Time-Critical Technology Identifies Deadly Bloodborne Pathogens

CONSIDER this scenario—an elderly man is brought to an emergency room confused, disoriented, and shaking severely with chills and fever. His heart is beating rapidly, and his blood pressure is dangerously low. The patient appears to be in a state of shock. What doctors suspect, but can't immediately confirm, is that he is suffering from extreme sepsis, a serious medical condition characterized by a whole-body inflammation and the presence of a bloodstream infection. Sepsis is the second-leading cause of death in noncoronary intensive-care-unit patients and the tenth-most-common cause of death overall in the U.S., according to the Centers for Disease Control and Prevention. If the patient does not receive swift medical intervention, death is imminent.

A new bloodborne-pathogen detection technology may in the near future allow doctors to diagnose serious bloodstream infections within an hour. Conventional lab tests typically take several days. Furthermore, instead of transporting a blood sample to a hospital, trained medical personnel could make a diagnosis at an emergency shelter or at the scene of a disaster. These and other advances are expected to result from a five-year, $8.5 million grant to a team of scientists, engineers, and physicians from Lawrence Livermore and the University of California (UC) at Davis that focuses on point-of-care testing (POCT). POCT is defined as diagnostic testing at or near the site of patient care.

"According to a recent study from the University of Manitoba in Canada, during the first six hours after the onset of septic shock, the mortality rate increases 7 percent for every hour that a patient doesn't receive effective antimicrobial treatment," says Livermore chemist Brian Baker, who works in the Laboratory's Physical and Life Sciences Directorate. "For treatment to begin without delay, health care workers need to quickly identify which bloodborne pathogen has infected the patient."

The National Institute of Biomedical Imaging and Bioengineering (NIBIB) awarded the grant in October 2007. The collaboration was funded as part of NIBIB's Point-of-Care Technologies Research Network, which includes three other medical research centers that are part of a larger cooperative research effort. The grant provides funds for developing and testing two single-channel prototype instruments, each of which can simultaneously detect five bacterial and fungal bloodborne pathogens. The first-generation POCT device measures about 40 by 50 by 50 centimeters and is designed for hospital settings, while a smaller, second-generation device will be designed for deployment to remote disaster sites.

Quick Detection Saves Lives

Five pathogens were selected based on their clinical significance, occurrence in hospitalized patients, threat to the community, and frequency of being found in wounds on victims of severe floods and other weather-related natural disasters. They are methicillin-resistant *Staphylococcus aureus* (MRSA),

a bacterium long associated with catheter-related infections and now being found in community settings such as schools; *Pseudomonas aeruginosa*, a bacterium often linked to hospital-acquired bloodstream infections and associated with pulmonary complications such as respiratory distress syndrome; *Escherichia coli*, one of the more common organisms found among patients who contract hospital-acquired infections; *Streptococcus pneumonia*, the most common cause of severe community-acquired pneumonia; and *Candida* yeast, which can affect people with weakened immune systems. Infections with any one of these five pathogens can lead to sepsis.

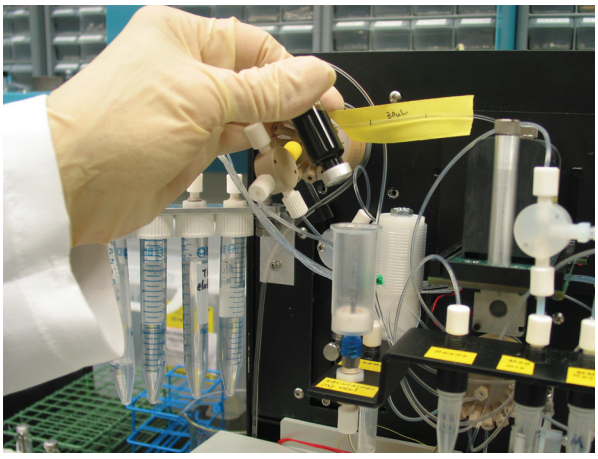
The detection process begins with loading a blood sample in the POCT instrument. The device then automatically handles all the remaining processing steps. Instead of relying on the polymerase chain reaction (PCR) technique used today—a time-intensive process that requires multiple cycles of heating and cooling blood samples—the compact instrument uses a new DNA amplification method called loop-mediated isothermal amplification (LAMP). The LAMP method uses a portion of *Bacillus stearothermophilus* DNA polymerase protein, an enzyme that splits the double strand of DNA and allows it to be copied at a single temperature (63°C, 145°F), saving precious time. The multichannel prototype can run a synchronized test for all five pathogens in the blood sample within one hour.

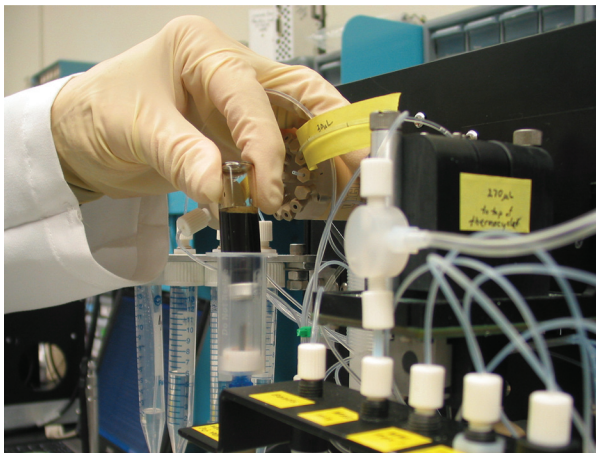
In 2005, emergency response efforts in New Orleans following Hurricane Katrina demonstrated the basic feasibility of POCT. However, according to Nam Tran, associate director of the Center for POC Technologies at the UC Davis Medical Center, follow-up laboratory experiments showed that current testing equipment is not adequate for field use by first responders. Rescues were impeded during the Katrina response because hospitals were out of commission and doctors did not have adequate tools for making fast diagnoses. Consequently, treatment was delayed. "Our preliminary survey work following Hurricane Katrina found disaster responders lacked evidence-based methods to diagnose and treat bloodstream infections," says Tran. "Bringing rapid pathogen nucleic-acid recognition technologies to the point of care will accelerate treatment decisions and potentially improve outcomes."

A Network of Solutions

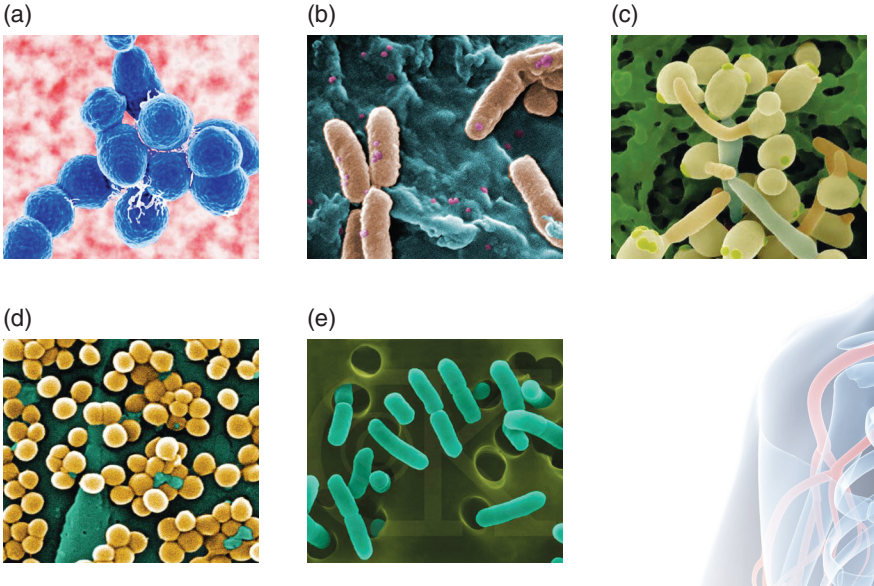
Previous Livermore-developed biodetection technologies—including the Autonomous Pathogen Detection System for protection against bioterrorism—will provide some of the foundational technologies for the new POCT instruments, according to chemical engineer John Dzenitis, who is leading the effort at Livermore. "Many of the techniques we use to detect bioterrorism can be adapted to detect bloodborne pathogens," says Dzenitis. "Before beginning this project, we had a good starting point in bioinformatics research, including tools for designing DNA tests, procedures for screening and optimizing the tests, and instrumentation to automate the process."

In addition to the center at UC Davis–Livermore, NIBIB has established three other centers that are part of the larger cooperative research effort. One center is at the University of Cincinnati and focuses on emerging neurotechnologies. Another center at Johns Hopkins University in Baltimore, Maryland, focuses on research involving sexually transmitted diseases. PATH Seattle partners with the University of Washington at the fourth center, which focuses on diagnostics for global health. Brenda Korte, program manager of NIBIB's Division of Discovery Science and Technology, oversees all four NIBIB centers.

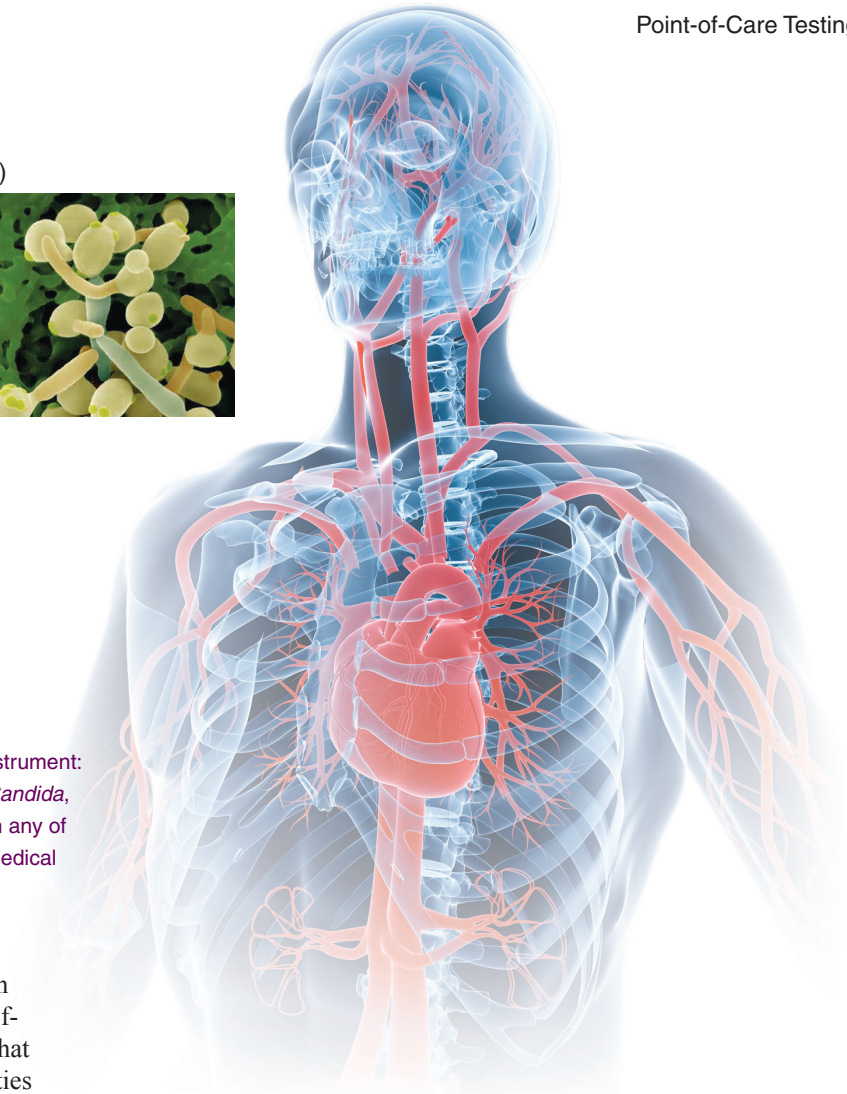




A blood sample is loaded in a prototype point-of-care testing (POCT) instrument designed for fast detection of high-priority bloodborne pathogens. The portable device uses a new DNA amplification method called loop-mediated isothermal amplification to detect multiple targeted pathogens at once.



Five pathogens were selected for testing in the prototype POCT instrument: (a) *Streptococcus pneumonia*, (b) *Pseudomonas aeruginosa*, (c) *Candida*, (d) *Staphylococcus aureus,* and (e) *Escherichia coli.* Infections with any of these pathogens can lead to sepsis. If a patient does not receive medical intervention within hours, death is imminent.



"The goal of the Point-of-Care Technologies Research Network is to drive the development of appropriate point-of-care diagnostic technologies through collaborative efforts that simultaneously merge scientific and technological capabilities with clinical need," says Korte. In the field, the need for rapid and accurate diagnosis of infectious disease is critical, given the dependence of therapeutic choices on pathogen identification and the time-consuming nature of alternate approaches. Korte notes, "The disaster setting presents specific environmental challenges that the team is addressing through ongoing needs assessments and through its expertise in both technology development and clinical applications."

A Healthy Future

The team plans to eventually develop an instrument capable of testing several blood samples at once. Initial tests have already begun at Livermore on bovine whole blood spiked with bacterial DNA and live bacterial cells. Future plans include testing similarly spiked human whole-blood samples on the POCT instruments at UC Davis Medical Center. These tests, expected to commence this year, will use whole blood from patient volunteers who are suspected of having a blood infection.

POCT devices also could be adapted to test for other types of pathogens, according to Dzenitis. "With a tweak in design, our device could be used to detect influenza viruses, or it could be used to test for certain types of DNA to determine if someone is predisposed to conditions such as Parkinson's or diabetes," he says. Whatever its purpose, one thing is certain—the POCT device has the potential to better prepare the nation for future disasters and enhance patient survival outcomes through rapid decision making at both hospital bedsides and in emergency field operations.

—Kristen Light

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