Commissioning the ABSL-3 Laboratory

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Synopsis

Airflow and pressurization relationships are adjusted and verified in most buildings with temperature / humidity control and energy optimization of main concern. In the Animal Biosafety Level 3 (ABSL-3) Laboratory, primary concern is the risk of respiratory transmission of serious and potentially lethal infections. This paper will discuss how primary and secondary barriers are designed and commissioned in the ABSL-3 laboratory which includes double-door entry through the use of an anteroom or airlock, air movement that is directional and single-pass, the importance of architectural tightness of walls, floors, penetrations, HEPA filtration of supply air, use of bubble tight dampers, and bag-in / bag-out filter exhaust air systems. This paper will also discuss standards and considerations set in CDC/NIH biosafety in microbiological and biomedical laboratories along with the actual design and testing of an ABSL-3 laboratory located at the Medical College of Wisconsin Biomedical Research Facility.

About the Author

Michael Rosenberg is a registered professional engineer and has been commissioning building systems and training countless owner's representatives in proper operation of their facilities for a span exceeding 28 years. He has extensive experience in commissioning systems in research facilities and vivariums. Mr. Rosenberg is currently employed by Stanley Consultants, Inc. in Chicago, Illinois as Senior Commissioning Engineer. Mr. Rosenberg has a degree in Electrical Engineering Technology from the Herzing Institute in Madison.

Biosafety in Microbiological and Biomedical Laboratories (BMBL)

The Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual was introduced in 1984 and is currently in its 5th Edition. The BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories. The principals for biosafety are containment and risk assessment. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAI).

Risk Criteria for Establishing Ascending Levels of Containment

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present distinctive hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol driven risk assessment.

The primary risk criteria used to define the four ascending levels of containment, referred to as Animal Biosafety Levels 1 through 4, are infectivity, severity of disease, transmissibility, and the nature of the work being conducted. Another important risk factor for agents that cause moderate to severe disease is the origin of the agent, whether indigenous or exotic.

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

Animal Biosafety Level 2 (ABSL-2) is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

Animal Biosafety Level 3 (ABSL-3) involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease.

Animal Biosafety Level 4 (ABSL-4) is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk or transmission.

Animal Biosafety Level 3 (ABSL-3)

There are three primary elements involved in operating a biocontainment laboratory.

- Safety Practices
- Safety Equipment (Primary Containment)
- Facility Requirements (Secondary Containment)

Safety Practices

The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergency situations. The workers are educated and trained to assure safety and health concerns are addressed. Consideration is given to specific biohazards unique to the animal species and protocol in use. Access to animal rooms is limited to the fewest number of individuals possible.

Safety Equipment (Primary Containment)

Protective laboratory coats, gowns, or uniforms are required to prevent contamination of personal clothing. Gloves must be worn at all times while in the animal rooms and removed when leaving. Eye and respiratory protection are worn as dictated by the risk assessment. Ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. All procedures involving manipulation of infectious materials, handling infected animals or the generations of aerosols must be conducted within biosafety cabinets (BSCs) or other physical containment devices when practical.

Facility Design (Secondary Barriers)

The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. All surfaces are designed and constructed to facilitate cleaning and decontamination. External windows are not recommended. The facility is supplied with 100% outdoor air and fully exhausted. The direction of airflow in the animal facility is inward. Animal rooms should maintain inward directional airflow compared to adjoining hallways. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision for effluent decontamination) are considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

Commissioning the ABSL-3 Laboratory

The steps involved in commissioning the biocontainment facility are not much different than commissioning any other facility. The difference in outcome however, can be enormous. Many facilities are commissioned to optimize energy savings and provide occupant comfort.

Commissioning practices for the biocontainment facility are primarily concerned with providing environmental and biological safety without compromising critical research, and only secondary to energy optimization and occupant comfort.

Design Phase

The initial role of the commissioning agent is to understand the facility design and requirements of the regulating agencies. This should be done early in the design phase by verifying the 'Design Intent' and 'Basis of Design' documents are complete and detailed. These documents should be referred to often as the facility and systems are designed. As the design documents are developed, checklists are developed which will form the basis of developing prefunctional and functional testing protocols.

Space Type	Minimum O.A. Ventilation Rate	Summer Design		Winter Design		Pressurization	Minimum Supply Air Filtration	Remarks
		Max. Temperature (F)	Max. Relative Humidity (%rh)	Min. Temperature (F)	Min. Relative Humidity (%rh)			
Animal Facility Spaces								
Animal Holding Rooms	100%	Note 2	55%	Note 3	30%	Note 3	HEPA	-
Animal Testing Rooms	100%	Note 2	55%	Note 3	30%	Note 3	HEPA	-
Animal Procedure Rooms	100%	Note 2	55%	Note 3	30%	Note 3	HEPA	-
Animal Bedding	100%	74	55%	72	30%	(-)	HEPA	-
Dirty Cage wash	100%	78	60%	72	30%	(--)	HEPA	-
Clean Cage wash	100%	78	60%	72	30%	(+)	HEPA	-
Sterile Cage wash	100%	78	60%	72	30%	(+)	HEPA	-
Cage wash Equipment Space	100%	85	70%	72	30%	(---)	HEPA	-

Note 1: Public spaces and office areas will have CO2 monitoring for ventilation control. Ventilation rate will be sized based on 20 cfm/person

Note 2: Animal holding and procedure spaces will have temperatures adjustable between 68F and 80F.

Note 3: Animal holding and procedure room pressurization will be adjustable from positive to negative.

Design features such as redundancy, standby power operation, pressure relationships, cleaning, sterilization, decontamination, animal watering, lighting, bedding disposal, and security are just some of the aspects required in designing the animal biocontainment facility. Systems required to be commissioned need to be identified along with methodology and acceptance criteria.

During the design phase, the commissioning agent should:

- Become knowledgeable in the design requirements of the biocontainment facility
- Review and provide written comments on design intent

- Review and provide written comments on design documents
- Attend design review meetings
- Prepare Commissioning Plan
- Prepare Commissioning Specifications
- Verify Basis of Design is updated

Construction Phase

During the construction phase, the commissioning agent makes periodic observations and meets with the installing contractors to discuss detail of installation, coordination of trades, documentation requirements, and completion schedule. The commissioning agent develops detailed prefunctional checklists and functional testing protocols that encompass the design considerations which are documented in the plans and specifications. Clear pass/fail criteria are essential. Activities executed in the construction phase are:

- Participate in bid/award process
- Review contractors startup plans
- Witness factory acceptance tests of major equipment
- Prepare prefunctional checklists
- Prepare functional performance test procedures
- Chair commissioning team meetings / issue minutes
- Participate in development of detailed commissioning schedule
- Review and comment on commissioning-related project schedules
- Perform construction observations of equipment and systems to be commissioned
- Witness startup of major systems and equipment to be commissioned
- Attend select jobsite coordination meetings
- Review TAB execution plan
- Review TAB documentation
- Participate in punch list effort
- Participate in development and review of operation and maintenance manuals
- Participate in training of owner's personnel
- Compile and maintain master commissioning record
- Provide final commissioning report with executive summaries for each system tested

Typical Systems Commissioned in the ABSL-3 Laboratory

Supply and Exhaust Air Systems

The ABSL-3 Laboratory contains caging systems, Bio-Safety Cabinets, HEPA filtration, and air control valves served by 100% outside air and fully exhausted. Airflow is inward in the animal holding room. The room maintains a negative pressure relationship with the corridor. Visual monitors are incorporated to provide personnel with audio and visual monitoring capabilities to verify pressure relationships.

Caging

Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage.

Class III Biohazard Safety Cabinet

Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. Filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA- filtered.

Bag-in / Bag-out Filters

An important aspect of the systems serving the ABSL-3 facility is redundancy. Systems are designed to provide safe operating conditions during both normal operation and failure or maintenance modes. Standby power systems and UPS backup should be considered for control systems operating equipment serving the facility. Systems are tested both in normal and failure or maintenance modes of operation.

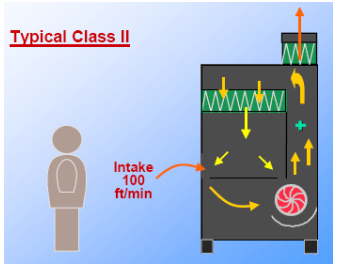
Suite	Room	Use	Room (Cubic Ft.)	Airflow		ΔP	Actual ACPH	Comments
				Design	Actual			
Suite 9	1530	Holding	3030	925	931	+	18	
	1535	Holding	4408	1450	1425	+	19	
	1540	Procedure	950	275	289	-	18	

Test and Balance Data

Pressure Monitor

Personnel must verify that the direction of airflow (into the animal areas) is proper. It is recommended a visual monitoring device indicates and confirms directional inward airflow be provided at the animal room entry.

Directional Airflow Monitor



Biosafety Cabinet

Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and when possible, animals.

Biosafety Cabinet

Process Equipment

The biocontainment laboratory incorporates process equipment such as bulk autoclaves, cage and rack washers, tunnel washers, and glass washers used to sterilize caging systems and laboratory equipment. The commissioning agent verifies operation of this equipment along with steam, electrical, compressed air, and water utilities. System design may incorporate bio-seals in conjunction with air pressurization relationships to separate dirty, clean and sterile partitions within the facility.

Tunnel Washer

Autoclave

Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.

Cage Washer

Decontamination

Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.

Chlorine Gas Decontamination

Animal Watchdog System

The animal biocontainment facility may incorporate a watchdog system to monitor and control lighting, watering, and environmental conditions. Critical research studies must be protected from accidents, contamination, and tampering. The access control system is designed to monitor and control personnel traffic in the animal rooms. The watchdog system may integrate and report to the building automation system or may operate as a stand-alone system.

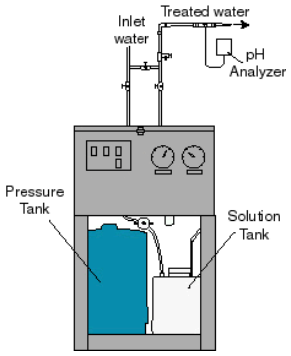
Animal Watchdog System

Water Purification

A facilities water source can vary over time. Pre-filtering will remove gross particles, but to control water quality, finer filtration using reverse osmosis (RO) is recommended. RO water removes >99.9% of most contaminants. Bacteria are one of the hardest contaminants to control. In fact, bacteria are such good survivors that they can live in RO water. Bacteria will attach to any surface that water contacts and develop a biofilm. Biofilm can occur in

the watering system piping. As the biofilm grows, pieces of it can detach and flow downstream to be consumed by animals.

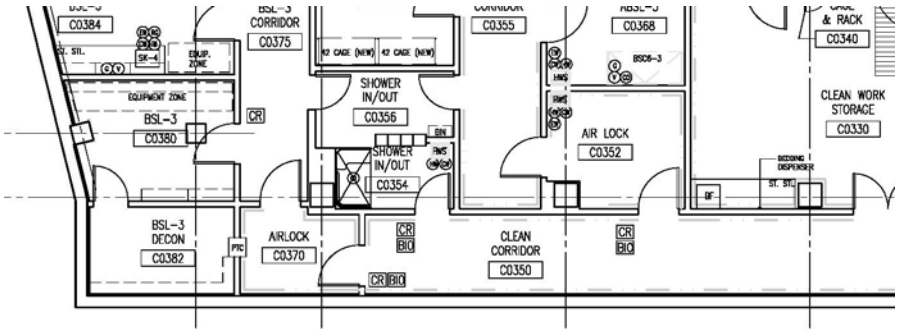
To control free floating bacteria, it is important to treat the water with a residual disinfectant. When combined with automated flushing, low residual chlorination (2-3ppm) or acidification (2.6 – 3pH) are very effective in controlling bacteria. The central proportioner shown to the right treats the RO water prior to transfer to the central storage tank.



Central Proportioner

Airlocks and Showers

Entry into the containment area is via a double-door entry which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.



Airlocks and Showers

Conclusion

This paper has been prepared to provide insight to the complexity involved in the design, startup, and commissioning of the Animal Biosafety Level 3 (ABSL-3) research facility. It is of utmost importance the commissioning provider understands the purpose and reasoning behind the design of systems and equipment serving the facility in addition to the design of the facility itself. Commissioning is much more than filling out checklists. It is a process in which the dynamic operation and integration of systems and equipment with the facility assures the researchers a safe, efficient, and conclusive atmosphere in which they conduct their work.

References

Biosafety in Microbiological and Biomedical Laboratories, U.S Department of health and Human Services, Public Health Services. Centers for Disease Control and Prevention and National Institutes of Health 5th Edition 2007