Autonomous Pathogen Detection System

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APDS team

- Assays
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- Software, control, comm.
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- Project
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BASIS and BioWatch:
Centralized testing of air filters for biological agents

Distributed Sampling Units
Continuous collection of aerosols

BASIS Operations Center
Links to public health and law enforcement agencies

Communications Network
Radio and Internet connections

Relocatable Field Laboratory
Aerosol testing and analyses

Receiving, Reloading, and Dispatch
Filter sample management

Lawrence Livermore and Los Alamos National Laboratories

Autonomous Pathogen Detection System:
Analysis at collector, networked reporting of results

- Aerosol collection
  - Up to 3,000 Lpm
  - Particle size selection
  - Samples are archived, can be cultured

- Sample preparation
  - Sequential injection analysis platform
  - Flexible and expandable

- Multiplexed detection and identification
  - Bead-based, Luminex™ immunoassay panels
    - bacteria, viruses, protein toxins
    - 11-plex + 4 controls
  - PCR confirmation of DNA sequences
  - Any antibody or sequence can be incorporated

- Data acquisition and control
  - Custom acquisition and analysis software
  - Wireless, Cellular, & Ethernet networking
Civilian & base protection differs from the battlefield

• Threat less known
  – Must test for more agents
• Operation is never-ending
  – Operating cost must be lower
• Different impact of alarms
  – Much less tolerance for false positives
• Treating victims vs. force protection
  – Some speed can be sacrificed for certainty

The APDS has advantages over the state-of-the-art

• APDS advantages vs. manual filter collection
  – Lower operating cost
  – Shorter time from collection to answer
  – Higher frequency reporting
• APDS advantages vs. triggered strip tests
  – Lower operating cost
  – Higher-quality antibody assays
  – PCR assay included
  – Assay upgrade/expansion is simple
• Issues for military applications
  – Ruggedization, equipment cost, speed vs. strip test
APDS gives lab-quality answers before a sample could get back to a lab

APDS software and communications allow remote command and control
**High flow-rate aerosol collection**

- 200 – 3,000 Lpm air sample in
- 4 mL liquid sample out
- Multistage
  - Prefractionator cap
  - Virtual impactor
  - Wetted-wall cyclone

**Deeply multiplexed immunoassays with Luminex™**

The sandwich fluoroimmunoassay is one of the most credible biodetection techniques.

Adding a color code (optical bar code) to the capture bead enables individual ID.

The Liquid Array enables flow cytometry.

Beads can be analyzed by flow cytometry.

Read ~ 10,000/min.

Different antibodies on each bead enables deeply multiplex detection.

Many different pathogens can be detected in a single assay.
Data-rich signals from flow cytometer are monitored to detect and identify biological agents

- Fluorescent intensities, numbers of beads, statistics
- Multiplex signals have extra information
- Internal controls are important for confidence
  - Instrument control (detector OK)
  - Fluorescent control (label OK)
  - Antibody control (labeling antibody OK)
  - Negative control (no nonspecific binding)

Example of multiplex immunoassay signals
Orthogonal identification using PCR

- Uses DNA instead of protein recognition
  - Looking for different signature, so “orthogonal”
  - Tremendous amplification gives great sensitivity
- TaqMan used for confirmatory PCR

APDS automated PCR is shockingly repeatable

24 consecutive PCR runs & 1 negative control
Proven in chamber testing at Dugway Proving Grounds

- September 2002
  - Multiplex immunoassay
  - Live-agent releases
    - *B. anthracis* (anthrax)
    - *Y. pestis* (plague)
- September 2003
  - Multiplex immuno. + PCR
  - Killed-agent releases
    - *B. anthracis* (anthrax)
    - *Y. pestis* (plague)
  - Simulant releases
    - Botulinum toxoid, *B. globigii*

Identification and confirmation of a *Ba* release
Identification and confirmation of a $Yp$ release

Identification and confirmation of a $Bg$ release
Identification of a botulinum toxoid release

Proven in fully autonomous testing

- Washington DC subway
  - June 2003
  - 1 unit, 7 days
- Albuquerque airport
  - December 2002
  - 2 units, 4 days
- Laboratory runs
  - $24 \times 7$ for 3 weeks
  - Many shorter runs
- Continuing tests in field
## Current work

- **Department of Homeland Security**
  - Field operation
  - Commercialization
- **Department of Defense (Tech. Transition Program)**
  - Triggered by early-warning detector (BAWS)