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Perspective

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# Research in the Hot Zone

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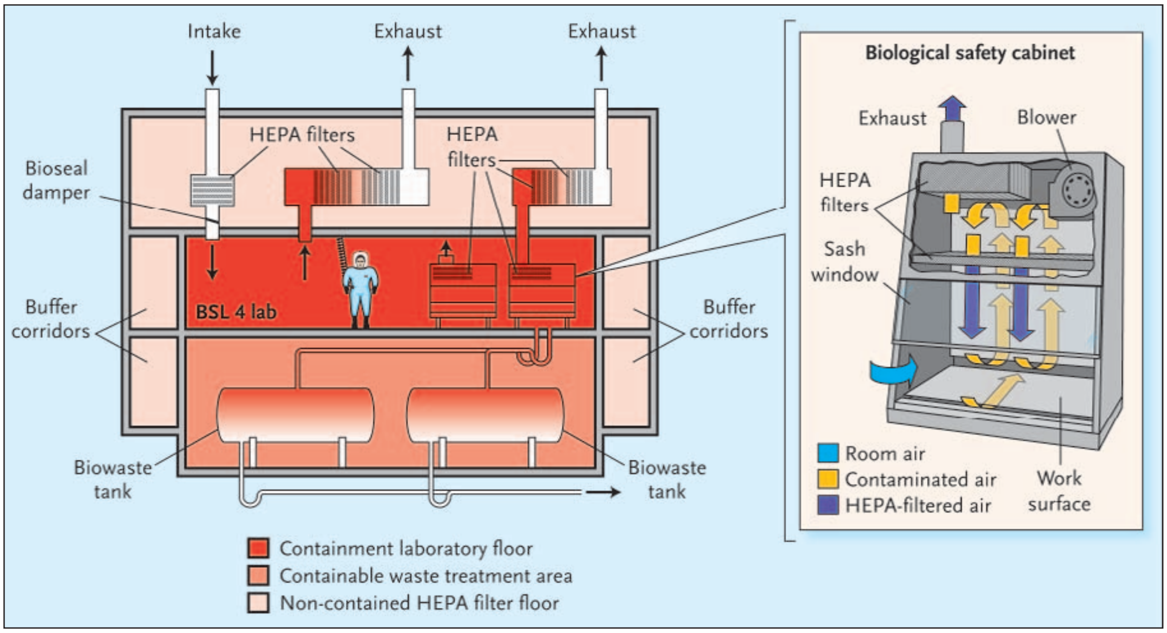
Research conducted at biosafety level 4, popularly known as the hot zone, continues to be both revered and feared. Biosafety level 4 laboratories are the places to study dangerous and exotic agents that cannot safely be studied anywhere else, such as pathogens that can be transmitted within a laboratory through the air. This level of biosafety is often required for the development of diagnostic tests, therapies, and vaccines and as the starting point for work on newly recognized agents whose risks have yet to be established.[1] At the same time, these laboratories are seen as potential targets of a terrorist attack or as possible sources of the very contagions that their researchers seek to prevent. Some cities and regions want no part of such laboratories (for example, a facility was built in Toronto that never went "hot") despite their extensive and redundant safety features.

A biosafety level 4 laboratory is a box within a box — a sealed environment within a larger building (see diagram), buttressed by strict security measures. Since mid-2004, one such laboratory, 2000 $ft^2$ in size, has operated in the middle of a medical school campus, at the University of Texas Medical Branch (UTMB) in Galveston (see photo). Led by David Walker, chair of the pathology department, planning for the facility began at UTMB in the 1990s — years before the attacks on September 11, 2001, and before emerging infectious diseases and bioterrorism became widespread concerns. Walker explained the rationale to me when I visited the university; it is to allow deadly pathogens to be studied with the same rigor as are other organisms.

The pathogens currently under study include the viruses that cause hantavirus pulmonary syndrome, a public health problem in North and South America; viruses that cause tick-borne encephalitis; Crimean–Congo hemorrhagic fever virus, a bunyavirus found in Asia and Africa; and Rift Valley fever virus, another bunyavirus found in Africa. Work is also focusing on avian influenza A (H5N1) virus, the arenavirus that causes Lassa fever, and other arenaviruses that are found in South America.

Named after Robert Shope, the eminent virologist who worked at UTMB from 1995 until his death in 2004, the laboratory contains areas for research involving cell cultures; research in animals, such as mice, hamsters, and guinea pigs; and necropsies. For example, H5N1's ability to combine with




**Cross-Sectional Diagram of a Biosafety Level 4 Laboratory (BSL 4 Lab).**

Safety cabinets may recirculate HEPA-filtered air to the laboratory air space or exhaust air directly through HEPA filters. Adapted from Smith Carter.

other influenza virus strains that infect humans will be studied in cell culture. Testing of its susceptibility to antiviral medications should begin this month in ferrets, the animals thought to best reflect humans in terms of influenza infection, according to Slobodan Paessler, the project leader for these tests.

Biosafety laboratories are found at many institutions. Biosafety level 2 is for agents presenting a moderate risk; level 3 is for pathogens of greater risk that can cause lethal infections and that can be transmitted through the air. The first biosafety level 4 laboratories in the United States were at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), at Fort Detrick in Frederick, Maryland, and the Centers for Disease Control and Prevention (CDC) in Atlanta. Today, there are five such laboratories (see map). Existing facilities are being upgraded, and additional facilities are under construction. New laboratories at the CDC will more than triple their biosafety level 4 space. A new USAMRIID facility is planned. In September 2003, the National Institute of Allergy and Infectious Diseases, using money appropriated by Congress after the anthrax attacks of 2001, funded two national biocontainment laboratories: the Galveston National Laboratory at UTMB and the National Emerging Infectious Diseases Laboratories at Boston University Medical Center, in Boston.[2]

The $167 million Galveston National Laboratory is being built on the UTMB campus, immediately adjacent to the Shope Laboratory (see photo), and is expected to open in 2008. It will contain 63,000 ft$^2$ of laboratory space, including about 12,000 ft$^2$ classified as biosafety level 4, and will be surrounded by a 200-ft security perimeter from which private vehicles will be excluded. In general, the Galveston project has had the support of the community, largely because of the extensive groundwork that had been laid during the planning and construction of the current laboratory. By contrast, there has been substantial community opposition in Boston. On December 9, 2005, the final environmental impact statement for the Boston University laboratories was published in the Federal Register. A “record of decision” is expected soon. If the decision is favorable — and a building permit and state building-code variances are approved — construction could start as early as February 2006 and be completed in 2008.

The city of Galveston is a barrier island in the Gulf of Mexico with a population of nearly 60,000. The Shope Laboratory is housed in a windowless three-story pavilion that is attached to a larger research building (see photo) and is designed to withstand the battering it might receive in a hurricane-prone area. The facility’s approximately 60 concrete pilings extend 120 ft into the ground, and both building and laboratory have concrete walls 10 in. thick.

The laboratory is surrounded by a secure "buffer zone," from which windows look into the lab. The air is under negative pressure so that it will not escape. All doors and other openings in the walls are sealed so that air would be contained even in the event of a complete power failure. Both intake and exhaust air are rendered noninfectious by means of high-efficiency particulate air (HEPA) filters: intake air is filtered once to protect against back drafts, and the exhaust air is filtered twice. Material that leaves the laboratory is rendered noninfectious through processes such as autoclaving or by "cooking" liquid waste. The exception is biologic materials that need to remain viable or intact. These are transferred in sealed containers, such as plastic freezer vials or glass ampules, which are enclosed in a second unbreakable container and removed through a "dunk tank" filled with disinfectant or passed through a fumigation chamber.

Charles Fulhorst, a veterinarian and associate professor of pathology at UTMB who has extensive experience with biosafety level 4, explained, "There is nothing that can go wrong in a lab like this that will ever get outside. The exception is if somebody intentionally takes something, if they put a vial in their pocket and keep walking. So we depend on the high integrity of those selected to work in biosafety level 4 laboratories."

In the laboratory, all personnel wear full-body positive-pressure "space suits" that are connected by tether hoses to a common air supply. Research is conducted within specialized areas known as "biological safety cabinets," which contain aerosols by creating a curtain of airflow away from the open laboratory space and by filtering any aerosols generated inside the cabinet. Not infrequently, researchers who pipette a virus create an aerosol. Infected animals are also constantly shedding viruses, so their cages are opened only inside a safety cabinet. In the animal room, each cage has an air supply with HEPA filtration so that animals in different experiments are prevented from infecting one another. The laboratory is designed to remain virtually sterile at all times and to keep the air safe for breathing.

**The Galveston National Laboratory under Construction on the Campus of the University of Texas Medical Branch, October 2005.**

The arrow shows the location of the Robert E. Shope Laboratory, a biosafety level 4 facility that has been operational since mid-2004.

John Glowczwski/UTMB.

In 1900, Galveston was largely destroyed by one of the deadliest hurricanes in U.S. history. So in September 2005, as Hurricane Rita bore down on the Gulf Coast, the UTMB research staff followed the procedures they use whenever the Shope Laboratory is brought down for annual maintenance. They secured and fumigated the laboratory after destroying all infectious cultures and euthanizing the animals. Culture stocks were maintained in plastic vials in padlocked freezers that were sealed with duct tape and protected by dry ice or liquid nitrogen and backup generators. In the end, the power remained on, and the laboratory sustained no damage, although the work that was in progress had to start over, at additional expense.

Biosafety level 4 research is slower, more physically demanding, and more expensive than comparable research that can be conducted at lower biosafety levels. The CDC designates many of the pathogens that are worked with at level 4 as "select agents," which are subject to strict legal requirements for possession, use, and transfer — rules that complicate the research process.[1,3] For example, researchers must register with the government and be approved. Merely entering or leaving the facility is not a trivial matter. The exit procedures take about 30 minutes and include a chemical shower to decontaminate the surface of the suit, followed by a body shower after the suit is removed. In the laboratory, the full-body suits and


**Biosafety Level 4 Laboratories in the United States.**

The laboratories in Atlanta are at the Centers for Disease Control and Prevention and Georgia State University; the latter is a "glove-box" facility, in which the researcher's body remains outside the part of the containment area that is classified as level 4. The laboratories in Frederick, Md., are at the National Interagency Biodefense Campus at Fort Detrick.

protective gloves, which are similar to dishwashing gloves, limit manual dexterity. Researchers use cameras and video monitors when direct observation is difficult, sometimes looking at a computer screen rather than into a microscope. As they move between different areas, researchers disconnect and reconnect their space suits from the central air supply. Extensive training is required — to master the environment and to perform research procedures safely. The researchers themselves face immediate risks, such as needle sticks, cuts during necropsies, or infections from aerosols.

In the Shope Laboratory, the air supply is sufficient for only four people at a time. The Galveston National Laboratory, which is expected to have annual operating costs of $20 million or more, will support a greater volume of research, provide space for more sophisticated research tools, and have a focus on applied research and product development. Stanley Lemon, the principal investigator for the laboratory, expressed the hope that in five years "we actually are doing the kind of research with agents like Marburg and Ebola that can lead to practical vaccines." According to Scott Weaver, a virologist and director for tropical and emerging infectious diseases at UTMB's Center for Biodefense and Emerging Infectious Diseases, perhaps the best example of the anticipated tools is magnetic resonance imaging of animals, such as macaques and other primates. This imaging will enable researchers to visualize disease processes in vivo over time. Such studies will replace some experiments in which animals are sacrificed daily or every several days so that necropsies can be performed to examine their tissues.

The rapid expansion of biosafety level 4 research in the United States has left unanswered questions. There is debate about whether the current federal investment in building and upgrading laboratories is excessive, inadequate, or on target, relative to spending on other public health priorities. There is uncertainty about the level of ongoing government support for operations, for the training of researchers, and for research. Training more researchers who can safely work with deadly pathogens may also increase the likelihood that one or more will be sympathetic to a terrorist organization or misuse their expertise in other ways.[4] The risks associated with biosafety level 4 laboratories, including those to the researchers and to the surrounding communities, can be greatly minimized, but they can never be eliminated entirely. Nonetheless, a research enterprise that seeks scientific understanding of deadly pathogens, as well as effective diagnostic tests, therapies, and vaccines, needs facilities that are up to the task.

Dr. Steinbrook is a national correspondent for the *Journal*.

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