KEYWORDS • Life Science • Lab Equipment • Safety

Biosafety Analysis Takes on Added Importance

Research into the agents of bioterrorism has become a fact of the 21st century that must be dealt with in a scientific manner in order to protect our citizens.

Ever since the terrorist events of Sept. 11, 2001, and the anthrax attacks soon thereafter, the public has been concerned about our preparedness for future attacks. For researchers working in biosafety labs (BSL), this increased "publicity," while not significantly altering the way they do their work, has raised the importance of their work. It also confirms the research they've been involved in for many decades. Research in manmade and naturally occurring biological agents, their identification, analysis, and diagnosis, and the development of vaccines or countermeasures has been strongly supported for more than 50 years.

A series of testing protocols, procedures, equipment designs, and laboratory configurations have been developed over this period of time to support research in biological agents considered harmful to humans. These procedures, equipment, and lab designs are well-documented and proven to be adequate to safely study every known biological agent from the common *E. coli* to the virulent Ebola virus.

Determining safety level needs

The Centers for Disease Control and Prevention (CDC), in Atlanta, is the lead government agency in the study of biological agents with its National Center for Infectious Diseases focusing on the most severe agents like anthrax, tuberculosis, and hemorrhagic viruses. Founded in 1946 as the Communicable Disease Center to investigate malaria, the CDC specifies four levels of biosafety precautions (see sidebar). Level 4 is the so-called "hot zone," the highest level of containment with pressurized space-suit personnel protection capabilities.

Procedures for operating a biosafety lab consist of detailed and documented microbiological practices, requirements for specific safety equipment, and descriptions of modifications for laboratory facilities.

BSL-1 offers basic containment and protection. Work is generally performed on open bench tops using standard

Comparing biosafety cabinetry

Type	Face velocity (fpm)	Airflow pattern	Radionuclides/ toxic chemicals	Biosafety levels	Product protection
Class I open front	75	In at front; rear and top through HEPA filter	No	2, 3	No
Class II Type A	75	70% recirculated through HEPA, exhaust through HEPA	No	2, 3	Yes
Type B1	100	30% recirculated through HEPA, Exhaust via HEPA and hard ducted	Yes (low levels)	2, 3	Yes
Type B2	100	No recirculation; total exhaust via HEPA and hard ducted	Yes	2, 3	Yes
Type B3	100	Same as IIA, but plena under negative pressure to room and exhaust air is ducted	Yes	2, 3	Yes
Class III	NA	Supply air inlets and exhaust through two HEPA filters	Yes	3, 4	Yes

Source: CDC

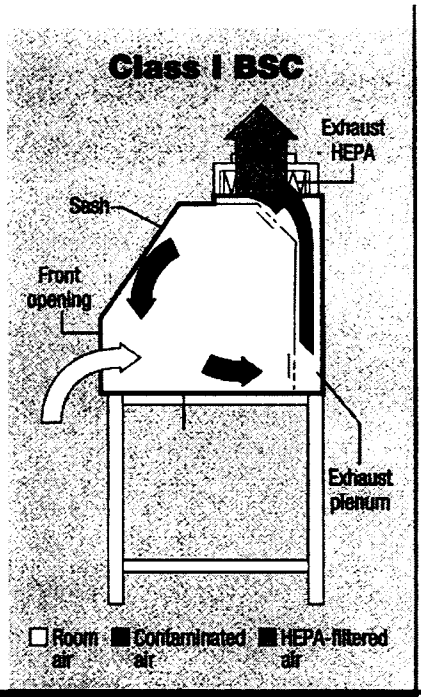
microbiological practices. Special containment equipment, like biological safety cabinets (BSCs) and modified facility designs are neither required nor generally used. Modified procedures to GLP for the specific task at hand, along with careful tracking and waste disposal procedures are generally acceptable for this level of containment and study.

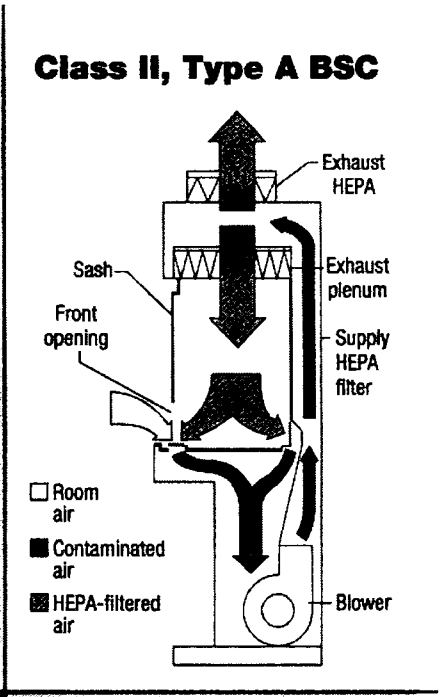
BSL-2 is similar to BSL-1 with the addition of 1) specific training for all personnel; 2) tighter access restrictions; 3) detailed precautions for the handling of sharps (objects having sharp edges or points, such as syringes, broken glass, and knives); and 4) safety procedures and equipment to contain the spread of aerosols and splashed working materials.

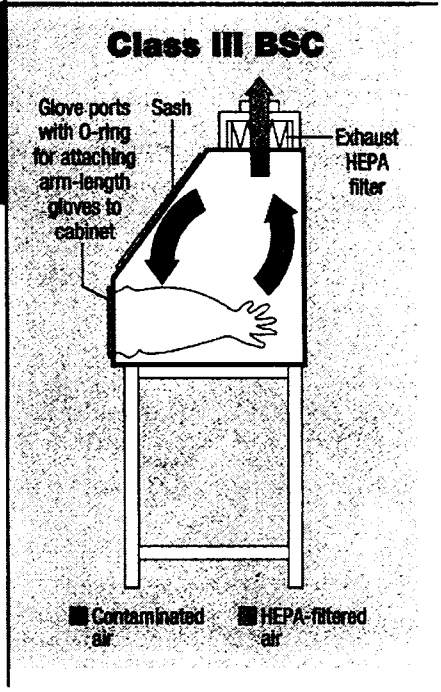
These labs require the use of BSCs, preferably Class II, or other appropriate protective clothing or equipment. BSL-2 facilities may require some modifications to restrict access, provide adequate cleaning, and accommodate specific equipment. There are no specific ventilation requirements for a BSL-2 facility.

BSL-3 offers the best protection available for research personnel and the environment, without going to the extreme procedural, equipment, and facility requirements—and certification—of a BSL-4 lab. BSL-3 requirements are applicable to clinical, diagnostic, teaching, research, and production facilities. All BSL-3 procedures involving infectious materials are conducted in Class II or III BSCs, or by personnel wearing protective clothing and equipment.

Documented procedures for the handling, processing, storage, and waste disposal are overseen by the lab director, who also is responsible for ensuring proper training programs. BSL-3 facilities must be isolated from other areas having open access. All surfaces and walls must be constructed to be easily cleaned and decontaminated, along with sealed access points and wall penetrations. Nonrecirculating ventilation systems that draw air from clean areas to the contaminated areas are recommended.







● Class I and II biosafety cabinets provide an effective containment system for handling moderate and high-risk microorganisms with up to 100 fpm face velocities to protect lab workers from infectious aerosols. Class III BSCs are totally enclosed ventilated cabinets for complete protection.

Kick it up a notch

BSL-4 facilities offer the best protection for personnel and the environment for handling all known biological agents. The facilities are similar to those in a BSL-3 lab except they are more restrictive and require more documentation. Additional features can include an active insect and rodent control program, an on-site safety administrator, immunizations for all personnel, double-door ingress/egress points with areas for removal of street clothing before entering and showering before exiting, in-lab decontamination processes for all equipment and waste materials, regular decontamination procedures, and isolated ventilation and utility supplies.

BSL-4 facilities are categorized as either cabinet labs or suit labs, referring to the extensive use of either BSCs or pressurized personnel "space suits."

"There's an exponential difference in the level of controls and procedures between BSL-3 and BSL-4," says Jon Crane, a biocontainment specialist at CUH2A, a laboratory architectural firm in Princeton, N.J. "The level of complexity in a BSL-4 facility has prevented labs from opening because they could not be certified."

Commissioning of a BSL-4 facility involves those agencies requesting or funding the research, such as the CDC or the US Dept. of Agriculture (USDA). "These agencies generally try to develop a coordinated policy for certifying facilities," says Crane.

Location is key

There are relatively few BSL-4 facilities compared to the numerous BSL-3 facilities, many of which are on university campuses. Those being operated or planned include the CDC's large facility in Atlanta (with an

Biosafety levels determined

Level	Description	Typical agents
1	Work involves well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to lab personnel and the environment.	Bacillus subtillis, E. coli, Infectious canine hepatitis
2	Work involves agents of moderate potential hazard to personnel and the environment.	Measles, Salmonella
3	Work done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation.	M. tuberculosis, St. Louis encephalitis, Zoonotic Q fever
4	Required for work with dangerous and exotic agents posing a high individual risk of aerosol-transmitted lab infections and life-threatening disease.	Marburg virus, Ebola virus, Congo-Crimean hemorrhagic fever

Source: CDC

expansion scheduled for 2004), the Univ. of Texas Medical Branch in Galveston (2003); the Southwest Foundation for Biomedical Research facility in San Antonio; the National Institutes of Health's Rocky Mountain Labs in Hamilton, Mont. (2004); another NIH lab in Bethesda, Md.; the US Army's Medical Research Institute of Infectious Diseases at Fort Dietrich in Frederick, Md.; a small facility at Georgia State University's Viral Immunology Center in Atlanta; and the Canadian Science Center for Human and Animal Health in Winnipeg, Manitoba.

There also are several foreign BSL-4 sites, including two in Germany, one in Spain, and one in South Africa. Russia's Soviet-era biowarfare program—supporting more than 10,000 scientists studying and weaponizing plant and animal diseases—resulted in the construction of numerous BSL-4 facilities. Many are still operational.

A planned upgrade from BSL-3 to BSL-4 to the US Dept. of Agriculture's Plum Island Animal Disease Center has encountered public resistance due to its close proximity (one mile) to densely populated areas of Long Island, N.Y., and Connecticut.

"Most BSL-4 facilities are located in urban areas and the rigorous commissioning and operating procedures make them safe labs," says Crane. There are no recorded incidents involving contamination from any US BSL-4 labs.

—Tim Studt

>> Resources

Biosafety Requirements and Policies, http://bmbl.od.nih.gov
Centers for Disease Control and Prevention, www.cdc.gov
CUH2A, www.cuh2a.com