

**Develop a Device to Rapidly and Sensitive Detect
and Identify Pathogens in an Environment or Population,
Spread either Naturally or Through Deliberate Acts**

WORKING GROUP DESCRIPTION

Background

If not detected and treated promptly, numerous emerging and reemerging infectious diseases (such as cholera, dengue fever, malaria, SARS, and West Nile virus), biological weapons (such as anthrax, smallpox, botulism, and bubonic and pneumonic plagues), and chemical weapons (such as ricin, sarin, and cyanide) have the potential to cause devastating public health crises that could result in the loss of millions of lives. Global travel by millions of people each year accelerates spread of disease, making it even more critical that new rapid-detection methods are devised and validated. To address this threat there is a need for rapid assay strategies for use in clinical diagnostics and environmental detection.

Conventional methods for identifying biological agents (such as immunologic assay and culture) and chemical agents (such as mass spectrometry) generally require high concentrations of the agent, involve complex labor-intensive processing, utilize several pieces of laboratory equipment, and must be executed by trained laboratory personnel. Such genomic methods as quantitative polymerase chain reaction (PCR) and microarrays improve the speed and sensitivity of diagnosis and increase the number of assayed markers (resolution) but still require trained personnel and are expensive.

The Department of Homeland Security and the Department of Defense are funding the development of environmental detectors that monitor outdoor air for biologic and chemical weapons. Sampling of air particles 1 to 10 microns in size is performed by vacuum, centrifuge, or tiny jets, whereas isolating and identifying bacterial, viral, or toxic particles use immunoassays, PCR, or mass spectrometry screens. These approaches have trade-offs that include speed, sensitivity, and cost (Brown, 2004). Fully automated systems capable of unattended collection, sensing, analysis, and reporting remain elusive.

The Problem

- Is it possible to build a device to rapidly and sensitively detect and identify such pathogens as bacteria, viruses, or toxins in an environment or population spread either naturally or through deliberate acts?
 - Can genomics help differentiate between natural and deliberate disease outbreak and provide evidence for attribution?
 - Can sensors quickly, cheaply, and accurately detect one of the dozens of bacteria, viruses, or toxins that could become aerosolized bioweapons? (Brown, 2004)
 - Is it more effective to perform syndromic surveillance of patients in hospital emergency rooms?
 - What is the role of such factors as cost, sensitivity, speed, complexity, minimum time needed to detect and identify a pathogen, and false positive rates, and what should be done with the information?

Initial References

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WORKING GROUP SUMMARY

Summary written by:

Jonathan Stroud, Graduate Science Writing Student, University of Southern California

Working group members:

- Mary Jane Cunningham, Associate Director, Life Sciences and Health, Houston Advanced Research Center
- George Dimopoulos, Assistant Professor, Molecular Microbiology and Immunology, Johns Hopkins School of Public Health
- Robin Liu, Manager, Microfluidics Biochip, Combimatrix Corporation
- Dan Luo, Assistant Professor, Biological and Environmental Engineering, Cornell University
- Deirdre Meldrum, Director of the NIH Center of Excellence in Genomic Science (CEGS) Microscale Life Sciences Center and Professor of Electrical Engineering, University of Washington

- George O'Toole, Associate Professor, Microbiology and Immunology, Dartmouth Medical School
- Jonathan Stroud, Graduate Science Writing Student, University of Southern California
- William Sullivan, Professor, Molecular Cell and Developmental Biology, University of California, Santa Cruz
- Joseph Vockley, Laboratory Director, Life Sciences Division, Science Applications International Corporation
- Debra Weiner, Attending Physician, Emergency Medicine, Children's Hospital Boston, Assistant Professor of Pediatrics, Harvard Medical School
- Lloyd Whitman, Head, Code 6177, The Surface Nanoscience and Sensor Technology Section, Naval Research Laboratory
- John Wikswo, Gordon A. Cain University Professor, Vanderbilt Institute for Integrated Biosystems Research and Education, Vanderbilt University

Summary

There are six questions to ask: Who, What, Where, When, Why, and How? Five out of six can be easily answered:

The what: detecting and identifying the biologic agents and toxins that cause disease

The where: anywhere there is the potential for disease

The when: as soon as possible

The who: the general public

The why: the easiest of all—to save lives

But how? That was the question that a 10-member focus group attempted to answer at the third annual National Academies Keck *Futures Initiatives* Conference in Irvine, California. The group, consisting of doctors, scientists, executives, and engineers,

worked to outline an approach to find the pathogens responsible for disease and effectively identify them, a technological challenge that if successful, could help prevent pandemics and minimize the effectiveness of future bioterrorist attacks.

Genomics, the science of deciphering or reading the genetic alphabet, was the focus of the conference, and so the group utilized this rapidly advancing field, building strategies and methods to detect and identify biologic and toxic agents capable of producing disease.

“Genetics isn’t the only thing we need to analyze,” Professor John Wikswo said. “But right now, it’s the only thing that has the requisite breadth. That’s because genetic sequencing is the only science to yield complete information about activity at the cellular level.”

Instead of focusing on one specific disease or pathogen class, the group decided to develop a matrix—a decision tree flowchart—for addressing all possible pathogens and diseases in a variety of scenarios. This would also include the capability to detect pathogens in their insect vectors, such as mosquitoes and ticks, stressed Dr. George Dimopoulos, an assistant professor at the Johns Hopkins School of Public Health.

The group laid out the broad gaps in current biomedical technologies, the proposed problem, and the various difficulties associated with broadening the application of genomics in disease detection. What do we need to measure? they asked. What technologies exist that we could use? What still needs to be developed or integrated? What might we consider in the distant future?

The group moved on to delineate systems criteria—a set of characteristics any device or method would need—and began to address the problem. Each member offered his or her opinion of ideal criteria and based on the conference call discussion, developed a matrix—or chart—to divide up and lay out what the group would delve into over the course of the conference.

“Ideally, we want to have something to detect the pathogens in 30 minutes or less,” said Dr. Robin Liu, the manager of a microfluidics biochip for the Combimatrix Corporation. Discussion ensued on the basic requirements, with each member in agreement on basic parameters.

”Ideally, we’d like a device that’s highly specific and sensitive, can be multiplexed to detect multiple markers, is fully integrated, automated, and portable, has low power consumption, and is disposable,” Wikswo reiterated. A modest goal, indeed.

The group decided DNA and RNA (nucleic acid) assays and miniaturized microarrays were the best option at present for analyzing the disease triggers in the body or environment. The problem with DNA-based assays is that most DNA assays require killing cells or organism samples to harvest their genetic material—somehow you have to go from an intact organism to DNA material.

Ultimately, direct nucleic acid assays won out over the more conventional amplification assays that require the PCR to obtain adequate genetic material, or immunoassays that require recognition of specific molecular structures of a particular pathogen or agent.

“You’re not easily going to be able to detect a signal pathogen in most samples with an immunoassay,” said Lloyd Whitman, a section head at the Naval Research Laboratory.

“I think it’s pretty clear that nucleic-acid-based detection will be more effective,” agreed Dan Luo, an assistant professor of biological and environmental engineering at Cornell University.

Ultimately, they focused on the three main knowledge gaps identified by Whitman that must be overcome in order to achieve the stated goal: (1) the development of ubiquitous, multivariate sensing, which means an all-encompassing sensor network that comprehensively monitors agents associated with disease; (2) the design of data processing and bioinformatics capabilities to analyze and integrate the information obtained from the sensors; and (3) the improvement of not only our understanding of disease transmission and immune responses but also how these both enable and limit pathogen detection.

Day 2 began with a discussion of whether detection should be clinic- or environment-based. The clinical approach would test people with infections—on the order of thousands of people. The environmental approach would measure agents in the environment, thus targeting entire populations—on the order of millions of people—who

were potentially at risk for disease but not necessarily infected. The group evaluated the relative importance, efficiency, and practicality of these two approaches.

The group viewed the problem from the perspective of biodefense, pandemic prevention, and improvements to health care, with particular attention being paid to existing technologies in biodefense and health care. The group ultimately identified a broad approach that would capture all these cases and address the current technology gaps in the process of treating an outbreak, a pandemic, or even the common cold.

To address and organize what any future system might require, they began to devise a decision tree—a basic flow chart—that would describe a comprehensive process for detecting disease-causing agents.

A broad detection strategy must address the possibility that pathogens might be found in any of four main target areas: (1) in the environment (drinking water, air, soil); (2) in people (at the doctor's or in the field); (3) in animal populations that serve as vectors for infectious agents (as with avian flu); and (4) airports, seaports, and border checkpoints that serve as entry portals.

“You find a bird that is sick and must ask whether it has bird flu or another bug, or a patient with a bad cough, an international traveler with a fever, or gray goo on the ground. Are there serious pathogens present?” asked Wikswo.

The group then refined its decision trees to outline how a researcher or physician might proceed when faced with an unknown symptom in people or animals, eventually covering both detection and response. By also analyzing possible procedures for pathogen detection in the environment or in the water or food supplies, they succeeded in identifying two technologies required to span existing technology gaps.

“For each of the four targets, you end up needing two things: a microbe identifier and a pathogenicity analyzer,” Wikswo said.

They then set to work discussing and outlining each object and its relevant challenges. First, a microbe identifier would identify genetic material and compare it to a database of known pathogens. Studying the host response to the pathogen includes analyzing changes in host gene expression. Since the most effective (but not necessarily the fastest) way to identify the pathogen would be through its genome, this effort would clearly benefit from the \$1,000 genome sequencer under consideration by other

conference working groups, a technology the whole group seemed to believe would be available in the near future.

Examples of pathogen identifier technologies considered by the group included Robin Liu's proposed Combimatrix continuous bioaerosol monitor and the Compact Bead Array Sensor System (cBASS) being developed by the Navy.

Second, in order to deal with a previously unidentified pathogen, the group discussed the capabilities required for a pathogenicity analyzer—a machine that would identify the ability of unknown or hacked pathogens to cause disease in cells, model organisms, animals, or people. The group considered a number of technologies, including gene arrays, electrochemical sensing of cellular metabolism, optical or magnetic immunoassays, cellular fluorescence, and flow cytometry—all techniques that could detect pathogen-induced changes in cellular function.

"I'd say you want to look for as many things as you can and then fuse them all together," said Whitman.

Coupled with the required advancements—ease of use, low rates of false positives and negatives, small size, ease of deployment, and relatively low cost—these two devices, it was determined, could revolutionize health care and disease detection and prevention.

"Screening this way could improve diagnosis and treatment of infections as well as prevent infection," said Debra Weiner, attending physician at Children's Hospital Boston and assistant professor at Harvard Medical School.

"Having this kind of approach would help the implementation and development of novel antibiotics that are not based on killing the bacteria," said George O'Toole, associate professor in the Department of Microbiology and Immunology at Dartmouth.

After the final presentation, the group members agreed that their work addressed a very important issue and that the presentation was a great cap to an important set of discussions.

In summary, the conference gave these researchers a chance to share knowledge and ideas and led to crosstalk that in the future may lead to an all-encompassing approach to a difficult problem.