



# USAMRIID Opens Advanced ABSL-3 Aerobiology Suite: Illustrates New and Unique Features for Animal Biosafety

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The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) recently opened its newly renovated aerobiology ABSL-3 suite at Fort Detrick in Frederick, Maryland. The 4,800-sf facility includes labs and animal holding rooms designed for high throughput animal bioaerosol efficacy studies conducted with Select Agents and toxins.

The project, completed in September of 2005, involved the demolition of existing BSL-2 lab space and renovation of the space into a new ABSL-3 lab, including innovative animal containment and testing equipment that was custom-designed for USAMRIID.

The renovation project also included replacement of the air handling unit and mechanical and electrical utilities; installation of redundant HEPA filtration, exhaust fans, and associated ductwork; upgrade of the fire alarm system and building steam capacity; installation of a reverse osmosis water purification system and clean steam generator for room air humidification; and upgrades to physical and electronic security components to meet the Select Agents rule.

USAMRIID researchers conduct animal studies to develop and test biodefense vaccines, drugs, and diagnostics for U.S. military personnel. Within USAMRIID is the Center of Aerobiological Sciences, which conducts and supports research on pathogenesis, prevention, therapy, or treatment of disease and intoxications or poisonings, caused by exposures to aerosols of biological threat agents through the respiratory tract to the lungs.

All testing is conducted in accordance with Food and Drug Administration Animal Rule 21 (Code of Federal Regulations, CFR) parts 3.14 and 6.01, which allows for testing to be done on animals because of ethics considerations or because of the lethal nature of the materials being tested.

Catherine Wilhelmsen, DVM, PhD, CBSP, a microbiologist for USAMRIID, who also serves as USAMRIID's Biological Safety Officer, points to the following design objectives as the overriding focus of the renovation project:

- Ensure safe, humane movement of animals.

- Optimize aerosol productivity and efficiency.
- Incorporate hands-free door operations.
- Stabilize the laboratory ambient temperature to prevent temperature fluctuations.
- Shorten sterilization time for soiled cages exiting the ABSL-3 facility.
- Provide flexibility for future changes in mission.

## Ensure Safe Transporting

"Secure and effective animal transporting is our top priority so that we can guarantee safe conditions for both animals and researchers," says Wilhelmsen. "With this in mind, we worked with Germfree Laboratories Inc. of Ormond Beach, Fla., to custom design mobile Class III-BSL animal transporters, the first such units to be used within USAMRIID."

Six mobile transporters provide HEPA-filtered ventilated containment for rodents, rabbits, and non-human primates. Each battery-powered rechargeable transporter is equipped with its own power pack so it can move easily between the animal holding rooms and the three free-standing stationary Class III biosafety cabinets in the lab.

A rapid transfer (alpha-beta) port on each mobile transporter allows animals to be moved directly into identical ports on the biosafety cabinets. Once the ports are docked together, lab personnel must rotate and lock the handles in place before the doors can be opened to complete the animal transfer. The transporter also docks directly to a custom-designed port within the doors of each of the four BSL-3 animal holding rooms.

The stationary biosafety cabinets also each have a second, smaller alpha-beta port and a bottom dunk tank as alternative ways for passing materials and supplies into the cabinets and for removing waste and supplies that require sterilization.

Wilhelmsen explains that prior to the renovation, aerosol-exposed animals were removed from biosafety cabinets through a double door pass box or autoclave while contained within biohazard bags or within rodent cages draped with disinfectant-moistened cloths. The con-

tained animals were then hand-carried or transported on a cart to the animal holding room, and passed through an open room door to a gowned technician wearing respiratory protective equipment.

## Optimize Productivity

“To maximize the new lab’s productivity, our goal is to increase aerosol exposure throughput and the number of different infectious agents and/or biological toxins that can be studied simultaneously,” says Wilhelmsen. “To do this we used an open lab design to improve traffic flow and worker efficiency.”

She adds that the layout includes two sets of hands-free operated double doors to encourage an orderly, directional traffic flow of people, materials, and animals.

Wilhelmsen also points to several ergonomic design elements within the stationary BSL-3 cabinets aimed at increasing worker efficiency by reducing fatigue. These features include angled viewing windows and slanted oval glove ports on both long sides of the cabinet.

In addition to the BSL-3 cabinets, the lab contains two open three-sided aerosol equipment preparation alcoves for setting up the aerosol generation equipment and four BSL-3 cell culture rooms each with its own Class II biosafety cabinet, incubator, tabletop centrifuge, and bench space.

Doorways to the cell culture rooms use transparent, powered sliding doors to minimize the footprint of the doors compared to conventional swinging doors. The sliding doors also enable hands-free operation since they can be opened by an elbow-operated pressure plate and automatically close with infrared sensors.

## Stabilize Temperature and Sterilizing Procedures

“Originally our design included double door autoclaves to be included as part of the Class III biosafety cabinets,” says Wilhelmsen. “However in the redesign we eliminated those autoclaves to create a more comfortable environment for animals inside the cabinets.”

USAMRIID installed instead a custom-designed bulk autoclave in the hallway just outside of the ABSL-3 lab. The chamber of the autoclave is over 7 feet high, 7 feet long, and 4 feet wide, which can accommodate a 2-over-2 nonhuman primate cage rack or comparably sized rabbit cage.

In addition to reducing the overall heat load in the suite, Wilhelmsen adds that the bulk autoclave has helped to control the amount of temperature fluctuations that must be corrected by the automated bioaerosol system. To further stabilize temperatures, USAMRIID installed exhaust ventilation hoods above the bulk autoclave and above the lab’s ultra-low freezers.

To sterilize laboratory equipment that cannot withstand the extreme heat of the autoclave, USAMRIID also has an airlock decontamination chamber that can be used for fumigating equipment and supplies and as an emergency backup to the autoclave. The airlock is equipped with air pressure resistant doors having inflatable gaskets, so the airlock can be made to be gas tight.

## Maximize Flexibility

“Currently this aerobiology lab is registered to work with select agents and toxins within BSL-3 containment,” says Wilhelmsen. “However we tried to design the facility to easily accommodate future shifts in our research mission or changes in protocol or procedure.”

The stationary biosafety cabinets are considered by USAMRIID to be flexible design elements since the cabinets can be easily relocated if necessary to a new facility. In addition, the rapid access ports on the cabinets can be resized, and decontamination ports are included so that vaporized hydrogen peroxide or vaporized formaldehyde or other sterilants can be used if available in the future.

USAMRIID also provided for capped-off steam piping above the dropped ceiling over the biosafety cabinets to allow for optional installation of integral double-door autoclaves on the cabinets if steam sterilization of waste is required in the future. Quick-clamp connections were also installed to make it easy to connect the biosafety cabinets to piped utilities if necessary.

## Safety Lessons Learned

Safety elements within the new suite include an emergency shower and eyewash plumbed in with a mixing valve to provide tempered water, an emergency phone, and a fire extinguisher. There is also an alarm that signals when pressure in the carbon dioxide gas system is low. The alarm is connected to a bank of CO<sub>2</sub> cylinders located outside of containment to supply the incubators and euthanasia chambers for the animals.

“A key safety challenge we face is maintaining a negative airflow gradient within this facility without getting any positive pressurization,” says Wilhelmsen. “It was difficult during the commissioning process to balance the laboratory variable flow damper system in this new lab with the building constant flow damper system in the rest of this containment building which consists of three floors of BSL-3 containment.”

Proper air balancing was achieved, however, and the laboratory has not experienced any incident of sustained positive pressurization since the start of aerosol operations in the new facility.

Wilhelmsen also points to the installation of dump valves within the airlock decontamination chamber in order to deflate the air pressure resistant doors so that the air-

lock can be used as emergency egress. Otherwise the airlock would not open, trapping anyone who was inside.

“Another critical safety feature is the establishment of a definitive one-way traffic flow of people, animals, and materials within the lab,” says Wilhelmsen. “Originally we had two sets of swinging doors, but we quickly learned it was necessary to designate one set for entry only and the other set for exit only in order to have an efficient flow.”

She also cites the last-minute addition of a hands-free handwash sink near the lab’s main exit as a safety element that was originally overlooked. According to the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 4th edition, the sink was necessary to receive approval for handling select agents and toxins.

## Biography

Catherine Wilhelmsen received her doctor of veterinary medicine (DVM) from Cornell University in 1978, and a PhD in veterinary pathology from Iowa State in 1989. She is a Diplomate of the American College of Veterinary Pathologists. Dr. Wilhelmsen is a 20-year veteran of the U.S. Army Veterinary Corps and served at the Armed Forces Institute of Pathology, Walter Reed Army Institute of Research, and the 10th Medical Laboratory in Landstuhl, Germany, USAMRIID, and the Armed Forces Radiobiology Research Institute. Since retiring from active military duty, she has worked as a civilian employee at USAMRIID, with successive appointments as microbiologist, biological surety officer, and microbiologist-biological safety officer. She is a certified biological safety professional (CBSP), and is licensed to practice veterinary medicine in the state of Maryland. She has consulted to the U.S. Department of Agriculture and CDC on biosafety and biosecurity, and does research and publishes on biological toxins and containment issues.

This article is based on a presentation given by Catherine Wilhelmsen at Tradeline’s *International Conference on Biocontainment Facilities* in March 2006.

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## Project Team

Air Pressure-Resistant Doors: Presray, Pawling, New York

Architectural Design: NuTec Design Associates, York, Pennsylvania

Autoclave: Getinge, USA Inc., Rochester, New York

Civil and Structural Engineer: NuTec Design Associates, York, Pennsylvania

Class II BSC’s: The Baker Company, Sanford, Maine

Class III BSC’s: Germfree Laboratories Inc., Ormond Beach, Florida

Commissioning Agent: USAMRIID Facilities Management/Safety

Engineering: NuTec Design Associates, York, Pennsylvania

Fire Protection Engineer: NuTec Design Associates, York, Pennsylvania

General Contractor: John J. Kirlin, Special Projects Division, Rockville, Maryland

Ultra Low Temperature Freezers: ThermoElectron doing business as Revco, Ashville, North Carolina

**Figure 1**

Three stationary free-standing BSL-3 biosafety cabinets are included in the new lab so that three different infectious agents and/or toxins may be aerosolized at one time. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)



**Figure 2**

Unique mobile BSL-3 biosafety animal transporters connect to the stationary biosafety cabinets through rapid access ports. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)



**Figure 3**

Dr. Wilhelmsen reads a printout of the data cycles in USAMRIID's custom-designed bulk autoclave outside of the ABSL-3 lab. The chamber of the autoclave is over 7 feet high, 7 feet long, and 4 feet wide, which can accommodate a 2-over-2 nonhuman primate cage rack or comparably sized rabbit cage. (Photo courtesy of USAMRIID, Photographer Larry Ostby.)

