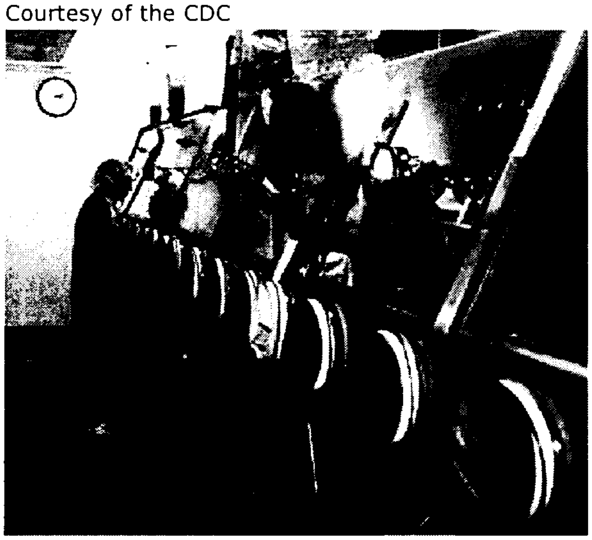
Science Under Class: Inside a Biosafety Level 4 Lab

What is it like to work with the world's most feared pathogens? | By Amy Adams

Courtesy of the CDC



PANDORA'S BOXES: Containment cabinets in a BSL-3 laboratory, prior to the construction of modern BSL-4 labs.

At first glance, the lab looks like any other. The refrigerators, centrifuges, and computers are all standard equipment, as are the ubiquitous work hoods. Even the fluorescent lighting and lack of windows are standard issue. It's the coiled air hoses, dangling from the ceiling--and maybe the air-locked, submarine style door that is the lab's only exit--that first betrays what goes on inside.

Suspended in the culture media behind that door are biologics whose very names are filled with menace: Ebola, Marburg, and Lassa fever, to name a few. Every day, scientists who work with such pathogens enter an environment unknown to others. Encased in bulky suits, they handle deadly specimens--protected by only a few pairs of gloves--in near isolation. They accept the ponderous equipment and safety regulations in order to work at the vanguard of pathogen research. It's just another day in a biosafety level 4 (BSL-4) lab, after all.

FAMILIAR TOOLS, RESTRICTED MOVEMENTS The high- security lab may contain familiar equipment, but working in it is quite a different experience, says Diane Negley, a micro-biologist at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) in Fort Detrick, Md. First and foremost is the lab wear.

Before entering the facility, scientists swap their street clothes for scrubs. Then they put on a full-body biosafety suit, which, weighing about 10 pounds and adding six inches of height, is "extremely cumbersome," says Negley. The constant airflow inflates the suit, making users resemble the Michelin Man; they occupy a lot more space and bump into things until they learn to accommodate for their new dimensions, says Negley. "Your movements are also restricted because you are hooking up and unhooking from the air," she adds.

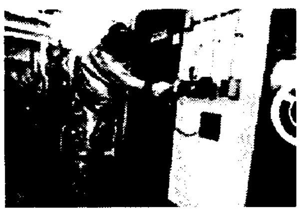
That air supply brings needed oxygen and keeps the temperature comfortable inside the suit. But it also makes for a thirsty environment. Don't quench that thirst too thoroughly before entering the lab, though. Leaving the lab midway through an experiment for an emergency restroom break is not an option. Negley says that unlike a normal lab situation, a researcher must plan out an entire experiment beforehand, and pack a cardboard box filled with prelabeled tubes, reagents and other equipment that the day's experiment will require.

Daryl Dick, a senior technician in the high-security lab at Health Canada's National Microbiology Laboratory in Winnipeg, equates the first time in a BSL lab with the first time scuba diving. "The first few times people go in the lab, the apprehension alone makes them breathe heavier." Some people never make it as far as the lab because of claustrophobia experienced inside the suit. But, he says, anyone who can tolerate wearing a full diving wetsuit with hood can handle the restriction of a biosafety suit.

Like scuba divers, researchers in the BSL-4 lab may have a buddy, but there won't likely be much talking; the airflow makes conversation difficult. The isolation of working alone adds to the physical strain of wearing the bulky suit for hours at a time, and spending those hours with nothing but plastic galoshes between tired feet and the concrete floor. Considering the intensity of the work, the physical exertion, and the isolation, Negley says working in a high-security lab is much more tiring than doing those same experiments on harmless microbes in a standard lab.

THE RIGHT TEMPERAMENT And then there's the mental strain of working with some of the world's most-feared organisms. Negley and Dick agree that, over time, scientists become accustomed to handling headline-grabbing bugs, but it never becomes routine. "Sometimes you stop and realize that you are holding a vial of pathogens only separated [from you] by three layers of gloves," Dick says. "But then you have to just get on with your work."

Courtesy of Southwest Foundation for Biomedical Research



IN THE VANGUARD: Scientists at the Southwest Foundation for Biomedical Research in San Antonio, Texas, have access to one of the safest laboratories in the world to conduct research on agents such as Ebola, Lassa fever, tularensis, and other lethal pathogens.

The ability to work carefully under dangerous conditions takes a certain temperament. An overly risk-averse person may be too cautious to get any work done, says Dick, whereas a risk-seeker may not take enough precautions and could endanger the lives of everyone in the lab. A person who can focus on detailed research for long periods of time and who doesn't need much company fares best.

Everyone needs training, though. Researchers hoping to work in a BSL-4 lab must first get the nod from the lab's director. They then spend several weeks learning the ropes under the tutelage of experienced researchers, before they are permitted to perform research on their own. Even experienced scientists receive mentoring for new procedures.

"Some people have realized that they need to change their research after trying to work the lab," Dick says. "It's not for everyone, but it's very interesting work. You are really breaking new ground with work that could change a lot of people's lives."

LEVELS OF SAFETY Labs handling microorganisms are categorized into four levels designated BSL-1 through 4. The Centers for Disease Control and Prevention and the National Institute of Allergy and Infectious Diseases collaborate on guidelines for working at each biosafety level, although neither the CDC nor the NIAID has regulatory control over the labs. Odds are, though, that if an organism requires a BSL-3 or BSL-4 facility, the organism falls under the purview of the "select agent regulations," a set of rules enacted in the wake of the Sept. 11, 2001, terror attacks that limits access to potential bioterror agents.

BSL-1 and BSL-2 guidelines pertain to labs that work with harmless organisms, such as common Escherichia coli cultures, or pathogens that have vaccines and are easily treatable. The truly nasty critters require BSL-3 and BSL-4 labs and have much more detailed handling procedures. The difference between the two categories of microorganisms comes down to how quickly they kill. Thus, BSL-3 labs would handle BSL-2 organisms occurring in a form that can be aerosolized and are therefore more toxic, or BSL-2 organisms that could cause serious illness but for which treatment is available.

Researchers who work with such pathogens in BSL-3 labs must wear booties, goggles, gloves, surgical gowns, and air filters. But they do not require the full Class A biosafety suit, according to Alan Casamajor, facility manager for biology and biotechnology research programs at the Lawrence Livermore National Laboratory in California.

BSL-4 labs are reserved for those organisms that would cause a person's untimely death upon accidental contact. According to Biosafety in Microbiological and Biomedical Laboratories (4th ed. published by the CDC and NIH) BSL-4 "is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-tranmitted laboratory infections and life-threatening disease." (http://bmbl.od.nih.gov) Usually, no vaccine exists and the infection is untreatable. Unknown pathogens that warrant extra care until more is known about them also receive BSL-4 status.

DISEASES STUDIED IN BSL-4 LABS

- Lassa fever
- Marburg hemorrhagic fever
- Russian Spring-Summer encephalitis
- Congo-Crimean hemorrhagic fever
- Omsk hemorrhagic fever
- Lassa fever
- Bolivian hemorrhagic fever
- Ebola hemorrhagic fever

According to Casamajor it's not the organism itself that is designated to a particular biosafety level but rather the type of experiment being done. "With some organisms you can go from level 2 to level 4 depending on what you are doing with it," he says. For example, many experiments with hantavirus can take place in a BSL-2 lab, but propagation should take place in a BSL-3 lab; preparing and handling the concentrated virus need to be done in a BSL-4 facility.

Though there are hundreds of BSL-3 labs located around the US, only three BSL-4 labs currently exist. They are located at USAMRIID, the CDC, and the Southwest Foundation for Biomedical Research in San Antonio, Texas. On Oct. 1, 2003, the NIAID awarded funds for two additional BSL-4 labs to be located at Boston University Medical Center and the University of Texas Medical Branch at Galveston. A sixth lab is under construction at the Rocky Mountain Laboratories in Hamilton, Mont. (NIAID recently funded nine new BSL-3 labs to supplement those in operation.)

Rona Hirschberg, senior program officer at NIAID, says the Institute awarded grants for new BSL-3 and BSL-4 labs based both on the scientific merits and on the ability of those labs to further NIAID's goals of finding vaccines and cures for emerging diseases. The new BSL-4 lab designs incorporate similar safety features to those already in operation, but will include state-of-the-art facilities. "Like any older lab, the existing BSL-4 labs aren't modern facilities," she says. New BSL-4 labs will be outfitted with modern equipment that may include imaging instruments and equipment for structural biology research.

BUILT FOR CONTAINMENT Dick calls BSL-4 labs "a totally self-contained box within a box." The labs are either housed in a separate building from other lab spaces or are located in a controlled, isolated section of a shared building. They have separate air and water supplies and a backup generator to keep the lab safe if it loses power.

Within the lab, researchers have several layers of protection from the deadly pathogens. The most obvious barrier is the biosafety suit, whose function is similar to that of a car's airbag: It's there if needed, but it doesn't play a first-line safety role.

All research takes place within the negative-pressure work hoods, which constitute the most important barriers against the bugs. "We're trained to a point where we can work in the hood without our suits and not get infected," Dick says. Such care takes patience. He says that although the equipment and techniques are identical to those of other labs, the experiments require at least twice as much time because of the careful handling that is needed.

Another key safety feature, the airflow system, draws air towards the most contaminated areas, such as the safety hoods and the animal facilities. The main lab remains at a negative pressure compared to a changing area, so that even if the air-locked door opened during a spill, clean air would rush into the lab room rather than contaminated air flowing out. Likewise, the safety suits are at a positive pressure to the lab and prevent contaminated air from entering. All the air leaves the lab through a HEPA filter designed to catch pathogens 85 times smaller than the smallest one known. This constant flow turns air over every three minutes. "If a spill does occur, once we clean it up we know within three minutes the room will not be contaminated," Dick says. He adds that all researchers must sign a form mandating that even if there is a spill, they will open their suit to perform first aid on a fellow researcher.

Together, these safety measures ensure that a pathogen safely housed in a BSL-4 lab is blocked at all paths leading to the outside world. All air ducts have HEPA filters to catch airborne organisms seeking to escape, and these filters get changed and decontaminated on a regular schedule. In the unlikely instance that a pathogen makes it into a drain, all wastewater from the lab is sterilized before leaving the facility. Even the wide-open door isn't a possible escape route, since all air flows away from doors and towards filtered air vents. Microorganisms on vials, needles, or other trash meet their end in an autoclave.

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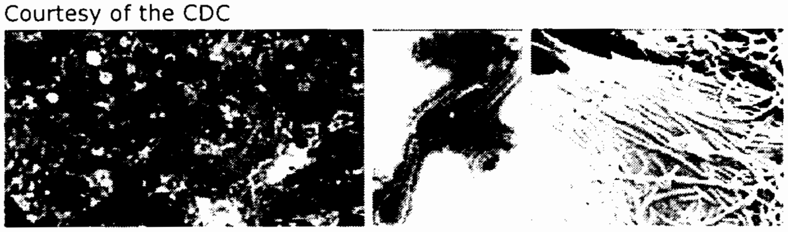

THEIR VERY NAMES FILLED WITH MENACE: Electron micrographs of the Lassa, Marburg, and Ebola viruses.

When large equipment such as a refrigerator or centrifuge breaks or needs to be replaced, the lab is completely decontaminated before a nonscientist is allowed in to make repairs or update the instrumentation, according to Michael Miller, chief of the lab response branch of the CDC's bioterrorism and response program. This process, which can take up to several days, ensures that no pathogens leave the lab with the old equipment. "When this work is going on, all samples are taken out of the lab to the storage location," Miller says.

That leaves the researchers as the only possible route out of the lab, and an unlikely one at that. Pathogens can't enter the sealed, positive-pressure suits as long as those suits are maintained hole-free. Stray pathogens on the suit's exterior face a five-minute, 200-liter decontaminating chemical shower, during which researchers sponge off the outside of the safety suit and clean any vials or equipment they may be carrying. Virus samples, even dead ones, stay with researchers while they change and take their personal showers. Any equipment then passes through an autoclave before returning to the low-security world.

"Because of what the agents are, you have to keep the samples in your possession," Negley says. She added that the benefits of working with killed virus in the comfort of her street clothes is worth the effort of transporting them out of the BSL-4 lab.

Some of the same physical and procedural barriers that prevent pathogens from escaping the lab also keep unauthorized people from getting in. Navigating from the street to the lab involves a gauntlet of security checks that include identity badges, visual inspections, personal identification numbers, and logbooks to record each researcher who comes and goes. Security personnel circle the lab to thwart anyone hoping to avoid those security measures.

These multiple layers of security ensure that researchers working with the world's most dangerous organisms remain safe from the pathogens they work with, as are those elsewhere in the building, and the public at large.

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