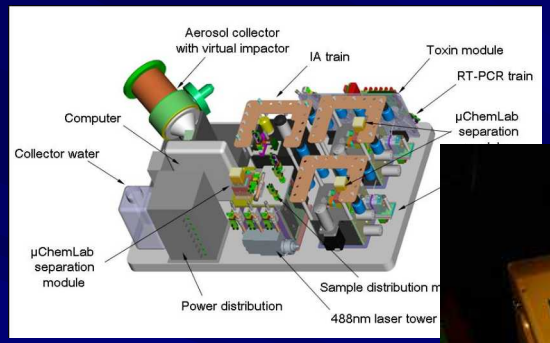
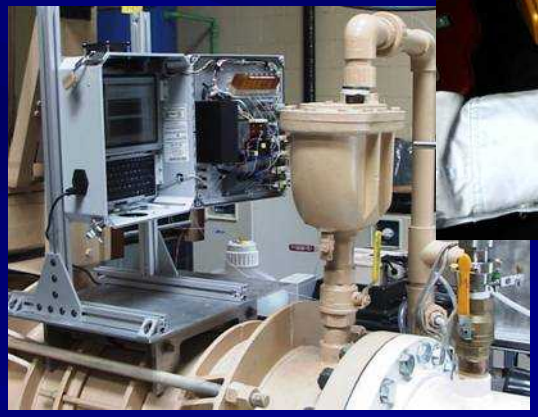
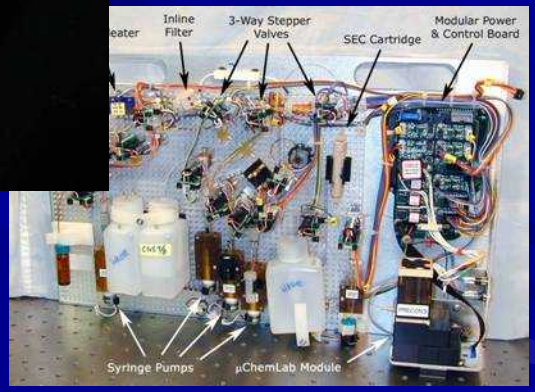
Micro-Total Analytical Systems and other Field capable Platforms: Challenges and Considerations

SAND2008-4124C







Victoria VanderNoot and Julia Fruetel
Sandia National Labs
Livermore, CA

AOAC Pac NW Meeting,
Seattle, WA June 18-19, 2008

Sandia National Laboratories

Why Develop Fieldable Detection Platforms?

…when there are established laboratory approaches and still significant technical hurdles to overcome?

- Automated operation
- Rugged performance
- Translating assays to a fieldable format
  - E.g., "gold standard" mouse assays

What could be gained from a portable device?

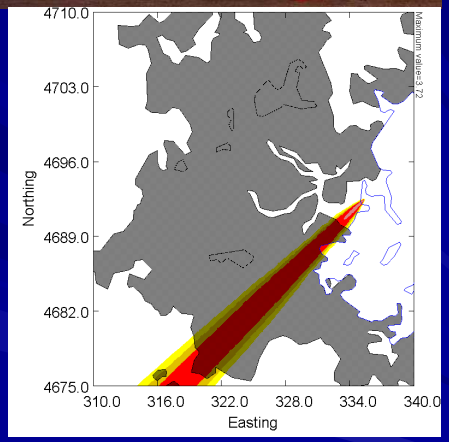
- Environmental (*water, air, soil*) ← Coastal monitoring
- Medical (*metabolites, infectious disease*) ← Bedside/Point of care
- Industrial (*manufacturing QC, effluents*) ← Inline Quality Control
- Food safety (*microbial, chemical*) ← Food Processing Plant

Enabling Sampling and Analysis to be in the same place has many advantages…

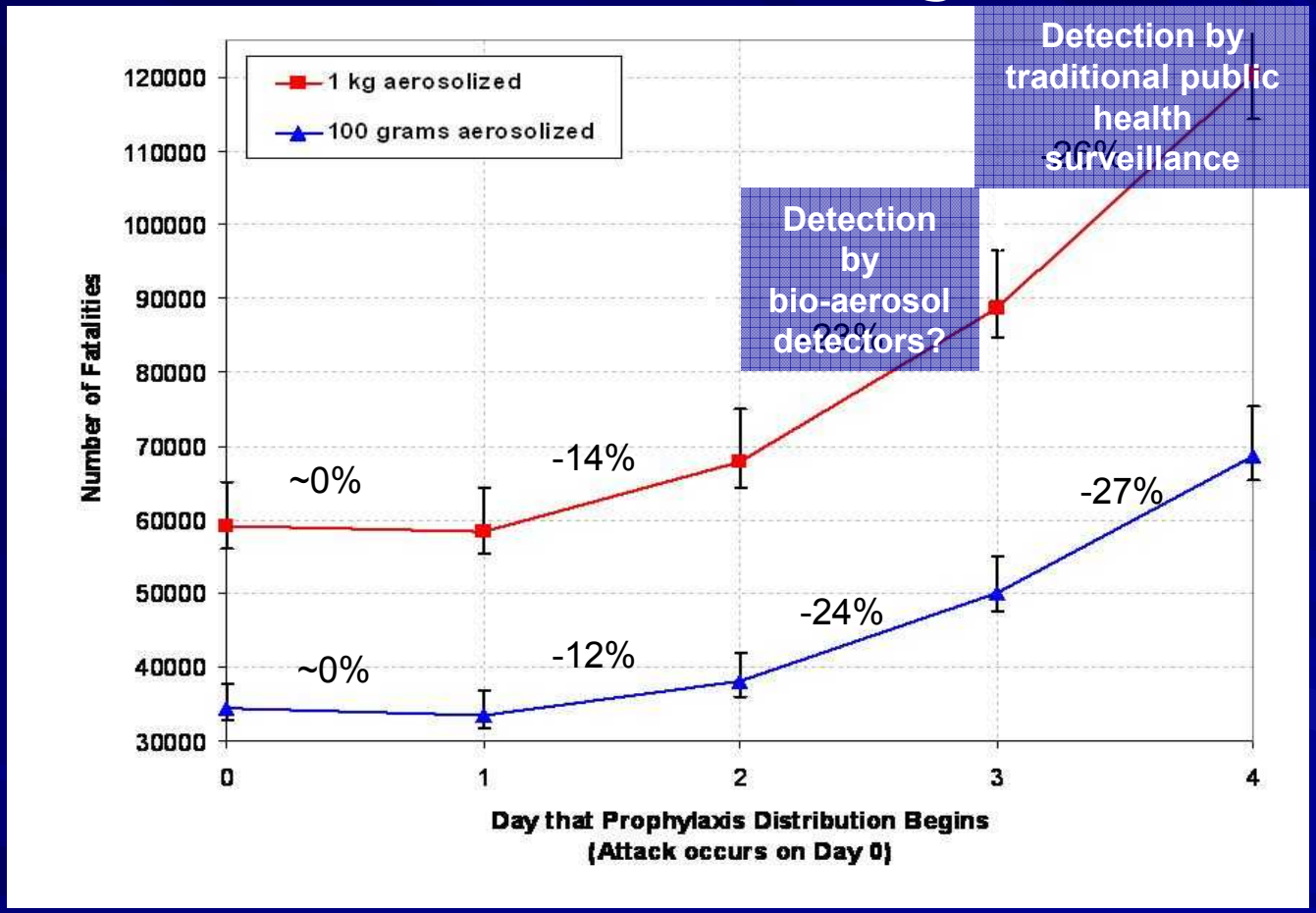
- Reduces labor, logistics, overhead
- Enables dramatically faster response (from days to hours or minutes)

Sandia's involvement in understanding and countering bioterrorism began with DOE/CBNP and then DHS

- Sandia has been actively engaged with DHS (and other sponsors including DoD):
  - To define the requirements of biodefensive architectures through systems studies
  - To meet those requirements through next-generation technology development
- Sandia is a National lab and homeland security is one of its core missions



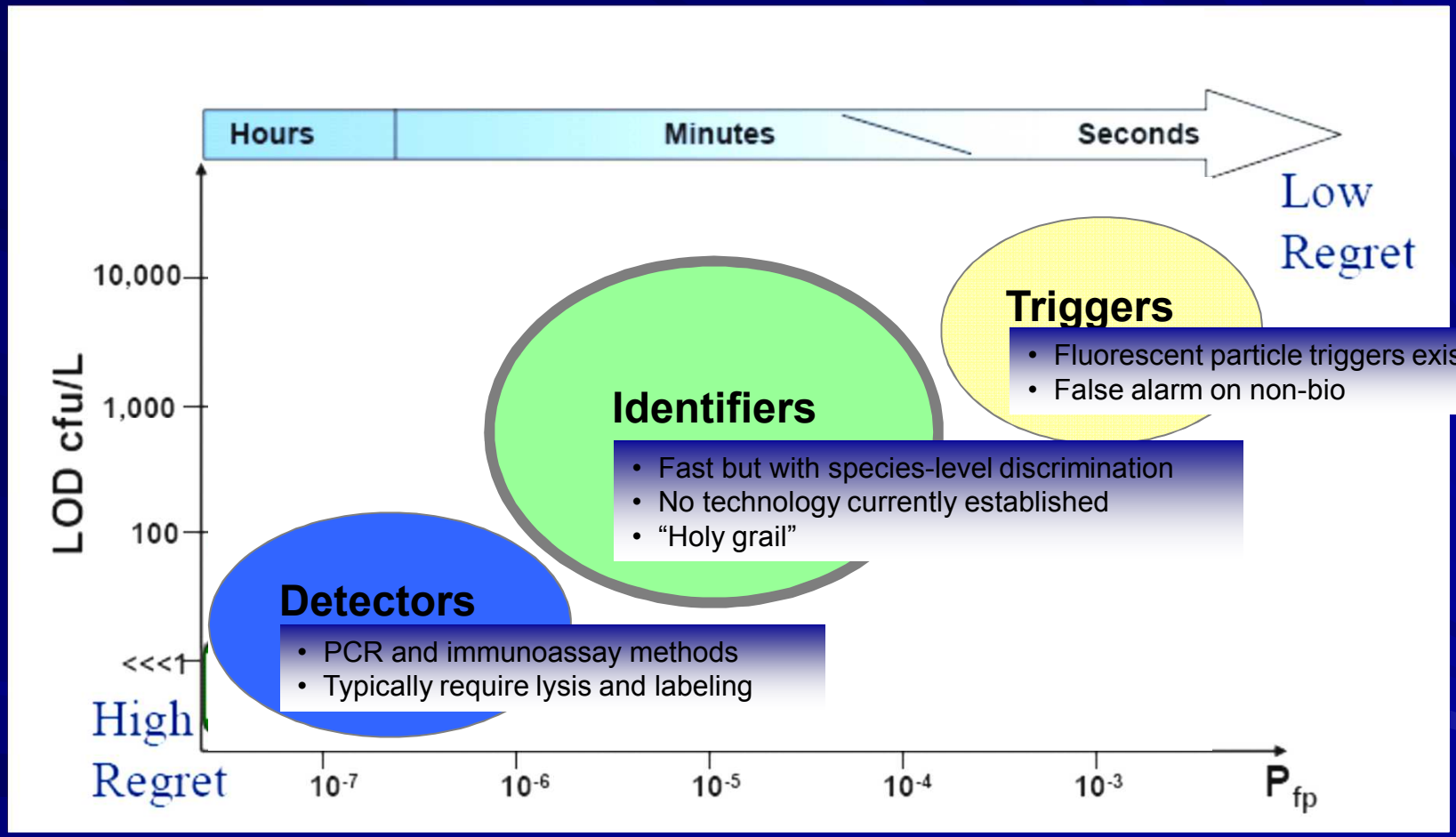
Study results indicate for every day's delay to the start of prophylaxis, the fatality rate grows at an increasing rate



- **Traditional public health surveillance used to detect natural disease outbreaks in a community will not recognize the attack in time for maximal treatment effectiveness**
- **Biodetectors can provide a significant advantage by enabling the response to begin more rapidly**

L. Yang, I. Chumfong, T. West, H. Ammerlahn and A. Yoshimura, Earlier Detection in Emergency Response to an Anthrax Attack, SAND2006-1740 (unlimited release), Sandia National Laboratories, Livermore, CA, 2006

Biological identifier requirements fall between particle triggers and high-confidence detectors



Detect to Treat systems | *Detect to Warn systems* | *Detect to Protect systems*

Military and Civilian Applications Have Different Needs and Requirements

Consequences of action/inaction are very different

- Systems analysis gives scope to the problem and can inform system requirements

http://www.edwards.af.mil/photos/index.asp

Logistics are often of highest concern

- Size & weight
- Field operation (eg. batteries)
- Automated operation
- Consumables

Accuracy and Cost are often of highest concern

- Extremely low false positives/negatives
- Sensitivity
- Operating costs

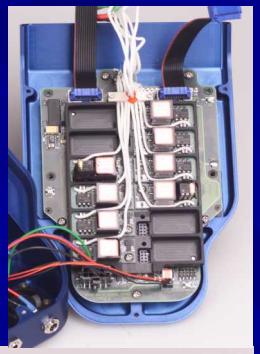
Core Technology: the μChemLab Analysis Platform

Development of the μChemLab was aimed at the civilian world
Designed with the first responder in mind

- Input from a large number of first responders from across the country
- Key observations:
  - Cost
  - Simple operation and training
  - Multi-use; upgrades as add-ons

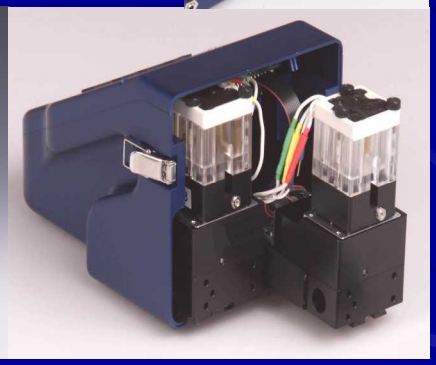
Lead to our modular design

- Hand portable
- On-board data analysis
- Push button operation
- Two analysis modules
- Battery operated



Also has become a flexible, reliable platform for routine laboratory R&D

- small and portable without compromising performance



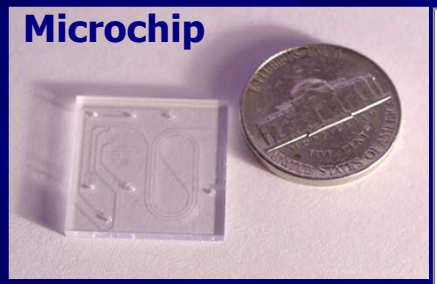
1) J.A. Fruetel, R.F. Renzi, V.A. VanderNoot, J. Stamps, B.A. Horn, J.A. A. West, S. Ferko, R. Crocker, C.G. Bailey, D. Arnold, B. Wiedenman, W-Y. Choi, D. Yee, I. Shokair, E. Hasselbrink, P. Paul, D. Rakestraw, and D. Padgen, (2005) *Electrophoresis*, **26,** 1144 – 1154.

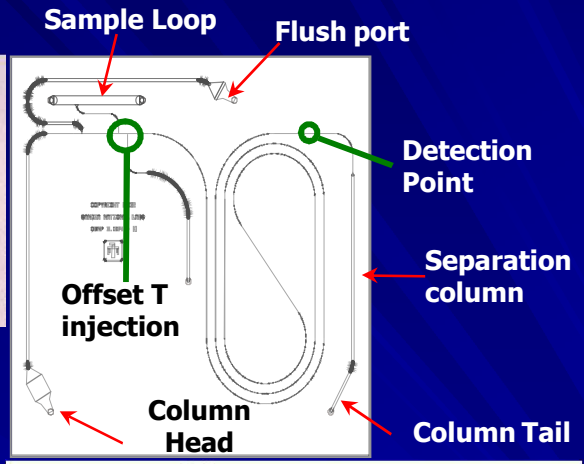
2) R.F. Renzi, J. Stamps, B.A. Horn, S. Ferko, V.A. VanderNoot, J.A.A. West, R. Crocker, B. Wiedenman, D. Yee and J.A. Fruetel, (2004) *Analytical Chemistry*, **77**, 435-441.

Chip-based Microseparations Provide Powerful Analytical Capability

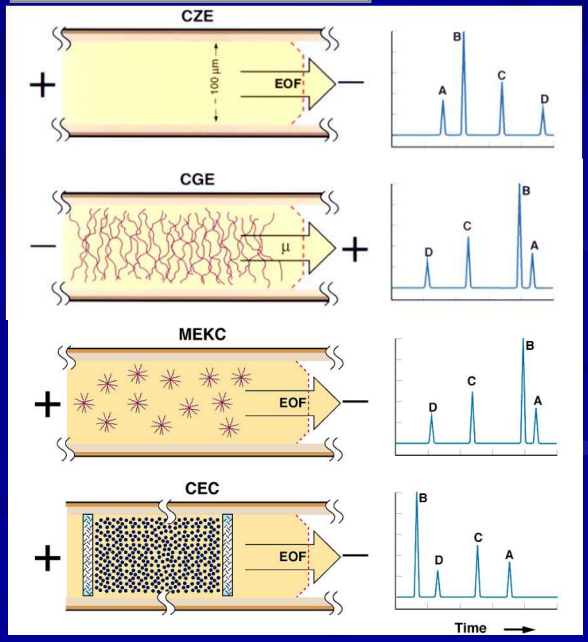
Chip-based core technology can be used to separate a wide range of molecules:

Proteins, Nucleic acids,
Reporters, Chemicals and drugs

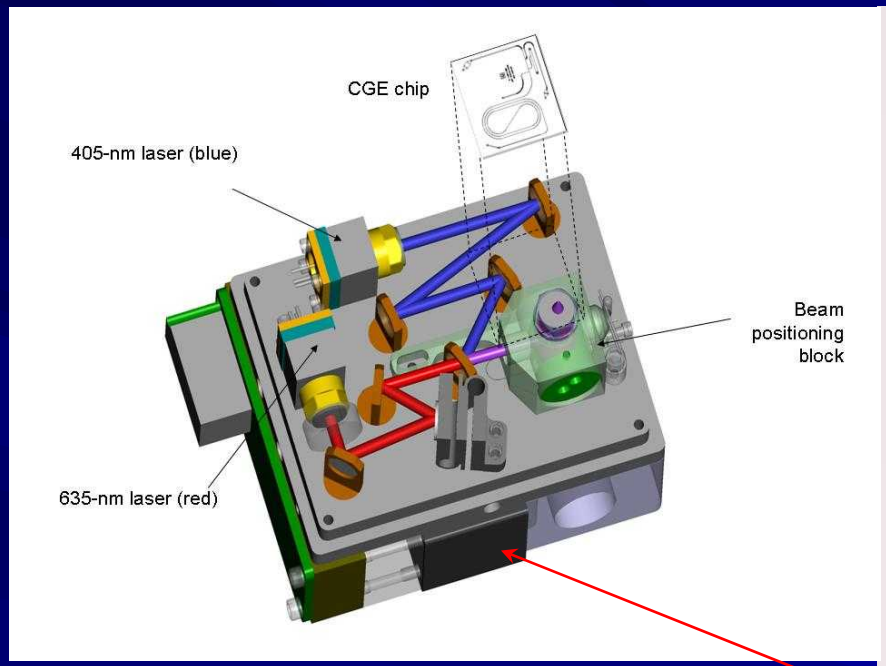


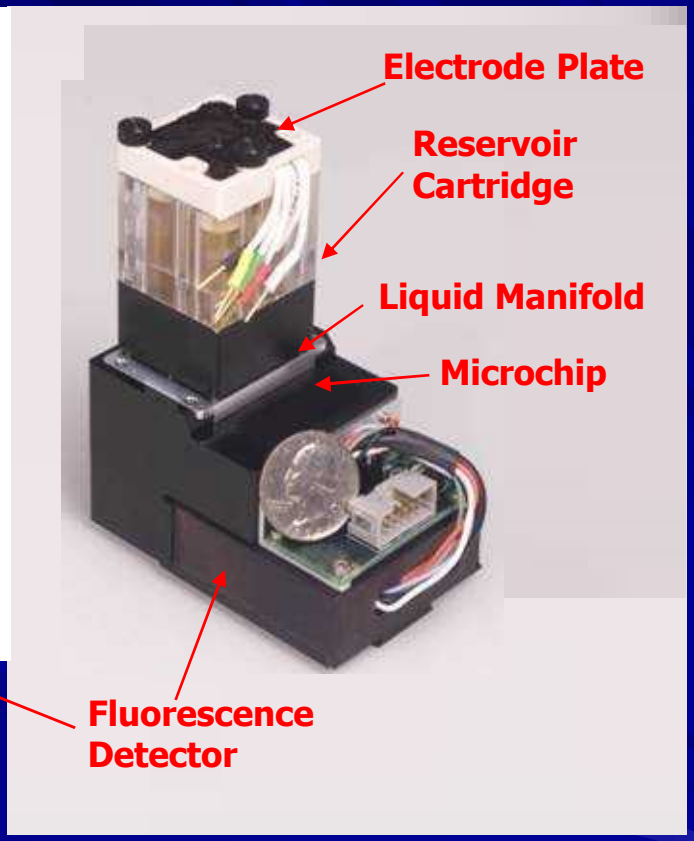


- **Microfluidics**
  - Time required for a separation is dramatically reduced
  - Reagent volumes are minimal
  - Parallel and sequential separations are facilitated
    - Provide differential selectivity
    - Improve detection reliability
    - Lower false alarm rates



Core Technology: Sensitive, Two-Color Optical Detection





Integrated Laser induced Fluorescence Detection

"Reference" channel allows correction of migration times

- Highly advantageous for ambient temperature variations

Transitioning Core Technology to the Field Also Requires Sample Preparation

- **Particularly important for biological samples**
- **Must be automated**
  - Cannot rely on the availability of highly trained personnel
- **Design of the sampling and sample preparation system will factor in compatibility with the core technology and be tailored to the specific target system requirements**
  - Logistics/Cost
  - Sensitivity/selectivity
  - Response time
- **Three integrated field detection platforms will be highlighted**
  - μChemLab coupled to automated sample collection and preparation
    - protein separations
    - PCR and immunoassay
  - Representing both the military and civilian sectors

Military and Civilian Applications Have Different Needs and Requirements

Consequences of action/inaction are very different

- Systems analysis gives scope to the problem and can inform system requirements

http://www.edwards.af.mil/photos/index.asp

Logistics are often of highest concern

- Size & weight
- Field operation (eg. batteries)
- Automated operation
- Consumables

Accuracy and Cost are often of highest concern

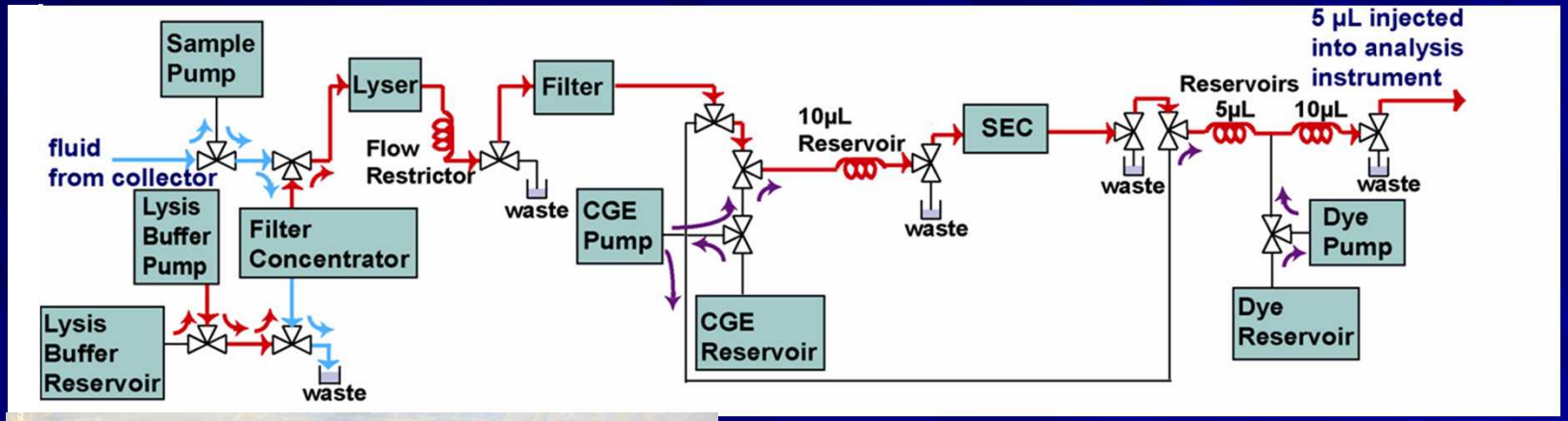
- Extremely low false positives/negatives
- Sensitivity
- Operating costs

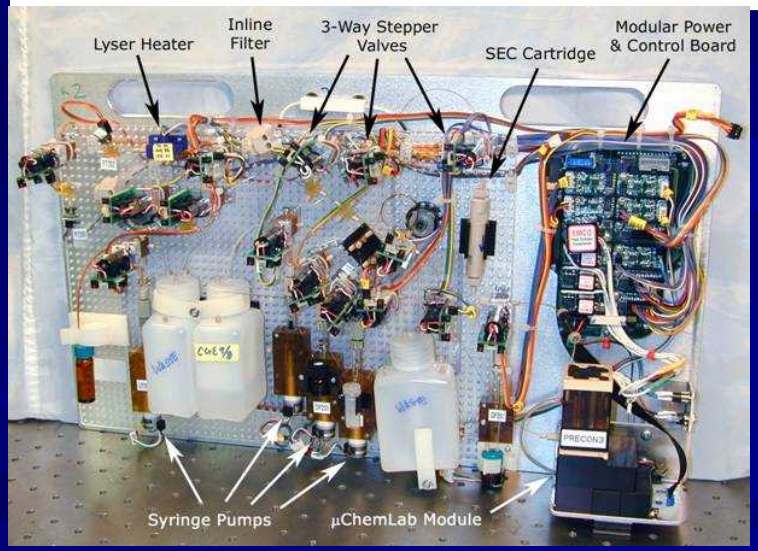
Our DoD Funding Sponsors Wanted Speed and Minimal Logistics Burden

- Performance
  - Rapid detection and identification of aerosolized organisms
  - Toxins, viruses, spores, and vegetative cells
- Logistical
  - Battery operation for 8 hours
  - Man portable (1 cu foot/50 lbs, including batteries)
  - Minimal reagent use
  - Continuous, autonomous operation for 8 hours

*Point Detection for JCBPDS (DoD DTO.50 program)

Automated Microfluidic Protein Profiling System for Bioagent Detection





Sample preparation system carried all steps needed to convert organisms to soluble proteins for analysis

- particle concentration, lysis and solubilization, sample clean-up (SEC) and fluorescent labeling

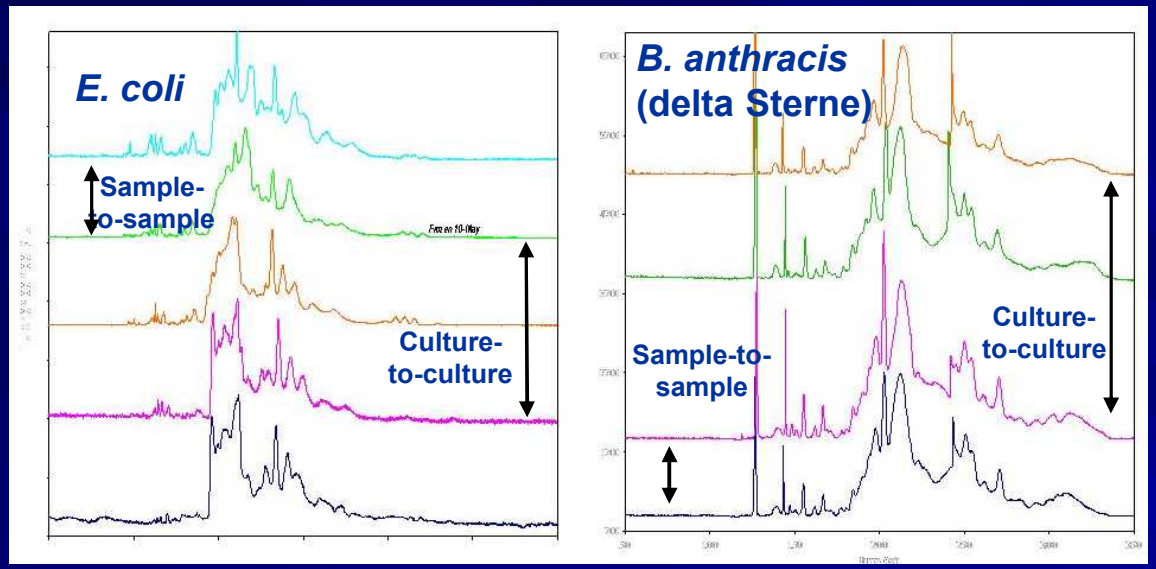
Field Testing of Integrated platform

- Commercial aerosol collector
- Autonomous operation for eight hours
- Redundant systems

1) Pizarro, S. A.; Lane, P.; Lane, T. W.; Cruz, E.; Haroldsen, B.; VanderNoot, V. A. *Electrophoresis* **2007**, *28*, 4697–4704.
2) Stachowiak, J. C.; Shugard, E. E.; Mosier, B. P.; Renzi, R. F.; Caton, P. F.; Ferko, S. M.; Van de Vreugde, J. L.; Yee, D. D.; Haroldsen, B. L.; VanderNoot, V. A. *Anal. Chem.* **2007**, *79*, 5763-5770.

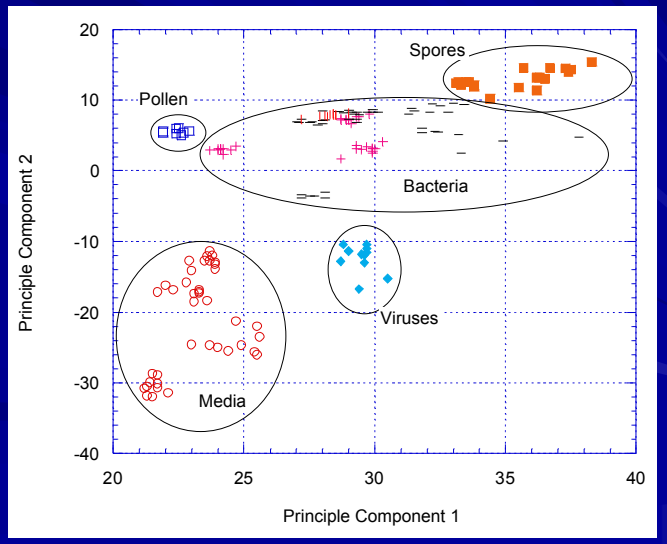
Principal Component Analysis Shows Discrimination Between Signatures

Viruses and Bacteria Are Detected From their Proteomic Signatures after automated sample preparation to solubilize the proteins



Analysis of Replicate Samples

Principal Component Analysis



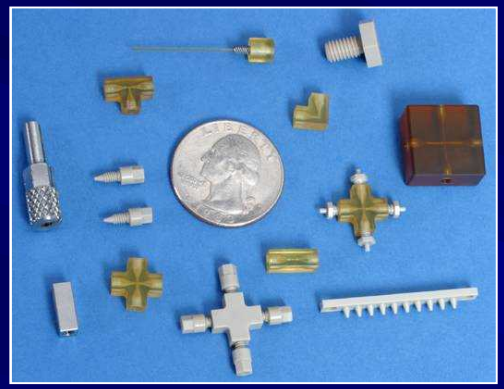
AOAC Pac NW Meeting,
Seattle, WA June 18-19, 2008

Sandia National Laboratories

Development of Integrated Detection Platforms Relies on a Variety of Enabling Technologies

Suitable microfluidic components were unavailable so a suite of components developed in-house

Designed for microfluidics, Enabled modular design



CapTite™ Fluidic Fittings

- One piece design
- Reusable
- Finger or tool tightened
- Up to 10 KSI

Syringe pumps

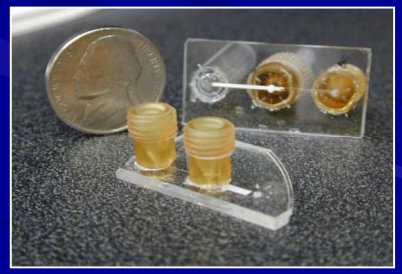
- Linear stepper motor
- Controlled small volume metering
- Bidirectional

Miniature valves

- 10 KSI capability
- Electrically actuated
- Low swept volumes (~10 nL)

EK pumps

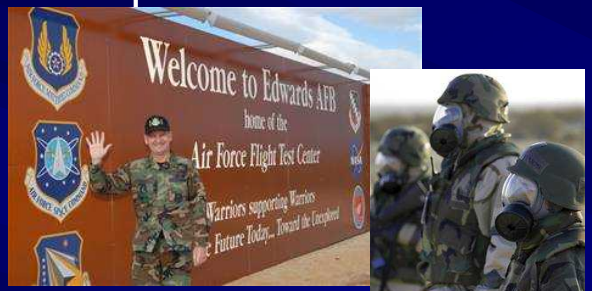
- no moving parts
- high pressure
- fast
- accurate



Military and Civilian Applications Have Different Needs and Requirements

Consequences of action/inaction are very different

- Systems analysis gives scope to the problem and can inform system requirements



http://www.edwards.af.mil/photos/index.asp

Logistics are often of highest concern

- Size & weight
- Field operation (eg. batteries)
- Automated operation
- Consumables

Accuracy and Cost are often of highest concern

- Extremely low false positives/negatives
- Sensitivity
- Operating costs

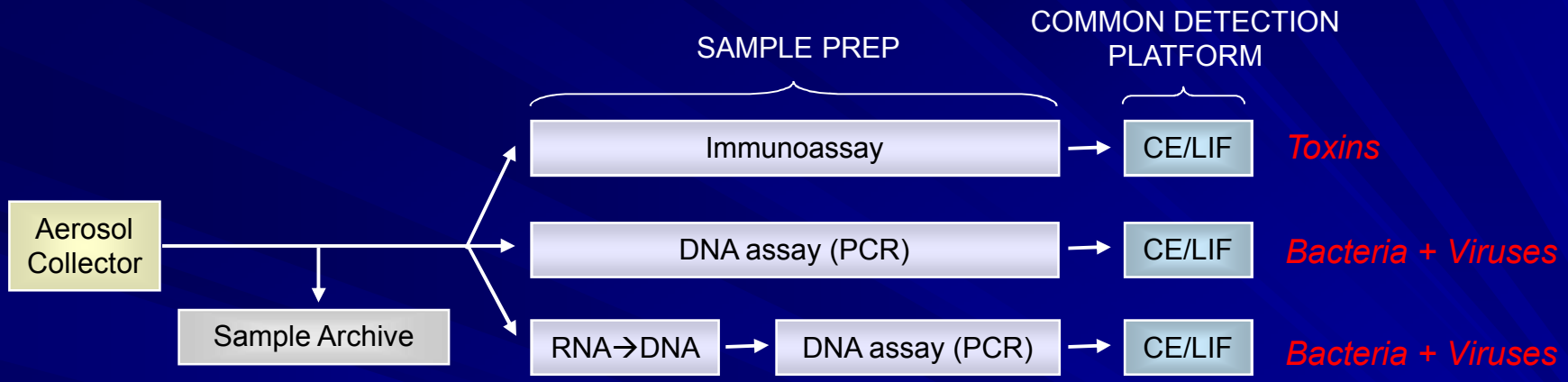
BioBriefcase (BBC) was Developed under the BAND Program of DHS

The BBC Program had strong performance and cost targets but more modest logistical requirements

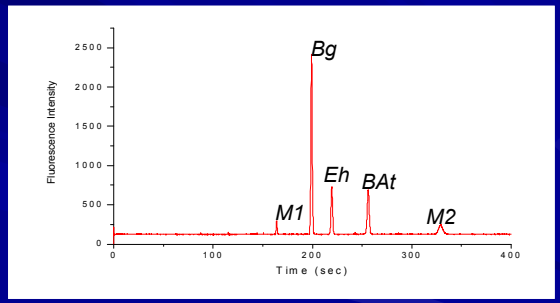
- **Performance**
  - Broad agent coverage > 20 agents
  - High sensitivity – Limit of detection of 100 organisms (10 ng toxin)
  - Single agent false positive rate of 10^{-7} with a goal of 10^{-8}
- **Cost of Ownership**
  - Unit acquisition cost of $25K per unit or less (mass production quantities)
  - Operating costs per unit of $10K per year or less inclusive
- **Logistical**
  - Continuous, fully autonomous operation
  - Fixed location (AC power)
  - Modest packaging requirements

The BioBriefcase was a collaboration between Sandia and Lawrence Livermore National Labs

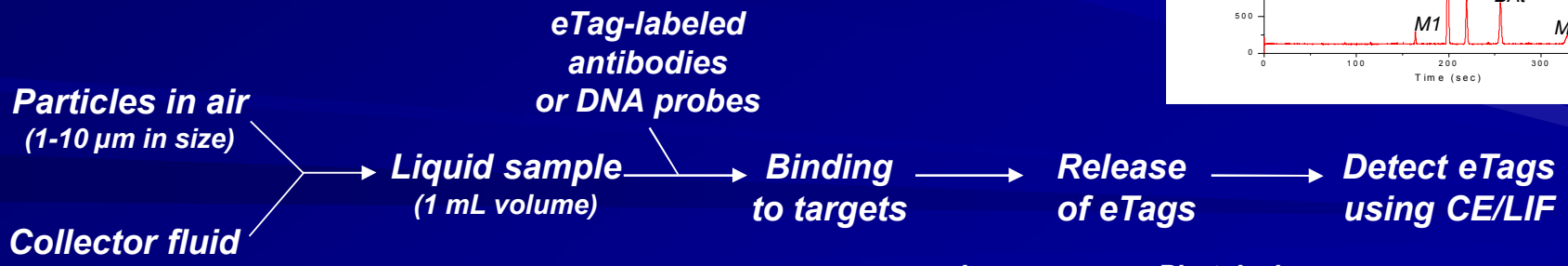
BioBriefcase employs three assay trains to detect the full biothreat spectrum (bacteria, viruses and toxins)



CE/LIF analysis of eTags



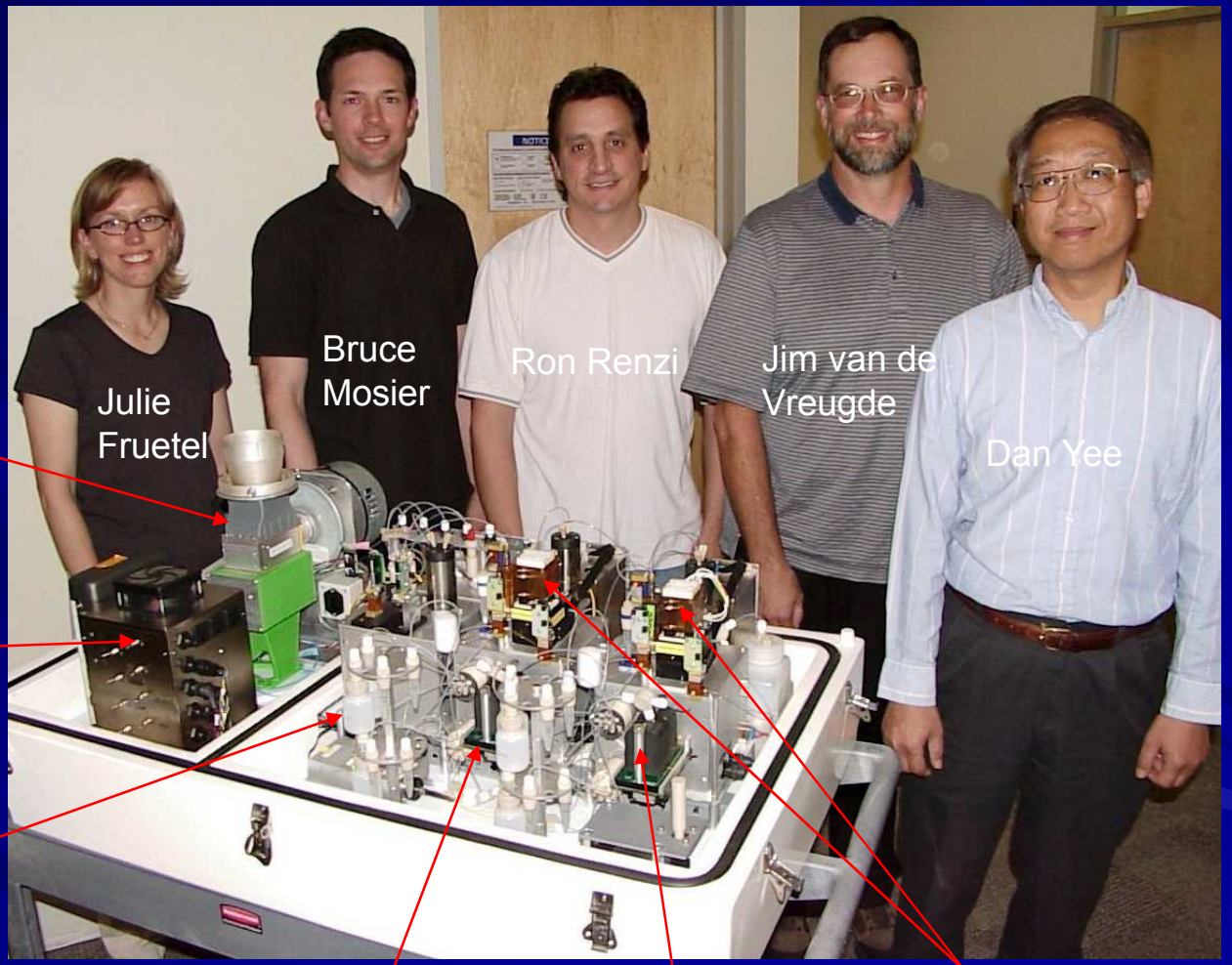
Assays use "eTags" (small fluorescent reporter molecules) to indicate when a pathogen is detected:



Immunoassay: Photolysis
DNA assay: PCR reaction

Sandia National Laboratories

BioBriefcase System in an Environmental Enclosure (no longer really a "briefcase"…)



Virtual Impactor/Wetted Cyclone Aerosol Collector

Laser and Power Modules

ImmunoAssay Train

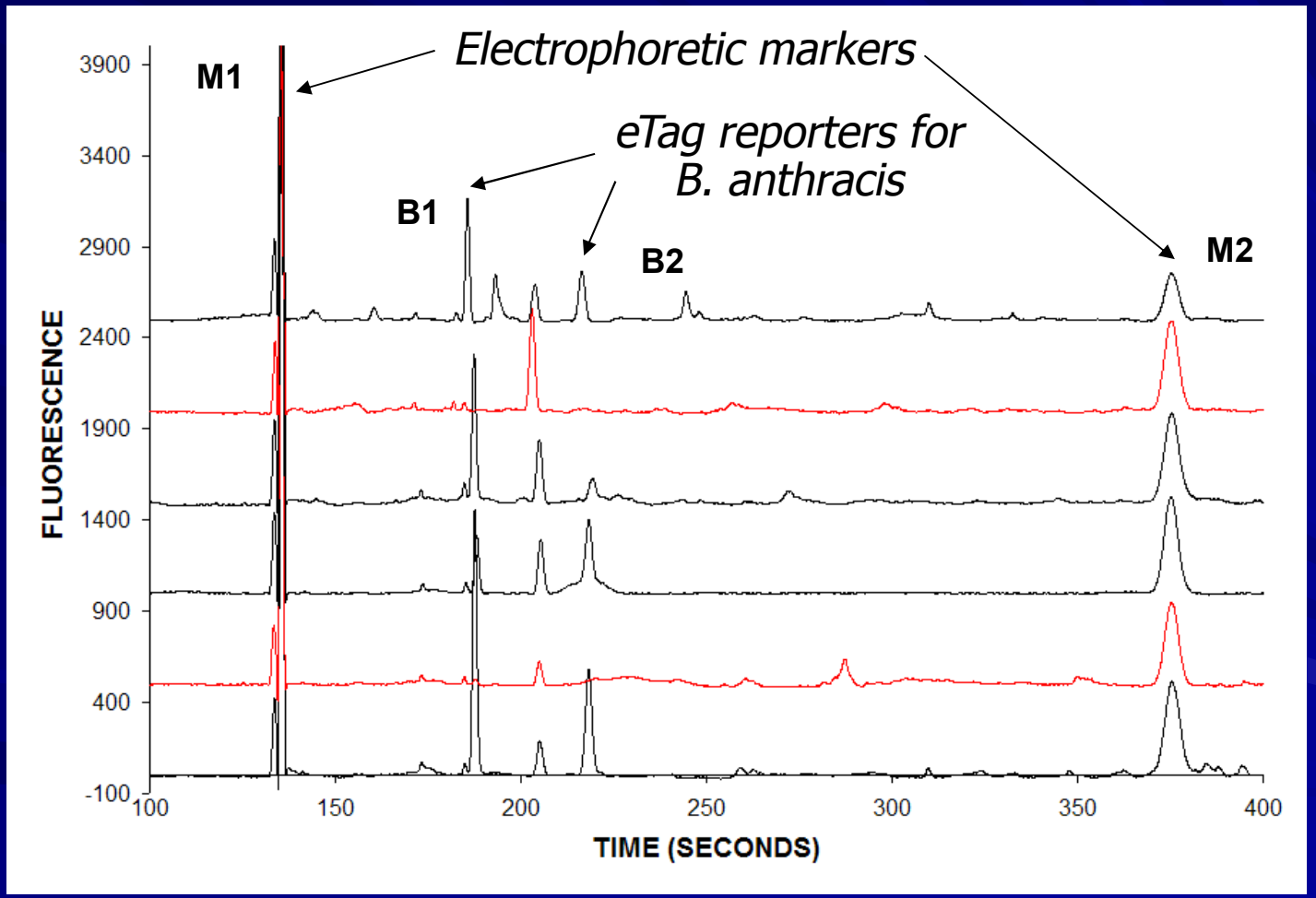
RT-PCR Train

PCR Train

µChemLab detectors

Sandia National Laboratories

Aerosol chamber testing results for 6 sequential samples correctly identified using Biobriefcase



Positive

Negative

Positive

Positive

Negative

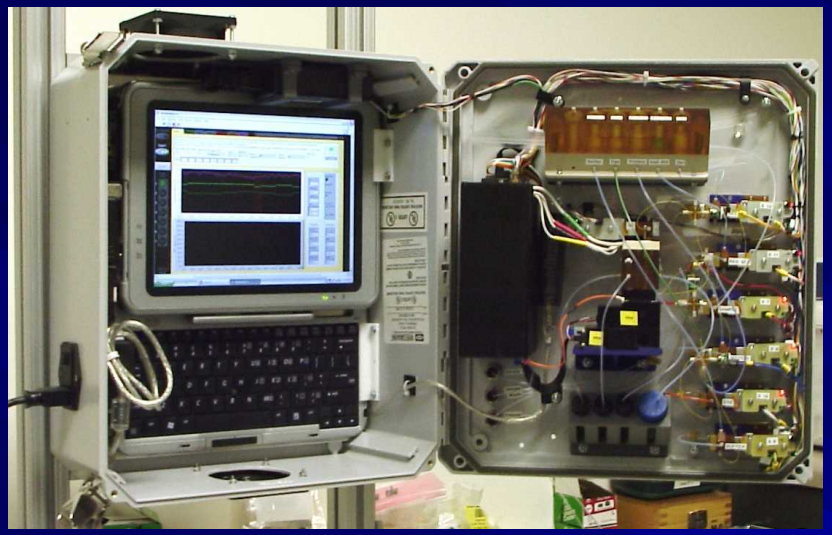
Positive

Developing Sustainable Technologies Requires Looking Beyond Biodefense

Defense against bioterrorism in either the military or civilian sectors warrants exceptional preparation *however*, in a market-driven environment, we *must* build capabilities for non-biodefense "everyday" applications

- **Dual functionality** is essential
- **Cost of a system** that may never be needed (we hope!)
- **Maintaining functionality and capability**
  - Will a system that has waited unused on a shelf for an indefinite period of time be operational the instant you need it?
    - Reagent lifetimes?
    - Other components?
  - Will a system that no one uses routinely be able to be operated?
    - Requisite know-how of operation
    - Trusting the information

The Unattended Water Sensor is an Example of this "Dual Functionality" Concept

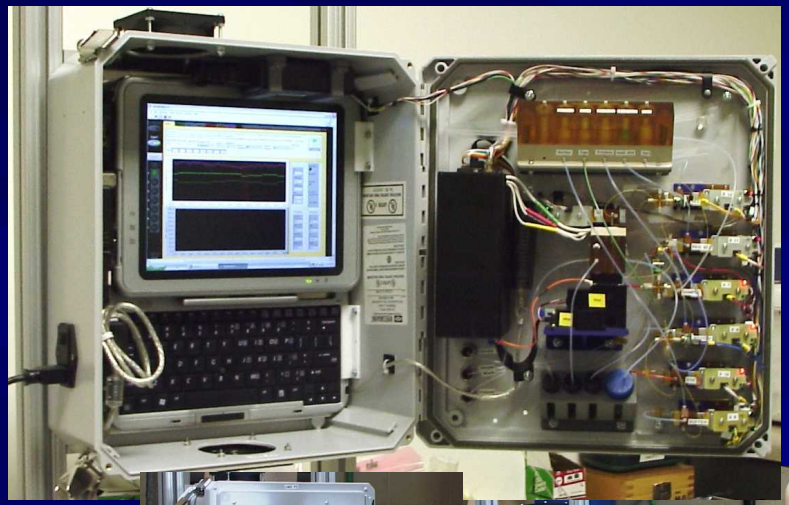


Utility managers like the idea of detecting acts of bioterrorism but cost is prohibitive unless it addresses standard water quality needs

- •effect of massive dilution working in their favor
- • realistically it is more important to test for the things that are currently regulated

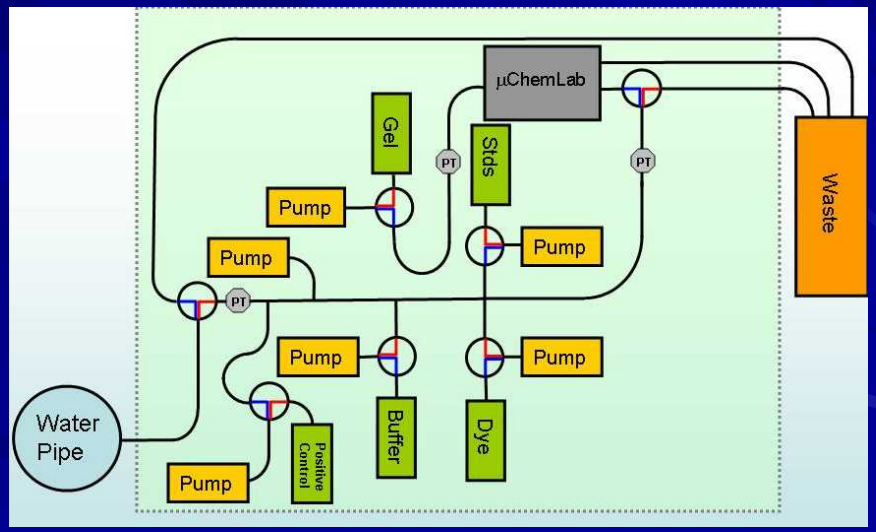
- **Continuous domestic water monitoring**
  - First Generation detects biotoxins
  - Planned second generation to test *E. coli*, protozoa
  - 30 day unattended operation
  - Analysis every 30 minutes
- **Cost is a major driver**
  - Focus is on everyday water quality issues
    - protozoa (chryptosporidia, giardia)
    - *E.coli* (regulated)
    - taste and odor (customer complaints)

The Unattended Water Sensor was Developed in Collaboration with the Australian Company Tenix Pty Limited



Continuous domestic water monitoring

- Extensive field testing in California and Arizona
- Wireless communication to researchers or an alarm system
- Modular design to speed maintenance
- Troubleshooting capabilities



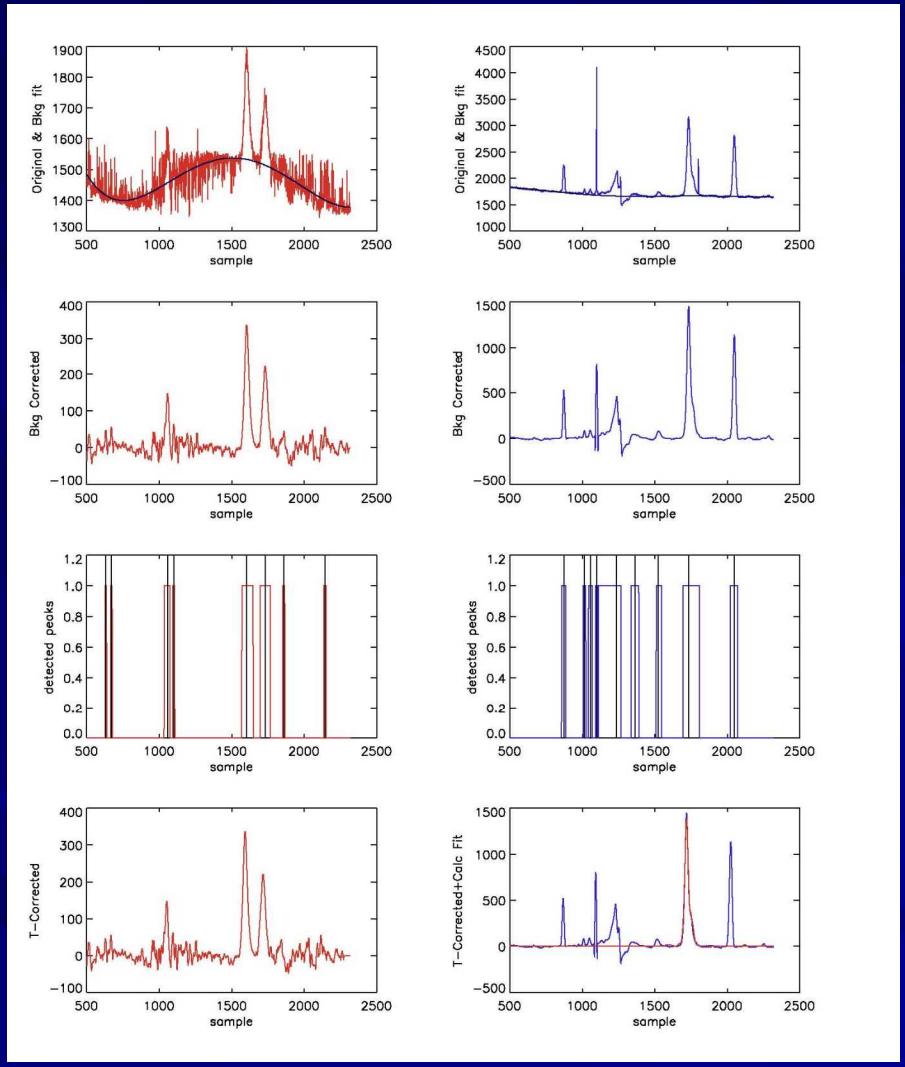
Victoria VanderNoot, Brent Haroldsen, Wayne Einfeld and Yolanda Fintschenko, *Environmental & Water Resources Institute Currents,* (2008) **9**, 6-7.

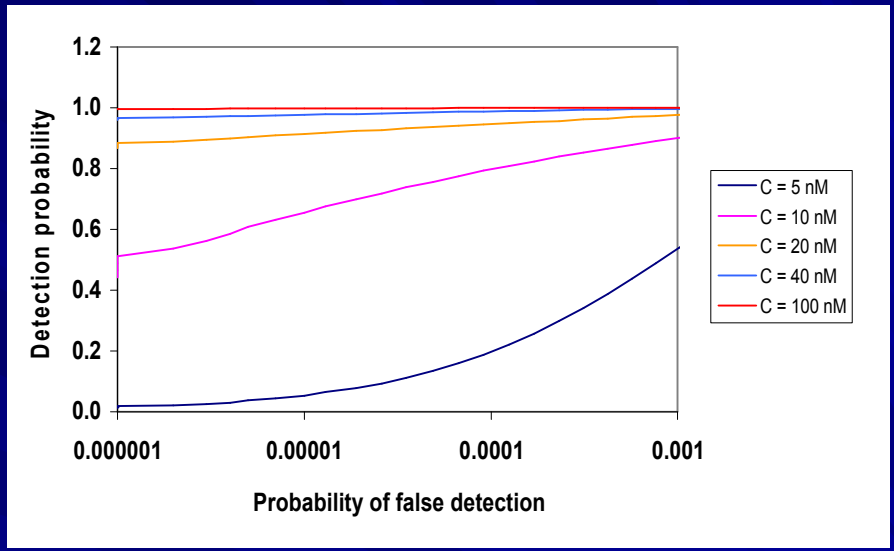
AOAC Pac NW Meeting,
Seattle, WA June 18-19, 2008

Sandia National Laboratories

Ricin Detection in Water

Ricin concentration of 369 nM





Preliminary UWS Receiver Operating Characteristic Curves for Ricin

Summary

- **Bringing uTAS systems to the field remains challenging**
  - Sample preparation
  - Especially important for biological samples
- **Fieldable uTAS systems will yield benefits in a wide area of analysis**
  - POC diagnostics, environmental monitoring and inline manufacturing QC
  - Benefits of reduced costs (labor and materiel) and response time
- **Strong advantages in the area of biodefense**
  - Military and civilian sectors generally have very different requirements
  - To ensure technology viability in a market-driven environment it must have benefit to a wider routine analysis space
- **We have developed a number of integrated field capable detection platforms based on electrophoretic separations**
  - Automated sample preparation
  - Modular design approach is flexible to adapt to system requirements

Acknowledgments

Sandia Contributors to uChemLab Programs

Mark Allendorf, John Brazzle, Pamela Caton, Wen-Yee Choi, Gabriela Chirica, Jose Cintron, Mark Claudnic, Robert Crocker, Evelyn Cruz, Rafael Davalos, Scott Ferko, Julie Fruetel, Brent Haroldsen, Brent Horn, Kyle Hukari, Jerry Inman, Susan Jamison, Bill Kleist, Karen Krafcik, Todd Lane, Marci Markel, Alf Morales, Bruce Mosier, Tom Raber, Ronald Renzi, Jimmy Ross, George Sartor, Isaac Shokair, Erin Shugard, Shane Sickafoose, Jeanne Stachowiak, James Stamps, Victoria VanderNoot, James van de Vreugde, Karl Wally, Jason West, Boyd Wiedenman and Dan Yee

Systems studies

Todd West, Lynn Yang, Isabelle Chumfong, Nate Gleason, Donna Edwards, Susanna Gordon

Lawrence Livermore National Laboratory (BBC):

Christopher Bailey, Perry Bell, Elizabeth Wheeler, Anne Erler, Kirsten Johnson, Bruce Henderer, Olgica Bakajin, Christine Hara, Mona Hwang, Shanavaz Naserabadi, Youngeun Kwon, Paul Butler, Robert Costa

Funding:

DHS BioCountermeasures Portfolio, Programs, Plans and Requirements DHS ORD and HSARPA (BAND Program), DTRA, Tenix PTY Ltd CRADA, DOE CBNP Program

**Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.*

Contacts

Automated Microfluidic Protein Profiling System (AMPPS):

Victoria VanderNoot *or*
vavande@sandia.gov
Brent Haroldsen
blharol@sandia.gov

BioBriefcase (BBC):

SNL: Julie Fruetel *or*
jafreut@sandia.gov
LLNL: Chris Bailey
bailey38@llnl.gov

Unattended Water Sensor (UWS):

Victoria VanderNoot *or*
vavande@sandia.gov
Brent Haroldsen
blharol@sandia.gov