Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases

How to Safely Work with Animals in Containment Laboratories

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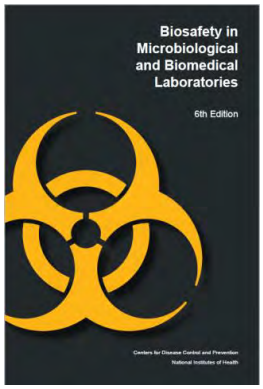


ChABSA Technical Seminar 3/15/22

Disclaimer

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Agenda

- Overview of select agent and toxins
- Review risk group classifications
- Discuss the biosafety program in containment labs
- Define containment levels
- Facility practices and procedures for different containment levels
- Working with animals in containment
- Disinfection

Select Agents and Toxins

"Select agents and toxins are biological pathogens or derivatives, respectively that have the potential to pose a severe threat to human, animal, or plant health."

Jonsson CB, Cole KS, Roy CJ, Perlin DS, Byrne G, members of the RBL-NBL Directors Network. Challenges and Practices in Building and Implementing Biosafety and Biosecurity Programs to Enable Basic and Translational Research with Select Agents. *Journal of bioterrorism & biodefense*. 2013;Suppl 3(15):12634-. doi:10.4172/2157-2526.S3-015.

Select Agents and Toxins

- **HHS Select Agents and Toxins**
  - Botulinum neurotoxins*
  - Coxiella burnetii
  - Ebola virus*
  - Francisella tularensis*
  - Marburg virus*
  - Monkeypox virus3
  - Ricin
  - Rickettsia prowazekii
  - SARS-associated coronavirus (SARS-CoV)
  - Staphylococcal enterotoxins A,B,C,D,E subtypes
  - Kyasanur Forest disease virus
  - Variola major virus (Smallpox virus)*
  - Yersinia pestis*
- **USDA Select Agents and Toxins**
  - African horse sickness virus
  - African swine fever virus
  - Avian influenza virus3
  - Classical swine fever virus
  - Foot-and-mouth disease virus*
  - Goat pox virus
  - Lumpy skin disease virus
  - Mycoplasma capricolum3
  - Mycoplasma mycoides3
  - Newcastle disease virus2,3
  - Peste des petits ruminants virus
  - Rinderpest virus*
  - Sheep pox virus
  - Swine vesicular disease virus

Risk Group Classification

Based on NIH and WHO Guidelines

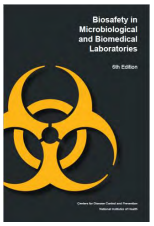
RG 1	RG 2	RG 3	RG 4
Agents that are not associated with disease in healthy adult humans	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

https://image.slideserve.com/309837/nih-guidelines-section-ii-l.jpg

Biosafety Program

A fundamental objective of any biosafety program is the containment of potentially hazardous biological agents and toxins.

- The term "containment" is used to describe safe methods for managing infectious materials in the laboratory environment where they are being handled or maintained.
- **The purpose of containment is to reduce or eliminate exposure** of laboratory workers, other persons, and the outside environment to potentially hazardous agents.
- A comprehensive biosafety risk assessment is a key component of a successful biosafety program and should be part of an all-hazards risk assessment; it should be conducted on a continual basis to address evolving risks within the laboratory environment. Detailed information on the biological risk assessment process is found in Section II of BMBL.



Final determination on the combination of containment measures required to address the relevant biosafety risk present at a facility should be based on a **comprehensive biosafety risk assessment**.

TABLE 27.1 A Matrix Commonly Used in the Risk Assessment Process When Determining the Level of Risk

	Probability of the event occurring				
Severity of the outcome	**Frequent**	**Likely**	**Occasional**	**Seldom**	**Unlikely**
Catastrophic	Extremely high	Extremely high	High	High	Moderate
Critical	Extremely high	High	High	Moderate	Low
Marginal	High	Moderate	Moderate	Low	Low
Negligible	Moderate	Low	Low	Low	Low

Fox, James G. *Laboratory animal medicine*. Elsevier, 2015. pg 1299

Primary Containment

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment.

- **The BSC is the standard device used to provide containment of hazardous biological agents and toxins when conducting microbiological activities.**
- Additional primary containment devices may include:
  - Sealed rotors and centrifuge safety cups which prevent aerosols, droplets, and leakage of hazardous biological agents and toxins that may result during centrifugation.
  - Sealed containers provide containment for transfers between laboratories.

Personal Protective Equipment

Personal protective equipment (PPE) helps protect the user's body from injury from a variety of sources (e.g., physical, electrical, heat, noise, chemical) or potential exposure to biological hazards and airborne particulate matter.

PPE is usually used in combination with other biosafety controls (e.g., BSCs, centrifuge safety cups, and small animal caging systems) that contain the hazardous biological agents and toxins, animals, or materials being handled.

In situations where a BSC cannot be used, **PPE may become the primary barrier between personnel and the hazardous biological agents and toxins**.

- Examples include fieldwork, resource-limited settings, certain animal studies, animal necropsy, and activities relating to operations, maintenance, service, or support of the laboratory facility.

Secondary Containment

Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices.

Such design features may include, but are not limited to the following:

- Ventilation strategies to ensure containment of the hazards;
- Effluent decontamination systems; and
- Specialized building/suite/laboratory configurations, including: controlled access zones to support the separation of the laboratory from office and public spaces;
- Anterooms; and
- Airlocks.

Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive-pressure suit[c]	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

a. Each successive BSL contains the recommendations of the preceding level(s) and the criteria in the cell.
b. Applies to Cabinet Laboratory
c. Applies to Suit Laboratory

BMBL 6th Ed. pg 68-69

Working with Animals in Containment

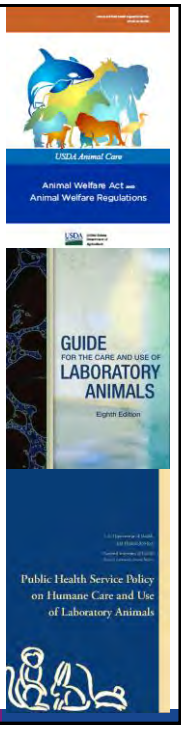
Considerations in Animal Model Selection

- Caging requirements
- Food and water
- Environmental Enrichment

Considerations in Animal Model Selection

Caging requirements

- Applicable laws and regulations in the Animal Welfare Act, the Guide, and PHS policy
- Ability to observe the animal
- Ease of sanitization
- Presence of a squeeze back mechanism
- The availability of battery backup and audible/visual alarms to alert when a cage rack is on battery backup
- Size



Considerations in Animal Model Selection

Food and water

- Feed and water that is brought into the containment area cannot be used for other studies or animals and is discarded.
- The feed and water must be free of agents that could compromise the study.

Considerations in Animal Model Selection

Environmental Enrichment

- AWA: The physical environment in primary enclosures **MUST** be enriched by providing means of expressing non-injurious species-typical activities.
- Species differences should be considered when determining the type or methods of enrichment.
- Examples of environmental enrichment include providing perches, swings, mirrors, and other increased cage complexities; providing objects to manipulate; varied food items; using foraging or task-oriented feeding methods; and providing human interaction *keeping in mind personnel safety precautions.*
- Environmental enrichment devices should be either durable, autoclavable materials or disposable materials.

Considerations in Animal Model Selection

Environmental Enrichment

Rodents

- Most commonly used animal in research
- Readily available
- Small size
- Breed variation
- Species behaviors

Non-Rodent Species

- Ferrets, rabbits, guinea pigs, unconventional species
- Vaccination status
- Serology status
- Group number
- Sex of the animals
- Specific pathogen free

Non-Human Primates

- Cynomolgous macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*)
- Full medical history
- Behavioral assessments
- Enrichment plans
- Group interactions

Agriculture Species

- Swine, goat, horses, others
- Secondary barriers may become primary barriers (BSL-3Ag)
- Waste removal
- Enrichment plans
- Size

Housing

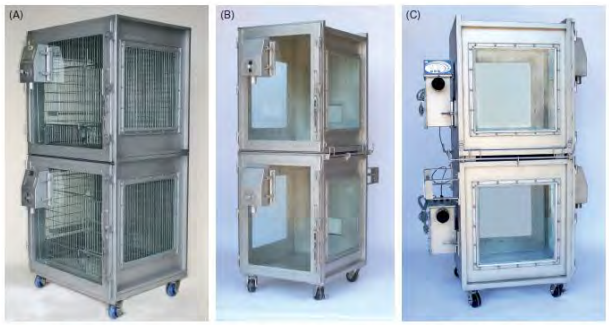


FIGURE 27.1 The primary containment cage shown is designed to house nonhuman primates. The clear Lexan® panels allow plentiful light penetration and a HEPA filtered air inlet box in the front of the cage (A) has a connection for a controlled warming system for animals recovering from anesthesia, or that otherwise become hypothermic. Cages are easily removed for cleaning the interior of the primary containment envelope (B). The blower motors and HEPA-filtered exhaust boxes are located on the back of each cage (C). *Photos provided courtesy of Carter Systems, Inc.*

Fox, James G. *Laboratory animal medicine*. Elsevier, 2015.

Housing

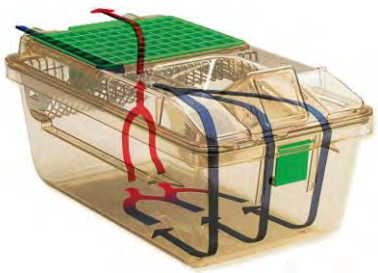


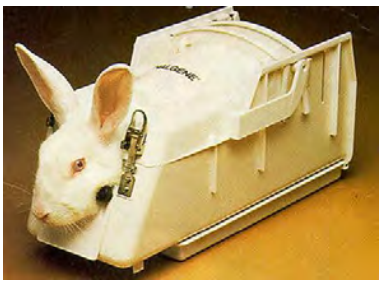
FIGURE 27.2 Negative pressure freestanding bioBUBBLE® enclosure. This unit is designed to be used for avian research requiring a primary containment system. Cages are located in the center of the enclosure and a bioBUBBLE® bedding disposal system is located in the front left corner of the enclosure. The disposal system is used for control of dust and allergens during cage waste disposal procedures. Two HEPA-filtered blower motors are present, one located in each of the back corners of the enclosure. *Photo provided courtesy of bioBUBBLE®.*

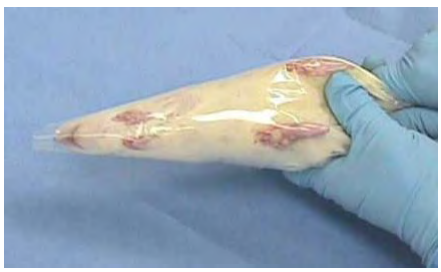
Fox, James G. *Laboratory animal medicine*. Elsevier, 2015.

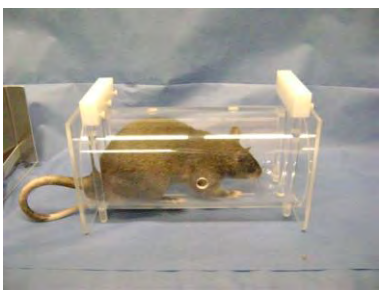
Trouble Shooting:

Bite-Resistant Gloves

Restraint Devices







Anesthesia

Sharps Use

Sharps Use

Sharps Use

- Keep fingers away from the needle at all times!!!!
- Use forceps as fingers
  - Uncap needles with forceps
  - Lift skin/stabilize with forceps
- **NEVER** recap needles
  - Before exposing a sharp, make sure everyone is aware
  - Once used, immediately place in sharps container

Workspace

Animals always kept in primary containment

Disinfection

Always clean and sanitize your workspace and any instruments used with an APPROPRIATE disinfectant for the APPROPRIATE contact time.

TABLE. Chemical compounds used for disinfection, effectiveness of chemical disinfectants and selected products against certain organisms, and selected properties of chemical disinfectants that should be considered when used for cleaning and disinfection

Chemical compounds	Chlorine* 0.01%–5%	Iodine iodophor 0.5%–5%	Chlorhexidine 0.05%–0.5%	Alcohol† 70%	Oxidizing agents 0.2%–3%	Phenol 0.2%–3%	Quaternary ammonium 0.1%–2%
Selected products	Clorox®	Tincture/ Provodine	Nolvasan®	Rubbing alcohol	Virkon-S®	pHisoHex®	Roccal-D®
Effectiveness of chemical disinfectants against certain organisms§							
Bactericidal	Good	Good	Good	Good	Good	Good	Good
Bacterial spores	Good¶	Poor	Poor	Poor¶	Fair to good	Poor	Poor
Virucidal	Good	Good	Poor	Fair	Good	Poor**	Poor
Envelope viruses	Yes	Yes	Limited	Yes	Yes	Limited	Limited
Nonenvelope viruses	Yes	Limited	No	No	Yes	No	No
Fungicidal	Good	Fair	Fair to good	Good	Fair	Fair	Fair
Protozoal parasites	Fair (concentrated)	Poor	Poor	Poor	Poor	Poor	Fair (ammonia)
Properties of chemical disinfectants††							
Effectiveness in organic matter	Poor	Poor	Fair	Poor	Poor	Good	Poor
Inactivated by soap	No	Yes	No	No	No	No	Yes
Effective in hard water	Yes	No	Yes	Yes	Yes	Yes	No
Residual activity	Poor	Poor	Good	Fair	Poor	Poor	Fair

Source: Adapted from the Nebraska Cooperative Extension and the U.S. Department of Agriculture, 2003.

* Bleach should be mixed fresh daily and replaced whenever contaminated with organic matter (1:32 dilution of 5.75% solution provides >1,500 ppm chlorine).

† Rubbing alcohol is flammable.

§ Effectiveness as a bactericidal, virucidal, or fungicidal agent and effectiveness in eliminating bacterial spores and protozoal parasites: good = effective; fair = moderate effect; and poor = inferior effect. Effectiveness in eliminating envelope and nonenvelope viruses: yes = effective; limited = moderate effect; and no = not effective.

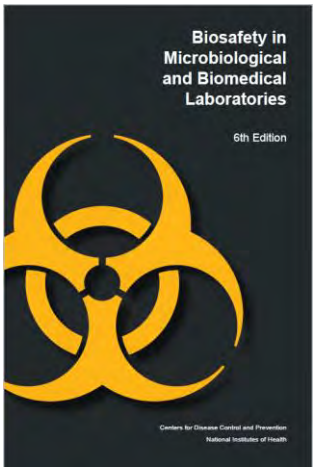
¶ Alcohol synergistically potentiates the sporicidal effect of hypochlorites (chlorine). Mix 5.75% solution of hypochlorite 1:1 with 50% ethyl alcohol/water. Mix fresh at the time of use and provide contact time of ≥30 minutes.

** The effectiveness of 2-phenylphenol (ortho-phenylphenol) is fair.

†† Effectiveness in organic matter: good = effective; fair = moderate effect; and poor = inferior effect. Inactivated by soap and effective in hard water: yes = chemical compound has this property; no = chemical compound does not have this property. Residual activity: good = chemical compound has residual activity; fair = moderate residual activity; and poor = inferior residual activity.

https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5404a2.htm

What Containment Level Should You Use?



- Pathogen name
- Pathogen summary
- Occupational infections
- Natural modes of infection
- Laboratory safety and containment recommendations
- Special issues
- References

- Tables in back

Example:

Macacine *alphaherpevirus* 1 (Herpes B Virus)

1. A PI wants to conduct HIV work in a rhesus macaque. What biocontainment level is needed?

Pg 255: BSL-2 practices and facilities are suitable for all activities involving the use or manipulation of tissues, cells, blood, or serum from macaques with appropriate personal protective equipment.

2. A PI wants to infect rhesus macaques to test therapeutics. What biocontainment level is needed?

Pg 255: Experimental infections of macaques as well as small animal models with B virus are recommended to be restricted to ABSL-4 containment.

Example:

Yersinia *pestis* (Plague)

1. A PI wants to infect Sprague Dawley rats with Y. pestis to study transmission factors. What biocontainment level is needed?

Pg 190: BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies.

In Conclusion

Determine exposure risk

Mitigate risk as much as possible

Use appropriate containment devices and PPE

Report any possible exposures to your immediate supervisor and Responsible Official.

THE END
Any questions?

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6348 www.cdc.gov