



Ask the Experts

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Do you have a biosafety question and you're not sure who to ask? Send your questions to the "Ask the Experts" column and I'll get them answered for you. Drawing from my own experience or that of other experts in the field we'll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

USDA BSL-3 Facility Requirements: What's the Concern?

We have had many questions and some fears with regard to the design and construction of containment laboratories for select agents that are on the USDA select agent list. There is concern among the scientific populace that their BSL-3 containment labs being built for use with USDA select agents and for small animal research with USDA select agents must meet the BSL-3 Ag requirements, and there are some USDA inspectors, I have been told, that are attempting to hold the research containment labs to that standard. I think it is time that everyone (researchers, administrators, inspectors, architects, etc.) to carefully review the requirements for working with infectious agents at BSL-3 and BSL-3 Ag, and should read it, pardon my pun, "Straight from the Horse's mouth."

The ARS Facilities Design Standards, published July 24, 2002 by the USDA Animal Research Service, Facilities Division, Facilities Engineering Branch, AFM/ARS, reads as follows: (I have taken the liberty of combining information on BSL-3 AG that was found in two sections of the document and

these are referenced as to the sections in which they were found.)

ARS Facilities Design Standards

The following material and Table 9.1 are excerpted directly from the ARS Facilities Design Standard. "Five biosafety levels are described below. Four are recognized universally (see the latest edition of the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*) and one (BSL-3Ag) is unique to ARS. These levels consist of combinations of laboratory practices and techniques, safety equipment, and facility design features appropriate for the dangers posed by the biohazardous materials, and by the procedures to be performed with these agents. These five biosafety level designations are applicable to all types of containment spaces, including laboratories, animal rooms, corridors, greenhouses, necropsy rooms, insect rearing facilities, carcass disposal facilities, etc.

The five biosafety levels, and the general types of biohazardous materials they are meant to contain are: Facilities

A. Biosafety Level 1 (BSL-1). Used with agents of no known or minimal potential hazard to facility personnel, animals, or the environment. They present no potential economic loss to the agricultural industries.

B. Biosafety Level 2 (BSL-2). Used with agents of moderate potential hazard to personnel, animals, and the environment, with minimal economic loss to the animal industries. Most research and diagnostics laboratories are at this level. It is the policy of ARS that any laboratory where research is being conducted on infectious agents will be designed, built,

and operated at a BSL-2 standard at a minimum.

C. Biosafety Level 3 (BSL-3). Used with agents which may be indigenous or exotic to the United States that can be contracted by the respiratory route, and may cause serious or lethal diseases to man, animals, or cause moderate economic loss to the animal industries.

D. **Biosafety Level 3 Agriculture (BSL-3Ag). Used with pathogens that present a risk of causing infections of animals and plants and causing a great economic harm. (Foot and Mouth Disease is the premier example.) Section 9.2.2** (Emphasis added)
In ARS, special features are required when re-

search involves certain biological agents in large animal species. To support such research, ARS has developed a special facility designed, constructed, and operated at a unique containment level called Biosafety Level 3 Agriculture (BSL-3Ag). Section 9.4.4 (Emphasis added)

E. Biosafety Level 4 (BSL-4). Used with highly lethal exotic agents which pose a high individual risk of life-threatening disease to man. Certain of these viruses also infect food animals and have the potential to cause severe economic loss to animal industries.”

Table 9-1
 General Containment Guidelines

| Biosafety Levels: | BSL-1 | BSL-2 | <i>BSL-3</i> | <u>BSL-3 Ag</u> | <u>BSL-4</u> |
|--|--------------------|--|--------------------|--|---|
| Facility Features: | | | | | |
| 1. Personnel Entry/ Exit through clothing change & shower rooms | n/a | n/a | <i>recommended</i> | <u>required</u> | required |
| 2. Materials, supplies, & equipment enter/ leave through double- door autoclave, fumigation chamber, or airlock | n/a | n/a | <i>required</i> | <u>required</u> | required |
| 3. Work conducted in primary containment equipment. | open bench tops | as required | <i>required</i> | <u>required</u> (If the space is a <u>lab.</u>) | required |
| 4. Hand washing station *(Foot, elbow or automatically operated) | required | recommended* | <i>required*</i> | <u>required*</u> | required* (not where a suit would be worn) |
| 5. Laboratory and animal room wastes from the containment area decontaminated or sterilized | n/a | recommended | <i>recommended</i> | <u>required</u> | required |
| 6. Lab clothing decontaminated before being washed | n/a | n/a; to be disposed of in the lab or washed by the facility | <i>required</i> | <u>required</u> | required |

Table 9-1continued...

Table 9-1 (Continued)
General Containment Guidelines

| | | | | | |
|---|---|---|--|--|---|
| 7. Animal cages autoclaved or thoroughly decontaminated before cleaning | cages washed, then rinsed at 180 degrees. | cages washed, then rinsed at 180 degrees. | <i>cages washed, then rinsed at 180 degrees.</i> | <u>required</u> | required |
| 8. Appropriate cautionary signs | n/a | required | <i>required</i> | <u>required</u> | required |
| 9. Separate building or isolated zone within a building | n/a | n/a | <i>required</i> | <u>required</u> | required |
| 10. BSC or other appropriate personal protective or physical containment devices | n/a | Class I or Class II BSC | <i>Class II or Class III BSC</i> | <u>Class II or Class III BSC</u> | Class III or Class I or II BSC with ventilated suit |
| 11. Suit room | n/a | n/a | <i>n/a</i> | <u>n/a</u> | As required |
| 12. Steam and/or ethylene oxide sterilizers: | recommended | required | <i>required (integral, double door)</i> | <u>integral, double-door</u> | integral, double-door |
| 13. Liquid effluent (bio-waste) treatment system | n/a | not required | <i>required</i> | <u>required</u> | required |
| 14. Personnel change room | n/a | n/a | <i>recommended for labs; required for animal facilities.</i> | <u>required</u> | required |
| 15. Shower available within facility | n/a | n/a | <i>recommended for labs; required for animal facilities.</i> | <u>required</u> | required |
| 16. Lab contiguous with shower | n/a | n/a | <i>n/a</i> | <u>as required for lab; required for "high risk" areas</u> | required |
| 17. Work surfaces: bench tops impervious to water, resistant to acids, alkalis, organic solvents and moderate heat. | required | required | <i>required</i> | <u>required</u> | seamless required |
| 18. Interior surfaces of walls, floors, and ceilings: monolithic, resistant to liquids and chemicals, all penetrations sealed. any drains in the floors contain traps filled with chemical disinfectant | n/a | walls, floors, and ceilings are monolithic, resistant to liquids and chemicals. | <i>required</i> | <u>required</u> | required |

Table 9-1continued...

Table 9-1 (Continued)
General Containment Guidelines

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|---|---|---|---------------------------------------|---|---|
| 19. Windows | not recommended for animal rooms. For other areas, if provided, fitted with fly screens | not recommended for animal rooms. For other areas, if provided, fitted with fly screens | <i>all windows closed and sealed.</i> | <u>no windows recommended (If with windows: breakage resistant and sealed)</u> | no windows recommended (If with windows: breakage resistant and sealed) |
| 20. Animal room: cages solid-sided, cages ventilated or filtered, restraining devices. | n/a | n/a | <i>as required</i> | <u>as required</u> | required |
| 21. Vacuum outlets (if provided) protected by HEPA filters & liquid disinfectant in traps | n/a | n/a | <i>required</i> | <u>required</u> | required if central vacuum systems are used |
| 22. Other liquid & gas services protected by backflow preventers | n/a | n/a | <i>required</i> | <u>required</u> | required |
| 23. Sewer & other vent lines protected by HEPA filters | n/a | n/a | <i>required</i> | <u>required</u> | required |
| 24. Ventilation (Facility): Individual supply & exhaust air systems. (for animal facilities, HVAC to be provided as per latest edition of <i>Guide for Care and Use of Laboratory Animals</i>) | ducted exhaust required | ducted exhaust required | <i>ducted exhaust required</i> | <u>required</u> | required |
| Single pass (no recirculation) | required | required | <i>required</i> | <u>required</u> | required |
| Directional air flow | required | required | <i>required</i> | <u>required</u> | required |
| Pressure gradient | recommended for animal rooms; n/a for other areas. | recommended for animal rooms; n/a for other areas. | <i>required</i> | <u>required</u> | required |
| Supply/exhaust coordination (exhaust confirmed before supply operates) | n/a | n/a | <i>required</i> | <u>required</u> | required |
| HEPA filtered supply and/or exhaust | n/a | n/a | <i>HEPA exhaust recommended</i> | <u>HEPA supply & exhaust for labs; HEPA supply and 2 in series HEPAs exhaust for high risk areas</u> | HEPA supply & exhaust for Cabinet Lab; HEPA supply and 2 in series HEPAs exhaust for Suit Areas |

Table 9-1 continued...

Table 9-1 (Continued)
General Containment Guidelines

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|--|------------------|------------------|---|--|---|
| 25. Ventilation (containment equipment): Class III BSC | n/a | n/a | HEPA supply filters & tandem (2 in series) HEPA exhaust filters | <u>HEPA supply filters & tandem (2 in series) exhaust filters.</u> | HEPA supply filters & tandem (2 in series) exhaust filters |
| Class I and II BSC | n/a | n/a | Class II; HEPA supply and exhaust | <u>Class II: HEPA supply and exhaust</u> | Class II; In suit lab, HEPA supply and exhaust |
| 26. DDC and building automation systems | to be considered | to be considered | required unless impractical | <u>required</u> | required |
| 27. Leak tightness testing & certification of critical components of the biological containment system prior to final acceptance of the completed work | n/a | n/a | BSC, HEPA filter assemblies (if required), welded ductwork (if required). | <u>BSC, HEPA filter assemblies, containment room, welded ductwork.</u> | BSC, HEPA filter assemblies, containment room, welded ductwork. |

Author's Note

Inconsistencies

Please note that the ARS document states that the BSL-3 requirements of the BMBL should be used for small animal research and that the "latest edition" of the BMBL should be used. In doing so, there are several inconsistencies that need to be addressed.

The ARS document requires the following for "Normal BSL-3" labs:

1. Liquid and gas services protected by backflow preventers
2. Sewer and other vent lines protected by HEPA filters
3. Liquid effluent (Bio-Waste) treatment system

These requirements are not required by the BMBL for BSL-3 facilities and not mentioned in the description of the BSL-3 facility in Section 9.4.3 of the ARS document. Since the ARS document defines two different BSL-3 facilities, one by the BMBL and the other the BSL-3 Ag for large animals, the inclusion of these requirements in this table appears to be a failure on the part of ARS to be consistent in the revision of its original document.

A careful review of these requirements would

provide an insight as to their necessity in any new laboratory.

Liquid and Gas Service Backflow Prevention

While it is most likely appropriate to provide backflow preventers in any laboratory water system that is connected to a potable water system, the use of backflow prevention (check valves) for laboratory gas systems in a BSL-3 laboratory is, in my opinion, significant overkill. For a hazardous microbial contamination to flow through a lab gas line back to a pressurized cylinder, a significant release of a highly infectious agent in the laboratory at the exact time that the gas cylinder became empty and by some unexplained circumstance became negative to the laboratory with the valve open would have to occur. The probability of such an event is so remote as to not warrant the expense of putting a check valve in the line, even assuming one could obtain such a valve designed for this particular purpose.

HEPA Filtration of Vents

If HEPA filtration of exhaust from the laboratory is not required, one would question why there would be a need to HEPA filter the exhaust from a sewer vent. This is particularly true when one considers that the procedures for the BSL-3 laboratory pre-

clude the disposal of any infectious material into the sinks. In addition, the probability of live organisms being aerosolized and being capable of traveling the significant distance from the laboratory to the outlet of the vent and being dispersed in an infectious state to the environment is, again, so extremely small as to not warrant the expense not only of installation, but also of maintenance.

Liquid Effluent (Bio-Waste) Treatment Systems

There is no requirement in the BMBL for a liquid effluent treatment system for a BSL-3 laboratory. All materials are easily treated either by chemicals or by steam sterilization, and the procedures, as noted above, preclude the disposal of wastes into the sewer system. BSL-3 small animal facilities should not have drains in the floor and cleaning should be performed using mops and sponges. Thus, there is no necessity for any liquid waste treatment system.

HEPA Filtered Supply

There has also been some misunderstanding as to whether or not the USDA Design Standards call for HEPA filtration of the supply for BSL-3 laboratories. As can be seen by reviewing Table 9-1, there is no requirement for HEPA filtration of the laboratory supply air. The misunderstanding, if there is one, is that a supply HEPA filter is required on containment equipment (i.e., biosafety cabinets). This is standard on such cabinets; however, some are evidently reading this as a requirement for such filtration on the room supply.

Summary

The most recent ARS Facilities Design Standards (FDS) define five levels of Biosafety Containment and these levels, with the exception of BSL-3 AG, are equivalent to those found in the CDC/NIH BMBL. BSL-3 AG is specifically designated for work with large animals (horses, cows, sheep, etc.). When working with these animals, it is difficult to contain the potential hazards associated with the work and stricter facility and procedural requirements are necessary.

While there are several inconsistencies in the ARS FDS table between the USDA BSL-3 and the BMBL, one would hope that these were simply an oversight during the revision process since the inconsistent requirements when carefully evaluated do not provide any additional level of containment integrity over the requirements of the BMBL. A well designed and certified BSL-3 laboratory facility, operated in compliance with the requirements of the BMBL, adequately meets the containment integrity requirements for research with any designated BSL-3 agent.

References

- U.S. Department of Agriculture. (July 24, 2002). *ARS facilities design standards*. www.afm.ars.usda.gov/ppweb/242-01m.htm.
- U.S. Department of Health and Human Services. (1999). *Biosafety in microbiological and biomedical laboratories, CDC/NIH guidelines* (4th ed.). www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Fund Donations

ABSA thanks the many of you have generously contributed to the Richard C. Knudsen Memorial Fund. The proceeds from this fund will be used to establish an award to honor Rich's memory. Those wishing to make donations to this fund should make their checks payable to the American Biological Safety Association. Please add a notation to the memo line that the check is to be used for the Richard C. Knudsen Memorial Fund. Checks should be mailed to ABSA, 1202 Allanson Road, Mundelein, Illinois 60060-3808.