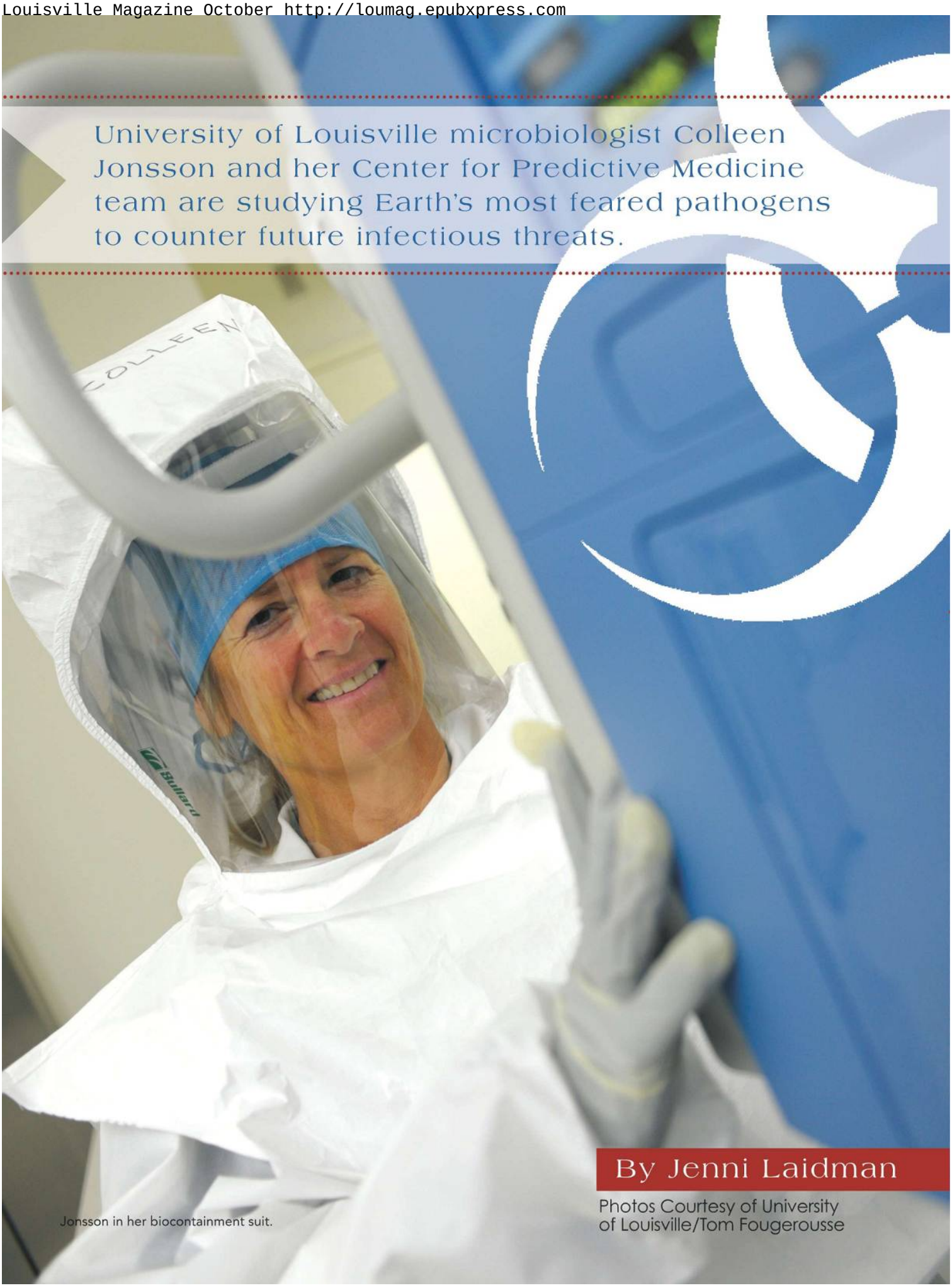
University of Louisville microbiologist Colleen Jonsson and her Center for Predictive Medicine team are studying Earth's most feared pathogens to counter future infectious threats.

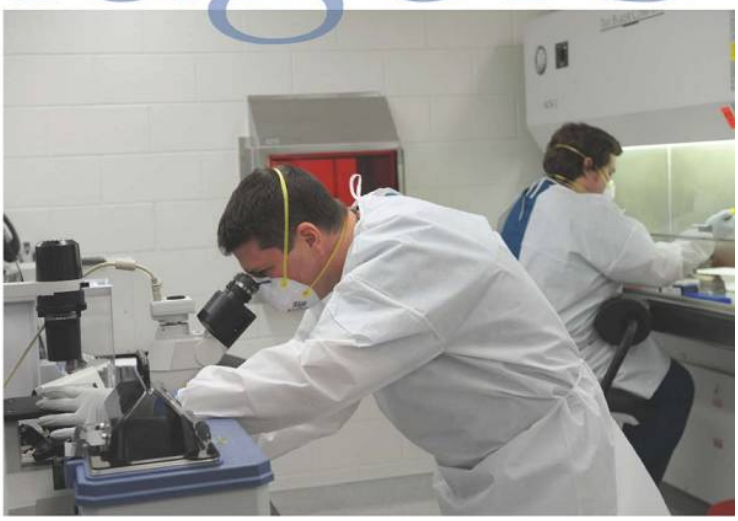


Jonsson in her biocontainment suit.

By Jenni Laidman

Photos Courtesy of University of Louisville/Tom Fougerousse

Microbe Managers



HER EYES ARE HAZEL. They open doors.

The iris scanning camera doesn't actually see their color; rather, it compares the unique angles within to a database. Only then can Colleen Jonsson activate the electronic access that lets her inside.

There are five doors, each with special security requirements, between the entrance to the University of Louisville's Regional Biocontainment Laboratory and the room where she leaves street clothes behind in favor of surgical scrubs. She tops the scrubs with a white Tyvek coverall and dons an air-purifying respirator, a hood, double gloves, and special shoes and socks. If the experiment under way is particularly risky, even underwear stays behind. After years of working in such laboratories, Jonsson, director of the Center for Predictive Medicine for Biodefense and Emerging Infectious Diseases — of which the laboratory is the major part — rarely wears jewelry. Why put on something you're only going to take off again in a little while?

This is biology's inner sanctum, soon to be home to Louisville's most dangerous inhabitants, lined up in tiny tubes inside locked freezers. There will be bubonic plague, and hantavirus — the respiratory infection that swept through the Four Corners region of the United States in 1993, killing 13. There will be commoners, such as influenza, and exotics, such as SARS (severe acute respiratory syndrome), which killed nearly 10 percent of its victims in 2003. The numbers of the frozen criminals could increase as new infectious diseases emerge, or as their danger as instruments of terror rises. The lab is waiting for the final OK from the U.S. Centers for Disease Control and Prevention to bring in these bad microbial actors sometime this fall.

Outside, the traffic on Hurstbourne Parkway rushes past, drivers unaware of the unremarkable building surrounded by a black fence and rangy meadow plants. The fence is guarded and crash-resistant. The building's specially coated windows won't break. It would take so long to penetrate the reinforced brick and concrete walls — even with the right tools — that arrest would precede entry. If someone shuts off the electricity to the building, a backup system launches. If they went after the water supply, a secondary water supply awaits. If the whole city lost power, laboratory operations would continue, fed by 25,000 gallons of fuel oil stored underground in a double-walled fiberglass tank ringed by leak monitors. "It's layers upon layers upon layers," says Brandy Nelson, biosafety manager for the Center for Predictive Medicine. Nothing is left to chance.

The laboratory, in the northeast corner of U of L's Shelby Campus, is one of 14 biosecure facilities around the nation commissioned by the National Institute for Allergy and Infectious Diseases. Although NIAID is part of the National Institutes for Health, its mission here has an air of defense: These emerging infectious diseases are also potential instruments of terror. If the threat of nuclear war hung over the 20th century like a mushroom cloud, the more stealthy threat of viruses and bacteria shadows the start of this century.

JONSSON HAS LONG, unfussy gold-brown hair. She is the owner of two mixed-breed dogs, both Humane Society rescues, and she looks like your next-door neighbor. She spends her spare time canoeing and learning to sail on the Ohio River. She also consorts with dangerous

The University of Louisville's new highly secured Regional Biocontainment Laboratory, located behind a black fence on the school's Shelby Campus.

disease. As the director of the Center for Predictive Medicine, her life's mission revolves around the laboratory's worst occupants — unspooling their mysteries and blunting their sting.

Jonsson is interested in the proteins in hemorrhagic Ebola virus — the chilling and incurable disease that turns bodies into bloody sieves. She regularly travels to the Amazon to look for rodent-carried hantavirus. In Paraguay she discovered that disturbed ecosystems led to outbreaks of the disease.

She was working at Southern Research Institute in Birmingham, Ala., when she was lured up I-65 to Kentucky in 2008 by the Louisville lab's resources, procured in recent years. Biochemist Eugenia Wang was one of the first to urge the university to compete for a slice of NIAID's millions. Wang, a trailblazer in the genetics of aging and director of the Gheens Center on Aging in the U of L School of Medicine, thought university research was a perfect match for the NIAID laboratory.

Influenced by a mentor who discovered that our own enzymes help influenza virus climb into our cells, Wang envisioned a center that would look at how our cells conspire with germs to cause infection. "We're always looking from the virus point of view," Wang says. "Why don't we look at the person at the core of the reaction?" With a focus on the disease host, the center could look for treatments that might disarm many pathogens at once, instead of finding a single weapon that could shoot only one enemy.

"Science is pretty much held captive by traditions," Wang says. Since the creation of the polio vaccine and earlier, researchers took a bug-by-bug approach, looking for a single medicine to combat a single disease. But the expansion of our understanding in genetics turned a host-based paradigm into the holy grail of individualized medicine, she says, and a Louisville laboratory could be positioned to pursue it. "This is a lesson I learned in my early career," Wang says. "To get ahead in science, you need a unique tool and a unique system." The system would be a host-based approach; the tool would be the latest in laboratory equipment.

Nancy Martin, in 2004 U of L's vice president for research, was persuaded by Wang's idea. University administrators envisioned lab construction on the underused Shelby Campus — 220 acres the university acquired in 1969 with the bankruptcy of the private Kentucky Southern College. The lab fit within the university's plan to transform most of the property into a research and office park. It seemed the perfect idea — unless you happened to live nearby.

JAY COMSTOCK WAS among those who didn't think much of the university's vision for his neighborhood. One of the owners of Comstock Brothers Electric Co., he lives in Bellemeade, just west of the Shelby Campus. He attended the first informational meeting about the lab.

It didn't go well.

"We made a huge mistake," U of L president James Ramsey said in August. "We tried to say, 'This is good for you.' We tried to sell it." In subsequent meetings, university personnel listened more and attempted to persuade less.

Talk to any U of L administrator and you'll hear the "mandate" mantra, a reference to Kentucky House Bill 1 of 1997. University officials say the bill "mandates" the University of Louisville to become a "premier metropolitan research institution." What better way to move toward fulfilling that mandate, they argue, than constructing a one-of-a-kind, 37,000-square-foot, $34.6 million facility? (The federal government picked up $22 million of the costs; the university paid $3 million and floated a bond for the remainder.) But neighbors felt no mandate. They felt a "shove-this-foregone-conclusion-down-our-throats" disruption. The meeting grew heated. Not as bad as a school board meeting to discuss school assignment, Comstock says, but people were upset. He stuck around afterwards to talk more.

Cheri Hildreth wanted to talk to Comstock, too. She also singled out other vocal critics from the meeting. Would you be on a citizen's advisory committee, she asked them. Would you tell us what we need to hear? Comstock signed on.

You don't hear much about Hildreth, the U of L director of environmental health and safety. She has the kind of job with enormous impact and nearly zero public presence, even though her role is critical to all kinds of university projects, including building Papa John's Cardinal Stadium and engineering the delivery of 19,000 H1N1 vaccinations in a day and a half last November. But the biosafety lab was quickly evolving into a monster on her agenda, and it wasn't just because of angry neighbors.

"Honey, let me tell you something — you don't even want to know all the stuff we've been through on this," Hildreth says. She had all kinds of new rules to absorb. She made trips to Washington, D.C., to "get us across the goal line." She helped select the architect/engineer, and the group that would test all building systems when the project was complete. She and two staff members were involved in design, construction and testing. Construction meetings were long and crowded with federally hired consultants. She helped the lab gain CDC approval to handle "select agents" — substances with the potential to cause severe health risks, including things like *Yersinia pestis*, which causes bubonic plague; Herpes B virus; and the bacterium that causes Rocky Mountain spotted fever. Not every NIAID lab has this clearance, and it's not easy to get. "It's a cumbersome process," Hildreth says. Everybody from Colleen Jonsson to the technicians who care for the laboratory mice go through a Department of Justice background check as part of the approval.

Then there were the bombs.

"They were driving us crazy," Hildreth says. A 35-pound satchel bomb right outside the lab door exploded; 500 pounds of explosives lay in wait only yards from the facility. These were just some of the imaginary scenarios lab management and local safety and emergency personnel worked their way through, deciding in detail how they would respond to any number of emergencies, from big explosions to someone passing out in a protected part of the laboratory.

Hildreth also attended every public meeting the laboratory required, and she worked with the ad hoc citizens committee for which she recruited Comstock. The ad hoc group suggested better, earlier meeting notification. They expressed reservations about how the neighborhood would be alerted to accidents. As a result, an elaborate warning system was devised that begins with the sound of sirens and ends with a computerized phone call placed to affected neighbors.

While the university's efforts didn't make objections to the lab disappear, it did ease some minds. "I think it would be nice if they built it in another place, but I am happy they have taken a lot of precautions," says Ed Wessel, a Bellemeade resident and president and CEO of LouChem Federal Credit Union. "They even took us on a tour to look at the safety factors, so I've been pleased with that."

The biggest fear, that deadly pathogens would easily escape and race through the neighborhood, lessened over the months of meetings.

"It took me two or three meetings before I was convinced this was safe," Comstock says. What finally cinched it were the blueprints.

"I swear I've never seen anything like it," he says. "It's built like a brick you-know-what. It's built literally like a bomb shelter, like a nuclear power plant, with redundant system upon redundant system. It's well-built, well-thought-out and well-planned. I was impressed. I've been in the construction business since about 1972, and it assuaged my fears."

Having plague in the neighborhood suddenly seemed doable.

UNDER A MICROSCOPE, *Yersinia pestis* inspires no shivers. Capsule shaped — long with rounded ends — its surface looks fuzzy, like a throw pillow discovered under the bed. Its common host, a flea, is a far more frightening beast under magnification. Brown and almost transparent, it has bristly, squirmy legs, hairs on its back and weird appendages protruding from its face.

Matthew Lawrenz, a researcher at the Center for Preventive Medicine, bypassed the flea's services in his controlled spread of black plague and injected the *Yersinia pestis* into mice with a needle. But first he mutated some of the bacteria. He wanted to know how the mutation would affect plague's killing powers. Would it reveal a possible target for new medications? Bubonic plague killed a third of the population of Europe in the 14th century and today is a potential bioterrorism weapon.

In 36 hours — a day and a half — he could already see a difference. Animals injected with mutated plague had fewer bacteria in their lymph nodes than those injected with normal plague. Alteration of a protein on the fuzzy surface of the bacteria crippled plague's attack, revealing, perhaps, a future drug target.

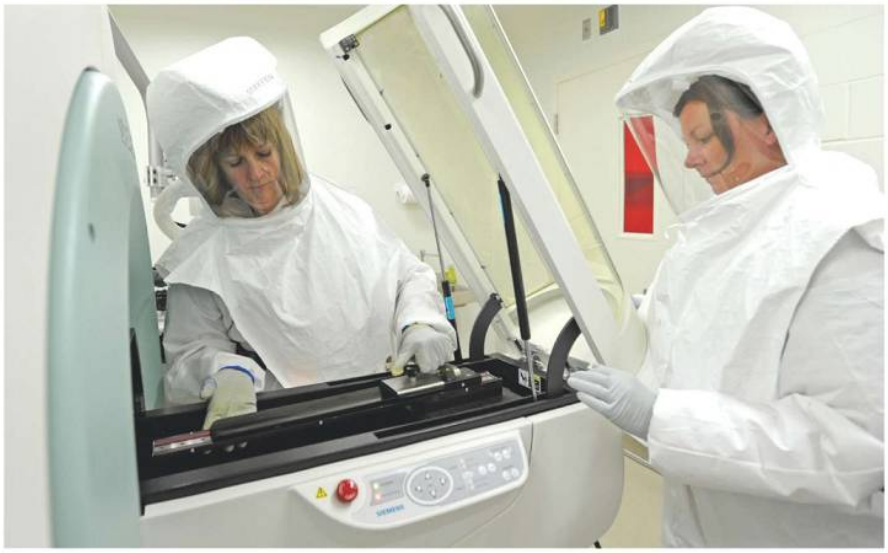
Lawrenz did that experiment during his postdoctoral studies at the Washington University School of Medicine in St. Louis. But now that he's at U of L, he can actually watch how the infection moves through the mouse, gaining fresh insights.

Lawrenz and the three other researchers now at the Center for Predictive Medicine all say the laboratory's equipment — the unique tool Wang foresaw — was the main reason they came to Louisville. Other facilities may have similar high-tech goodies, but Louisville is one of the few that has the equipment in biosafety level 3 protection, meaning it can be used with some of the meanest organisms on the planet. Perhaps chief among these technological wonders is a machine that looks like a CT scanner for a Barbie doll. It's called a Siemens tri-modality scanner and it very nearly is what it looks like — just substitute "rodent" for "Barbie doll" and add positron-emission tomography and single-photon computerized tomography to regular computerized tomography. Think of it as, "Honey You Shrunk the Really Expensive Hospital Technology!" The $1 million unit combines the equipment physicians use to look for cancer, or watch brain function, or create images of internal organs, in a machine designed for a rodent. And that's what Lawrenz would use to watch the plague in real time.

Even putting something so basic as a microscope into the lab's protected area makes a significant difference to research. If the microscope is outside the safe area, scientists could only use it for dead organisms — and that's generally how such studies are done. But the BSL3 has a special high-resolution confocal microscope in a protected area.

"This is a unique resource," Jonsson says. "Not many labs in the nation have this available to them, to look at cells alive." This imaging power played a persuasive role in her decision to leave Southern Research, where she created a successful infectious-disease program.

"It was really hard to leave," Jonsson says. "But I really wanted a tool that would allow me to see the effect of my therapeutics in real time in animals. With this imaging, I can do that."



The lab's compact Siemens tri-modality scanner looks like a CT unit for a Barbie doll, and it very nearly is — just substitute "rodent" for "Barbie doll."

The move to Louisville also gives her a third opportunity to build a program from scratch. At Southern, which historically focused on cancer, she created an infectious-disease initiative, and at New Mexico State University before that, she fostered a statewide network to offer college students research opportunities. Under her leadership, the new Center for Predictive Medicine will add at least three more researchers in the near future. She will supervise the addition of another 13,000 square feet to the biocontainment facility, for a total of 50,000 — an expansion made possible by the infusion of $9.8 million in federal stimulus money. She will collaborate with institutions all over the country and work with local hospitals in the event of infectious outbreaks, and she could help the lab become an engine for biotechnology business in Louisville.

The size of any economic boost from the lab is at best a guess, but a study conducted

for the University of Texas Medical Branch at Galveston concluded that its new biocontainment lab would add $1.4 billion to the state's economic production over 20 years, as well as 22,500 person-years of employment in those two decades. The Galveston lab is a biosafety level 4 facility, one of just a handful in the country. The difference between what's studied in a BSL3 like Louisville's and a BSL4? There are cures to the infectious agents in Louisville's freezers. There are no cures for BSL4 agents.

HOW JONSSON GOT to this point in her life, at the leading edge of infectious disease research, is a winding story, full of determination and uncanny adaptability — not unlike the viruses she studies, with their swift ability to mutate when conditions change.

She started as a plant biologist, working for Monsanto for two years after college on fungal diseases in soybeans. In graduate school she studied how some plants resist a fungal assault. But she wanted to study a simpler organism than fungi. There is nothing much simpler than a virus, a strand of genetic material that can't even reproduce without hijacking another creature's cell. She switched to virology.

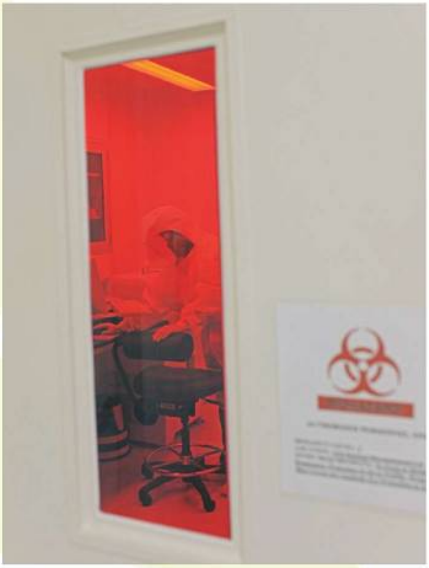
A virus may be a simpler organism, but its behavior isn't. Jonsson spent a decade studying how some types of viruses, called retroviruses, use an enzyme called integrase to plug their genetic material into your DNA. That led her to an interest in hantaviruses, a retrovirus that emerged in New Mexico right before she started teaching at New Mexico State University. Hantavirus — which first brings on flu-like symptoms, then constricted breathing, respiratory failure and, sometimes, death — got her into fieldwork, testing farm workers to see who had been exposed to hantavirus. (Eventually this would lead her to Honduras, Paraguay and Brazil.) Along the way, she took up the objective of drug discovery, forming a partnership with University of New Mexico chemist Jeffrey Arterburn. Their work led to a patent on a more easily tolerated antiviral drug.

When she left New Mexico State for Southern Research in 2003, she and microbiology colleague Dong Hoon Chung began screening thousands of small molecules as possible drugs against SARS, influenza, West Nile virus, and respiratory syncytial virus. This approach to drug discovery, called high-throughput screening, uses a plate the size of an index card with 384 depressions. Into each tiny well goes a cell under attack by a particular virus or bacteria. A different small molecule is dropped into each well. A computer records which molecules affect pathogen activity and by how much. Chung will direct high-throughput drug discovery at the Louisville laboratory.

But without a doubt the biggest adaptation of Jonsson's life took place when she was 18 and a student at a community college in St. Louis. It is unusual to find Ph.D. scientists who ever attended community college. But Jonsson hadn't planned to become a scientist. She intended to be an artist, to the exclusion of everything else. "I didn't want to do anything except art," she says. "I didn't have very good grades."

Then she encountered Jon Hawker, her first college biology teacher.

"All of a sudden the light went on," she says. "That was it. He opened up the whole world and I wanted to know about everything."



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JONSSON AND BIOSAFETY manager Nelson lead a visitor through the lab's still empty animal area, the future home of rodents to be used in experiments. The floors here are shiny, so reflective they look wet. Five layers of resin coat them, making it impossible for any spill to disappear. Animals will move into this area soon.

The sound of rushing air is loudest here, although it is the background music through most of the BSL3. Step inside a secure laboratory and a steady breeze rushes in under the door. It's because all the laboratories are maintained under negative pressure so that no lab air ends up in the hall. In here, the air is changed 15 times each hour. Even the rodent air is sequestered. The air that caged mice breathe doesn't mix with the air in the rest of the lab, and it, too, is filtered.

Everything is meticulously planned, down to how deliveries will be made to the building and what to do with trash.

Despite all the sterility, and all the rules and regimentation, for the researchers the BSL3 is the promised land. It's clear as Jonsson unconsciously strokes the confocal microscope as she explains its benefits. Who knows what might be discovered here?

For instance, when bacteria infect us, we generally know, they slip around our immune defenses and then suppress them. Jonathan Warawa, an investigator with the Center for Predictive Medicine, wanted to know how a bug called *Burkholderia pseudomallei* sneaks behind enemy lines and takes up residence in the human body, where it causes the disease melioidosis. The pathogen is common in Southeast Asia and northern Australia and is a possible weapon in bioterrorism. In fact, its near-identical relative, *Burkholderia mallei*, which causes the equine disease glanders, has a long history in biological warfare. Glanders can also infect humans. In World War I German troops sent glanders-infected animals across battle lines, and in the Second World War, Japanese military employed the bacteria against civilians, prisoners of war and horses, according to the *Journal of the American Veterinary Medical Association*. There is also evidence that the former Soviet Union deployed the pathogen against Afghan soldiers in the 1980s.

To find out just what happens when melioidosis bacteria invade, Warawa exposed mice to an altered form of the pathogen, one stripped of its sugar coating — a chain of sugars embedded in the bacteria's outer membrane. Warawa did this work when he was at Rocky Mountain Laboratories in Montana. Although lung cells mounted a spirited defense against this mutated invader, cells in the liver and spleen missed the alarm call.

"This gives us clues, additional questions to ask," Warawa says.

Warawa also participated in a study in which, not only melioidosis, but plague and the bacterium *Francisella tularensis*, lost virulence under the assault of a single treatment — an artificial bubble called a liposome carrying a fragment of *F. tularensis* membrane. The germ causes tularemia, sometimes called rabbit fever. It's considered among the most infectious bacteria known; inhalation of only a few organisms can cause serious disease.

This is the kind of single-bullet multi-target weapon that center researchers hope to find as the Center for Predictive Medicine matures.

"You can just think about science as full of opportunities," says Eugenia Wang. "You just grasp them when they show up."

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