



Autonomous Pathogen Detection System

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CHI Systems Integration in Biodefense
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The APDS Team

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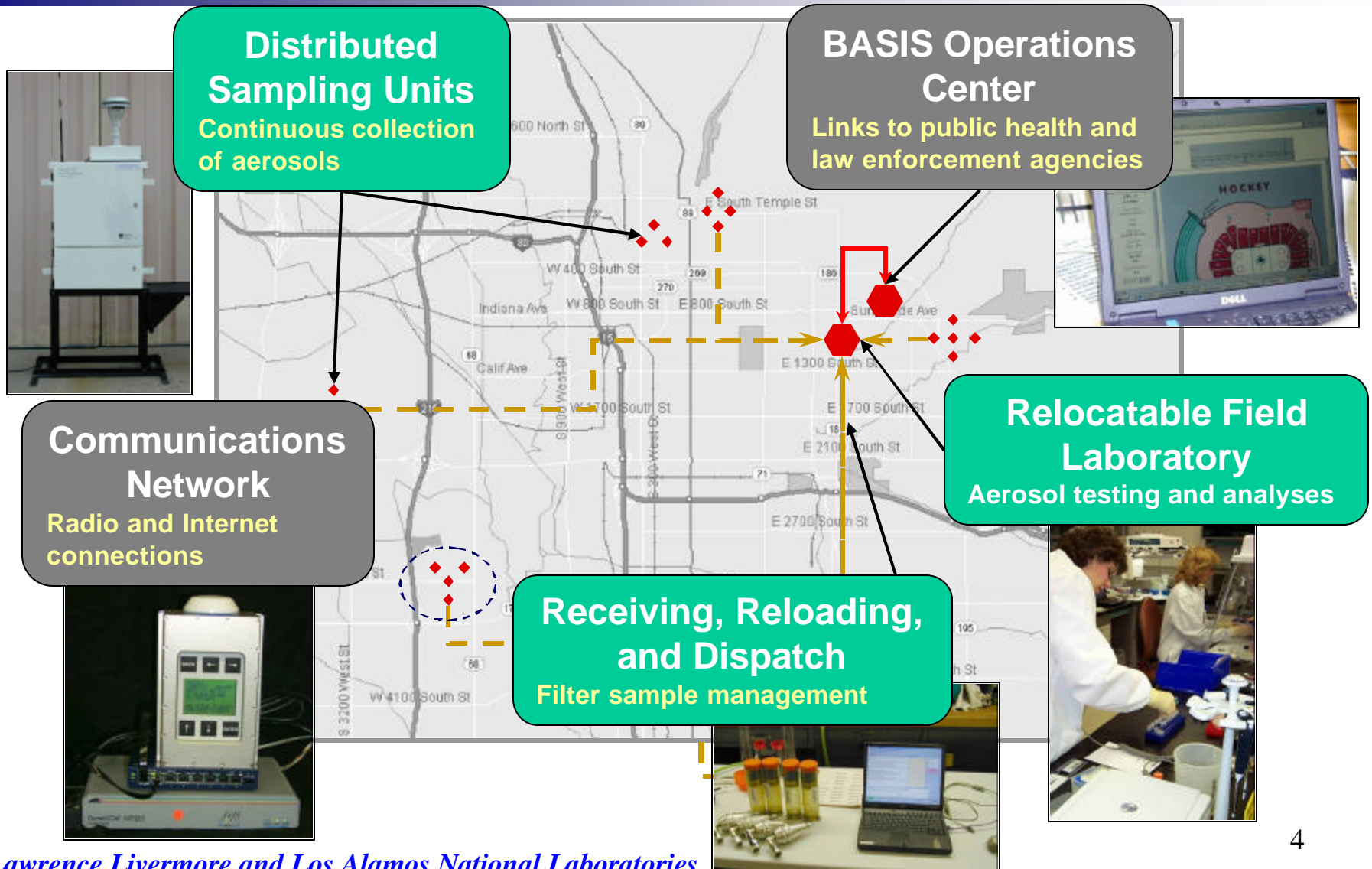


Characteristics of civilian defense against bioterrorism

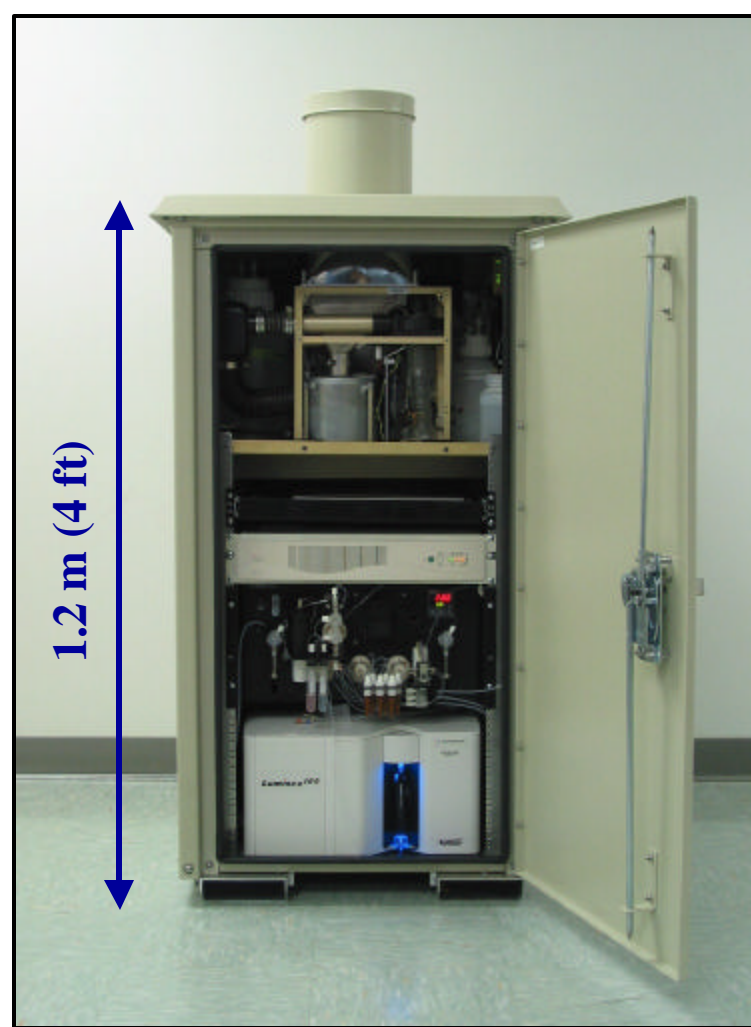
- Many possible threat agents
 - Assays must be multiplexed
- Operation is never-ending
 - Operating cost must be low
- High impact of alarms
 - Frequency of false positives must be low
- Response time includes public health action
 - Speed in initial detection traded for certainty

BASIS and BioWatch:

Centralized testing of air filters for biological agents



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



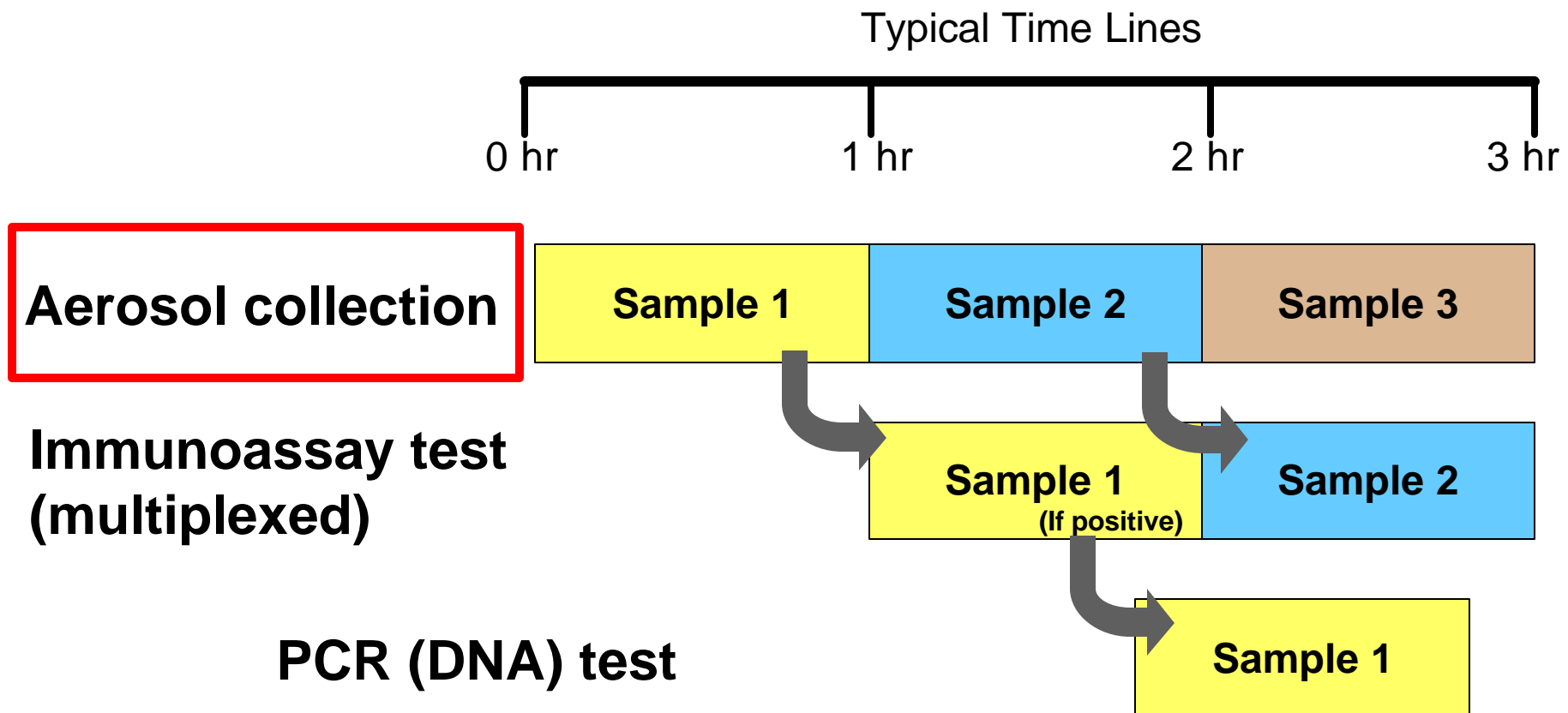
- Aerosol collection
 - Particle size selection
 - Samples are archived, can be cultured
- Sample preparation
 - Sequential injection analysis platform
 - Flexible and expandable
- Multiplexed detection and identification
 - Bead-based, Luminex read-out
 - Any antibody or DNA sequence can be incorporated
- Data acquisition and control
 - Automated, centralized monitoring
 - Wireless, Cellular, & Ethernet networking
- One-week autonomy at 1 sample per hour

APDS provides significant improvements in monitoring capabilities

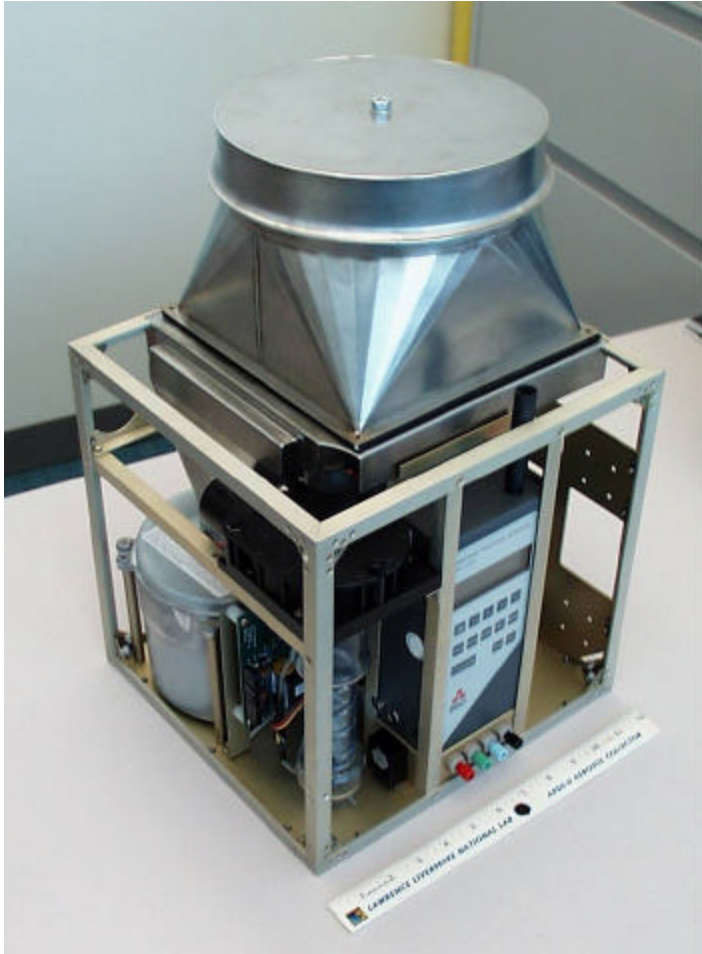
- Higher temporal resolution for rapid detection and response
- Two distinct (orthogonal) testing methods for high-confidence results
- Larger number of assays to detect more threat agents
- Lower labor requirements to minimize operational costs

APDS provides laboratory-quality answers within hours of exposure

Past “PCR confirmation” process



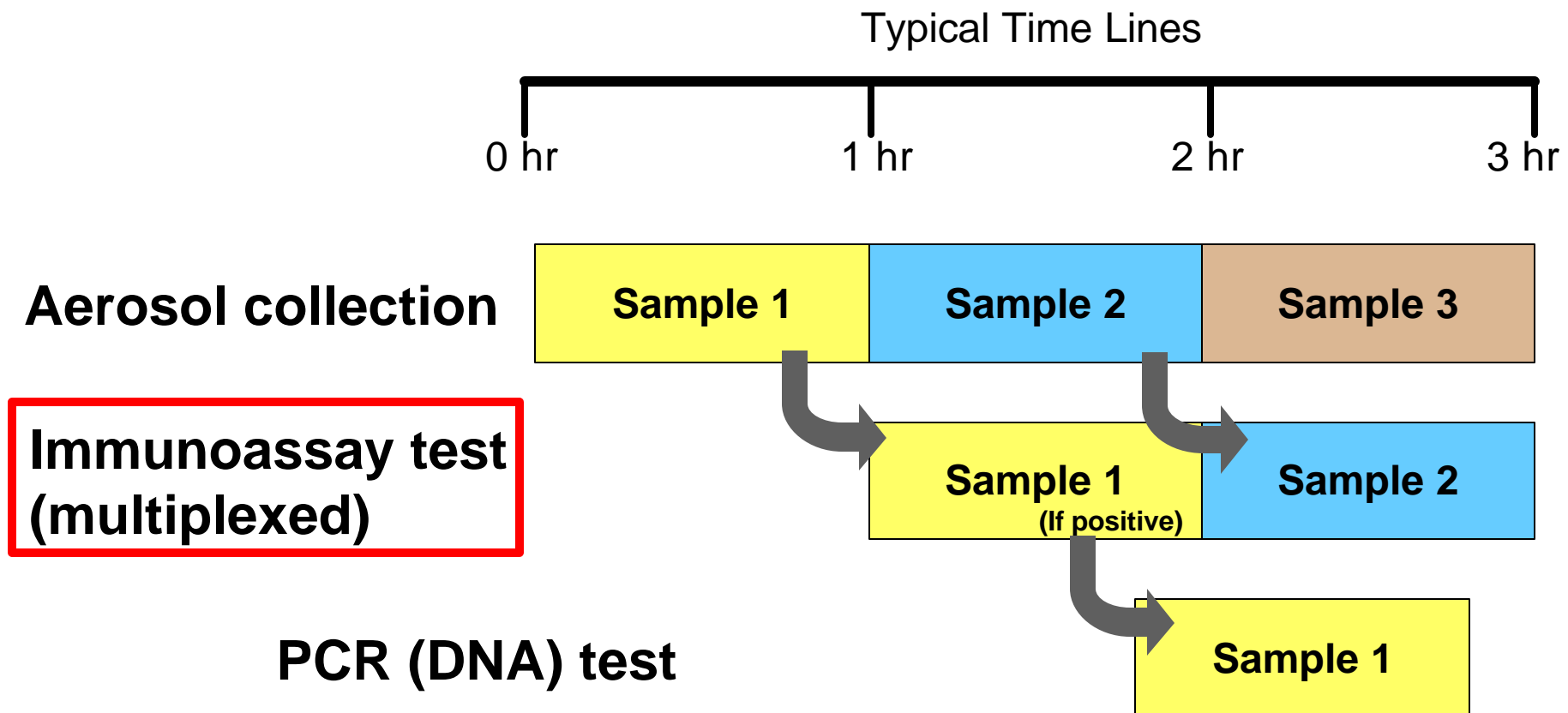
High flow-rate aerosol collection



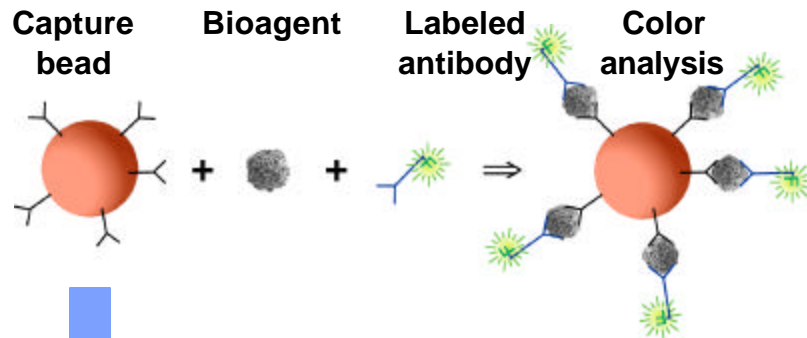
- 300 – 3,000 Lpm air sample in
- 4 mL liquid sample out
- Multistage
 - Prefractionator cap
 - Virtual impactor
 - Wetted-wall cyclone

APDS provides laboratory-quality answers within hours of exposure

Past “PCR confirmation” process

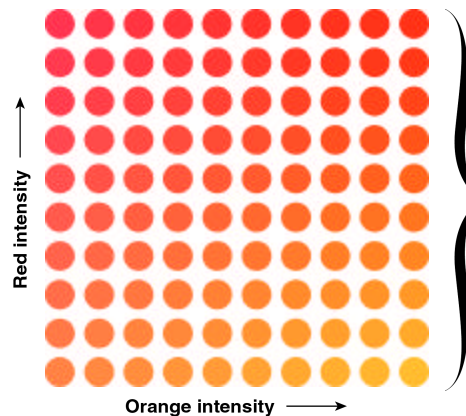


Luminex immunoassay platform permits screening for dozens of agents

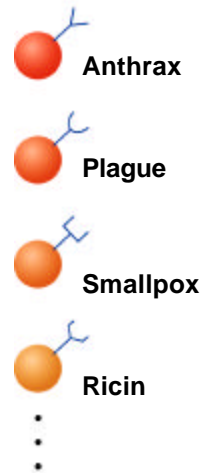


The sandwich fluoroimmunoassay is one of the most credible biodetection techniques

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



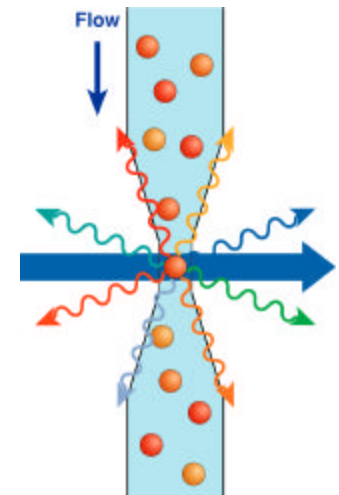
Liquid Array



Different antibodies on each bead enables deeply multiplex detection

Beads can be analyzed by flow cytometry

at rates up to 10,000/s



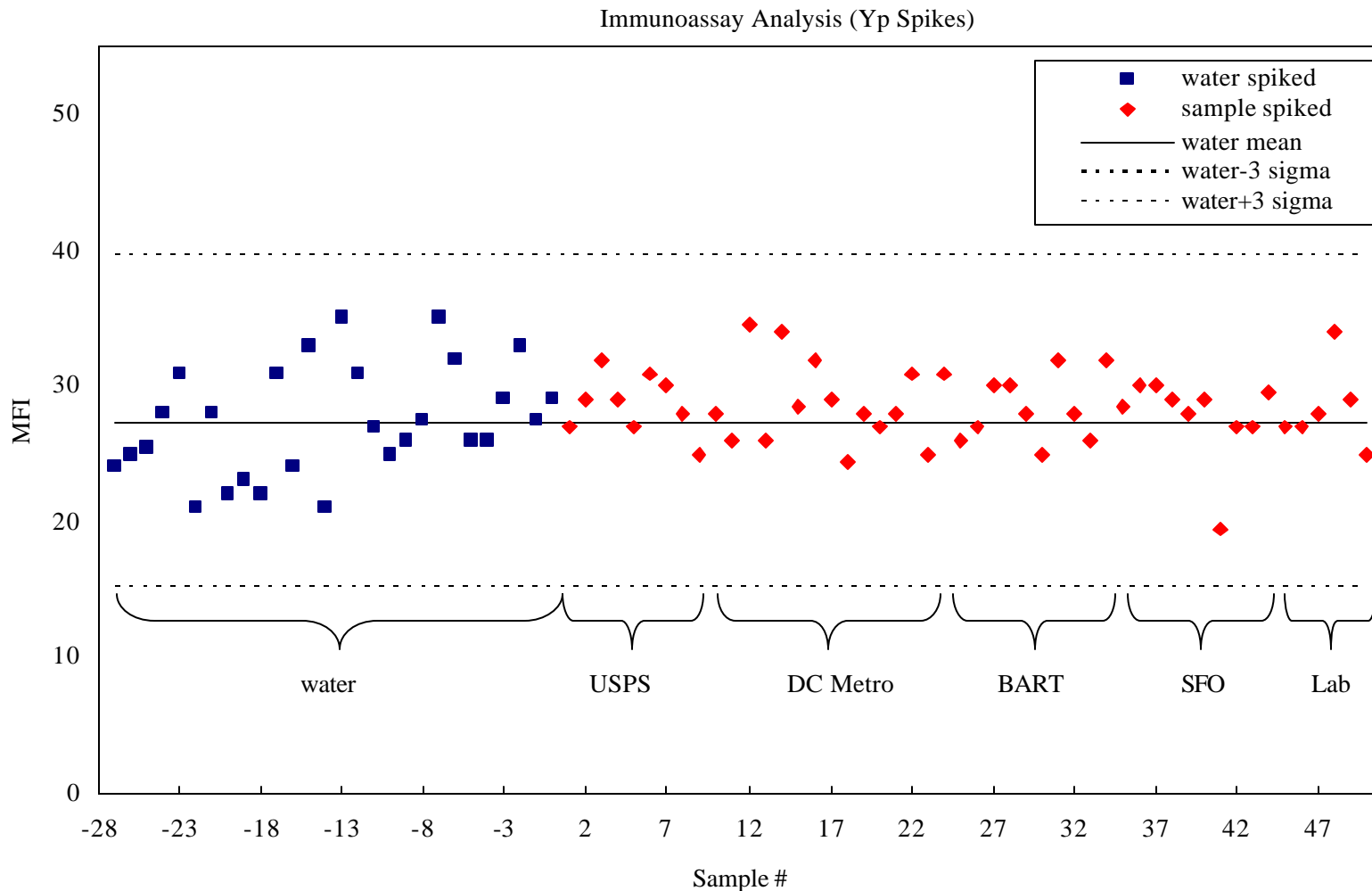
Many different pathogens can be detected in a single assay

Testing with environmental samples shows that the assays are robust

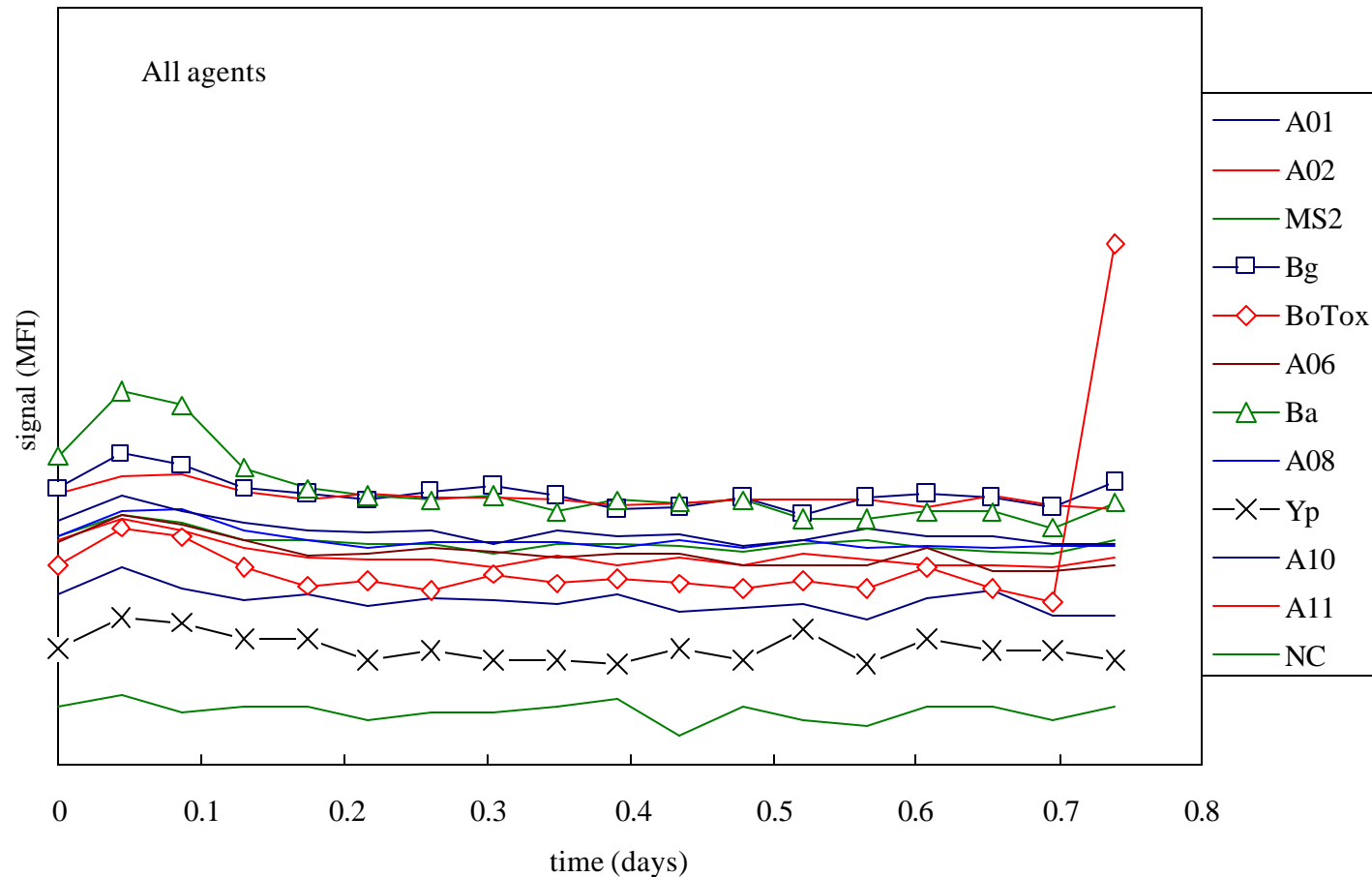
- Wetted-wall cyclone samples obtained in the field...
 - Subways: DC Metro and BART
 - Airports: ABQ and SFO
 - Other: U.S. Postal Service (non-APDS), lab
- ...and compared quantitatively to plain water
 - Immuno and PCR assays run on bench-top
 - Unspiked samples
 - *Yersinia pestis* and *Bacillus anthracis* spiked samples

Immunoassays robust in even the worst environmental samples

- All spiked with same thing, no change in sensitivity.

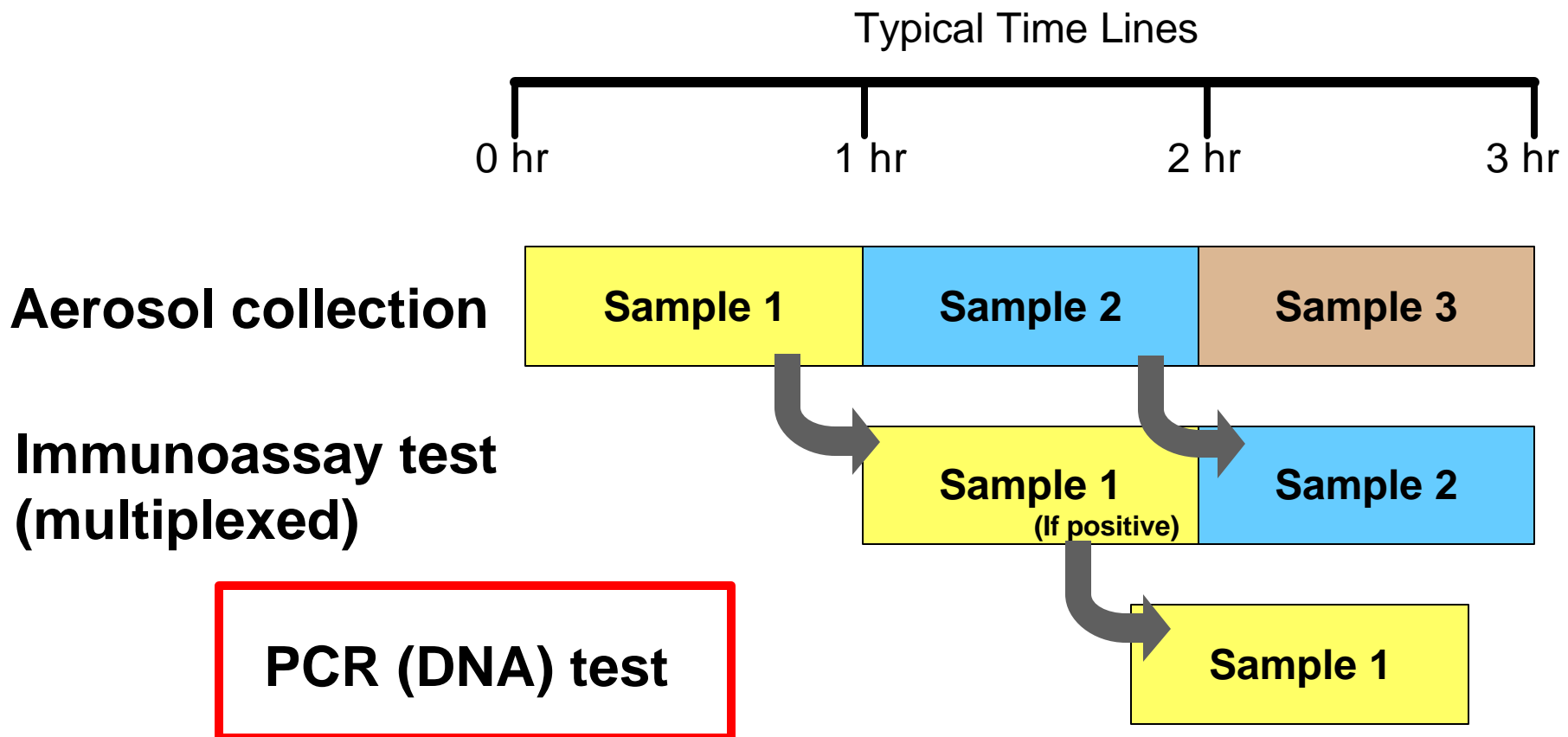


Example of multiplex immunoassay signals from the APDS with a BoToxoid aerosol chamber release



APDS provides laboratory-quality answers within hours of exposure

Past “PCR confirmation” process



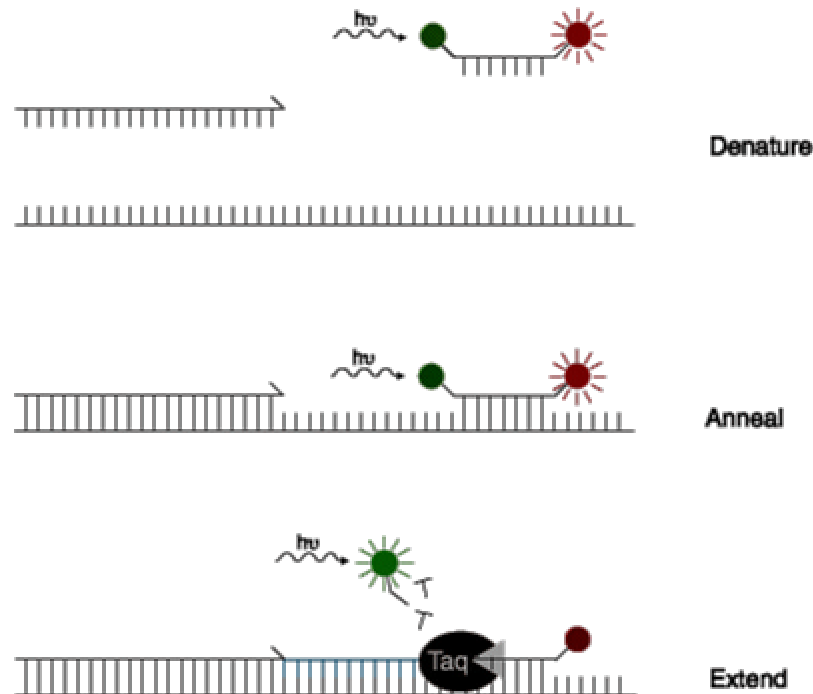
TaqMan PCR is used for confirmation of identified pathogens

- Excellent selectivity and sensitivity
- BASIS and CDC/ LRN signatures currently used
 - Cross-reactivity has been screened out
- Internal controls are used on every sample for high-confidence results
- System error rate is minimized by having orthogonal tests

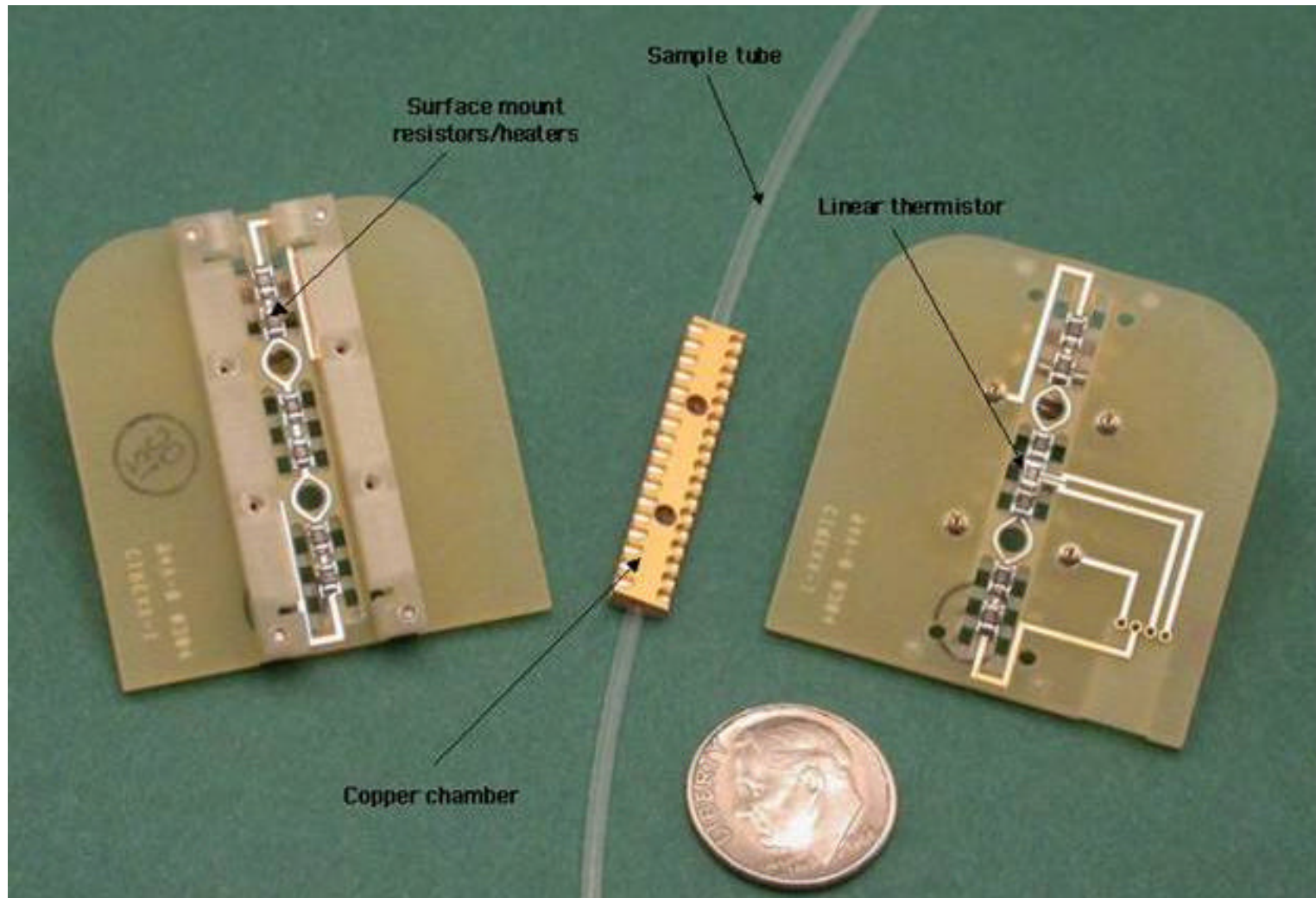
$$p_{\text{FalsePositive}} = p_{\text{FalseIAResult}} p_{\text{FalsePCR}}$$

Orthogonal identification using TaqMan PCR

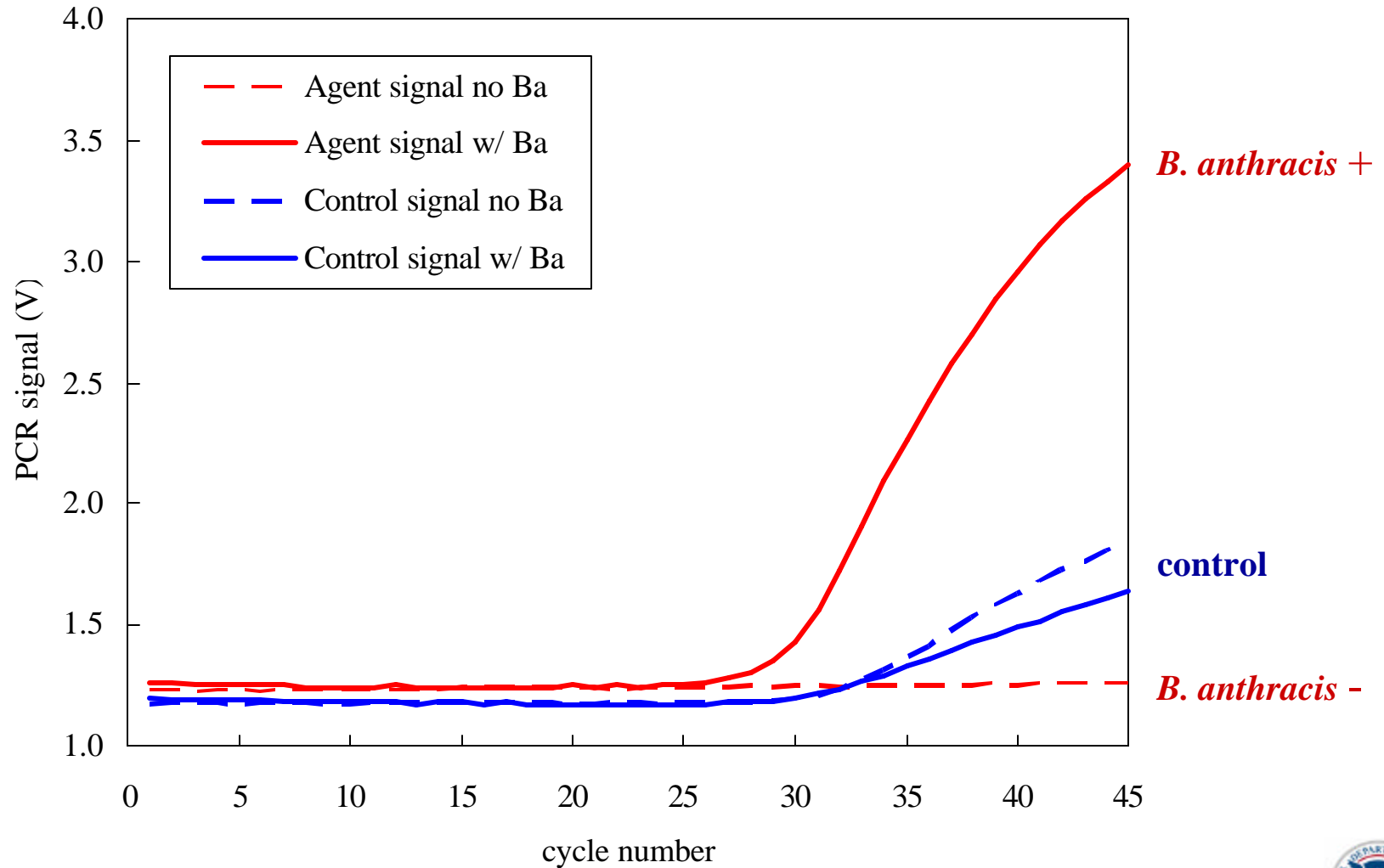
- Uses DNA instead of protein recognition
 - Looking for different signature, so “orthogonal”
 - Tremendous amplification gives great sensitivity
- TaqMan used for confirmatory PCR



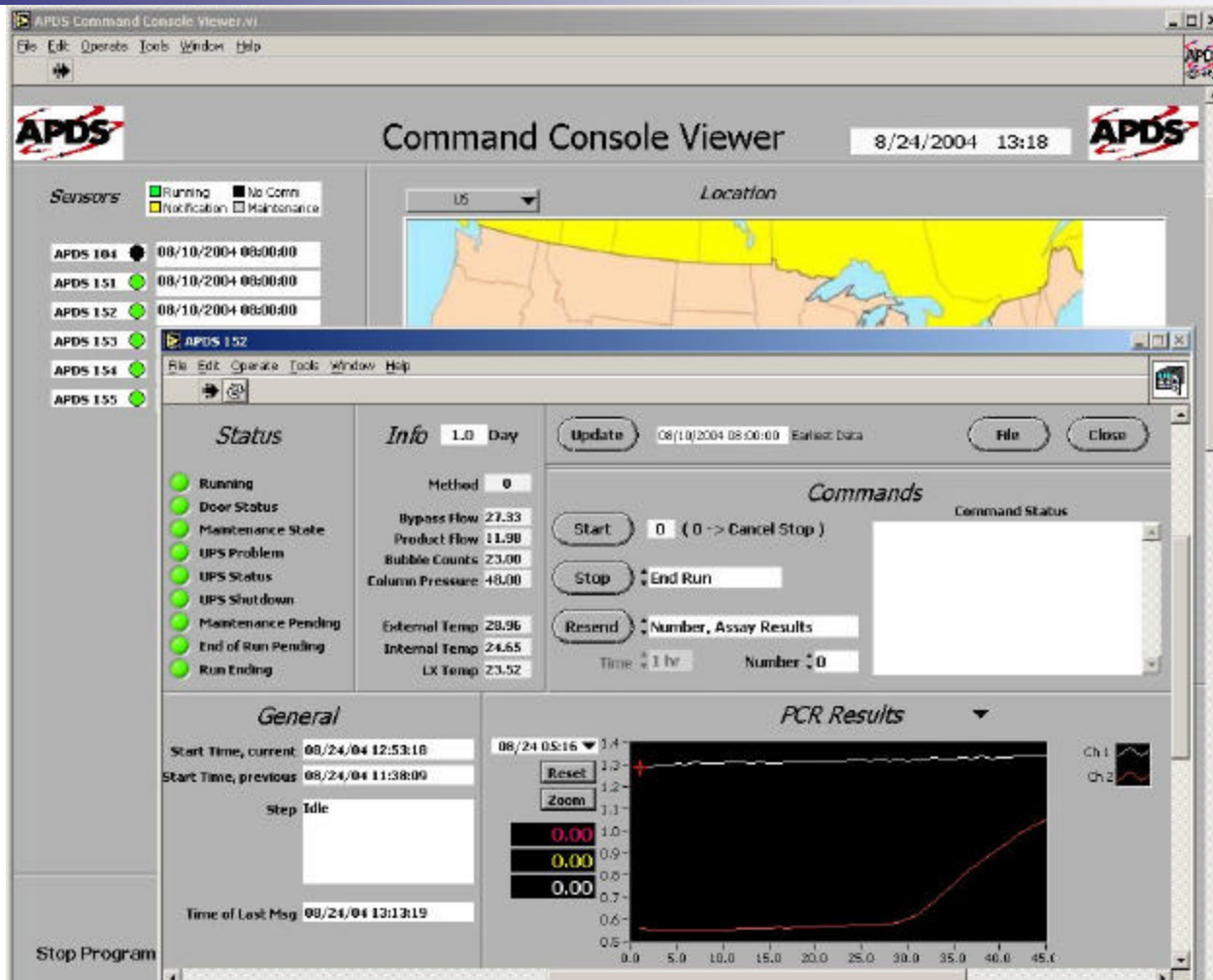
Flow-through PCR module uses a custom but simple surface-mount approach



An internal positive control gives confidence in PCR negatives



APDS network allows remote access to a wide range of system functions and reports



Data-rich command/control helps evaluate alerts

APDS and its components have undergone extensive performance testing on the bench and in the field

- Laboratory
 - Aerosol collection
 - Immunoassay, including environmental samples
 - PCR, including environmental samples
- Dugway Proving Ground
 - Live-agent challenges of immuno. system
 - Killed-agent challenges of immuno.+PCR system
- In-field
 - Airports
 - Subways
 - Facilities

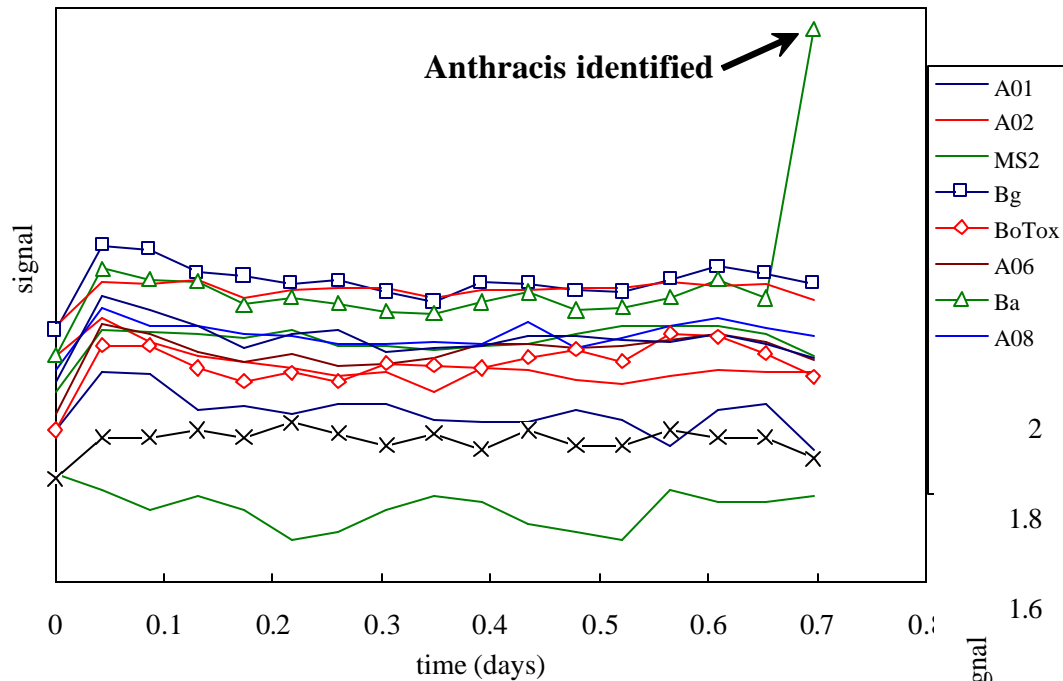
Dugway testing in 2002 and 2003 demonstrated multiplexed immunoassay and PCR confirmation capabilities



- Objectives
 - End-to-end system tests with aerosolized agents
 - Identify multiple agents with a single panel
 - Automatically confirm DNA with PCR
 - Demonstrate DNA extraction module in system

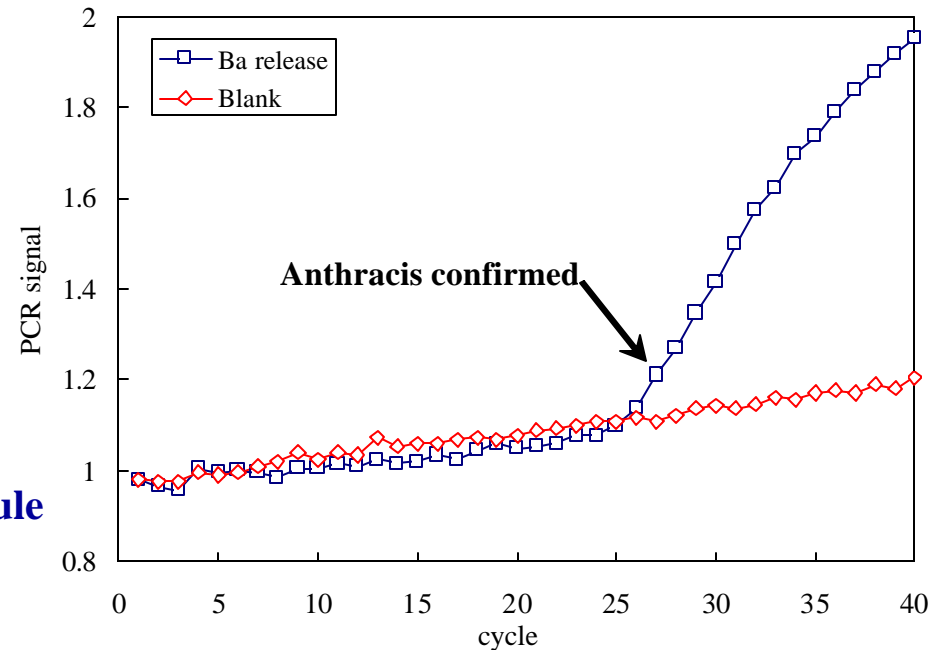
Identification and PCR confirmation of a *B. anthracis* release

Immunoassay



Included DNA extraction module

PCR

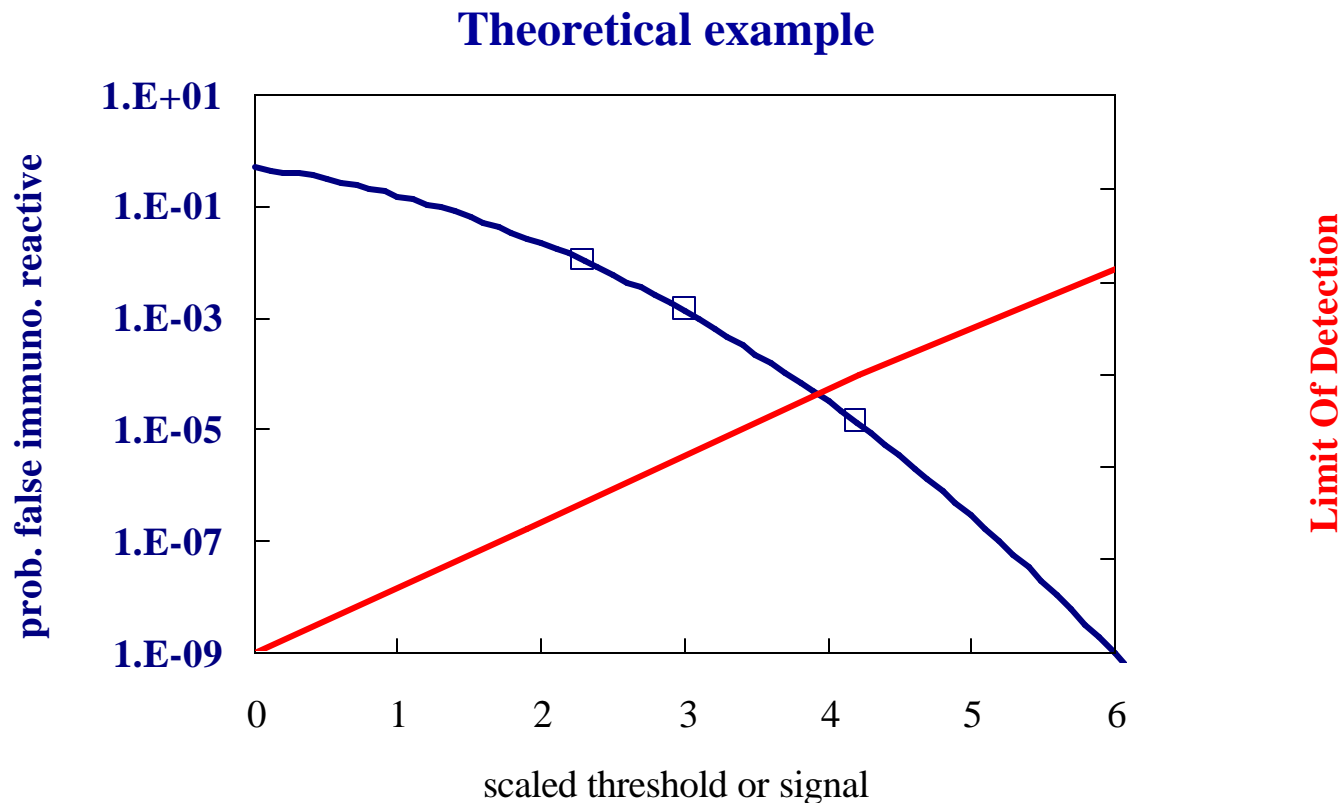


Proven in fully autonomous field testing



- 3 subways
- 2 airports
- Other facilities
- Continuing tests across the country
 - Over 19,000 field samples
 - Over 95,000 assays run

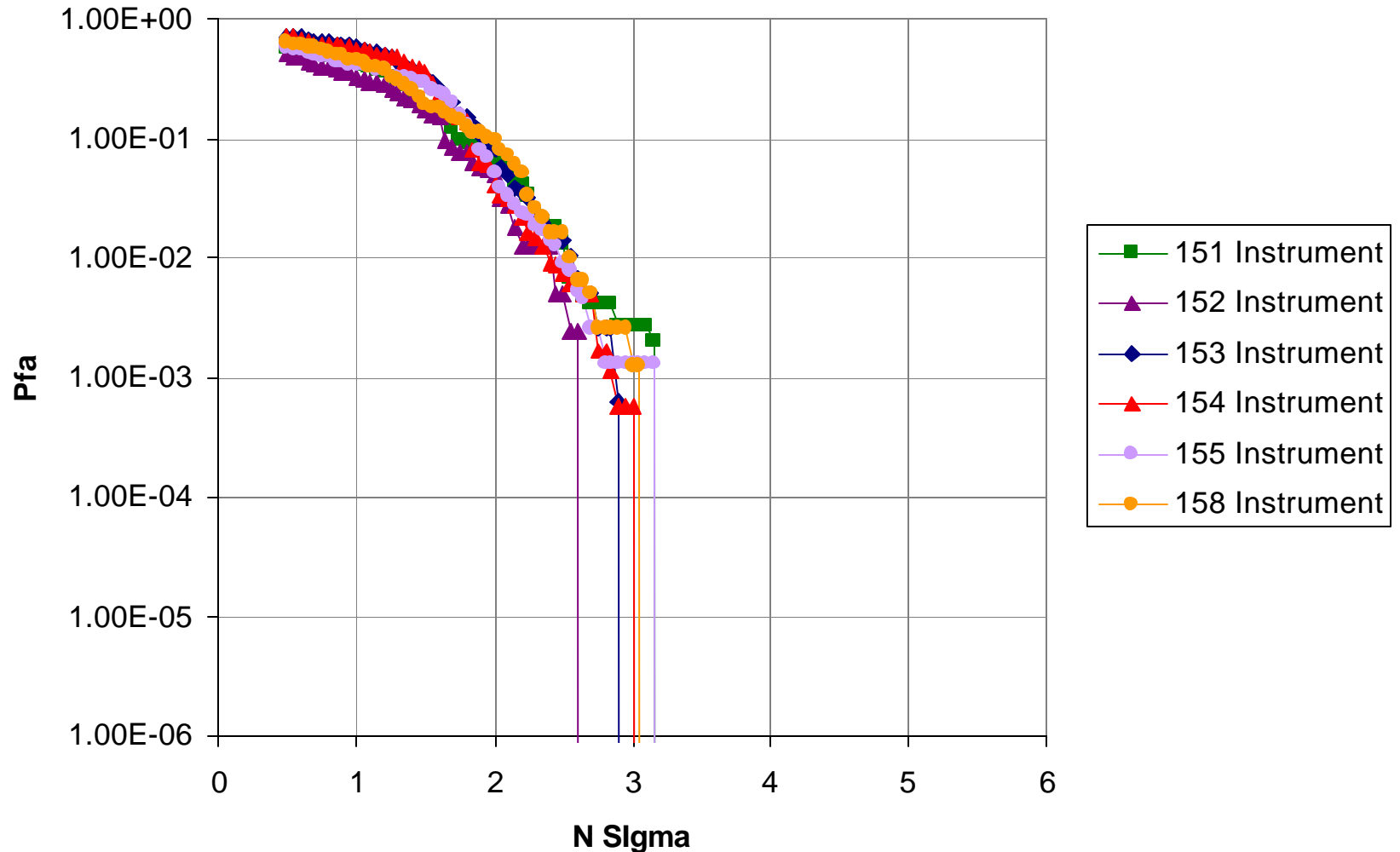
Sensitivity is inextricably linked to error rate through Receiver Operating Characteristic (ROC) curves



Note: Immuno. reactive would normally be checked with PCR

$$p_{\text{FalseSystem}} = p_{\text{FalseImmuno}} \cdot p_{\text{FalsePCR}}$$

Comparing instrument data by analyzing immunoassay positives vs. threshold



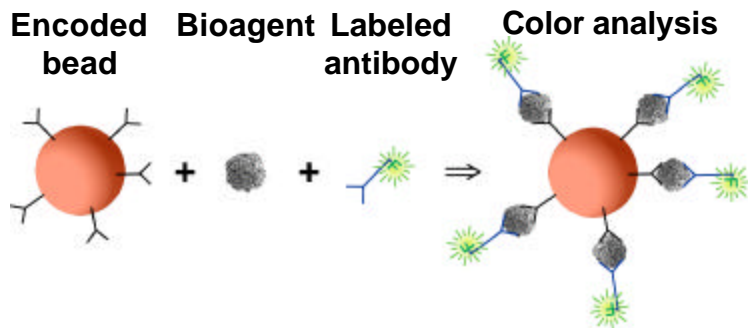
High-quality assays are the foundation of the APDS

- Luminex multiplexed immunoassay screening test
 - CDC Laboratory Response Network assays
 - Includes internal controls for high-confidence results
- TaqMan PCR secondary test
 - CDC Laboratory Response Network signatures
 - Includes internal positive control for high-confidence results
- New assay developments are being added...

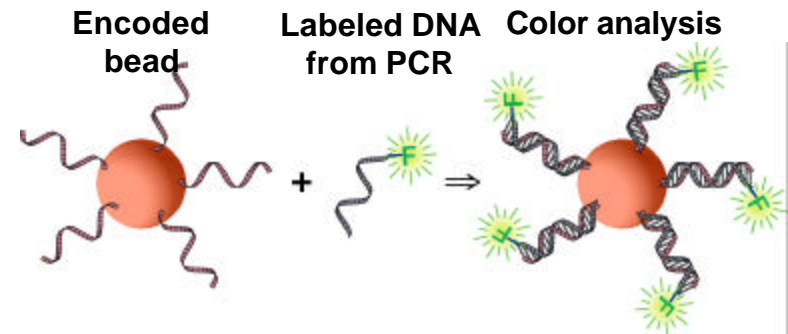
We are integrating a new Multiplex PCR (MuxPCR) capability

- Tests for many DNA signatures at once
 - Signature sets can be based on TaqMan probes & primers
- Readout by hybridization to Luminex beads instead of electrophoresis or microarrays
- Much of the process and hardware are similar to the immunoassay

Current Multiplex Immunoassay process



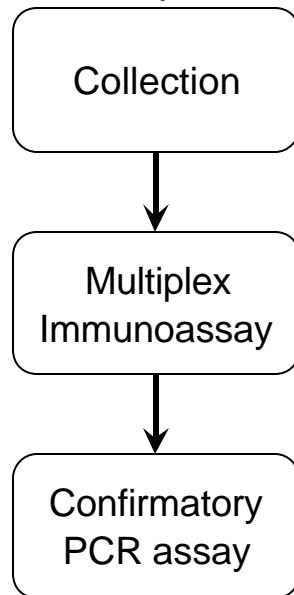
Multiplex PCR process



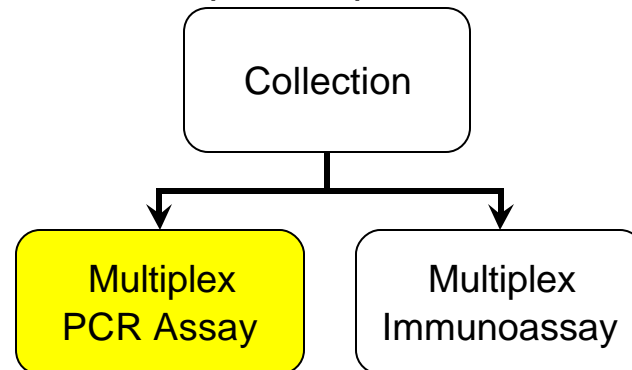
MuxPCR will be a step-change in monitoring capability for the APDS

- Allows running full CDC/ LRN PCR panel on every sample
- Assay development and validation already underway for BioWatch
- Better sensitivity
- Easier reagent sourcing
(chemical synthesis, not animals)
- APDS already has all required types of hardware and software

Current process



Improved process



The APDS Team

