



Operating a BSL-4 Laboratory in a University Setting: Georgia State University Lab Studies Deadly Alpha Herpes Virus

Tradeline Publications

Orinda, California

The Viral Immunology Center at Georgia State University (GSU) is recognized as the national resource facility for research related to the early diagnosis and effective treatment of dangerous viral diseases, most notably B virus (*Cercopithecine herpesvirus 1*), herpesviridae. Funded by the National Institutes of Health, the Center operates the National B Virus Resource Laboratory aimed at learning more about the deadly alpha herpes virus and preventing its transmission to laboratory workers.

The GSU Center, located in downtown Atlanta, was the first BSL-4 facility in the country operating in a university setting. Under the direction of Julia Hilliard, the goal of the laboratory's research is not only to diagnose viral diseases, but also to study the agents and use their infrastructure to design vaccines.

The B Virus laboratory had been located at the Southwest Foundation for Biomedical Research in San Antonio, Texas, and operations were proceeding well in the BSL-4 environment. However, space constraints limited the size of the staff to 10 people and restricted the amount of work that could be done.

Research officials at Georgia State University called upon Hilliard to move her facility to downtown Atlanta in 1997, noting the University's proximity to Emory University, the Centers for Disease Control, and the Medical College of Georgia. The retrofitted lab at the University offers three times the amount of space that Hilliard was using at the Texas facility.

"The thrill of working in a rich milieu and having a new facility was enough to engage my interest

in moving the laboratory," recalls Hilliard. "We now know what it's like to build a BSL-4 laboratory in a highly concentrated downtown area. We were told this would not be a big problem because the residents were already aware that maximum-containment laboratories were operating in the city."

Maximum-containment BSL-3 and -4 suites are necessary due to the nature of the alpha herpes virus, or B Virus, which is transmitted by the *Macaca* species of monkeys often used in biomedical research. Although there is a low frequency of infection among people, the virus is considered a severe occupational hazard for laboratory workers as a result of its lethal consequences if not diagnosed quickly after infection.

"The virus causes death in 80 percent of the infected individuals (after it literally attacks the cervical spinal cord)," says Hilliard. "There have been five fatalities in the last 12 years at four different institutions in four states. The survivors are our first introduction to what happens when you put a pathogen like B virus into the human population. The reason we work with this agent is because we know that rapid identification means we can successfully treat the individuals."

The cost of moving to Atlanta was approximately \$200,000, which included expenses for shipping the laboratory equipment, moving staff, and flying back and forth between the new lab and the Texas lab to verify that the performance standards of the new facility matched those of the well established lab. The cost to renovate the space to accommodate the

Center was approximately \$1 million, not including the daily maintenance costs of the hot lab. Both laboratories had to be operated concurrently in order to preserve the integrity of the testing until Hilliard's team determined that the new Center could function efficiently on its own.

The architectural firm of Lord, Aeck and Sargent designed the first phase of the Center, while construction of the BSL-4 lab was supervised by the architectural firm of HOK in cooperation with The Baker Co., which customized the 32-linear-foot glovebox cabinet.

Designing a BSL-4 Laboratory

The Viral Immunology Center, housed in the University's six-floor, 500,000-sf natural sciences building, features a departmental animal facility in the basement and BSL-3 and -4 laboratories on the third floor, including a specialized BSL-4 for small animal models of disease. The Center, which occupies about 5,000 sf and employs 30 people, also includes two support BSL-2 laboratories, a biochemistry lab, robotics facility, offices, a fermentation lab, and shared departmental resources, including electron and confocal microscopy areas, a sequencing core, and a FACS-analysis core for cell sorting.

The activities that take place in the Natural Science Center are of particular interest when designing a BSL-4 laboratory in a university setting. This building typically houses the departments of biology, chemistry, biochemistry, physics, and anatomy in addition to the BSL-4 lab. Research activities that are not part of the viral laboratories must also be taken into consideration when deciding where to locate the BSL-4 suites. Other activities to consider include teaching sessions and seminars.

Providing access to the laboratories in a manner that protects visitors is critical to the efficient operation of the facility. Serious consideration is given to the type of barriers that are erected between common access hallways and restricted areas to ensure that lay visitors and research supporters can view the work taking place without being exposed to potential risks. Access is provided via keypad entry for students, faculty from other disciplines, visiting scientists, security and maintenance personnel, and inves-

tigators. The BSL-3 and -4 laboratories have a magnetic card access so that entry and exit into the facilities can be tracked by a computer. With the additional restrictions imposed by the new Homeland Security Program, all individuals who work within the BSL-3 and -4 labs must first have federal security clearance after submission of extensive documentation to the FBI. The facilities must also meet Clinical Laboratory Improvement Amendments and Select Agent inspection requirements to carry out the research and diagnostic missions of the laboratory.

Key Components of a Maximum-Containment Facility

Each day Hilliard's facility receives up to 20 boxes from all over the world from individuals who may have been exposed to the secretions of a Macaca monkey. Minimizing the transport of materials going to high-containment areas is critical. When the samples arrive, they are triaged to determine what tests must be done so the results can be sent to the concerned individuals as quickly as possible.

The packages, transported through an isolated hallway that is sectioned off from other areas of the facility, are opened in the Level 2 unpackaging room, attached to the BSL-3 and -4 suites. Each of these areas can be accessed only by computer-controlled keypad entry. Any materials that leave the BSL-4 facility undergo an intensive search for evidence of any live virus, using virus plaque assays to verify that all virus has been inactivated. If the assay is negative, the chain of custody for removal begins.

Items that leave the BSL-4 laboratory must go through two sets of autoclaves or two sets of cross-linked glutaraldehyde dunk baths. Dunk baths enable chemical sterilization of the outside wall of submersed containers. This allows a live virus to be removed from the cabinet for ultra-low freezing and storage within the BSL-4 and passage of inactivated materials to the Level 2 labs. Live virus never leave the suite.

The disposal system consists of two redundant 150-gallon tanks that are released into a boiler-decontamination unit. A chilling mechanism cools the water, which is released into the public system

once it is steam disinfected and verified as inactivated. A drain is situated under the tanks to contain leakage, should that occur.

The specialized HVAC unit dedicated to the independent air system of the BSL-3 and -4 suites is located on the roof of the Natural Sciences Center, while the decontamination facility is positioned under the BSL-4 laboratory. Computers are used to analyze temperatures, airflow, and the rooftop exhaust systems, which are actively engaged. In case of power failures, a natural gas backup system is in place to maintain air purification, negative pressure, and equipment power.

It is critical to be able to monitor the temperature and air supply for each of the high-containment suites by computer on site or from remote locations by the extensively trained and certified engineer required to maintain the facility. The outer hallways are kept at negative pressure assessed by magnahelics, and with each step further into the biocontainment areas the negative pressure gradient increases according to the specifications outlined in the CDC's *Biosafety in Microbiological and Biomedical Laboratories*.

"The goal of the BSL-3 rooms is to isolate viruses from small samples. In order to process the samples, pull out the virus, and identify unusual agents, we have to have incubators that are fed five percent carbon dioxide and 95 percent air," explains Hilliard. "We can't bring tanks in and out of these rooms, so we have a separate alcove which houses the gas tanks feeding the incubators in the BSL-3 and BSL-4 labs."

It is challenging to keep the BSL-3 laboratories stocked and tidy at the same time, keeping in mind that many of the items that come into the labs are not going to leave until they are thoroughly melted down by repeated autoclaving. Disabled equipment cannot be removed from the laboratory for maintenance or repair. Tools for minor repairs are decontaminated with gas sterilization before they are removed from the maximum-containment suites.

Keeping the Scientists Safe

Necessary precautions are taken to protect the researchers while they are working in the laboratories. Scientists usually work in a cabinet or a suit lab,

which relies on the standard biocontainment gear, in the BSL-4 facility. Working in a cabinet lab is extremely challenging, but considered safer than a suit lab because the individual uses large glove ports and is separated from the pathogen by thick stainless steel. The cabinet lab at the Center is 32-foot long and can accommodate three or four researchers at a time. A nearby animal section houses mice, rats, and rabbits, and a screen built into the cabinet allows investigators to visualize materials analyzed by an inverted, phase-contrast microscope.

Working in the cabinet lab can sometimes lead to fatigue because scientists must do all of their work using arm-length gloves to maneuver materials within the stainless steel cabinet while working in a line with other researchers. Working in the suit lab allows scientists to use the traditional lab layout and to move around more freely. However, breathing the positive-pressure air in the suit can also cause fatigue.

Barrier protection masks are worn at all times in the BSL-3 and -4 labs. Only 10 people on Hilliard's staff are trained to work in the BSL-3 lab, while only five of these can enter the BSL-4 facility.

"The masks are basically a reminder for us not to put our double-gloved hands up to our faces. If we relied on them as filtration or personal protection devices, we would go to something different," says Hilliard. "All agents are handled in a Class 2 cabinet in the BSL-3, so there is no time at which an agent is uncapped in the room surrounding the cabinet."

Important Considerations

Careful consideration must be given to the installation of all systems and how they will interact. The HVAC system at GSU had been repaired at an annual cost of \$12,000 every year since Hilliard's lab moved into the facility. A new HVAC system specifically engineered after analysis of previous failures was installed at a cost of \$250,000 and 2003 marked its first year of operation.

"This is an existing building that we modified. It wasn't meant to be under constant negative pressure and that has caused substantial stress on the walls and ceiling," says Hilliard. "Careful consideration of the systems that are linked is very important. We are

right in the middle of downtown on a rooftop so there are many issues to address.”

Cabinet labs should be designed so they are ergonomically comfortable for researchers of varying physical stature. The cabinet labs at the Center were designed largely with the input of the former associate director, who is more than 6-feet tall, creating a challenge for other lab staff to do their work. Smaller gloves, extenders, and a few other modifications enable the other investigators to work relatively comfortably.

It is also crucial to properly position the laboratories in a manner that is most conducive to the type of work being done. For example, a West Nile BSL-3 is located adjacent to the robotics facilities that service the sera and viral culture laboratories.

The BSL-2 labs have standard virology for lower containment agents like HSV-1 and HSV-2. The sum total of the work performed in these labs is to take core resources of the BSL-3 and -4 labs, combine them with the research missions, maintain a high-containment robotics lab for high-throughput virus identification, as well as serologic screening, perform microarray analysis, and be flexible enough to look at new agents as they come to the scientists’ attention.

“All of our work would be for naught if we didn’t have the supportive BSL-2 labs,” says Hilliard.

The robotics unit can perform multiple tests and process thousands of sera daily. The viral culture robot can plate samples and screen them at multiple times during the day for the presence of a virus. This allows mass processing of thousands of diagnostic samples. If a disturbance in cell culture is noted, a pager is sounded and an investigator is called to the room for further analyses and to notify the institution submitting the sample for analysis.

“In the BSL-4 laboratory, the goal of our work is the production of large amounts of virus and containing the supply very securely,” says Hilliard. “We can then look at drug sensitivities and how the virus

behaves in cells in order to understand the pathogen and how to control it.”

Biography

Julia Hilliard is director of the Viral Immunology Center at Georgia State University in the Department of Biology. She is also the Georgia Research Alliance Eminent Scholar in Molecular Biotechnology and Director of the National B Virus Resource Center for global diagnostic resources. She has worked with the BSL-4, newly classified Select Agent, B Virus (Cercopithecine herpesvirus 1) for 23 years, providing diagnostic resources to the biomedical community for the last 16 years.

This report is based on a presentation given by Julia Hilliard at Tradeline’s *International Conference on Biocontainment Facilities* in May 2003.

For more information, please contact: Julia Hilliard, Director, Viral Immunology Center, Georgia State University, Box 4118, Atlanta, GA 30302-4118, 404-651-0811, jhilliard@gsu.edu.

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Resources

Biosafety in Microbiological and Biomedical Laboratories:

www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Clinical Laboratory Improvement Amendments:

www.cms.hhs.gov/clia/

Select Agents:

www.cdc.gov/od/sap/docs/salist.pdf

Viral Immunology Center:

www.gsu.edu/~wwwvir/index.html

Figure 1

The entry area for the maximum-containment labs includes a pass-through shower. Scientists prepare for their work by donning either a suit lab or working in the cabinet lab.

(Photo courtesy of Julia Hilliard.)



Figure 2

Materials that leave the BSL-4 lab go through two sets of autoclaves or two sets of cross-linked glutaraldehyde dunk baths. A decontamination facility is situated under the lab. The disposal system consists of two redundant 150-gallon tanks released into a boiler-decontamination unit.

(Photo courtesy of Julia Hilliard.)



Figure 3

When pathogen samples arrive, the packages are transported through an isolated hallway that is cordoned off from other areas of the facility. They are opened in a Level 2 unpackaging room, which is attached to the BSL-3 and -4 labs. (Photo courtesy of Julia Hilliard.)

