

Tech Notes

CANARY: Technology for rapidly identifying biological agents

The incidents of anthrax-laced letters, the emergence of SARS (Severe Acute Respiratory Syndrome), and repeated occurrences of illnesses caused by food-borne pathogens highlight the need for rapid, sensitive identification of the responsible biological agents. Lincoln Laboratory has developed instruments for rapid bioidentification and has transferred the technology to industry.

Growing threats posed by pathogens—whether in intentional bioagent attacks or through unintentional spread of disease—have intensified the demand for a pathogen detection and identification method that can provide a warning before the target population is infected.

To meet this need, MIT Lincoln Laboratory has developed biosensors that significantly improve the speed and sensitivity of bioagent identification. These biosensors use a Laboratory-developed technology called CANARY (for Cellular Analysis and Notification of Antigen Risks and Yields).

- CANARY uses nature's bioidentifiers, B cells, the fastest pathogen identifiers known. B cells are a type of white blood cell that binds to and recognizes pathogens.
- The Laboratory genetically engineered B cells to bind specifically to pathogens of interest and then, within seconds, to emit photons that indicate that binding and therefore recognition have occurred.

- Prototype sensors were built and validated and are now used in fielded applications. The technology has been transferred to industry for manufacturing.

Speed and Sensitivity

CANARY can detect <50 particles of pathogen in less than 3 minutes, including the time required to prepare the pathogen samples. In contrast, competing technologies take between 15 minutes and 4 hours, time spans that are inadequate for warning a population.

The speed demonstrated by CANARY makes it the only technology that can provide true detect-to-warn operation.

CANARY Applications

The sensitivity and speed of CANARY, along with the multiple-sample capability and the sensor's portability, make the technology valuable for rapid onsite detection/identification needed in emergencies. CANARY could provide an excellent first screen

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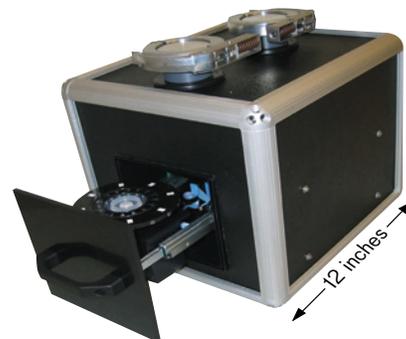
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For liquid samples, the Laboratory developed a 16-channel sensor that processes ~100 samples in an hour. This unit has higher throughput than that of any competing technology.



For aerosol samples, the 37 lb, 1 ft³, portable PANTHER sensor called CUB (for Compact Unit Biosensor) has shown identification of pathogens in a record time of <2 minutes, and its modest cost, less than \$30,000, makes it viable for widespread biodefense use.

for people who may have been exposed to aerosolized spores, thus enabling treatment to be started sooner.

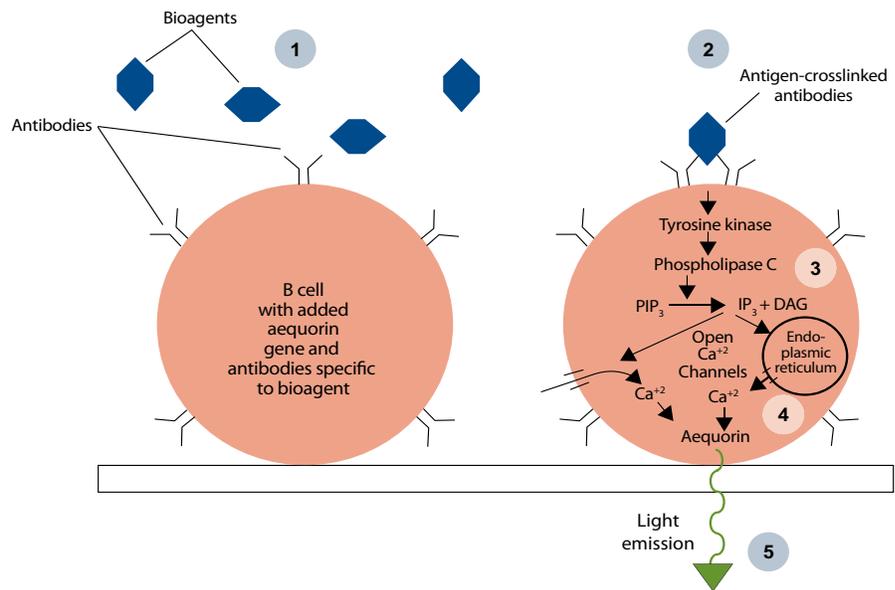
Lincoln Laboratory has tested CANARY's ability to identify bio-agents contaminating a number of sample types. Tests on produce and meat have demonstrated that CANARY can rapidly detect small amounts of pathogens such as *E. coli*.

Technologies for collecting dry aerosols have also been developed. The dry-aerosol format collects particles directly from the air by exploiting the relatively high momentum of particles to force them to impact on a dry surface (e.g., inner surfaces of a test tube), where a fraction of the impacted particles are retained. The advantages of the dry-impaction

CANARY could provide an excellent first screen for people who may have been exposed to aerosolized spores, thus enabling treatment to be started sooner.

method are that (1) almost all consumables (e.g., liquid media) are eliminated from the CANARY process, (2) the system is not impacted by the low-temperature freeze-out experienced by liquid-based collection systems, and (3) the dry impaction localizes the bioagents to the tube surface, eliminating the need to pre-spin samples for maximum concentration and thereby speeding up and simplifying the identification process.

An early proof-of-concept demonstration using the dry-impaction method to detect *Bacillus subtilis* spores showed that the method yielded a B-cell response similar to that of liquid samples and that CANARY had the potential to improve the combined speed and sensitivity for bioaerosol identification by more than an order of magnitude compared to all other automated bioaerosol identification sensors. This demonstration motivated the rapid development of automated bioaerosol sensors for operation in real-world environments.



The fundamental components of the CANARY biosensor include genetically engineered B cells that emit photons upon binding to specific bioagents and a photodetector that measures the luminescence. (1) B cells are exposed to bioagents in test sample, (2) bioagents bind to B-cell antibodies, (3) the biochemical signal transduction cascade is triggered, resulting in Ca²⁺ release, (4) Ca²⁺ makes aequorin emit photons, and (5) emitted photons are detected.

CANARY Bioaerosol Sensors

The Laboratory developed three bio-aerosol sensors in which CANARY technology has been integrated with the dry-aerosol-collection architecture – the Biological Agent Warning Sensor CANARY (BCAN), the Triggered CANARY (TCAN), and the Pathogen Analyzer for Threatening Environmental Releases (PANTHER). BCAN was designed to provide 30 automated sampling and analysis cycles, with sensitivity sufficient to detect low-concentration threats. Extensive field testing demonstrated that BCAN could identify biologically relevant concentrations in as little as 90 seconds and that its rate of false positives was low enough to assure high reliability. TCAN was designed to be a simple, cost-effective biosensor for real-time monitoring of bio-aerosols in indoor environments. As part of a network of sensing devices, TCAN has delivered high-confidence identification of suspect particles in less than 5 minutes.

Most recently, the CANARY technology has been incorporated into PANTHER, a flexible bioaerosol sensor platform. This biosensor

incorporates the key functions of the CANARY technology in simple-to-handle, inexpensive, disposable plastic disks preloaded with B cells. PANTHER enables 16 simultaneous tests for the presence of up to 48 agents to be performed on a single aerosol collection. PANTHER, which has shown high-confidence identification in less than 2 minutes, is intended for use in building/site protection, emergency response, and environmental monitoring.

With minor modifications, the smallest PANTHER, the CUB, could dramatically improve biological detection capabilities for emergency responders. The technology could be adapted for medical diagnostics to test patient samples and give rapid results without having to send samples to a laboratory. ■

Additional Reading

Petrovick, M., J. Harper, et al., "Rapid Sensors for Biological Agent Identification," *Lincoln Laboratory Journal*, **17(1)**: 63–84, 2007.

Rider, T., M. Petrovick, F. Nargi, et al., "A B Cell-Based Sensor for Rapid Identification of Pathogens," *Science*, **301**: 213–215, 2003.