Detecting Bioaerosols When Time Is of the Essence

ABOUT seven years ago, Livermore researchers received seed funding from the Laboratory Directed Research and Development Program to develop an instrument that counters bioterrorism by providing a rapid early warning system for pathogens, such as anthrax. (See *S&TR*, January/February 2002, pp. 24–26.) That instrument, the Autonomous Pathogen Detection System (APDS), is now ready for deployment to better protect the public from a bioaerosol attack, and the development team has been honored with a 2004 R&D 100 Award.

The lectern-size APDS can be placed in airports, office buildings, performing arts centers, mass transit systems, sporting arenas—anywhere an attack might be launched. APDS was designed to get results fast and get them right, without false positives. Biological scientist Richard Langlois, who spearheaded the APDS development effort, explains, "The system provides results on the spot. Faster results allow a faster emergency response, which in the end means saving lives."

The Autonomous Pathogen Detection System (APDS) monitors the air continuously for biological threat agents and uses two identification technologies to reduce the probability of false alarms. It can measure up to 100 different agents per sample and reports identified agents within an hour.

Responding Rapidly, Reliably

One of the methods a terrorist might use to disperse a biowarfare agent is through an aerosol attack. In fact, the anthrax mail room release in 2001 and the ricin release in 2004 involved relatively small amounts of deadly material. Countering such threats in an effective manner requires an automated system that continuously monitors the air, quickly analyzes samples, and identifies a wide range of agents without false positives.

APDS is designed to meet that need. It monitors the air for the three types of biological threat agents: bacteria, viruses, and toxins. Because it operates continuously, the system can detect low concentrations of bioagents that might go undetected by a system that is triggered only when the overall number of particles in the air is high. APDS collects aerosol samples, prepares them for analysis, and tests for multiple biological agents simultaneously. This automation reduces the cost and staffing that would be required to manually analyze samples.

The current system is configured to test simultaneously for 11 agents and can be expanded to 100 agents without a change in instrumentation. "Given the number of pathogens potentially available to terrorists," says Langlois, "the ability to detect and analyze large numbers is critical." APDS also identifies particles within 1 hour—faster than comparable systems, which can take 4 to 20 hours. Having results promptly is crucial for emergency-response efforts, as is being certain that the results are real. "Our goal was to have two independent, autonomous, 'gold-standard' assays to provide the highest confidence in detection results in the shortest possible time," says Langlois.

Checking It Twice

As APDS collects air samples, it first runs them through an immunoassay detector. If that detector returns a positive result, APDS performs a second assay based on nucleic-acid amplification and detection. Having two different assay systems increases system reliability and minimizes the possibility of false positives.

The immunoassay detector incorporates liquid arrays, a multiplexed assay that uses small-diameter polystyrene beads (microbeads) coated with thousands of antibodies. Each microbead is colored with a unique combination of red- and orange-emitting dyes. The number of agents that can be detected in a sample is limited only by the number of colored bead sets. When the sample is exposed to the beads, a bioagent, if present, binds to the bead with the appropriate antibody. A second fluorescently labeled antibody is then added to the sample, resulting in a highly fluorescent target for flow analysis. Preparing the sample and performing this first analysis takes less than 30 minutes.

System software compares the result with preset threshold criteria for a positive identification. A positive immunoassay result triggers the second test—a DNA analysis using the rapid polymerase chain reaction (PCR) technique. For this test, an archived sample is mixed with reagents for the target organism and introduced into the flow-through PCR system, which consists of a Livermore-designed, silicon machined thermocycler mounted in line with the sample preparation unit. Specific nucleic-acid signatures associated with the targeted bioagent are amplified up to a billionfold and detected as a change in fluorescence. The PCR analysis is completed within 30 minutes.

Results are transmitted every hour to a control center, where the instrument's performance is monitored. "The architecture of wireless communication with a command center works well with existing building safety and security systems," says Langlois. "Because malfunctions and failures are rare, a small command staff can easily oversee a network of 10 to 100 instruments and still provide maintenance, scientific interpretation of assay results, and communication with the appropriate authorities."

Saving Time, Saving Lives

In September 2003, APDS passed a series of pathogen exposure tests at a high-containment laboratory at the Dugway Proving Ground in Utah. In these trials, the system clearly demonstrated that it could detect real pathogens and confirm the identifications with a fully automated second assay method. APDS units were also deployed at the Albuquerque Airport in New Mexico and at a Washington, DC, Metro station, where they provided continuous monitoring for up to seven days, unattended.

The system can be adapted for situations where environmental or clinical pathogens require monitoring. For example, APDS could test for mold or fungal spores in buildings or for the airborne spread of contagious materials in hospitals. It also could identify disease outbreaks in livestock transport centers or feedlots. "Basically, there are no fully integrated systems with the capabilities of APDS commercially available in the civilian or military market," notes Langlois. "The system offers ongoing environmental monitoring and rapid detection of harmful pathogens, allowing emergency workers to respond immediately to decontaminate areas and, most importantly, save lives."

—Ann Parker

Key Words: anthrax, Autonomous Pathogen Detection System (APDS), biological agents, bioterrorism, multiplex immunoassay, polymerase chain reaction (PCR), R&D 100 Award, ricin.

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