSmithKline, Research Triangle Park, NC).
  - Arenaviridae and bunyaviridae (including the viruses that cause Lassa fever, Argentine hemorrhagic fever, and Crimean-Congo hemorrhagic fever): ribavirin. This material can be used under an investigational new drug protocol (in the United States)

only for empirical treatment of hemorrhagic fever virus patients while awaiting identification of the etiological agent.
  - Poxviridae (including the viruses that cause smallpox and monkeypox): in addition to the Dryvax (Wyeth Inc, Philadelphia, PA) vaccine variant derived from Vero cells, ACAM2000 (Acambis, Canton, MA), two small molecule poxvirus inhibitors are currently in clinical trials: the Cidofovir lipid conjugate CMX001 (Chimerix Pharmaceuticals, Durham, NC) and ST-246 (Tecovirimat; SIGA Technologies, New York, NY).[17–19]
  - Retroviridae (including human immunodeficiency virus): the latest highly active antiretroviral therapy recommendations for postexposure prophylaxis are available through the US Public Health Service.[20]
  - There are currently small-molecule therapeutics under development for treating potential filoviradae (including Marburg and Ebola viruses) infection, but no products have yet been approved by the US Food and Drug Administration for clinical use.[21]
  - Additional vaccines and antiviral treatments for flaviviradae and togaviradae infections are in varying stages of development or clinical trials. A US Food and Drug Administration licensed vaccine is available as a preventative treatment or prophylaxis against such agents as Japanese encephalitis virus and Yellow Fever virus, while other vaccines remain under investigational new drug status for Venezuelan equine encephalitis, Dengue fever, and tick-borne encephalitis virus.[22–25]
- **Extent to which infected animals transmit the disease**. This discussion involves the zoonotic diseases or diseases that can be transmitted from animals to humans. These diseases include, but are not limited to, the following:
  - those transmitted directly from animals to humans (eg, rabies);
  - those that can be acquired indirectly by humans through ingestion, inhalation, or contact with infected animal products, soil, water, or other environmental surfaces that have been contaminated with animal waste or a dead animal (eg, anthrax); and
  - a disease that has an animal reservoir, but requires a mosquito or other arthropod to transmit the disease to humans (eg, St Louis encephalitis virus and Rocky Mountain spotted fever).

  Exposure risks in laboratories that use animals may differ from the exposure risks encountered in microbiology laboratories. Within microbiology laboratories, hazardous conditions may arise from human activities or from equipment within the laboratory. In animal facilities, the animals themselves may create hazards for the laboratory workers via:
  - generation of infectious aerosols;
  - animal bites or scratches to the person handling the animal; and
  - shedding of infectious known or unknown zoonotic agents in animal secretions and excretions, contaminating the animal holding room, cage, bedding, equipment, or other fomites. For example, in addition to usual activities in the laboratory, handling materials contaminated with hantaviruses is a concern because viruses are spread as aerosols or dusts from rodent urine, droppings, or by direct contact with saliva through cuts or mucous membranes.
- **Stability of the agent**. An agent's (microorganism's) stability to environmental conditions and susceptibility or resistance to disinfectants results from its internal and external chemical composition. For instance, spores of the genus *Bacillus* are resistant to adverse environmental conditions and disinfectants in part because of the presence of dipicolinic acid (pyridine-2,6-dicarboxylic acid) in their spore coat. Dipicolinic acid plays a significant role in the survival of *Bacillus* spores exposed to wet heat and ultraviolet radiation.[26] Many viruses and bacteria are sensitive to environmental conditions and disinfectants because of the high lipid content in their outermost layer.

Biosafety Levels

BSLs are guidelines that have evolved to protect laboratory workers from biological hazards. They do not take into account additional hazards found within the laboratory, including chemical, physical, or radiological hazards. These guidelines are based on data from laboratory-acquired infections and on an understanding of the risks associated with various manipulations of many agents transmissible by different routes. These guidelines operate on the premise that safe work sites result from a combination of engineering controls, management policies, work practices and procedures, and, occasionally, medical interventions. The different BSLs developed for microbiological and biomedical laboratories provide increasing levels of personnel and environmental protection.[1] BSL descriptions comprise a combination of facilities, equipment, and procedures used to handle infectious agents to protect the laboratory worker, the environment, and the community. This combination is proportional to the potential hazard level (risk group) of a given infectious agent. Equipment serving as primary barriers includes but is not limited to BSCs, centrifuge safety cups, and containment animal caging. Facilities also consist of secondary barriers, such as self-closing/locking doors, hand-washing sinks, and unidirectional airflow from the least hazardous areas to the potentially most hazardous areas. Procedures consist of standard and special microbiological practices. Finally, PPE includes dedicated laboratory clothing and respiratory protection.

There are four BSLs described in *Biosafety in Microbiological and Biomedical Laboratories*.[1] These levels range from a basic level (BSL-1) through maximum containment (BSL-4). BSL-1 consists of facilities, equipment, and procedures suitable for work, with infectious agents of no known or of minimal potential hazard to healthy laboratory personnel. BSL-1 represents a basic level of containment that relies on standard microbiological practices, with no special primary or secondary barriers recommended, other than a sink for hand washing.

BSL-2 consists of facilities, equipment, and procedures applicable to clinical, diagnostic, or teaching laboratories; suitable for work involving indigenous moderate-risk infectious agents present in the community; and associated with human disease of varying severity for which vaccines or therapeutics are usually available.[1]

Primary hazards to personnel working with these agents are accidental percutaneous or mucous membrane exposures and ingestion of infectious materials. (Inhalation exposure to agents at the BSL-2 level is uncommon; the main risk with aerosol generation is potential contamination of the laboratory with infectious agents that can result in exposure through breaks in the skin, ingestion, or injury. Therefore, all aerosol-generating procedures should be performed in a BSC or other primary containment equipment, but respiratory protection to mitigate aerosol exposure is rarely recommended unless there are other circumstances involved.) BSL-2 differs from BSL-1 in five ways:

1. Laboratory personnel receive specific training in handling pathogenic agents.
2. Scientists experienced in handling specific agents direct the laboratory.
3. Access to the laboratory is limited when work is in progress.
4. A laboratory-specific biosafety manual is prepared or adopted.
5. Procedures capable of generating potentially infectious aerosols are conducted within class I or class II BSCs or other primary containment equipment. Personnel receive specific training in the proper use of primary containment equipment and adhere strictly to recommended microbiological practices.

BSL-3 includes facilities, equipment, and procedures applicable to clinical, diagnostic, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease, especially after inhalation exposure, and vaccines or therapeutics may be available.[1] Hazards to personnel working with these agents include autoinoculation, ingestion, and exposure to infectious aerosols. BSL-3 differs from BSL-2 in four ways:

1. At BSL-3, laboratory personnel receive more extensive training in handling potentially lethal pathogenic agents than the degree of training received at BSL-2.
2. All manipulations of infectious or toxin-containing materials are conducted within class II or class III BSCs or other primary containment equipment. Personnel are trained to use this safety equipment properly.
3. The laboratory has special engineering and design features that include access zones with two self-closing and locking doors, sealed penetrations or penetrations capable of being sealed, and directional airflow (from areas of low-hazard potential to areas of high-hazard potential). Laboratory personnel are trained to understand these special design features.
4. Only the laboratory director can approve a modification of these BSL-3 recommendations.

BSL-4 comprises facilities, equipment, and procedures required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease transmitted by the inhalation route and for which a vaccine or therapy are not usually available.[1] Hazards to personnel working with these agents include autoinoculation, mucous membrane or broken skin exposure to infectious droplets, and exposure to infectious aerosols. BSL-4 differs from BSL-3 in six ways:

1. Laboratory personnel receive specific and thorough training to handle extremely hazardous infectious agents. Their supervisors are competent scientists who are trained and experienced in working with these agents.
2. Laboratory personnel understand the function of primary and secondary barriers and laboratory design features. They are trained in standard and special microbiological practices and the proper use of primary containment equipment.
3. The laboratory director strictly controls access to the laboratory.
4. The laboratory is in a controlled area within a building, completely isolated from all other areas of the building, or is in a separate building.
5. All activities involving agent manipulation within the work areas of the laboratory are conducted within a class III BSC, or within a class I or class II BSC used in conjunction with a one-piece, positive-pressure protective suit that is ventilated by a life-support system.
6. The BSL-4 laboratory, or maximum containment laboratory, has special engineering and design features to prevent dissemination of microorganisms to the environment.

BIOSAFETY PROGRAM ELEMENTS REQUIRED FOR CONTAINMENT AND MAXIMUM CONTAINMENT LABORATORIES

Measures Taken in Research to Protect Laboratory Workers

Although BSL-3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, and production (large-scale) facilities, where work is done with indigenous or exotic agents with the potential for respiratory transmission and lethal infection, this section will emphasize BSL-3 research laboratories. BSL-4 practices, safety equipment, and facility design and construction apply to work in a reference diagnostic or research setting with dangerous and exotic agents that pose a high individual risk of life threatening disease. These agents may be transmitted by aerosol, and there may be no available vaccine or therapy. BSL-4 research facilities, both class III BSC laboratories and protective-suit laboratories, will be covered in this section. Due to the Biological and Toxin Weapons Convention of 1972, legitimate production (large-scale or greater than 10 L of culture) BSL-4 facilities do not currently exist (most BSL-4 operations are small scale only because of the working conditions inherent to a BSL-4 suit or cabinet laboratory; large-scale facilities would be used only in very special circumstances and do not exist in the United States).

Documenting Safety Procedures

A laboratory's biological safety program manual is a laboratory-specific guide that should include safety standards and standing operating procedures (SOPs), guidelines, and documents for the containment laboratory (see the Lawrence Berkeley National Laboratory's *Biological Safety Program Manual*[27] as an example). These safety SOPs identify the special hazards of the laboratory and the procedures to abate or mitigate the associated risk. SOPs or documents specify the following:

- laboratory entry and exit in detail;
- proper use of laboratory-specific safety equipment (eg, BSCs, sterilizers, pass boxes, and dunk tanks);
- decontamination procedures for the specific laboratory;
- maintenance of laboratory safety and maintenance-related records (access logs, drain flush logs, emergency deluge shower, and eyewash periodic test logs);
- floor plan with hand-wash sinks and all other safety features annotated;
- emergency and routine communication procedures for the specific laboratory; and
- laboratory and agent-specific training for all laboratory personnel.

A compilation of existing SOPs, specifying how a laboratory worker would access the SOPs (online, paper copy in a binder, or both) is suggested. To meet the specific training and proficiency requirement, trainers should provide documentation for standard safety and laboratory essential training, with specific additions for the laboratory that cover orientation for workers new to the laboratory and laboratory-unique procedures and operations. Trainers should consider including in the manual material safety data sheets for the chemicals used in the laboratory. Material safety data sheets for chemicals can be obtained from vendors' websites or from the institutional chemical hygiene officer.

Assessing Individual Risk

For each person working in a BSL-3 and BSL-4 research laboratory, a supervisor conducts a detailed, thorough, individually tailored job hazard analysis or workplace hazard analysis (risk assessment). During this analysis, each task the individual intends to perform within containment is evaluated in terms of its inherent risk (as described in Risk Groups and Biosafety Levels, above). Each task is considered in terms of a potential laboratory exposure to the infectious agent (and its associated toxins for toxin-producing [toxigenic] agents). Considerations include use of sharp instruments and animals that could potentially result in puncture injuries, operations that may generate infectious aerosols, and direct handling of infectious agents versus observing (auditing) others working with biological materials. The hazards, once identified, are mitigated, preferably by isolating operations that pose a risk within primary and secondary containment devices (barriers), by substituting unbreakable plastic laboratory vessels for glassware and blunt instruments for sharp instruments, and by chemically or physically immobilizing animals to prevent or reduce the risk of sudden or unpredictable behavior leading to bites and scratches. Once the risk assessment is written, this document is approved by the second-line supervisor and reviewed by both the biological safety officer and the occupational health physician for accuracy and completeness.

The preferred means to mitigate risk is by using engineering controls (eg, BSCs, chemical fume hoods, sealed centrifuge rotors, and safety cups) and partial containment caging for animals (eg, micro isolator cages; ventilated cage racks; and ventilated, negative-pressure, HEPA-filtered rigid cubicles or flexible isolators). Where the hazard cannot be eliminated by physical means, it can be managed by administrative controls that provide specific training on procedures. Examples of such procedures include disposing used injection needles without recapping them or using an approved, one-handed practice to recap needles, either the one-handed scoop technique or a one-handed technique using a recapping device (an engineering control that holds the cap in place). Specific training is provided to encourage workers to use safe methods and operations to prevent aerosol generation, skin and mucosal contact with infectious agents, and handling of sharps where they cannot be eliminated.

If the hazard cannot be eliminated by engineering or administrative controls, it may be mitigated by using PPE to protect against contact, as well as mucosal and respiratory exposure. Vaccinations, when available and where medically indicated, may serve as an adjunct to PPE, but never as a substitution for PPE. Once all the tasks an individual will perform have been assessed and all the infectious and toxic agents the individual will work with have been identified, the tasks and agents are recorded in a document that the worker and the supervisor prepare together. The mitigating controls are then chosen with input from safety professionals and occupational health and medical staff to form a collection of primary barriers, approved practices, PPE, and vaccinations. Based on an individual worker's current educational and experience levels and state of health, certain controls may not be feasible. High-risk tasks may have to be avoided, on a spectrum that may range from observing high-risk tasks (in-vivo work, such as manipulations of exposed animals) and performing low-risk tasks (in-vitro work with infected cell cultures in a BSC), to the extreme that the individual may not be granted access to the containment laboratory.

Physical Barriers

Primary barriers include class II and class III BSCs, PPESs, and containment animal housing. Class II BSCs are open-fronted cabinets with HEPA filtered laminar airflow. Class II type A1 and type A2 cabinets may exhaust HEPA-filtered air back into the laboratory or may exhaust the air to the environment through an exhaust canopy. Class II type B1 cabinets have HEPA-filtered down-flow air composed of uncontaminated, recirculated in-flow air (30%) and exhaust most (70%) of the contaminated air through a dedicated duct with a HEPA filter to the atmosphere. Class II type B2 (total exhaust) cabinets exhaust all in-flow and down-flow air to the atmosphere after passing through a HEPA filter located in a dedicated exhaust duct. To verify proper operation, all class II BSCs must be field certified in accordance with National Sanitation Foundation International Standard/American National Standard for Biosafety Cabinetry Class II (Laminar Flow) Biosafety Cabinetry Standard 49[28] on initial installation, at least annually thereafter, or after every major repair or relocation of the cabinet.[1,29,30] It is recommended that accredited certifiers be engaged for provision of class II BSC certification and repair service. Class II cabinets may be used in BSL-3 laboratories, when supplemented by use of PPE (gloves, gowns, and respiratory protection when warranted by a risk assessment), and may be used in BSL-4 laboratories in conjunction with wearing a one-piece, positive-pressure, ventilated suit with a life-support system, an in-line HEPA or high-purity filter, and supplied with grade D breathing air.[31]

When working within a class II BSC, the equipment and materials are arranged in a clean-to-dirty layout, with clean materials in the center of the workspace, potentially contaminated materials at one end of the workspace within the cabinet, and potentially contaminated waste materials at the other end of the workspace.[32] Class III cabinets are totally enclosed, ventilated, gas-tight cabinets. They provide the highest level of product, personal, and environmental protection against respiratory exposure to infectious or toxic aerosols and are most suitable for work in BSL-3 and BSL-4 laboratories. Operations are conducted using shoulder-length gloves or half-suits connected to the cabinets. Air is supplied to the class III cabinet through a HEPA filter, and air exhausted from the cabinet to the atmosphere passes through two HEPA filters in series (or one HEPA filter and an exhaust air incinerator). Materials are removed from the cabinet by passing them through an interlocked, double-door sterilizer or through a chemical dunk tank filled with an appropriate disinfectant for the infectious agents or toxins in use at BSL-4, but some class III cabinets interlock with a class II BSC for removal at BSL-3. Several class III cabinets, housing a refrigerator, cell culture incubator, centrifuge, or aerosol-generating equipment, may be connected in a cabinet line as an integrated system for use in a BSL-3 laboratory or in a BSL-4 cabinet laboratory. A complete change of clothing is required for workers, including a dedicated laboratory scrub suit, jumpsuit or gown, shoes, and examination gloves for hand protection in case of a puncture or if a pinhole develops in the cabinet shoulder-length gloves, or half-suits.

Primary barriers for animal housing include the following: (*a*) micro isolator cages with filter tops for rodents; (*b*) ventilated rodent cage racks; (*c*) ventilated, negative-pressure, HEPA-filtered cubicles; (*d*) ventilated, negative-pressure, HEPA-filtered flexible film isolators; and (*e*) rigid, ventilated, negative-pressure, HEPA-filtered isolation cages.[32] Rigid, ventilated, negative-pressure, HEPA-filtered, mobile animal transport carts have been developed at US Army Medical Research Institute of Infectious Diseases to isolate animals during transfer between containment animal facilities.[33] Other primary containment devices include ventilated, filtered enclosures for continuous flow centrifuges and use of sealed rotors and centrifuge safety cups in conventional centrifuges. Primary containment devices used in necropsy rooms include downdraft necropsy tables, specially designed class II cabinets for conducting necropsies, and HEPA-filtered vacuum shrouds for oscillating bone saws.

Personal Protective Equipment

In BSL-3 containment, laboratory workers wear protective clothing, such as solid-front or wraparound gowns, scrub suits, or coveralls. This protective clothing is not to be worn outside the laboratory. To aid in enforcement of this rule, laboratory clothing may be color-coded, so that it can be readily identified if worn outside the laboratory. Scrub suits are typically two-piece ensembles composed of trousers and tunics. Tunics with long sleeves that terminate in knit wrist cuffs aid in donning protective gloves. Gloves are drawn over the cuffs and may be secured in place using tape. Long-sleeved tunics are favored over short-sleeved tunics because long sleeves with gloves taped to the sleeves can provide a physical barrier to protect the skin of the wrists and arms from potential exposure to infectious agents, including bacterial spores.[33] Disposable clothing should not be reused. Reusable clothing is decontaminated, usually by autoclaving, before being laundered to prevent an exposure hazard to laundry workers.[30,34] Clothing is changed when overtly contaminated or after every work session, depending on facility policy. The wearing of dedicated laboratory shoes or safety shoes may be required in BSL-3 facilities. Otherwise, disposable shoe covers should be worn. Wearing dedicated laboratory socks provides comfort to the feet and extra skin protection to exposed ankles if trousers are not long enough to cover the legs fully. Not all biocontainment facilities in the United States require workers to have a change of clothes. If a clothing change is required, dedicated socks and shoes are indicated. In the absence of a clothing change requirement, the dedicated shoes and socks may not be used in lieu of shoe covers, coveralls, or no additional PPE, depending on a risk assessment.

Protective gloves must be worn when handling infectious materials, animals, and contaminated material. Gloves are selected to meet the needs of the risk assessment. Nitrile or latex gloves may be appropriate if they provide the worker with protection from the infectious agent being handled. However, gloves manufactured from other materials (eg, neoprene [DuPont Performance Elastomers LLC, Wilmington, DE], butyl rubber, and Hypalon [DuPont Performance Elastomers LLC]) may be indicated to protect against exposure to other contaminated materials, such as toxins, organic solvents, and caustics, or to serve as an alternative to personnel who may have allergic reactions or sensitivities to latex or nitrile. Gloves should be changed frequently, followed by thorough hand washing. Disposable gloves should never be reused. To ensure protection when working with highly hazardous materials, double gloving (wearing two pairs of gloves) should be practiced, with the inner glove taped to the wrist cuff to minimize potential contamination. If the outer glove is punctured or torn, the protective skin barrier should still be maintained by the inner glove if it was not breached (provision of redundant protection). If working with contaminated sharps (eg, needles, scalpels, glass slides, capillary tubes, pipettes) or with infected animals that may bite or scratch, laboratory workers should consider wearing cut-resistant over-gloves (eg, Kevlar [EI Du Pont de Nemours and

Company, Wilmington, DE]; armored, stainless-steel mesh; or leather gloves) for additional protection.[35] If working with materials where there is a splash hazard, the use of safety goggles or face shields and head covers (bonnets, caps, hood) may be indicated, unless the individuals are using a full-face respirator, such as a powered air-purifying respirator (PAPR).

When entering rooms housing infected animals, additional PPE (wraparound gowns or Tyvek [DuPont Tyvek, Richmond, VA] coveralls, foot covers or boots, head covers, eye and respiratory protection, etc) is required. These PPE requirements will be indicated on the warning sign posted on the door of the animal's cage. Respiratory protection is provided by using properly fitted respirators approved by the National Institute of Occupational Safety and Health (NIOSH).[36] Surgical masks or nuisance dust masks do not meet the NIOSH definition of a respirator. NIOSH-approved respiratory protection systems are commonly used in BSL-3 laboratories and animal rooms when the respiratory hazard cannot be completely engineered out through the use of primary containment devices. Respirators used to filter particulates are classified into three series, corresponding to resistance to oil mist particles: (1) *N*, or least resistant, (2) *P*, or partially resistant, and (3) *R*, or resistant. They are further differentiated based on their efficiency at removal of 0.3 μm aerosol particles, similar to HEPA filters (95%, 99%, and 99.97% or –100).[37] Useful and comfortable negative-pressure respirators include disposable N-100 filtering face pieces with integral exhalation valves and tight-fitting, half-face, negative-pressure respirators fitted with N-100 particulate filters. These respirators have an assigned protection factor of 10, meaning there are 10-fold fewer particulates at the breathing zone inside the respirator than outside the respirator, providing the respirator is properly fitted and worn. A properly fitted and worn full-face piece, negative-pressure respirator has an assigned protection factor of 50 to 100 and also provides eye protection. All users of respirators must be enrolled in a respiratory protection program in accordance with the Occupational Safety and Health Administration (OSHA) Respiratory Protection Standard.[31] Users of tight-fitting respirators must be fit tested annually or when significant physical changes occur (weight gain or loss) using an approved qualitative or quantitative fit test. Wearers of tight-fitting respirators must not have facial hair that could interfere with the fit of the respirator, nor should eyeglasses interfere with the tight seal. Users of full-face, tight-fitting respirators who wear eyeglasses will need special optical inserts that may be worn inside the respirator face piece. Individuals fit tested for respirators must ensure that they only use respirators that they have been trained and certified to use during annual fit testing.

When working in a BSL-3 environment, such as a room housing infected animals in open cages or a necropsy room equipped with a downdraft table and an oscillating bone saw, greater respiratory protection might be needed. A NIOSH-approved PAPR with a loose-fitting hood or a tight-fitting full-face piece is often used and provides an assigned protection factor of 1,000. Benefits of wearing a loose-fitting hood include comfort, no requirement for fit testing, and amenability to use by individuals with facial hair. Reusable turbo blowers for PAPRs are powered by rechargeable batteries. The blowers may be equipped with N-100 particulate filters or with combination cartridges that incorporate a particulate filter with activated charcoal or other chemical absorbent for use in atmospheres of between 19.5% and 23.5% oxygen that have contaminated particulates and low levels of organic or other specified chemical vapors. The airflow in cubic feet per minute, with cartridges installed, must be checked with a flow gauge before each work session. Because there are no OSHA standards or end-of-service life indicators for particulate filters when used with infectious agents, institutes have to develop local criteria for determining when to replace particulate filters. As a complete protective ensemble, PAPRs with loose-fitting hoods may be worn in conjunction with Tyvek suits or long-sleeved scrub suits, gloves, laboratory socks, and shoes with shoe covers or over-boots. All NIOSH-approved respirators are approved as a complete system, so components cannot be switched between different manufacturers' products without negating the approval. For example, a NIOSH-approved PAPR system consists of the turbo blower unit, battery, belt, hose, filters or cartridges, and loose-fitting hood or tight-fitting face piece, all assembled and marketed by the manufacturer as a complete system. Only approved, compatible replacement components from the same manufacturer may be used with a given respiratory protection system.

To be approved to use a respirator, a user must be medically cleared based on a health history questionnaire and a pulmonary function test or other relevant medical examinations on a case-by-case basis; be enrolled in an employer-provided OSHA-compliant respiratory protection program[31]; receive initial and annual training on the use of the assigned respirator or additional training when a different type of respirator is assigned; and undergo annual fit testing for negative-pressure, tight-fitting respirators. Proper fit testing procedures are available in Appendix A of the OHSA Respiratory Protection Standard.[31]

In a class III BSC operation (BSL-4 cabinet laboratory), personnel must remove all personal clothing and undergarments and shoes. Complete laboratory clothing, including undergarments, pants, shirts, shoes, and gloves, is provided and worn by laboratory workers.[1] Workers wear nitrile or latex examination gloves for extra protection when working in class III BSCs, just in case the shoulder-length box gloves develop pinholes, punctures, or tears.

In BSL-4 protective suit laboratories and BSL-4 animal facilities, personnel must remove all personal clothing, including undergarments, socks, shoes, and jewelry. Personnel at USAMRIID may ask for an exemption for wedding bands, but only eyeglasses in addition to exempted wedding bands may be worn in the BSL-4 suites. Complete laboratory clothing, including undergarments, pants, shirts, jumpsuits, socks, and gloves, is provided for, and used by, laboratory workers. Workers don a fully encapsulating positive-pressure protective suit supported by an umbilical-supplied air system. It is common practice in BSL-4 laboratories for individuals to periodically verify PPES integrity prior to donning by taping the exhaust valves of the suit and inflating it to a set pressure point. This test is performed at USAMRIID at a minimum when the individuals change their gloves on a weekly basis, but practices vary at other BSL-4 facilities. In addition, annual pressure decay testing is conducted at USAMRIID on all PPES used in the BSL-4 laboratory. The suit can be fitted with integral protective over-boots or with legs terminating in soft booties. If a suit of the latter design is used, the worker dons protective over-boots inside the BSL-4 suit facility, after passing through an airlock equipped with a decontaminating chemical suit shower. When not in use, protective over-boots are stored inside the BSL-4 facility. As of this writing, PPES for use in a BSL-4 environment are not federally regulated by OSHA as level A chemical suits or as respirators, and such suits are not currently NIOSH approved. However, the compressor and filter system must provide minimum grade D breathing air to the PPES.[31]

Medical Surveillance

Medical surveillance, if indicated, may comprise baseline and periodic (usually annual) studies, including the following:

- complete medical history,
- urinalysis,
- hematology (complete blood count),
- serum chemistry panel,
- serum protective antibody titers for specific disease agents,
- physical examination, and
- ancillary studies.

Ancillary studies can include the following:

- periodic chest radiograph,
- periodic electrocardiogram,
- annual audiogram,
- annual visual acuity testing,
- annual evaluation of respiratory capacity, and
- mental fitness, neurological examinations, and random testing for illicit substance use (as needed).

An effective occupational health program benefits both the employee and the employer and may reduce time lost to injuries. This occupational health program will comply with OSHA and other applicable federal and state laws and regulations. Medical surveillance is a critical part of a comprehensive occupational health and safety program. An occupational health and safety program has the following objectives[38]:

- protect workers against health and safety hazards in the work environment;
- properly place workers according to their physical, mental, and emotional abilities;
- maintain a pleasant, healthy work environment;
- establish preplacement examinations;
- establish regular, periodic health examinations (medical surveillance);
- diagnose and treat occupational injuries, exposures, and diseases;
- consult with the worker's personal physician, with the worker's consent, regarding other related health problems;
- provide health education and counseling for workers;
- provide safety education for workers;
- identify hazardous situations or find the means to prevent or mitigate hazardous situations; and
- establish surveys and studies of the industrial environment to protect workers, their families, and the community.

Laboratory workers employed in a BSL-4 suit facility are enrolled in a medical surveillance program, and they should be medically evaluated for fitness to use a PPES. At USAMRIID, workers in the BSL-4 suit laboratories are enrolled in a hearing protection program. When the 8-hour, time-weighted average level is 85 dB (decibels) or greater, workers must be enrolled in an employer-provided hearing protection program to comply with OSHA regulations.[39] The program requires employees to undergo initial baseline and annual surveillance audiometry, fitting, and training to use hearing protectors (ear plugs or muffs).

Personnel are required to receive initial familiarization training on how to wear the protective suit, as well as receive extensive, documented, tailored training provided by an assigned mentor before being considered proficient to work independently in BSL-4 containment. Currently, USAMRIID enrolls new personnel who plan on working in BSL-4 containment in a specialized 3-day training course teaching them the fundamentals of the BSL-4 environment, suit use, entry and exit procedures, movement and dexterity exercises, and emergency response. Once employees complete the basic training course, they are then mentored for a set period of time before they may apply for independent access to BSL-4. After demonstrating proficiency, laboratory workers can begin independent work in the BSL-4 containment suite.

During normal operations in the BSL-4 containment suite, workers may disconnect briefly from the breathing air supply to move about and then couple to an airline in a new location within the suite. One manufacturer advises that up to a 5-minute residual air supply may remain in the suit if there is an unanticipated loss or interruption of the breathing air supply.[40] In regular operations, it is prudent not to remain disconnected from the air supply for more than 2 or 3 minutes, because the carbon dioxide concentration and humidity level will quickly rise within the suit space. It is recommended as a best practice to remain connected to the air supply as much as possible when completing work tasks in BSL-4. Generally, the visor fogs ups before the carbon dioxide concentration builds up to a hazardous level, thus prompting the user to connect to the air supply expeditiously.

It is important that personnel are fit for the physical challenges of working in a BSL-4 PPES laboratory. An ongoing medical surveillance program ensures that, in the event of occupational exposure to an infectious agent or toxin, the medical needs of the worker will be met immediately. If a laboratory worker should become ill without obvious exposure to an agent, the individual will be assessed to determine whether the illness is related to an unknown laboratory exposure.

At USAMRIID, all potential biological exposures are assessed through the combined efforts of the Safety, Radiation, and Environment Division; personnel supervisors; and the Medical Division. All employees are instructed to notify the Safety, Radiation, and Environment Division of any mishaps occurring either inside or outside containment suites. For mishaps in the containment suites, all personnel involved in the incident are instructed to report to the Medical Division in the absence of a life-threatening emergency for an initial briefing. The initial briefing is conducted with the affected personnel, supervisors, a safety representative, and the competent medical authority. After the briefing, initial exposure and disease risk are determined and postexposure prophylaxis is administered (as determined by subject matter experts).[41,42] In the event of a medical emergency or potential exposure in BSL-4 containment, the Department of Defense currently has a memorandum of agreement with the Department of Health and Human Services for potential exposure monitoring, care, and treatment of inpatients enrolled as clinical research subjects. Local arrangements are made for laboratories outside the United States.

Protecting the Community and the Environment

Secondary barriers are the elements of laboratory facility design and construction that (*a*) contribute to protection of laboratory personnel, (*b*) provide a barrier to protect persons outside the laboratory, and (*c*) protect persons and animals in the community from infectious agents in the event of an accidental release within the laboratory.[1] Secondary barriers in BSL-3 containment facilities at USAMRIID include entry vestibules or personnel airlocks that feature two self-closing and lockable doors, clothes change rooms and shower facilities based on a risk assessment, and a hand-washing sink in each laboratory room. The sink is located near the room exit door and can be operated using foot pedals or knee or elbow paddles, or is automatically activated by an infrared sensor. Other secondary barriers include floor, wall, and ceiling finishes constructed for easy cleaning and decontamination; sealed penetrations in floors, walls, and ceilings; and sealable openings to facilitate decontamination. Laboratory furniture has waterproof and chemical-resistant bench tops, and chairs are covered with nonfabric material to permit easy decontamination. An autoclave is available in the facility. The facility is equipped with a ducted exhaust ventilation system that creates inward directional airflow from areas of lower potential hazard to areas of higher potential hazard (negative-pressure gradient) without recirculation of air or airflow reversals under failure scenarios.[1] To confirm inward airflow, a visual monitoring device (eg, a magnehelic differential pressure gauge [Dwyer Instruments, Michigan City, IN]; photohelic gauge [Dwyer Instruments, Michigan City, IN]; rodimeter; or "telltail") should be available at the laboratory entry.

In animal biosafety level 3 (ABSL-3) facilities, room fittings and ventilation should be in accordance with the Institute of Laboratory Animal Resources Commission on Life Sciences and National Research Council's *Guide for Care and Use of Laboratory Animals*[43] and

Biosafety in Microbiological and Biomedical Laboratories.[1] If the ABSL-3 facility has floor drains, the drain traps are always filled with an appropriate disinfectant. Additional environmental protection design features (enhancements) in BSL-3 laboratories and animal-holding spaces (including provision of personnel showers and effluent decontamination, HEPA filtration of exhaust air, and containment of piped services) may be indicated, depending on the nature of the infectious agents to be used (eg, arboviruses, highly pathogenic influenza viruses and high-consequence animal pathogens); the risk assessment or maximum credible event analysis of the site (eg, laboratory to be located in a highly populated urban center or in a remote region having a low-density population); and applicable federal, state, and local regulations.

Secondary barriers required in BSL-4 laboratories and ABSL-4 animal-holding spaces are all those specified for BSL-3 laboratories and ABSL-3 animal holding spaces, with additional provisions. Other required secondary barriers include a dedicated, non-recirculating ventilation system with supply and exhaust components balanced to ensure directional airflow from areas of lower potential hazard to areas of higher potential hazard. HEPA filtration of supply air and double HEPA filtration of exhaust air, with redundancy (backup exhaust duct with fan and in-line double HEPA filters), are also required, as is alarm and daily monitoring to prevent positive pressurization of the laboratory or animal-holding space. In large, complex operations, a supervisory control and data acquisition system (also known as a building automation system) may be installed to monitor and control room pressures automatically. An automatically starting emergency power source (usually a diesel-powered generator) is required as a minimum for the redundant exhaust ventilation systems, redundant life-support (breathing air) systems, alarms, lighting, entry and exit controls, and BSCs. Laboratories using PPES are required to have primary and backup breathing air compressors along with a secondary breathing air system capable of supporting the egress of all personnel in the BSL-4 suites in the event of a breathing air compressor failure. In practice, the freezers and other laboratory equipment (incubators and refrigerators) are generally also on circuits that can switch to emergency backup power. Other infrastructure elements that contribute to the secondary barrier include change rooms, personnel showers, effluent decontamination by a proven method (preferably heat treatment), and containment of piped services. Floor and sink drain traps must be kept filled with an appropriate disinfectant (one with proven efficacy for the microorganisms handled within the BSL-4 facility). An autoclave with two interlocked doors, with the outer door sealed to the outer wall (a so-called "bioseal"), is required at the containment barrier. The autoclave is automatically controlled so the outer door cannot be opened until a sterilization cycle has been completed. A dunk tank, fumigation chamber, or a ventilated equipment airlock is also provided so materials may pass into the containment area. Materials that cannot be steam sterilized may be safely decontaminated either through a fumigation cycle in a ventilated airlock or by passage through the chemical shower cycle or dunk tank and removed from the containment area. The walls, floors, and ceilings are constructed as a sealed internal shell (the containment envelope) capable of being decontaminated using a fumigant. Bench tops have seamless surfaces impervious to water, resistant to chemicals, and free of sharp edges. Appropriate electronic communications are provided between the BSL-4 containment area and the noncontainment area, which may include a telephone, facsimile, two-way radio, intercom, and a computer system on a local area network or wireless network. BSL-4 protective suit laboratories also have a dedicated area for storing suits and boots, and a double-door personnel airlock equipped with a chemical shower for surface decontamination of protective suits. Animal-holding rooms need to meet the standards specified in the *Guide for Care and Use of Laboratory Animals*.[43] Containment operational parameters are inspected and verified daily before work is initiated in the BSL-4 facility.

Solid and Liquid Waste Inactivation and Disposal

The US Environmental Protection Agency (EPA) defines antimicrobial pesticides as substances or mixtures of substances used to destroy or suppress the growth of harmful microorganisms (eg, bacteria, viruses, or fungi) on inanimate objects and surfaces. Public health antimicrobial products are intended to control microorganisms infectious to humans in any inanimate environment. These products include sterilizers (sporicides) and disinfectants.[44] Sterilizers (sporicides) are used to destroy or eliminate all forms of microbial life, including fungi, viruses, and all forms of bacteria and their spores. Sterilization is widely used in hospitals for infection control. Types of sterilizers include steam under pressure (autoclaves), dry-heat ovens, low-temperature gas (ethylene oxide), and liquid chemical sterilants. All types of sterilizers are also applicable for use in microbiological and biomedical laboratories. In laboratories, autoclaving is used to prepare sterile instruments, equipment, and microbiological nutrient media and to render microbiologically contaminated liquid and solid waste sterile before it enters the waste disposal stream. Laboratory glassware is dried, sterilized, and depyrogenated (rendered free of endogenous pyrogens) in dry-heat ovens. Ethylene oxide sterilization is used to sterilize materials such as delicate instruments and laboratory notebooks, which cannot withstand steam sterilization, but is seldom used to sterilize solid waste. Liquid sterilants, used to sterilize delicate instruments by immersion and to sterilize impervious surfaces by surface application, can be added to suspensions of infectious materials to chemically inactivate them. Disinfectants, according to the EPA, are used on hard inanimate surfaces and objects to destroy or irreversibly inactivate infectious fungi and bacteria, but not necessarily their spores. The EPA divides disinfectant products into two major types: hospital and general use. Hospital disinfectants are most critical to infection control in hospitals and are used on medical and dental instruments and on hospital environmental surfaces. General disinfectants are products used in households, swimming pools, and water purifiers.

The decision about the type of biological inactivation required depends on a number of factors; the type of biological agent requiring inactivation along with whether the agent is present in a large amount of organic material can initially narrow the choice. In some cases where either large spills or large amounts of organic material are present, a detergent solution may be used prior to disinfectant to enhance efficacy at the site of cleanup. Other variables, including but not limited to pH, temperature, type of materials requiring biological inactivation (eg, neoprene, metals, or plastics), age of disinfectant, humidity, concentration, and contact time requirements can also affect the choice of inactivation method.

An example of a liquid sterilant-disinfectant is Alcide EXSPORE (Alcide Corporation, Redmond, WA) 4:1:1 base concentrate (1.52% sodium chlorite; EPA Registration No. 45631-3), which comes with a separate activator concentrate (9.5% lactic acid) as a set. This sterilant-disinfectant must be freshly prepared by diluting the base with water per the manufacturer's instructions before adding activator to generate chlorine dioxide.[45] The prepared sterilant-disinfectant should be used immediately and must be freshly prepared daily.

An example of a hospital disinfectant is Micro-Chem Plus (National Chemical Laboratories, Inc, Philadelphia, PA; EPA Registration No. 1839-95-2296), a proprietary mixture of two quaternary ammonium compounds and inert ingredients that is labeled to kill listed microorganisms (specified viruses, fungi, and nonspore-forming bacteria) when mixed at the rate of 2 ounces of the concentrated product per gallon of water.[46]

An example of a general disinfectant used at USAMRIID is Clorox Ultra Germicidal Bleach (The Clorox Company, Oakland, CA; 6.15% sodium hypochlorite [5.84% available chlorine]; EPA Registration No. 67619-8). When mixed at the rate of 12 ounces per gallon of water (5,000 ppm), it is labeled to kill listed microorganisms (specified viruses, fungi, and nonspore-forming bacteria).[47] Bleach is not registered by the EPA as a sterilant. During the subsequent cleaning and decontamination of spore-contaminated postal facilities after the 2001 anthrax-by-mail incidents, the EPA issued crisis exemptions on a case-by-case basis to use bleach for emergency decontamination subject to adherence with specified conditions of application (see http://www.epa.gov/pesticides/factsheets/chemicals/bleachfactsheet.htm). Bleach solutions (1:10 dilution) are now sold premixed, as standard 1:10 dilutions must be made fresh daily. Clorox disinfecting wipes (EPA Registration No. 5813-79) also come ready-made with an extended shelf life compared to fresh 1:10 solutions. Wipes may be used in the laboratory or in the field with the same efficacy as fresh daily 1:10 solutions.

In BSL-4 laboratories and in BSL-3 and ABSL-3 facilities, if indicated by the risk assessment, liquid effluent (laboratory sewage) must be inactivated by a proven process, generally heat treatment under pressure.[1] Effluent decontamination systems are available as six different types. Systems may be batch-based chemical systems using peracetic acid, sodium hypochlorite, quaternary ammonium compounds, or chlorine dioxide. Heat treatment effluent decontamination systems may be either continuous flow or batch process and can run at high temperature (>121°C) or sub-boiling temperatures. Batch process effluent decontamination systems models can also be designed to run as a heat treatment system augmented by chemicals depending on the demands of the laboratory. Solids suspended in the liquid waste are comminuted (finely ground). The effluent is heated to specified temperature and held at that temperature for a certain period of time. Then it is cooled, sampled for sterility testing, and released to a municipal or nonpublic sewer system. The time–temperature relationship for the selected process depends on the inactivation profile of the infectious microorganisms that could be present in the liquid waste. The current process at Fort Detrick holds the heated effluent at 132°C (270°F) for a minimum of 12 minutes, sufficient to inactivate fungal and bacterial spores. The standard liquid biowaste process used at the Canadian Science Centre for Human and Animal Health (Winnipeg, Manitoba, Canada) heats the effluent to 121°C (250°F) for a 30-minute holding time, but has the capability of achieving a temperature as high as 141°C (286°F).[48] The standard process is sufficient to inactivate fungal and bacterial spores. The higher temperature is available, if needed, to inactivate prions (heat-resistant infectious proteins).[49]

Animal carcasses exposed to biological agents inside BSL-3 or BSL-4 laboratories require decontamination prior to removal from the containment suites. Per SOP, carcasses are bagged and decontaminated with the appropriate disinfectant prior to autoclaving. The autoclaved carcasses can be incinerated subsequently or disposed of in accordance with applicable local, state, and federal regulations. Animal carcasses can also be inactivated with an alkaline hydrolysis-based tissue digester. Digesters can be run either at boiling or sub-boiling temperatures using potassium hydroxide or sodium hydroxide alkali for a set cycle time. Cycle time depends on weight, composition, and surface area of animal carcasses, percent of alkali, temperature, and amount of water.[50–52]

After infectious materials have been inactivated by an appropriate method of sterilization or disinfection, they may be removed from the laboratory and disposed of in accordance with applicable federal, state, and local regulations. In the United States, disposal of several categories of solid waste (regulated medical waste, perceived medical waste, and pathological waste) is regulated at the state level. Many states have strict regulations that require that such waste be sterilized and rendered unrecognizable (by processes such as incineration, shredding, or grinding with steam sterilizing or irradiating) before final disposal in a sanitary landfill.

Standard and Special Microbiological Practices

Standard and special microbiological practices universal to all BSLs are as follows:

- The laboratory director limits or restricts access to the laboratory when experiments are in progress.
- A biohazard sign may be posted at the entrance of the BSL-1 laboratory if infectious agents are present or stored in the laboratory. A biohazard sign is posted at the entrance of BSL-2, BSL-3, and BSL-4 laboratories and animal rooms when infectious agents are present.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of aerosols.
- Work surfaces are decontaminated at least once daily and after any spill of viable material.
- All infectious waste is decontaminated by an approved process (eg, autoclaving before disposal).
- A pest (insect and rodent) control program must be in effect.
- Personnel wash their hands after handling viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, taking medication, and storing food for human consumption in the laboratory or animal-holding facility are not permitted. If contact lenses are worn in the laboratory or animal-holding area, goggles or a face shield should also be worn. Personnel should refrain from applying cosmetics or lip balm, chewing gum, and taking oral medications while in the laboratory or animal-holding facility.
- Mouth pipetting is prohibited. Only mechanical pipetting devices are to be used.

There are no special practices for the BSL-1 laboratory. The following special practices apply to BSL-2, BSL-3, and BSL-4 laboratories, as well as to ABSL-2, ABSL-3, and ABSL-4 animal-holding areas:

- Secure all laboratories registered for select agents and toxins.[53] Keep BSL-2 and BSL-3 laboratory room doors closed when working with infectious agents. Keep doors in BSL-4 laboratories and in ABSL-2, ABSL-3, and ABSL-4 animal-holding areas closed and locked.
- Only individuals advised of the potential hazards who meet specific entry requirements may enter the laboratory or animal-holding room.
- In ABSL-2, ABSL-3, and ABSL-4 animal-holding facilities, the Institutional Animal Care and Use Committee and the Institutional Biosafety Committee approve special policies and procedures.
- Along with the biohazard sign, post the following information at the entrance to the laboratory or animal-holding room: the agents in use, the BSL, required vaccinations, any PPE required, the name and phone number of the principal investigator, and any procedures required to exit the laboratory or animal-holding room.
- At-risk individuals entering the laboratory or animal-holding room may receive appropriate vaccinations if available for the agents being handled or agents potentially present in the room.

- A tuberculosis skin test or other tuberculosis surveillance procedures are indicated on an annual basis if personnel are working with or around nonhuman primates.
- Describe biosafety procedures for BSL-2 and ABSL-2 facilities in SOPs. Describe biosafety procedures for BSL-3 and BSL-4 laboratories and ABSL-3 and ABSL-4 animal-holding facilities in a biological safety manual specific to the laboratory or animal-holding facility. Advise personnel of the specific hazards, require them to read and ensure they understand the manual, and make certain that they comply with it.
- The laboratory director must ensure that laboratory and support personnel receive appropriate initial training and annual training, and additional training on potential hazards in the laboratory or animal facility; precautions to take to prevent exposures; and procedures on evaluating potential exposures. The laboratory director is also responsible for ensuring that the previously described training is appropriately documented.
- Use caution with needles and syringes. In BSL-3 and BSL-4 laboratories and in ABSL-3 and ABSL-4 animal-holding facilities, use only needle-locking syringes or disposable syringe–needle systems in which the needle is integral to the syringe. Also consider tools that allow for one-handed recapping of syringe needles or systems without needles. Dispose of used sharps in conveniently located puncture-resistant containers.
- Place all potentially infectious materials in covered, leak-proof containers during collection, manipulation, storage, transport, or shipping. Place viable material to be removed from a class III BSC or a BSL-4 facility in an unbreakable, sealed primary container that is enclosed in an unbreakable, sealed secondary container. Pass this enclosed material through a chemical disinfectant dunk tank, fumigation chamber, or airlock with a chemical suit shower (in the case of a BSL-4 suit facility).
- Decontaminate work surfaces and laboratory equipment with an effective disinfectant routinely, after work with infectious materials is completed, and after any spills. Contaminated equipment must be appropriately decontaminated before repair or maintenance or packaging for transport.
- Immediately report to the laboratory director (supervisor) any spill or accident that results in exposure to infectious materials. Institute medical evaluation, surveillance, and treatment as appropriate and document this medical care in writing. In BSL-3 and BSL-4 containment facilities, develop and post spill procedures and conduct drills on an annual basis. Professional staff or other appropriately trained personnel must decontaminate, contain, and clean up any spill of infectious material. In BSL-4 containment, establish practical and effective protocols for emergency situations, including the evacuation of incapacitated staff.
- Animals and plants unrelated to the work conducted are not permitted in the laboratory.
- In BSL-3 and BSL-4 containment facilities, the laboratory director must ensure that all personnel are proficient in standard microbiological practices, laboratory-specific practices, and operations before they begin work with microorganisms.
- In BSL-3 and BSL-4 containment facilities, conduct open manipulations of infectious agents in BSCs or other primary containment devices. Conducting work in open vessels on the open bench is prohibited. Vessels with tight-fitting covers (gasketed caps, O-ring seals) should be used to hold viable cultures within water baths and shaking incubators. Use sealed rotors or centrifuge safety containers fitted with O-ring seals to contain centrifuge tubes. Use plastic-backed paper towels on nonperforated surfaces to facilitate cleanup. Use plastic vessels in place of glass vessels.
- In BSL-3 and BSL-4 containment facilities, autoclave or decontaminate all materials other than materials to be retained in a viable state before removing them.
- At BSL-4, maintain a physical or electronic log of all personnel, with the time of each person's laboratory entry and exit recorded. This requirement also applies to all personnel who have access to areas in which select agents and toxins are used or stored.[53]
- In BSL-4 containment (and in BSL-3 containment, if indicated by risk assessment, site-specific conditions, or applicable regulations), enter and exit the laboratory only through the clothing change and shower rooms. Remove and leave personal clothing in the outer change room. Change completely into laboratory clothing. On exiting the laboratory, remove and leave all laboratory clothing in the inner change room. Take a decontaminating (soap and water) personal wet shower for a minimum of 3 minutes on exit from the laboratory.

Autoclave soiled laboratory clothing before laundering. Use the equipment airlock to enter or exit the laboratory only in an emergency.
- Bring supplies and materials into the BSL-4 facility through the double-door autoclave, fumigation chamber, or equipment airlock, which is decontaminated before and after each use. Secure the airlock outer door before the inner door is opened. Secure the airlock inner door after materials are brought into the facility.
- In BSL-4 containment, institutes are required to establish a system to report laboratory accidents and exposures, employee absenteeism, and medical surveillance of a potential laboratory-acquired illness.[1]
- Make available a facility for quarantine, isolation, and medical care of personnel who work in BSL-4 containment and who are affected with a potential or known laboratory-acquired illness.

ROLE OF MANAGEMENT IN A BIOSAFETY PROGRAM

Management must consider safety a top priority and, on a daily basis, work closely with and support safety personnel. Although management must provide a biosafety program as well as engineering features and equipment designed to reduce the risks associated with the research conducted at the institute, safety is also an individual responsibility. To illustrate this point (Figure 30-1), consider the mission or purpose of an institute as the hub of a wheel. All personnel, regardless of education, experience, or job description, are the spokes of the wheel and must be reminded regularly of the importance of their contributions to an institute. If one (or more) of the spokes is not functioning as designed, the wheel does not operate smoothly. Consequently, it takes longer to meet not only personal goals and objectives, but also institute goals and objectives. All personnel (each spoke of the wheel) in an institute must be considered important, regardless of their perceived contributions.

The goals of a biosafety program include the following: (*a*) prevention of injury, infection, and death of employees and the public; (*b*) prevention of environmental contamination; (*c*) conformance to prudent biosafety practices; and (*d*) compliance with federal, state, and local regulations and guidelines. The ultimate objective of these goals is to keep everyone healthy while supporting productive research. Appropriate personnel training is paramount. Both initial and refresher personnel training must address the institutional biological safety program and the elements of biosafety. Training can be conducted as a discussion rather than as a formal lecture to promote audience participation. This technique allows individuals to have ownership over policies through an integrated program of safety engineering, vaccination, health surveillance, and medical management of illness. Risk encompasses awareness, assessment (or evaluation), mitigation, and management of the risk. Communication is a fundamental part of risk assessment and training. The US government has developed a 5-step risk management process (Figure 30-2).[54] The five sequential steps of the risk management process include the following:

1. **Identify hazards**. What is the hazard?
2. **Assess hazards**. What is the danger of this hazard?
3. **Develop controls and make risk decision**. What controls can be used to remove this hazard, or make a decision to accept some risk?
4. **Implement controls**. Controls developed for the risk are implemented (or put into operation or practice).
5. **Supervise and evaluate**. After a period of evaluation as new data becomes available, the controls implemented are reviewed to determine whether they were adequate, or if additional controls must be added.

Figure 30-1. Institute personnel are depicted as the spokes of a wheel that work together to accomplish a common mission. *maintenance staff

The philosophy of a biosafety program is based on an early estimation of risk, followed by application of appropriate containment and protective measures.

Figure 30-2. Five steps of the risk management process. Adapted from: US Army Safety Center, Fort Detrick, MD.

It is very important to investigate and review safety incidents at the institute because presentation of this data will heighten the awareness of individuals that accidents do happen despite safeguards.

Laboratory Safety Audits

An audit is a methodical examination and review. In the present context, it is a systematic, critical review of laboratory safety features and procedures. The terms "survey" (comprehensive view) and "inspection" (a critical appraisal, description of some obvious hazards and how safety personnel try to minimize the risk of these hazards, an official examination, or checking or testing against established standards) are often used interchangeably with the term "audit." Safety personnel must emphasize that their role is to try to identify hazards, conduct risk assessments, develop risk management strategies, and evaluate the effectiveness of those strategies over time while minimizing impact on the research. Safety personnel must actively engage with and seek the help of all administrative and laboratory personnel in hazard identification. It is important for safety personnel to remain actively engaged with laboratory personnel outside of laboratory audits to minimize potential negative associations that may be encountered with inspections. It must be understood that a safety department cannot provide absolute safety, but strives to provide reasonable safety. Safety personnel advise, guide, provide limited training, and implement institute and regulatory policies (in conjunction with the institutional biosafety committee). The safety department, with continued support from management and all facility personnel, can minimize the risk of hazards by implementing institute and regulatory guidelines. During the laboratory safety audit, safety practices and equipment are evaluated. General safety, life safety, biological safety, chemical hygiene, and radiation safety are topics covered in a typical laboratory safety audit. Laboratory audits should be scheduled on a regular basis and may be announced or unannounced.

Self-audits of required safety practices provide a measure for achieving compliance with safety rules and regulations.[55] Designated safety specialists can conduct regular safety audits at quarterly intervals, accompanied by the laboratory supervisor and a facilities management representative. Deficiencies can be pointed out during the audit. Later, a written report with suggestions for corrective action may be sent to the laboratory supervisor. The supervisor reports progress on remediation to the safety specialist within a mutually agreed on, fixed-time period. Safety personnel should follow up on any deficiencies noted during a laboratory audit periodically to ensure laboratory personnel have taken the appropriate corrective actions. Support from higher management is essential for an audit to have the desired effect of improving employee safety, as well as instituting compliance with applicable regulations.[56]

Use of a checklist ensures a systematic, standardized audit, thus reducing the chance of missing critical items. Citing the pertinent requirement or applicable regulation on the checklist provides a ready reference and justification for each item listed on the checklist. Within the overall laboratory safety audit, the following list of biosafety elements should be covered[57]:

- autoclave repair and operational records where applicable,
- proper use of PPE,
- appropriate laboratory clothing,
- no food or drink in the laboratory,
- proper use of sharps and sharps disposal containers,
- decontamination of infectious materials before disposal,
- proper disposal of laboratory waste,
- proper signage (laboratory, equipment, materiel),
- current certification of BSCs and fume hoods, and
- use of in-line HEPA filters on laboratory vacuum outlets where applicable.

Additional biosafety elements audited at USAMRIID include: (*a*) weekly flushing floor and sink drains and recording the action in a drain flush log; (*b*) flushing the eyewash weekly and recording the action in an eyewash flush log; (*c*) testing (flushing and measuring the flow rate) the emergency deluge shower at least weekly and recording the action in an emergency shower test log; (*d*) recording during the audit differential pressures for laboratory rooms as displayed on the magnehelic and photohelic gauges; (*e*) checking documentation that emergency communication devices have been tested at least monthly; (*f*) testing and recording during the audit operating status of alarms, emergency lights, and emergency exit lights; and (*g*) spot checking laboratory SOPs, laboratory biosafety manuals, and laboratory personnel training records.

Four events that warrant conducting a formal, unscheduled audit of a laboratory include the following[57]:

1. accident or injury in the workplace,
2. follow-up to implementation of new biosafety regulations or procedures,
3. a new funding source requesting documentation of workplace safety, and
4. new infectious agents proposed for use in the laboratory.

An important time for evaluation of biosafety SOPs may be before a major outside organization or agency conducts a site visit.[57] Two examples of organizations conducting site visits are the Joint Commission on Accreditation of Healthcare Organizations and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Examples of agencies that conduct inspections of laboratories registered for select agents are the US Centers for Disease Control and Prevention and US Department of Agriculture Animal and Plant Health Inspection Service Select Agent Program Laboratory Inspection Programs. Laboratories that do not work on select agents may be subject to a US Department of Agriculture inspection for specific biological agents or an NIH Office of Biotechnology Activities audit if they have a functioning Institutional Biosafety Committee.[58] For subordinate laboratories of the US Army Medical Research and Materiel Command, safety office personnel conduct periodic safety site assistance visits.[30] For Department of Defense (DoD) research, development, test, and evaluation (RDTE) laboratories, the director of Army safety conducts biological defense safety evaluation site visits.[30]

In DoD RDTE facilities, health and safety professionals must conduct internal inspections (audits) of BSL-1 and BSL-2 laboratories at least quarterly and must conduct internal inspections of BSL-3 and BSL-4 laboratories at least monthly.[34] Inspections must be documented, deviations from safe practices recorded, and recommended corrective actions taken. If deviations are life threatening, access to the laboratory area is restricted until corrective actions have been taken. New RDTE efforts involving biological agents must be evaluated and inspected before startup. Any Department of the Army headquarters agency can recommend special studies or reviews when (*a*) conditions or practices that may affect safety have changed, (*b*) major system modifications to facility design and physical configuration are made, and (*c*) safety, health, and environmental protection standards and requirements have changed significantly.[30] Safety officials maintain safety inspection records for 3 years, and they review records annually to note trends that require corrective actions.[30] Laboratory supervisors review their work areas at least weekly and take any needed corrective actions promptly.

At USAMRIID, safety professionals assigned to the Office of Safety, Radiation and Environment conduct semiannual comprehensive inspections of BSL-1 and BSL-2 and quarterly inspections of BSL-3 and BSL-4 laboratories to identify potential problems. These quarterly inspections augment the monthly inspections conducted by laboratory suite supervisors or their designees. Inspections, which may be announced or unannounced, include coverage of general safety practices and safety practices specific to a particular BSL.[57]

Biological Defense Research Program Laboratories

All laboratories involved in DoD RDTE operations must comply with the Department of the Army Pamphlet, *Safety Standards for Microbiological and Biomedical Laboratories*.[34] These regulations specify safety policy, responsibilities, and procedures for military and contract laboratories conducting operations at BSL-2, BSL-3, and BSL-4 in support of the US military biological defense program. The DoD Biological Surety (Biosurety) Program is a new program implemented in DoD biological defense RDTE laboratories that use DoD-provided biological agents.[59] This biosurety program is patterned after existing nuclear and chemical surety programs, and its purpose is to ensure the safe and secure use of biological agents. The program encompasses physical security, biological safety, biological agent accountability, and personal reliability as measures to prevent unauthorized access to agents of bioterrorism (select agents).[59,60]

Laboratory Animal Care and Use Program

Federal animal welfare regulations[61–63] from the US Department of Agriculture Animal and Plant Health Inspection Service, state and local laws, and the Public Health Service Policy on Humane Care and Use of Animals[64] regulate the care and use of laboratory animals used in research. Many of the applicable regulations and policies are summarized in the *Guide for the Care and Use of Laboratory Animals*.[43] The responsible administrative official at each institution using laboratory research animals must appoint an Institutional Animal Care and Use Committee representative to oversee and evaluate the institution's animal program, procedures, and facilities on a semiannual basis to ensure they are consistent with the animal welfare regulations, Public Health Service policy (for those institutions that receive NIH funding), and recommendations specified in the guide.[43] The guide covers many aspects of an institutional animal care and use program, including the following:

- policies and responsibilities,
- monitoring care and use of animals,
- veterinary care,
- qualifications and training of personnel who work with animals, and
- occupational health and safety of personnel working with animals, physical facilities, and animal husbandry.

Under the heading of occupational health and safety, critical topics in an effective animal care and use program include the following:

- hazard identification and risk assessment;
- personnel training, hygiene, safe facilities, and procedures;
- health monitoring;
- animal experimentation involving biological and other hazardous agents;
- use of PPE;
- medical evaluation; and
- preventive medicine for personnel working with animals.

A voluntary program exists for the assessment and accreditation of institutional animal care and use programs. At the request of a given institution, the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) will send laboratory animal technical experts to the institution to conduct a site visit and evaluate all aspects of an institution's animal care and use program. If all aspects of the program meet the high standards of AAALAC, the institution may be granted the coveted designation "AAALAC accredited," which is effective for 3 years. Triennial renewals require a complete, comprehensive reassessment of an institution's animal care and use program. Accreditation by AAALAC is mandatory for DoD organizations and facilities maintaining animals for use in DoD programs.[64]

THE BIOSAFETY PROFESSION

Many biological safety professionals begin their careers as bench scientists in the biological sciences, particularly microbiology, or as professionals in medicine or the allied health sciences, and subsequently transfer into the biological safety field to work as biological safety officers, occupational health and safety managers or specialists, or in closely related positions. With the quickening tempo of biological defense research and the establishment of new, high (BSL-3 or BSL-4) biocontainment laboratories, the demand for competent biological safety professionals is increasing. Academic institutions and government agencies are beginning to recognize the need to establish didactic and practical training opportunities in biological safety. For example, the Division of Occupational Health and Safety and the National Institute of Allergy and Infectious Diseases of the NIH have jointly established a National Biosafety and Biocontainment Training Program offering 2-year postbaccalaureate and postdoctoral fellowships at the NIH campus in Bethesda, Maryland. This program specifically trains fellows to support BSL-3 and BSL-4 research environments by acquiring the necessary knowledge and skills to meet scientific, regulatory, biocontainment, biosafety, engineering, communications, management, and public-relations challenges associated with conducting research in such facilities.[65] Education is carried out through extensive mentorship and training in pertinent safety and regulatory guidelines within the 27 institutes and centers at the NIH-Bethesda campus. Second-year fellows then apply their knowledge through a series of developmental assignments at external facilities outside the NIH system to better develop a well-rounded understanding of prudent safety practices that they may apply after departing the fellowship. Examples of academic fellowship programs include the biosafety fellowship program at Washington University School of Medicine in St Louis, Missouri, or the 1-year internship program at the Great Lakes Regional Center for Excellence at the University of Chicago.

Credentialing biological safety professionals is not currently mandated or regulated. A formal, voluntary credentialing process exists to enable biological safety professionals to meet minimum set standards of expertise and proficiency. The American Biological Safety Association (ABSA), the national organization of biological safety professionals, has established two levels of credentialing: (1) the Registered Biosafety Professional (RBP) and (2) the Certified Biological

Safety Professional (CBSP). The RBP is an individual with a documented university education or specialized training in relevant biological safety disciplines who has submitted an application and has been found to be eligible for registration by the ABSA RBP Evaluation Review Panel.[66] The RBP has sufficient understanding of cell biology, pathogenic microbiology, molecular genetics, host immune responses, and concepts of infectious agent transmission to enable the RBP to apply safeguards when working with biohazardous materials.

The CBSP is an individual who has a combination of documented university education, specialized training, and experience in relevant biological safety disciplines, and has further demonstrated knowledge and proficiency by passing the Specialist Microbiologist in Biological Safety Microbiology examination administered by the National Registry of Certified Microbiologists of the American Society for Microbiology. Every 5 years, qualification as a specialist microbiologist may be renewed by submitting to the National Registry of Microbiologists evidence of acceptable continuing education credits or by retaking and passing the examination. The CBSP also participates in a certification maintenance program administered by ABSA in which the individual submits a certain number of acceptable certification maintenance points every 5 years to maintain certification.

SUMMARY

A successful biosafety program is based on an early estimation of risk and application of appropriate containment and protective measures. It is important to review safety incidents that occur in the institute, because these data will heighten individual awareness that accidents do happen despite implementing safeguards. The goals of a biosafety program are to:

- facilitate safe, productive research,
- prevent environmental contamination,
- conform to prudent biosafety practices, and
- comply with federal, state, local, and institutional regulations and guidelines.

To achieve the goals of the biosafety program, information pertaining to the program must be conveyed to the workforce, along with how it benefits the workforce. Presentation of concepts must be expressed in understandable terms. Initial and refresher training of personnel must address elements of biosafety and the institute's biological safety program. To promote audience attentiveness, participation, and retention of information, training is best conducted in an informal discussion format. Training success is gauged by how well the workforce collectively internalizes the biosafety program, as evaluated within the overall context of a positive safety culture that permeates all work attitudes and operations. Elements of a positive safety culture include the following[34]:

- applying (regularly) safety practices and using safety terms in the workplace;
- including safety practices in the employee's job description and performance appraisals;
- specifying and monitoring safe behaviors in the workplace;
- providing tangible rewards for promoting safety;
- articulating safety concerns in interactions with management, peers, and subordinates;
- emphasizing safety procedures when starting new tasks;
- briefing employees on safety procedures and the consequences of ignoring safety practices or engaging in unsafe behaviors;
- observing, reporting, and correcting hazards promptly;
- keeping staff up to date on regulatory and institutional changes; and
- using PPE appropriately (always).

Management must consider safety a top priority and work closely on a daily basis with safety professionals, who need their support on policies to be implemented. Management must provide a safety program, engineering features, and equipment designed to reduce research-associated risks in the institute. Biosafety professionals strive to provide reasonable assurance of biological safety, but cannot guarantee absolute safety. In the end, the success of the safety program depends on the employees themselves. Safety is as much an individual responsibility as any other assigned performance objective.

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